Yashwant Pathak *Editor*

Genomics-Driven Healthcare

Trends in Disease Prevention and Treatment

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ISBN 978-981-10-7505-6 ISBN 978-981-10-7506-3 (eBook)
https://doi.org/10.1007/978-981-10-7506-3

Library of Congress Control Number: 2018945057

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Printed on acid-free paper

This Adis imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Dedicated to the loving memory of my parents, and to Param Pujaniya, Dr Keshav Baliram Hedgewar, and Mananiya Madhukar Limaye, who gave me proper direction; my wife Seema, who gave a positive meaning to my life; and my son Sarvadaman, who has given my life a golden lining.

Foreword

Two general treatment approaches are employed in the pharmacotherapeutic management of disease. One is the “trial and error” approach, employed for drug treatments of diseases such as hypertension, arrhythmias, diabetes, esophageal reflux, depression, and schizophrenia. For these diseases, a small number of drugs provide evidence-based first-line therapies. Finding the most effective drugs for a given patient is often done through “trial and error”, and it can often take months to accomplish the desired therapeutic outcome.

The other approach to the drug management of disease is a “per-protocol” approach, where the treatment for a given disease is essentially the same for everyone with that diagnosis. Examples of diseases treated by this approach include most cancers, heart failure, myocardial infarction, and organ transplantation. In both scenarios, a certain percentage of patients will have no benefit from a given medication, or may experience serious adverse effects.

One of the major reasons for inter-individual variations in drug responses is the presence of genetic variations that result in proteins with variable activities. Polymorphism is the occurrence, in the same population, of two or more alleles at one locus, each with a frequency of >1%. Genetic variations can be a result of RNA splicing; gene transcription; and nucleotide repeats, insertions, deletions, and single nucleotide polymorphisms (SNPs) that can alter the amino acid sequences of the encoded proteins.

Pharmacogenomics is the study of how changes in the genome-wide DNA sequence affect drug responses. This book systematically updates our current pharmacogenomic knowledge of important drug targets, drug transporters, and drug-metabolizing enzymes to better understand currently available evidence for this recently established discipline and its potential application in personalized medicine.

Recent advances in molecular research have revealed many of the genes that encode drug targets that demonstrate genetic polymorphism. These variations, in many cases, have altered the target’s sensitivity to the specific drug molecule, and thus have a profound effect on drug efficacy and toxicity. For instance, the β_2-adrenoreceptor, which is encoded by the *ADRB2* gene, illustrates a clinically significant genetic variation in drug targets. The variable number tandem repeat polymorphisms in the serotonin transporter (*SERT/SLC6A4*) gene are associated with response to antidepressants. With respect to drug transport polymorphisms, the

most extensively studied drug transporter is P-glycoprotein (P-gp/MDR1), but the current data on its clinical impact is limited. Polymorphisms in drug transporters may change a drug's distribution, excretion, and response. Polymorphisms in the cytochrome P450 (CYP) family may have the most impact on the fate of drugs. *CYP2D6*, *CYP2C19*, and *CYP2C9* gene polymorphisms and gene duplications account for the most frequent variations in the phase I metabolism of drugs, since nearly 80% of the drugs in use today are metabolized by these enzymes. Extensive polymorphism also occurs in a majority of phase II drug-metabolizing enzymes. One of the most important polymorphisms is thiopurine *S*-methyltransferase (TPMT), which catalyzes the *S*-methylation of thiopurine drugs. The promise of pharmacogenomics lies in its potential to identify the right drug at the right dose for the right individual.

The role of genomics can be helpful in understanding disease occurrence and progression, the medical needs of specific ethnic/racial groups, and the medical needs of distinctive populations with specific gene pools. Genomics can assist in effective disease management, and finally it can help to achieve optimal care for both healthy populations and patient populations.

The 18 chapters of this book cover various aspects of the application of pharmacogenomics for disease prevention and for better health care.

This area of research is growing significantly and there was a need for such a book. I am sure the scientific community will welcome the efforts of Dr Yashwant Pathak, who has edited this book, and I would like to congratulate him for his hard work in bringing out his volume.

I wish all the best for the book and I am sure it will contribute to the wealth of knowledge in this area.

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Preface

Omics-Driven Trends in Disease Prevention and Better Healthcare

With the advent of "Omics" sciences, such as proteomics, pharmacogenomics, pharmaco-genetics, metabolomics, transcriptomics, and bioinformatics, their application in genomic studies has initiated new pathways in the areas of drug design, discovery, delivery, and disposition, thus creating a knowledge base that will be helpful to build strategies for disease prevention and better health for all.

This book addresses various trends in science that can or will lead to disease prevention, disease mitigation, and better health for the masses, based on the Omics revolution we have been witnessing since the publication of genome studies in the early part of this century. The book covers Omics-driven trends not only in drugs but also in pharmaceuticals for disease prevention and better healthcare. There is also a special focus on nutrition and nutraceuticals.

This book has 18 chapters written by leading researchers in this field and they have addressed various issues related to genomics-driven trends in disease prevention and better healthcare. The chapters deal with pharmacogenomics and pharmacoepigenetics and their impact on therapeutic strategies; drug discovery after the genomic revolution; genomics and transporters in drug discovery, delivery, and disposition; SNP molecular approaches; genomics and metabolizing enzymes; the impact of genomics on drug discovery and clinical medicine; neurodegenerative diseases and genomics; genomics and lung diseases; bioinformatics approaches for better health care, and new approaches for the prevention of diseases, based on genomic knowledge.

I feel this book will be a significant contribution toward the understanding of genomics and its implications in disease prevention and better healthcare.

I would like to thank personally all the contributors whose hard work led to this book—their hard work in providing the chapters on time is greatly appreciated.

My special thanks to Dean Kevin B. Sneed for providing the foreword for this book, and I thank the group of Springer publishers who have worked hard to make this project successful. I also thank the University of South Florida and the College of Pharmacy for providing me with support to complete the book project.

My personal thanks to my family, who always have to sacrifice their time for such endeavors; I always owe them a lot.

My special personal thanks to Carolyn Honour, Cameron Wright, Ellen Blasig, Palani Murugesan, and all the other *Springer Nature* staff who helped to get the book ready for production.

If the readers find any mistakes or have suggestions about improvements, kindly forward these to me and I will try to incorporate any changes in the second edition.

Tampa, FL, USA Yashwant Pathak

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1 Rethinking Drug Discovery and Targeting After the Genomic Revolution

Dharmesh R. Chejara, Ravindra V. Badhe, Pradeep Kumar, Yahya E. Choonara, Lomas K. Tomar, Charu Tyagi, and Viness Pillay

Abstract

The study of the entire genome provides for a complete and better understanding of functional relationship of different genes, genes coding for protein and other regulating sequences, as phenotypic expression is a complex interplay of these and much more. The genomic revolution, in its practically applicable form, is yet to arrive. This genomic sequence information for various organisms, including humans, is now influencing drug discovery which provides opportunity for researchers to develop new drugs/medicines. Major challenges in new drug discovery are to identify targets that are essential for the organism to survive. Several latest technologies have allowed us to understand the mechanisms of disease with respect to biological system concepts, and therefore therapeutic intervention has been developed using informative database and technologies. Therefore, with developing therapeutic interventions, it is imperative for pharmaceutical researchers to rethink about new drug discovery and targeting employing information obtained from the genomic revolution. The chapter is summarized with an outline on the brief introduction on genomic revolution followed by changing scenario in drug discovery and targeting and paradigm shift in the treatment of certain major conditions such as cancer, cardiovascular diseases and tuberculosis in postgenomic era.

Keywords

Drug discovery and targeting · Therapeutic interventions · Pharmacogenomics · Personalized medicine

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_1

1.1 Introduction

The genomic revolution has heralded a new era in pharmaceutical research since its beginnings with the Human Genome Project's (HGP) initiation in 1990 [1]. The genome provides information on life at a molecular level, which can direct drug discovery and development by identifying new targets and categorizing differences between individuals that determine individual reactions to disease and treatment. Databases of this information represent invaluable tools in this endeavour [2].

The cost of DNA sequencing dropped by approximately 14,000-fold between 2000 and 2010, and the genomes of 14 mammals have been sequenced [3]. With the advent of improved profiling technologies and databases, particular genetic studies can be concluded in a matter of days [3, 4]. Genome-wide association studies (GWAS) have exposed variants in DNA that are associated with common autoimmune, cardiovascular and metabolic diseases [3]. The genomic revolution has illuminated the basis upon which molecular interaction facilitates biological function. Determining this molecular activity is the key pursuit in the analysis of genomic data [5]. However, once the activity is known, the application of that knowledge is what becomes important. In the context of drug development, the genomic revolution has allowed unprecedented new insight into how treatments can be tailored or how genetics can be manipulated in order to maximize positive and minimize deleterious effects of substances. With this in view, this chapter focuses on the rethinking of drug discovery and drug-targeting strategies after the genomic revolution. The first section describes the revolution itself; the second describes how this has changed the scene of drug development; and the final section outlines the paradigm shift in the treatment of diseases in the "postgenomic" era.

1.2 Genomic Revolution

The genome represents the physical heredity of life, holding the absolute blueprint for the structure and maintenance of a complete organism. This information is coded in pairs of nucleotide bases (commonly referred to using four letters: A, T, C and G), arranged in sequence in a double helix structure that was first discovered 50 years ago. The human genome comprises three billion such pairs, and the "reading" of the first complete sequence involved an investment of 13 years and US$3 billion by the HGP before its final presentation in 2003. This breakthrough sets the stage for advancing DNA sequencing technology in terms of speed and cost: a genome can now be sequenced in a week for approximately US$10,000, and this is expected to reach as little as US$1000 in the near future [6]. The resulting availability of complete genetic sequences, and the ability to compare these via computer-driven analysis, is sure to provide ever-increasing insights that impact quality of life.

Gene sequencing had been around for a long time prior to the HGP but was generally limited to decoding fragments of DNA at a time, usually related to a known disorder. Analysing the complete genome has yielded a much better understanding of the relationship between genes and regulating sequences; however, the revolution *in its truly applicable form* has only just begun. The HGP provided a definite order

of three billion letters that still needed to be decoded into a language that could be understood and translated for revolution to strike, and that is a challenge for researchers in academia and industry to achieve. As explained aptly by E. Birney while heading a DNA sequencing group, it is like "being given the best book in the world, but it is in Russian and it's incredibly boring to read" [7]. However, the decoding holds much promise in improving diagnosis, prevention and treatment of diseases. Many projects have been unfurled, all laying the road from this big ocean of information towards its interpretation for healthcare benefits.

The HapMap project provided a list for commonly existing variations within human genome [3]. The Roadmap Epigenomics Program of the National Institute of Health (USA) is directed towards deciphering the role and function of genes that regulate the expression of protein-coding genes, controlling their on-off mode at specific times and in tissues [7]. The project Encyclopedia of DNA Elements (ENCODE) is working towards building a comprehensive database of human genome sequences detailing identity and location of all the genes (protein coding and nonprotein coding) [3, 7]. The Cancer Genome Atlas is dedicated towards matching tumour and blood samples from 20 common cancer types [7]. The UK NHS recently announced the opening of 11 Genomic Medicine Centres committed to the completion of the 100,000 genome projects [8]. This project aims to collect and analyse DNA samples of patients with cancer and rare diseases vis-a-vis the medical information of these patients. Such studies will cumulatively provide new insights into disease, hopefully leading to breakthroughs in treatment strategies for generations to come. The research approach of the GWAS combines genomic technologies with traditional epidemiological investigations in order to understand the complexity of environmental factors interacting with genetic factors, to identify genes associated with or acting as risk factors for a given disease.

Genomic studies have broadened and strengthened our understanding of the evolution of man. The genome of Neanderthal man has been mapped using DNA bone fragments. Comparison with the modern human genome revealed 99.5% similarity between the two. Some of the genome segments of Neanderthal man have been identified in humans.

Data and analyses collected over time by naturalists and ecologists can now be correlated to genomic findings to further understand similarities and differences in the pattern of adaptation to environmental changes by different organisms, including humans. To this end, genome drafts of many microbes, plants and animals are already prepared; indeed, sequences of 14 mammals are ready for detailed comparisons [3]. Study in plant genomics has enabled development of crops with resistance to viruses, pesticides and herbicides and with tolerance to drought and flood. This same technology has been applied to microorganisms in the manufacture of foods and therapeutics. The sequencing of microbial genomes has informed the development of novel treatment strategies and diagnostics based on newly discovered targets.

While care must be taken to address ethical, legal and social issues, future generations can be expected to witness a transformed world of healthcare, where medicine may be optimized based on the genome, in coordination with the characteristics of the drug and disease, and preventative lifestyle changes are encouraged to improve overall health.

1.3 Changing Scenario in Drug Discovery and Targeting

Upon completion of the HGP in 2003, a new era for biomedical research has begun. The study of genetics has produced a huge quantity of data, and this has provided the basis for understanding numerous complex diseases [9]. This understanding has, in turn, encouraged the development of technology and medicines that address these diseases. Parkinson et al. reported the impact of the genomic revolution on drug discovery with special reference to the anti-infectives [10]. We will take a similar approach here to discuss changes in the approaches to this development for diseases more generally. Target identification is key to drug discovery, and genomic techniques can also guide drug development in terms of clinical trial inclusion criteria, but genomic techniques can also be applied throughout treatment to tailor drug selection or dosage. As such, considering pharmacogenomics has become critical from drug discovery through to clinical use.

Selecting new targets to treat infectious diseases has presented a major challenge in drug discovery. Despite great investment by the pharmaceutical industry, many drug candidates fail at the point of phase II efficacy trials suggesting a failure to adequately select a chemical that has real-world activity at the target [4, 11].

Several genomic technologies that can be used in target selection are mentioned in Fig. 1.1 and summarized in Table 1.1. These approaches for targeting each come

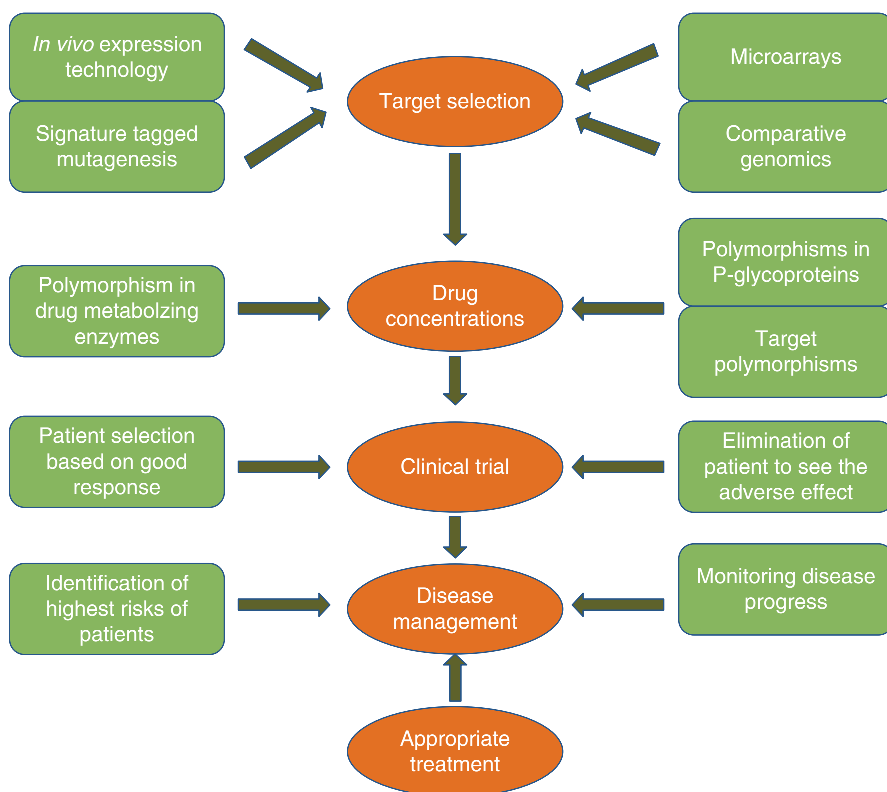

Fig. 1.1 Genomic technologies/approaches and impact thereof in new drug discovery and development

Table 1.1 Genomic technologies/approaches for target selection

Technology/ approach	Use for	Limitation	Modification	Reference
Signature-tagged mutagenesis	Detection of essential genes that are responsible for survival of the pathogen in animals	To identify genes that are essential in vitro	Designing a library of genes under the control of a regulatable promoter or temperature-sensitive mutants	[12]
In vivo expression	Detection of pathogenic genes induced in vivo	Limited to some genes that are essential in vivo	Constructing a combination of the available genome sequences and novel advanced technologies, e.g. bioinformatics, microarrays and proteomics	[13]
DNA microarrays	Identification of in vitro and in vivo grown pathogens as well as host response to pathogens	Limited to only correlative information about gene function	Constructing a combination data derived from high-throughput cell-based assays on existing genome sequence information and other functional genomic techniques	[14]
	Identification of the molecular target of antimicrobial drugs	Limited to identify drug targets		
		Technical use is difficult for the pathogens that are grown in vivo		
Comparative genomics	Identification of the molecular target of antimicrobial drugs	Limited to provide a 3D structure for every protein in an organism	Industries and academic research institutions designed a representative structure for every existing protein fold (~1000)	[15]
	To identify aminoacyl-tRNA synthesis targets in pathogenic bacteria			

with advantages and disadvantages but offer much improved efficiency and over traditional techniques. Several approaches have been modified further according to the requirements, e.g. signature-tagged mutagenesis, in vivo expression, microarrays and comparative genomics (Table 1.1).

1.3.1 A New Pattern for Drug Development in the Form of Pharmacogenomics

Pharmacogenomics affords a new paradigm in drug development. It is a key factor in the personalized medicine concept, as the genomic sequence of an individual provides a lot of information on the anticipated response to a drug [4]. It serves to reduce the risk of unpredictable response to medicinal treatment resulting from genetic polymorphisms and can identify targets for treatment, for example, selecting patients with HER2+ cancers for trastuzumab treatment. As such, pharmaceutical companies have made considerable investment in pharmacogenomics [16]. However, pharma companies and physicians alike are still waiting for the true potential of the field to be realized, in terms of rapid cheap pharmacogenomic testing, capacity for drug discovery, facilitating fast and easy trials in specific patient groups and revival of old drugs for patient subsets with improved adverse event or efficacy profiles [17].

1.3.2 Personalized Medicine for Individual Genomes

Researchers have developed numerous strategies to personalize medicine, and working to identify specific genetic variants within an individual in order to optimize their treatment has had major successes in terms of prevention and cure of disorders. Risk prediction and risk factor modification in cardiovascular diseases, diabetes and cancer, in particular, have been revolutionized by such an approach [18]. Robust biomarkers to inform primary prevention are key in this endeavour, and ongoing developments in pharmacogenetics, pharmacogenomics, pharmacoproteomics and pharmacometabolomics will continue to enhance individualized medicine.

Pharmacogenetics involves the identification of associations between genetics and drug efficacy or toxicity, commonly as a result of drug-metabolizing enzymes such as the cytochrome P450 family, with a view to predicting and preventing side effects when treating individual patients [19]. Pharmacogenomics assesses the mechanism of action of a drug on a cell based on gene expression patterns and is more concerned with drug discovery. Pharmacoproteomics personalizes medicine through a further functional representation of patient-to-patient variation versus genotyping alone. Similarly, pharmacometabolomics observes the functional outcomes of a drug treatment within an individual (Fig. 1.2).

1.4 Paradigm Shift in the Treatment of Disease in the Postgenomic Era

Genomics allows for prediction of disease, identification of disease-influencing loci, evaluation of the function of these loci and elucidation of the resulting mechanisms that can represent therapeutic targets. In the postgenomic era, two new types of treatments have arisen: those where an active compound is delivered in order to

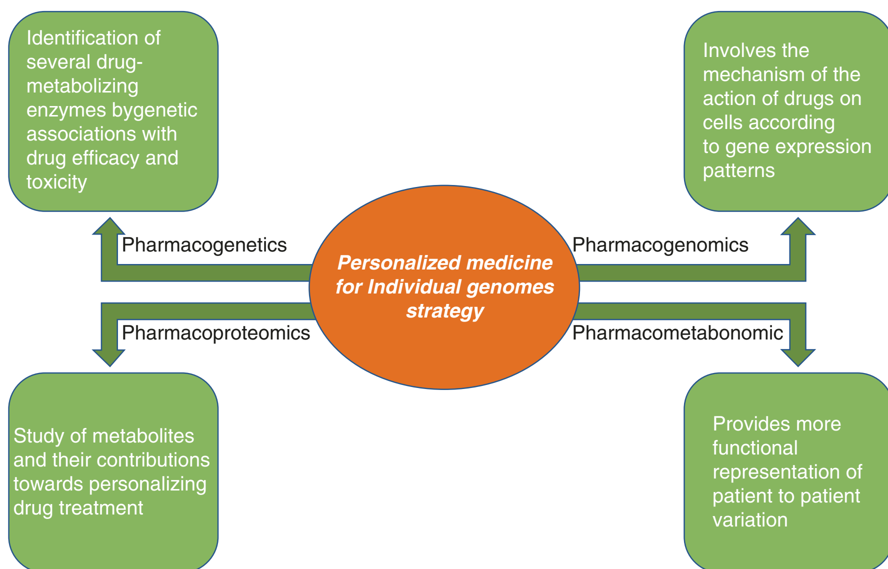

Fig. 1.2 Contribution of the different genomic approaches towards personalizing medicinal treatment

improve body function based on a genetic profile and those where a treatment changes the genes themselves. These have the potential to save millions of lives. Since the genomic revolution, researchers have been hunting for mutations that are responsible for disease and using genome data to inform the development of new treatments. Below, we describe the paradigm shift for therapeutic treatment that occurred after the genomic revolution in terms of a few major diseases.

1.4.1 Shift in Treatment and Drug Development for Cancer

In the 15 years since the revealing of the first human genome, great progress has been made in terms of drug discovery and targeting. In the field of cancer research, mechanisms of tumorigenesis underlie a focus for improved therapeutic efficacy. Genes are followed to their encoded receptor function to inform drug development, and the complete genome sequence revealed a number of such genes to target. Mutagenic changes lead to the growth and proliferation of cancer cells, and often these changes occur in growth factor receptors resulting in abnormal function. Development of cell signal transduction pathway inhibitors targeted towards the translational products of these genes has been studied and achieved successfully. Tyrosine kinase (TK) receptors are an example of this where much progress has been made. These can be membrane bound or cytoplasmic. EGFR is a membrane receptor involved in cellular proliferation. Gefitinib is a small molecule inhibitor of EGFR, which was developed to target EGFR+ lung cancer, and has a response rate

of 71% in these patients versus 1% in those with EGFR- cancer [20, 21]. Chronic myeloid leukaemia (CML) commonly results from a reciprocal translocation between two genes on the long arm of chromosome 22 and chromosome 9 resulting in the formation of BCR-ABL fusion protein with constitutive ABL kinase activity. Imatinib was approved in the USA for CML patients in 2001, and studies have reported a survival rate of approximately 90% [22, 23]. Given its general TK inhibitor mechanism, imatinib has subsequently been used in gastrointestinal stromal tumour patients with c-Kit, a receptor tyrosine kinase mutation, with a response rate of 50%, compared with a 5% response rate to conventional chemotherapy [24–26]. A list of targeted cancer therapeutics with gene and genetic alteration information is summarized in Table 1.2.

Overexpression of a gene can result from amplification, whereby the copy number of a gene is increased. A tandem amplification in the Her2 gene, leading to overexpression, is often seen in breast cancers. Trastuzumab is a monoclonal antibody that targets HER2 and has efficacy in this cancer [27]. Ras genes are thought to be mutated in approximately one third of cancers, with Ras mutations observed in 30–40% of thyroid cancers, 50% of colon cancers and 90% of pancreatic cancers, respectively [28]. There has been little success in targeting Ras genes to date, but current strategies focus on inhibiting the FTase [29]. Lonafarnib and tipifarnib are selective inhibitors with this mode of action and have shown efficacy in preclinical testing, with hopes they might proceed into later-stage clinical trials [30–32].

Table 1.2 List of targeted therapeutic agents with gene and genetic alteration information for the treatment of cancer

Genetic alteration	Gene	Therapeutic agent
Overexpression	Ras	Lonafarnib
	Hsps	Ansamycin, geldanamycin
Overexpression	Aurora A and B kinases	MK-5108 (VX-689)
Overexpression	Polo-like kinases	BI2536, GSK461364
Mutation, amplification	*EGFR*	Gefitinib, erlotinib
Mutation, amplification	*EGFR2*	PKC412, BIBF-1120
	ALK	Crizotinib
Mutation	*Cox*	Celecoxib, rofecoxib
	PDGFRA	Sunitinib, sorafenib, imatinib
	c-KIT	Sunitinib, imatinib
	BRAF	SB-590885, PLX-4032, RAF265, XL281
	BRCA1 and *BRCA2*	Olaparib, MK-4827 (PARP inhibitors)
Amplification	*ERBB2*	Lapatinib
	c-MET	Crizotinib, XL184, SU11274
Translocation, mutation	*FGFR3*	PKC412, BIBF-1120
	RET	XL184
	JAK2	Lestaurtinib, INCB018424
Translocation	*FGFR1*	PKC412, BIBF-1120
	PDGFRB	Sunitinib, sorafenib, imatinib
	ABL	Imatinib

Endothelin is a small peptide produced by endothelial cells and plays an important role in differentiation and proliferation of cancer cells. Atrasentan targets the endothelin receptor and has undergone phase III evaluation for prostate cancer [28, 33, 34]. Bevacizumab inhibits vascular endothelial growth factor-A (VEGF-A), which promotes angiogenesis within tumours [35]. Cyclooxygenase-2 (COX-2), an endoplasmic enzyme that mediates the conversion of arachidonic acid into inflammatory prostaglandins (PGs), has also been targeted as a potential cancer treatment. PGs enter the cell nucleus and can impact disease progression. Celecoxib, rofecoxib and valdecoxib are examples of selective COX-2 inhibitors that have been evaluated in clinical trials [36, 37]. Heat-shock proteins (HSPs), often referred to as molecular chaperones, help to prevent nonspecific aggregation of protein and maintain cell architecture under stress. While many studies report no difference in expression of HSPs between normal and cancerous cells, cancer cells appear more susceptible to HSP inhibition due to interaction with various cofactors. Ansamycins such as geldanamycin inhibit the function of HSP90 by binding to the N-terminal pocket. Alvespimycin was developed as an oral analogue, with improved bioavailability and longer plasma half-life. Retaspimycin reached phase III trials before being discontinued due to side effects. Over 50 trials of HSP inhibitors are ongoing [38, 39].

1.4.2 Shift in Treatment and Drug Development for Cardiovascular Disease

Cardiovascular disease is one of the most common causes of morbidity and mortality, accounting for approximately 30% of deaths worldwide [40]. However, recent years have seen the development of impressive therapies for reducing risk of cardiovascular disease. Statins, in particular, have dramatically reduced disease risk, especially in the secondary prevention context. Beyond statins, few drug classes exist for the treatment of cardiovascular disorders (angiotensin receptor blockers, brain natriuretic peptide mimetics, glycoprotein IIb/IIIa inhibitors and direct rennin inhibitors [41]), and, of course, novel therapies to remediate the remaining risk are still actively sought. Primary obstacles to drug development in this field have historically included a scarcity of accepted surrogate biomarkers for cardiovascular outcomes and a lack of tools to directly observe the vascular effects of promising drug candidates [42]. In the postgenomic era, significant advances have emerged in terms of genotyping, mRNA profiling, proteomic maturation and metabolomic methodologies, which helped to overcome these issues [11].

The genomic revolution allowed for the honing of preclinical mouse models of atherosclerotic disease, using candidate gene approaches like transgenic and gene knockout mice, and open system approaches such as gene expression profiling, proteomics and genetics [43, 44]. This opened up a myriad of opportunities for target identification and validation for CVD drugs based on mouse and human genetic profiling and in developing surrogate biomarkers. Ganesh et al. have reported several new findings for Mendelian diseases, and the clinical treatment thereof, with special reference to the inherited arrhythmias, aortic aneurysms, cardiomyopathies

and congenital heart defects [45]. Coronary artery disease, stroke, hypertension and hypercholesterolemia have also been discussed [46]. GWASs and next-generation sequencing methods have been used to identify novel genetic determinants of CVD (Table 1.3) [51–55].

Table 1.3 Targeted genomic approaches identified by the whole genome sequencing

Name of new approaches	Use	Limitation	Modification	Reference
Pharmacogenetics	For idiosyncratic, unpredictable type B reactions, for instance, abacavir, and the hypersensitivity syndrome	Some drugs were never approved because of drug-induced liver injury	Identification of a high-signal	[47, 48]
			Marker and identification of such genetic risk factors offer the possibility of improving the safety profile of prescribed drugs	
			Advances have been made in identifying pharmacogenetic biomarkers	
Drug-gene interactions predicting efficacy	Used for candidate's gene study where a genetic variant in gene modified the gene response to specific drugs in GWAS	Effect sizes are very small and much difficult to separate from random variation in individual patients	Finding of such a drugs like metformin which is less important for its potential clinical applications than for the biological insight provided by this link between glucose control and a gene involved in the response to DNA damage	[49, 50]
Guidelines for genetic testing	Used for key criteria which include analytic validity, clinical validity and clinical utility	Gives selected outcomes for several drugs, e.g. clopidogrel, warfarin, etc.	Determination of genetic tests in patients with the positive care and less side effects for those who are taking clopidogrel and warfarin	[45]
			Recommendation for use of some drugs for those having the same indication along with similar risk-benefit profile	

In the postgenomic era, several research articles have explored the complexity of cellular and molecular interactions involved in cardiac remodelling [56]. However, a limited understanding of the mechanisms of myocardial recovery had meant that little information could be taken from clinical trials. Systems biology has recently been used to speed up target identification and improve clinical trial success (Fig. 1.3a).

Reductionist experimental approaches are used to create formal associations between different molecular/cellular moieties and phenotypes, and systems biology is implemented to understand these interactions. Network theory describes the nature of how the interactions between genes, proteins and metabolites mediate functional changes at the level of cells, tissues and organs. In visualizing a network, individual components can be represented by circles and lines used to connect those components that interact with one another (Fig. 1.3b). Some may have few interactions, but others may have many, creating a hub-like structure that suggests an important role in regulatory processes may be present (Fig. 1.3c). Where these components are found to be not currently modulated by existing therapeutics, they can represent a new target. The approach has been applied in pursuit of new treatments

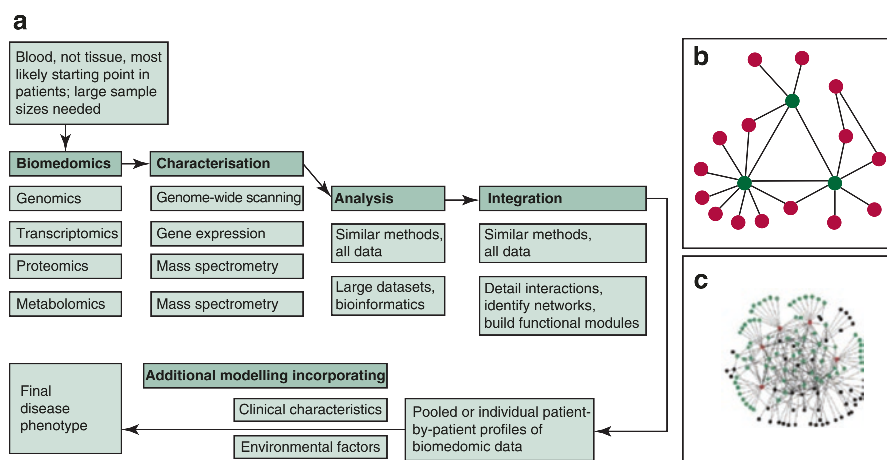

Fig. 1.3 Application of a systems biology approach for the discovery and development of cardiovascular disease. (**a**) Systems biology entails a series of steps, beginning traditionally with advanced characterization of genomic, transcriptomic, proteomic and metabolomic datasets, which are then analysed by various bioinformatic approaches. Systems biology places emphasis on definition of interactions, delineating networks linking proteins, genes or metabolites and describing functional units or sets to provide testable mechanistic models of clinical phenotypes. (**b**) A simple, scale-free, gene network composed of nodes (depicted by circles) with many edges (depicted by lines) that represent the interaction between nodes. (**c**) A complex scale-free network, with most nodes having one or two edges and a few nodes (shown in red) having many (termed hubs). This high degree of connectivity guarantees that the system is fully connected [Adapted from Shah and Mann, 2011 [57] with permission from Elsevier B.V. Ltd. © 2011]

for heart failure but does have some limitations [58, 59]. It needs to be advanced using new datasets, afforded by new sequencing technologies, so that researchers have a more reliable "guidebook" to inform their development decisions wherein all biological components of the disease pathway are accounted for and understood. Once the economic constraints and the multidisciplinary skillset required for the approach are addressed, systems biology will become invaluable in developing new treatments for heart failure.

1.4.3 Shift in Treatment and Drug Development for Tuberculosis

Tuberculosis is one of the most deadly infectious diseases on the planet, with nine million new cases and 1.5 million deaths reported annually (WHO). With whole genome sequencing of the causative bacteria (*Mycobacterium tuberculosis*, Mtb) completed in 1998, expectations were high for the development of novel therapies. Biochemical screening failed to produce effective candidates due to poor penetration or bacterial drug efflux, when target inhibitors were applied to live bacteria. Whole-cell screening has the advantage of interacting with the entire bacterial genome/proteome/metabolome at once. The pharmaceutical company GlaxoSmithKline recently identified 177 active compounds with activity against Mtb using whole-cell screening [60]. With the improved capacity for detecting SNPs or other mutations that confer resistance to specific strains of bacteria, new targets can be identified for novel therapeutics. Some targets that whole genome sequencing has yielded are listed in Table 1.4.

There are several structural and therapeutic classes of anti-TB compounds which are capable of inhibiting Mtb and even the multidrug-resistant (MDR) Mtb infections. Below is a list of some leading anti-TB compounds with promising Mtb intervention profiles:

Table 1.4 List of the targets identified by whole genome sequencing for TB drug discovery

Target gene	Drug/inhibitor	Sequence technology
atpE	Bedaquiline (TMC207)	454
Ddn	PA-824	NimbleGen
Ddn	Delamanid (OPC67683)	Not specified
dprE1	BTZ043	ABI-Sanger
dprE1	DNB1	ABI-Sanger
dprE1	VI-9376	ABI-Sanger
dprE1	377790	Illumina
dprE1	TCA1	Illumina
inhA	Pyridomycin	Illumina
mmpL3	SQ109	Illumina
mmpL3	AU1253	SOLiD
mmpL3	THPP	Illumina
mmpL3	Spiro	Illumina
mmpL3	BM212	Illumina
mmpL3	C215	Illumina
qcrB	Q203	Illumina
qcrB	IP3	Illumina

1. Bedaquiline (TMC207), a diarylquinoline drug, was approved by FDA in 2012 for the treatment of multidrug-resistant (MDR) Mtb infections. The drug targets a SNP in the bacterial gene atpE, which encodes the C chain of the ATP synthase of Mtb [61].
2. The benzothiazinones (BTZ) are sulphur-containing heterocyclic compounds that have been evaluated in vitro and in animals for their anti-TB activity. BTZ043 targets DprE1, which is involved in arabinan synthesis for the Mtb cell wall. Inhibiting DprE1 results in the discontinuation of arabinogalactan synthesis which leads to cell lysis [62].
3. Imidazopyridines are amides that inhibit the growth of Mtb by targeting the respiratory cytochrome bc1 complex. Promising results have been obtained using these compounds in murine models of Mtb infection [63, 64].
4. SQ109 inhibits the synthesis of mycolic acid and the recently identified transmembrane transporter of trehalose monomycolate, MmpL3 [65, 66].
5. Thiophenes inhibit polyketide synthase (Pks13), which is an essential enzyme for the synthesis of mycolic acid [67].
6. Pyridomycin is a natural prodrug that is activated by KatG to form the active isonicotinic acyl-NADH complex. This inhibits the FASII enoyl-ACP reductase (InhA) in a different manner than the classic Mtb drug isoniazid and shows activity in isoniazid-resistant strains.
7. Ethionamide is another prodrug that inhibits InhA and is activated by the mycobacterial monooxygenase EthA [68]. EthA is controlled by the Mtb repressor gene EthR. Willand et al. described improved ethionamide activity when co-administered with an inhibitor of EthR, BDM31343 [68].
8. EccB3 is a conserved protein component of the ESX-3 secretion system of Mtb, which is essential for growth in vitro and as such represents a future target [69, 70].

These new strategies might come up with novel unconventional targets for the development of new classes of anti-mycobacterial compounds that may act under in vivo conditions in synergy with conventional drugs (Table 1.5).

Table 1.5 List of identified new chemical entities with the development of antituberculosis drugs

Drug/ inhibitor	Class of drug	Hit identification strategy	Mechanism(s) of action	Mechanism(s) of resistance	Target(s) confirmed
PA-824	Nitroimidazoles	Whole-cell screening of metronidazole derivatives	Inhibition of cell wall synthesis and interference with cell respiration by NO production	Mutation in the nitroreductase Ddn required for prodrug activation	No
OPC-67683	Nitroimidazoles	Whole-cell screening for mycolic acid biosynthesis inhibitors	Inhibition of mycolic acid synthesis and NO production	Mutation in the nitroreductase Ddn required for prodrug activation	No

(continued)

Table 1.5 (continued)

Drug/ inhibitor	Class of drug	Hit identification strategy	Mechanism(s) of action	Mechanism(s) of resistance	Target(s) confirmed
TMC207	Diarylquinoline	Whole-cell screening from quinolone derivatives	Inhibition of ATP biosynthesis	Mutation in the c subunit of ATP synthase, other(s)	Yes
Q203	Imidazopyridine amide	Phenotypic screen in infected macrophages	Inhibition of the cytochrome bc1 complex	Mutation in the b subunit of the cytochrome bc1 complex	Yes
SQ109	Diethylene diamine	Whole-cell screening of ethambutol derivatives	Inhibition of mycolic acid biosynthesis, other(s)	Mutation in MmpL3	Yes
BTZ043	Benzothiazinone	Whole-cell screening	Inhibition of arabinogalactan biosynthesis	Mutation in DprE1	Yes

Conclusion

Genomic technologies and approaches are chief in order to develop biological understanding and medicine after the genomic revolution. The present summary has demonstrated the challenges in drug discovery and identification of new targets for these therapeutics. It has also described several of the latest technologies and approaches with potential application to understanding the mechanisms of disease, in pursuit of true biological system concepts. The development of new drugs will likely be accelerated through the practical implementation of these technologies and approaches. It may take a long time for novel genomic drugs to enter into the market. However, there has been much improvement in drug development using genomic technologies, and open access to genomic data profiles has had a positive effect on the progress of drug development.

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2 Living Between Sickness and Health: Where Is the Human Genome Leading Us?

Amy Broadwater and Yashwant Pathak

Abstract

In recent years, much research has been done on genetic links to disease risk and development. The human genome contains thousands of SNPs or single nucleotide polymorphisms. These SNPs account for much of the variance between people; therefore, it makes sense that the SNPs could also reveal the variance in health between people. This chapter delves into common diseases that appear later in life and potential genetic risk factors for the diseases. Recent research has created ties between genome polymorphisms and cancer, cardiovascular health, and mental health. Identification of the polymorphisms could lead to a future of personalized medicine, where a patient's genome would be used to assess risk of illness and prevent complications to the fullest extent. In some disease states, such as breast cancer, genomic research is already being used clinically to identify women at high risk of development. In other disease states, research is only just beginning to identify genetic loci that could be responsible for development. This chapter is meant to answer the following question: how much of our health can we control, and what role does genetic variation play in disease development?

2.1 Introduction

Genetics is by far one of the most interesting components of cellular and molecular biology. DNA is the instruction manual for the cell. It controls how proteins are made, and it creates the different types of RNA needed for cellular function. By controlling these components, it can control the phenotypic variations of the

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_2

organism as a whole. Why does a person have brown eyes? Because the DNA has coded for proteins that produce a brown pigment in the iris. A person with blue eyes would have DNA that could not create this pigment: their blue eyes are actually a lack of pigment in the iris. The most interesting and important component of this example is *why* their eyes are different colors.

Humans share 99.6% of their genetic makeup. This means that 99.6% of one person's DNA is *identical* to everyone else in the world. Every difference between humans, be it skin color, eye color, hairiness, or facial structure, can all be attributed to the 0.4% difference in genomes. The thought of this is astounding! More miraculously, these variations in the genome are not all clumped together on one piece of DNA. Humans have 23 pairs of chromosomes, and on each of these chromosomes, thousands of genes could be encoded. On a gene, the entire sequence may be identical between all humans, with the exception of one base pair that could be different. These one base-pair variations are referred to as SNPs, or single nucleotide polymorphisms, and these are much of the cause for variation among humans. Please refer to Fig. 2.1 for a visual example of SNPs.

Back to the eye color example, let us assume that eye color is very basic genetically, and one gene, called the eye color gene, controls the color of the iris, either brown or blue. On the eye color gene, there is one known SNP with two variations. Variation 1 (Fig. 2.1, Man 1) creates a functional pigment protein; thus the person's eyes are brown. Variation 2 (Fig. 2.1, Man 2) codes for a nonfunctional pigment protein; thus the person's eyes are blue. Although this is a very basic example, this is the foundation for human variation: in reality, complex phenotypes such as eye color are the result of combinations of SNPs on various genes.

Now, let us apply this concept to human diseases. A researcher can determine the color of an individual's eyes simply by looking at their DNA and at which SNPs they have on certain genes. Why can't diseases be the same? Can a researcher look at a patient's SNPs and determine what diseases that person is at a higher risk of

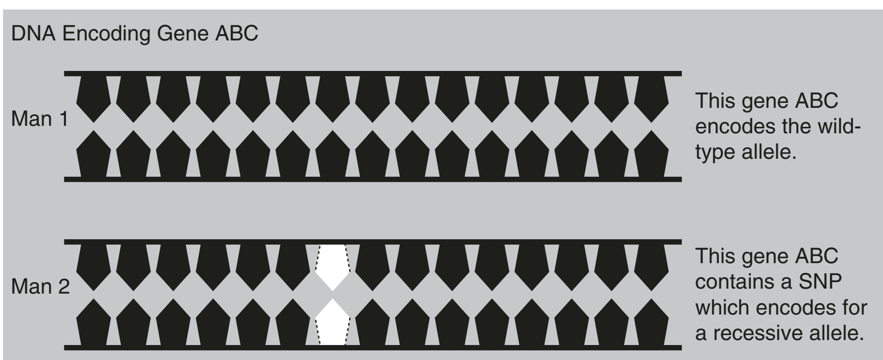

Fig. 2.1 Man 1's DNA for gene ABC contains the more common base pairs encoding the gene. Man 2's DNA contains a SNP, or single nucleotide polymorphism, indicated by the white base pair. Because of this SNP, the expression of the gene in Man 2 will be different than the gene in Man 1. SNPs cause variation in phenotypes

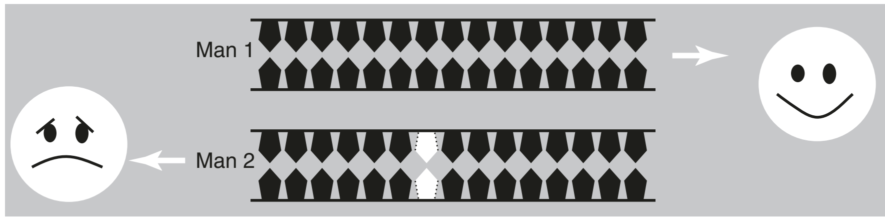

Fig. 2.2 The wild-type, dominant SNP in Man 1 has resulted in normal health. However, the variation in genetic code for Man 2 has resulted in higher risk of disease development and poor health. This shows how a single nucleotide change in an important gene can have profound effects on the body and overall health

developing (Fig. 2.2)? That is the topic of the following chapter: **Living Between Sickness and Health: Where Is the Human Genome Leading Us?**

This chapter will go over recent research on a variety of diseases with genetic links from 2011 to 2016. The focus of the chapter is on diseases that are undetectable at birth and develop later in life. Examples include various cancers, cardiovascular diseases, and mental illnesses. The goal of the chapter is to elucidate how much control the genome has over one's health, a sort of variation on the classic nature versus nurture question. Is a disease caused by a patient's environment/experiences, or is the disease inevitable as it is written into the patient's DNA? The answer is not so black and white.

In the not-too-distant future, physicians will be able to look at the SNPs in an individual's DNA and determine for what diseases they are at risk. This chapter will go over what potential SNPs those future physicians may look for when determining a patient's risk. Many of these SNPs are still in the preliminary stages of proving their relations to a disease, while some others are already being used clinically for risk assessment, such as BRCA1 and BRCA2 for breast cancer risk.

The language of SNPs in some of the following studies may be confusing or complicated. The typical SNP language used in research is rs_____, where the blank is a string of numbers. Additionally, the researchers may refer to a specific gene, such as the BRCA1 gene listed above, or an area of a chromosome, such as 3q26.2. There are several references that can be used for further personal research and clarification on the SNP, gene, additional studies, and possible clinical uses. PharmGKB (at pharmgkb.org) and SNPedia (at snpedia.com) are excellent resources that can be used to expand on the knowledge stated in the chapter [1, 2].

Hopefully, this chapter will be thought-provoking as it delves through various diseases with potential genetic ties. The information is as much fact as it is philosophical. Do humans have control over their fate, or is it written into their DNA from the beginning? Even if we could determine what ailments we may develop later in life, is that the information that we *should* know? Once we have found a genetic cause of disease, is prevention even possible with today's technology? Only time will tell where the human genome is leading us.

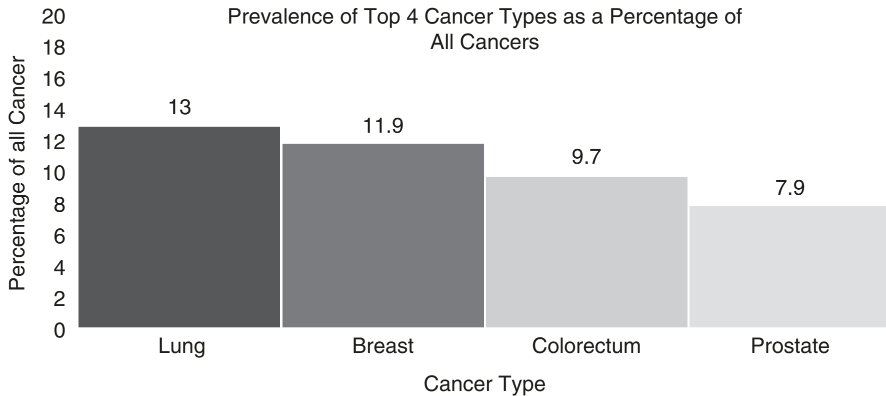

Fig. 2.3 A graphic representation of the top four cancer types according to the National Cancer Institute [4]. The frequency of cancer types is listed as a percentage of all cancers

2.2 Cancer

Cancer has affected the life of almost every person: one does not have to look too far to find someone who has suffered from cancer. According to the American Cancer Society, the risk of developing any type of cancer is 42.05% and 37.58% for males and females, respectively; the risk of dying from cancer is 22.62% and 19.13% [3]. Recent evidence has emerged that shows a link between cancer and genetic predisposition. This section will focus on various genotypes that can change the risk of cancer development, specifically of lung, breast, prostate, and colorectal cancers: four of the top common cancer types according to the National Cancer Institute (Fig. 2.3) [4].

2.2.1 Lung Cancer

Lung cancer is the most common type of cancer today [4]. It accounts for 19.4% of cancer mortalities, with about 1.6 million deaths every year [5]. While lung cancer is typically thought to be developed because of exposure to chemicals, as in smoking or construction, genetic sequencing has shown that an individual's genome can have a profound effect on the development of lung cancer. Within the past decade, a large number of genome-wide assessment studies (GWAS) and candidate gene studies have identified 241 SNPS thought to be involved with lung cancer development [6].

With 241 identified SNPs, there is no doubt about the complexity of the genome's contribution to lung cancer development. Researchers work to find links between the genetic variation and the RNA or protein polymorphism and then a link to how these polymorphisms could affect normal physiology of the cell. miRNA, for example, is involved with regulatory processes in lung cancer cells. The SNPs in the coding region for miRNA, rs9660710 and rs763354, have a significant effect on the odds of developing lung cancer: the first SNP increasing the odds of development and the second decreasing the chance of development. However, these SNPS do not have an effect on the survival rate of lung cancer [7].

Lung cancer may also be caused by other diseases, like COPD. In COPD, the lungs are chronically inflamed, genetically unstable, and susceptible to infection. Often, COPD can lead to lung cancer in the long run. Out of the patients who smoke, those with COPD have three to ten times the risk of developing lung cancer compared to patients without COPD [8]. The impact of COPD on lung cancer is too great to ignore. Researchers wonder: do the underlying genetic factors in COPD development contribute to the eventual development of lung cancer? One possible explanation is the PARK2 gene, which has been shown to have such a link. PARK2 encodes Parkin, which has a role in inflammation, and a PARK2 deficiency may have a significant role in genomic instability. A case control study revealed that the PARK2 SNPs, rs577876, rs6455728, and rs9346917, had a link between COPD and lung cancer development. PARK2 polymorphisms may soon be an important tool for early diagnosis and prevention of lung cancer development in patients with COPD [9].

2.2.2 Breast Cancer

Breast cancer has a very strong link to genetics: being related to a direct female who develops breast cancer almost doubles the likelihood of development, and every relative who is affected by the disease increases the risk even more [10]. To summarize, the more direct relatives develop or have developed breast cancer, the higher the risk of breast cancer, and the earlier in life development may occur. The high stakes of the disease being inherited makes genetic markers an excellent and important tool to assess the risk of development in a patient.

The most important genetic markers identified are the breast cancer susceptibility genes: BRCA1 and BRCA2 (Fig. 2.4). These genes are responsible for reconnecting a double-strand break, when both strands of DNA are broken, through homologous recombination [11]. These are high penetrance genes, meaning that the presence of this gene mutation increases the chances of development of cancer more

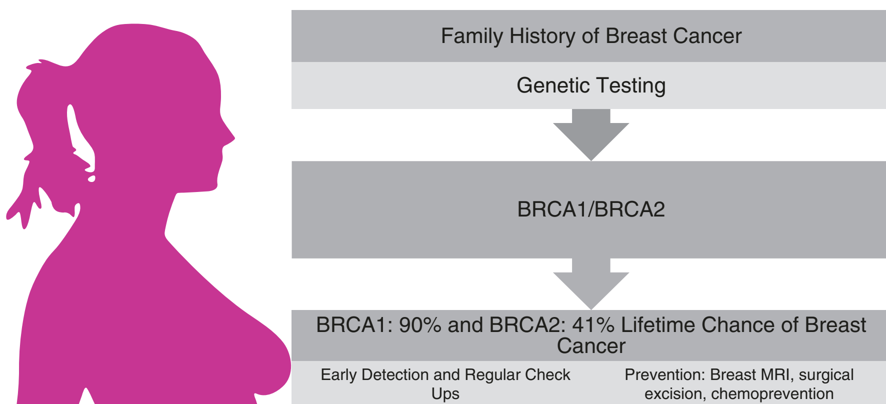

Fig. 2.4 While BRCA1 and BRCA2 are only the cause of 3–6% of breast cancer instances, genetic testing in a woman with a family history of breast cancer in multiple women is an important aspect of prevention. Once diagnosed with these genes, the patient should be treated more aggressively, as the lifetime rates of cancer increase drastically

than four times. These genes account for 3–6% of breast cancer, and the cumulative risk up to the age of 80 for cancer is 90% for BRCA1 and 41% for BRCA2. These genetic mutations may also increase the risk of developing other types of cancers, such as fallopian tube, periodontal, ovarian, colorectal, and pancreatic cancers [12].

In patients with BRCA1 and/or BRCA2 mutations, early detection and regular checkups are key to preventing negative outcomes. MRI scans of the breasts, surgical excision as a preventative measure, and chemoprevention, using antiestrogen drugs, are some of the ways to decrease the risk of the patient developing cancer. The most effective preventative measure that can be taken is a bilateral mastectomy, which reduces the risk to nearly zero. Additionally, the genetic mutations have a drastic increase on the risk of developing ovarian cancer. Precautions should be used to ensure the patient is not only being assessed for breast cancer but also for other cancer risks [12].

While BRCA1 and BRCA2 are the most major and well-known gene mutations that may cause breast cancer, other genetic mutations can increase the risk to a lesser extent or create an additive effect to the risks of development. These genetic mutations include TP53, CDH1, PTEN, and STK11. These genes are high penetrance, similar to BRCA, but are very rare to inherit. The CHEK2 gene is another gene that can be inherited; however, this gene has only intermediate penetrance [12].

A 2016 study found another genotype that can cause an increased risk of breast cancer. RAD51B promotes the binding of RAD51 to a double-strand break in DNA to begin the repair process. The study tested the coding region on the DNA for RAD51B and included the introns and exons in patients with breast or ovarian cancer. It was found that the alleles rs2588809, rs1314913, and rs999737 were associated with breast cancer risk, with the highest risk for cancer when all three SNPs were observed [13].

2.2.3 Prostate Cancer

Prostate cancer is one of the most common cancer types in men. It begins when the prostate begins to enlarge uncontrollably, and some of the cells become abnormal [14]. The risk of prostate cancer increases with a family history of the disease: 5–10% of all prostate cancer diagnoses are from inheritance [15]. This suggests a strong genetic component to developing the disease.

In recent years, genome-wide assessment studies have identified over 100 possible genetic associations with prostate cancer development. However, the International Consortium for Prostate Cancer Genetics performed a study that identified six previously identified SNPs as being strongly associated with prostate cancer. These SNPs were located in regions 3q26.2, 6q25.3, 8q24.21, 10q11.23, 11q13.3, and 17q12. The most common SNP found in patients in the prostate cancer cohort was rs138042437 in the 8q24.21 region [16].

Another study found that microRNA (miRNA) could be associated with a higher risk of prostate cancer. miRNA is important for posttranslational modifications and other biological processes in the body. It has been suggested that a SNP in a miRNA-coding region may affect how well the RNA can participate in its biological role; the differing alleles may even increase the risk for cancer development. The CC genotype of miR-499 rs3746444 was shown to increase the risk of developing prostate cancer as opposed to the TT genotype. Other polymorphisms of miRNA did not show any association with risk of prostate cancer [17].

Further testing needs to be done to verify and strengthen the results of the above studies. Both research groups stated that a more diverse and larger cohort should be used in order to have an adequately powered study that usable results can be obtained from. Nevertheless, although the genetic influences on prostate cancer are difficult to elucidate, the current information on the subject is an excellent stepping stone to discovering clinically useful testing and potential treatment sites.

2.2.4 Colorectal Cancer

Colorectal cancer is the cancer of the large intestine and/or rectum. It is the third most common type of cancer affecting both men and women in the United States [18]. There are two types of hereditary colorectal cancer: familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome. These types of colorectal cancer make up 5–10% of incidences [19]. Currently, testing can be done to determine a patient's predisposition to developing colorectal cancer by detecting MLH1, MSH2, APC, MSH6, PMS2, and MUTYH mutations, which have been proven to be linked to colorectal cancer development [19].

FAP testing is done by detecting the APC gene. Inactivation of one APC allele is known to drastically increase the chance of cancer formation, and the inactivation of the second APC allele is the rate-limiting step to cancer development. Once the cancer has begun developing, it progresses at a rapid rate. HNPCC testing is done by detecting the genes MSH2, MLH1, PMS2, and MSH6; major mutations in these genes further push the rate of HNPCC development [20].

A recent genome-wide assessment study identified additional risk loci for colorectal cancer, at 6p21.1, 8q23.3, 10q24.3, and 12p13.3, as well as two new variants at 10q25.2 and 20q13.3. Using this data, researchers discovered potential new colorectal cancer genes: TFEB, EIF3H, SPSB2, and PRS21. The discovered variants and loci have effects on the aforementioned genes that control expression of the genes in ways which can contribute to the risk of developing cancer. The genes control biological functions that range from homeostasis to translation, protein synthesis, and degradation. This shows the range of homeostatic processes that can contribute to colorectal cancer if not working properly [21].

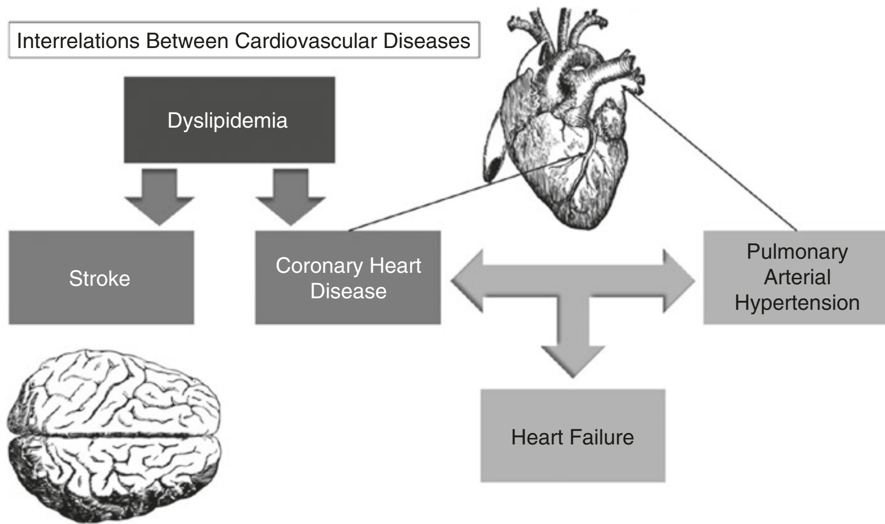

Fig. 2.5 Diseases of the cardiovascular system are reliant on one another; development of one cardiovascular disease can cause a cascade effect if left unchecked. The above cardiovascular diseases are explored in detail in this chapter. Because an individual may have genetic risk factors for these diseases, early detection through genetic testing is an important component of prevention and treatment

2.3 Cardiovascular System

Previously, lifestyle choices, such as poor physical health and diet, would have been to blame for a heart attack or stroke. However, recent studies have shown that this may not be the case with certain patients. While lifestyle does come into play with these diseases, genetic predisposition to development of the disease may also be to blame. Cardiovascular diseases, such as coronary artery disease (CAD), pulmonary arterial hypertension (PAH), stroke, and dyslipidemia, have been shown to have strong connections to the genome; an individual with a certain genotype may have a much higher risk of developing these diseases (Fig. 2.5). In this section, recently discovered genetic connections to the aforementioned disease states will be discussed.

2.3.1 Coronary Heart Disease

CHD occurs when plaque builds up in the coronary arteries, the arteries that supply the heart muscle with oxygen. As the plaque deposit increases in size, the artery becomes progressively narrower, resulting eventually in a complete blockage and causing a myocardial infarction [22]. Risk factors for development of CHD include genetic predisposition, smoking and alcohol consumption, stress, diabetes, hypertension, and low levels of physical activities [23]. To combat the blockage of

essential arteries, angiogenesis, or the development of new blood vessels, occurs to ensure that the blocked area continues to receive blood flow. Recent research has suggested that VEGFA (vascular endothelial growth factor A) and VEGFR2 (vascular endothelial growth factor receptor 2) have a significant impact on the neovascularization involved in CHD [24].

A 2016 study in China sought to discover the connections between various SNPs on the VEGFA and VEGFR2 gene and CHD, while also adjusting for the compounding variables, or additional risk factors as discussed above. The researchers compared 810 CHD patients to 805 healthy individuals with the goal of clarifying which genomes were more susceptible to the development of CHD and identifying appropriate treatment strategies. The VEGFA SNPs demonstrated a higher risk of CHD in patients with T allele of rs3025039, A allele of rs1570360, and C allele of rs699947, as well as the genotype TT/CT of rs3025039. The VEGFA genotypes of rs1570360 and rs699947 demonstrated a decreased risk of CHD. As for the VEGFR2 polymorphisms, rs2305948 (C > T) and rs1870377 (T > A) showed significant correlations with the development of CHD, while the polymorphism rs7667298 (A > G) showed a decreased risk of CHD development. The confounding factors, including smoking, alcohol consumption, diabetes, and hypertension, were all studied in comparison to the varying SNPs. The results varied between the SNP, confounding variables, and an increase or decrease in risk. Overall, the risk factors were found to aggravate CHD development and complications. Polymorphisms in VEGFA and VEGFR2 had significant correlations with the risk of development of CHD and therefore would make excellent clinical markers for the disease [24].

Another recent 2016 study found a different suspicious gene that could be potentially involved with CHD risk. MnSOD, or manganese-dependent superoxide dismutase, is found in the mitochondria and prone to polymorphisms; this enzyme helps to control and relieve oxidative stress. Because some of the underlying factors of CHD are from oxidative stress, the various polymorphisms of MnSOD can determine the underlying genetic risk for development of CHD. It was found that the Val/Val genotype of MnSOD showed a higher risk of CHD than the controls. The author noted that the study had a low power; therefore, further testing should be done before this enzyme could be considered an appropriate clinical tool to assess the risk of development of CHD [25].

2.3.2 Pulmonary Arterial Hypertension

Pulmonary arterial hypertension, or PAH, is a condition in which there is high blood pressure in the artery connecting the lungs and the heart. The blood in this vessel flows with oxygen-rich blood from the lungs into the left atrium of the heart. This condition places a lot of strain on both the heart and the lungs; the higher the pressure, the greater the strain. Eventually, the stress on the heart results in right heart failure [26]. PAH can be idiopathic or hereditary, which points to a genetic cause.

It has been recently uncovered that epigenetics may have a major role in the risk and development of PAH. Epigenetics are changes in the expression of a gene

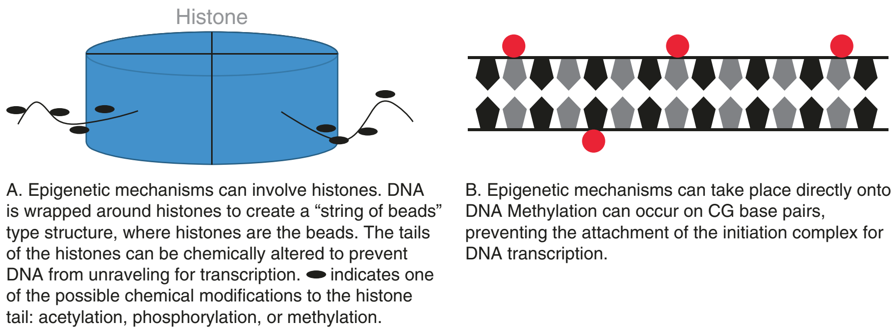

Fig. 2.6 Two mechanisms of epigenetics are outlined above. Through these methods, cells can alter gene expression without changing the content of the DNA sequence

without changing the sequence of nucleotides that can be inherited or created as a result of interactions with the environment (Fig. 2.6) [27]. While the epigenetic model of PAH is still considered only a hypothesis, recent findings of epigenetic influence on the genes involved with superoxide dismutase 2, granulysin, histone 1 levels, and others (genes involved with the progression of PAH) have all supported and strengthened this hypothesis. Despite these findings, PAH is incurable, and evidence points to a more complicated etiology, including predisposing factors and injury in addition to epigenetics. However, the usage of epigenetic knowledge of PAH may provide a path to treatment and a cure in the future [28].

2.3.3 Stroke

Ischemic stroke is usually caused from the same root health issue as coronary heart disease: a blockage of a blood vessel cuts off circulation to, in this case, the brain. After a certain period of time without blood flow, the neurons in the affected region in the brain begin to die. For this reason, a stroke of any kind can be extremely debilitating, resulting in handicap or death. The high stakes of having a stroke make this disease an ideal candidate for determining genetic links: if a patient is at high risk, they can take precautions to decrease their risk of stroke.

A study published in 2016 worked to elucidate which genes and SNPs could put a patient at high risk for developing an ischemic stroke. It was noted that many of the genetic mutations that could put a patient at risk for developing CHD also put them at risk for developing an ischemic stroke. ABCA1, or adenosine triphosphate-binding cassette transporter A1, was chosen as the target of the study: this protein is involved with moving cholesterol across a membrane from peripheral tissues to apolipoprotein acceptors [29]. It was found that a SNP on 9p21, rs4977574, was associated with a higher risk of ischemic stroke, while the SNP for the ABCA1 protein, rs2740483, was found to have a decreased risk of ischemic stroke.

Additionally, carriers of the rs4977574G allele had a higher risk of stroke, while noncarriers of the rs2740483C allele had a higher risk of stroke [30]. These finding would prove useful for assessing the risk of ischemic stroke by finding the genotype of the patient.

Another 2016 study performed a genome-wide association study to identify new markers for ischemic stroke risk, focusing on large artery atherosclerosis, or large-vessel ischemic stroke. It was found that five SNPs in the PTCSC3 (papillary thyroid carcinoma susceptibility candidate 3) were significant with regard to stroke risk. These SNPs were rs2415317, rs934075, rs944289, rs2787417, and rs1952706 [31]. While no conclusions were made about risk associated with each SNP, the most important finding was that the PTCSC3 pathway could have an effect on the risk of ischemic stroke. The study opened up the door for further studies to expand on the information found and pinpoint the effects that each allele has on ischemic stroke risk.

2.3.4 Dyslipidemia

Dyslipidemia, or as it is commonly known, high cholesterol, is one of the biggest contributing factors to heart attack and stroke. In 1% of Caucasians, dyslipidemia can be inherited: when two or more direct relatives have dyslipidemia, it is called familial combined hyperlipidemia [32]. This type of dyslipidemia is defined as an elevation in total cholesterol, triglycerides, or both.

The inheritance behind familial combined hyperlipidemia is polygenetic in nature. Many different genes contribute to hyperlipidemia, including APOE, LIPC, and APOA5, which contributes to lipid metabolism. Variants of these genes may cause a diminished efficacy of lipid metabolism in an individual. Additional genes that have been found to contribute to dyslipidemia include the UBRI, MTHFD2L, and PIGV-NR0B2 region, although the roles of these genes in lipid control are still unknown [33].

2.4 Mental Illness

When it comes to mental illnesses such as depression or bipolar disorder, it is already well known by healthcare professionals that there is some level of heritability with the disease. In depression, a patient may have receptor polymorphism which could affect how serotonin works in their brain. However, when it comes to specific polymorphisms, there is still much more research and discovery that needs to be done. The same could be said about other mental illnesses, such as Alzheimer's, Parkinson's, and schizophrenia. Researchers devote their entire career to elucidating the genetic causes of these mental diseases, but this field of study is underfunded and therefore difficult to progress. The following section will go over some of the recent findings of genetic causes in the mental illnesses mentioned above.

2.4.1 Depression/Bipolar Disorder

The origin of depression is still relatively unknown. According to the Mayo Clinic, a variety of factors could be involved, including biological differences, brain chemistry, hormones, and inherited traits [34]. Although researchers are still unsure of the etiology, depression is a complex disease with widespread effects. Major depressive disorder affects more than 15 million adults ages 18 and older in any given year [35].

There are a variety of medications available to treat depression, including selective serotonin reuptake inhibitors and tricyclic antidepressants, to name a few. Recent research has been working toward the goal to clarify not only the etiology of depression but the wide range of responses in patients using common antidepressant medications. A recent hypothesis points to receptor polymorphisms in the brain. The ABCB1 gene, which encodes P-glycoprotein, is responsible for transporting molecules such as xenobiotics across membranes; of particular interest, P-glycoprotein is also responsible for transporting antidepressants across the blood-brain barrier [36].

The C3435T polymorphism in the ABCB1 gene points to a predisposition for ineffective treatment in patients with the CT or TT genotypes; however, the severity of the depression symptoms is higher in the CC genotype. The more T alleles a person has, the lower the severity of their symptoms, but treatment options will also be less effective. More research needs to be done to assess the other SNPS on the ABCB1 gene and potentially identify a connection between them and depression [37].

Major depressive disorder and bipolar disorder are very similar, with one exception. In bipolar disorder, the patients have periods of major depression, broken up by periods of mania. According to the National Institute of Mental Health, the prevalence of bipolar disorder among adults in America is 2.6%, with 82.9% of these cases being classified as severe bipolar disorder [37]. As in depression, the etiology of bipolar disorder is unknown; however, some genetic links to bipolar disorder may have been elucidated.

Eight hundred three SNPs that could be potentially related to bipolar disorder were found. These SNPs belonged to genes that were shown to be related to transmission of signals in the brain [38]. Of these SNPs, specific combinations were more common in patients with diagnosed bipolar disorder. Bipolar patients all had the SNP YWHAH_rs10495832 as well as a combination of three other SNP genotypes. Complex diseases are expected to have multiple genes contributing to the illness, and delving into combinations of SNPs as a link to a disease is intensive and difficult. These combinations of SNPs were ones only found in patients with bipolar disorder, but studies involving multiple SNPs are new and rare. These polymorphisms may be the genetic link to bipolar disorder that researchers are seeking, but more testing is needed before the information can be taken to the level of risk assessment and diagnosis [39].

2.4.2 Parkinson's Disease

Parkinson's disease can be attributed to a familial cause or a sporadic cause. Family history has already been identified as a risk for development of Parkinson's disease. Over 30 different gene loci have been identified as contributors to disease development, even across different ethnicities. Some of the most notable genes include PARK16, SBT1, SNCA, LRRK2, GBA, and MAPT [40]. Of these genes, it is possible that combinations of their SNPs contribute to not only the development of Parkinson's but the severity of the disease as well.

The most notable gene-gene interaction found thus far has been between LRRK2 R1628P × GBA L444P. This combination showed significant effects on the Unified Parkinson's Disease Rating Scale, including effects on behavior/mood, activities of daily living, and motor skills. There were also effects on the Hoehn-Yahr stage, showing an increase in severity of symptoms. Other gene-gene interactions may have similar effects, but the confounding variables of studies so far have rendered that data insignificant [41].

Regardless, many SNPs have been proven independently to be linked to an increased risk of Parkinson's development. SNPs in LRRK2 have been shown to be the most common genetic link to the disease in both sporadic and familial Parkinson's [42]. This gene, along with the GBA gene, plays key roles in the pathology of Parkinson's disease and the formation of Lewy bodies [43].

As mentioned above, GBA, or glucocerebrosidase, has a strong link to Parkinson's disease etiology. GBA encodes a lysosomal protein involved in glycolipid metabolism. Mutations in this gene can lead to a dysfunctional protein and cause susceptibility to Lewy body formation. The variants N370S and L444P occurred more often in patients with Parkinson's disease versus in patients without. The utilization of these variants may be useful for early diagnosis and preventative treatment in the future [44].

2.4.3 Alzheimer's Disease

The polymorphism of the APOE gene is the strongest genetic risk factor for Alzheimer's disease thus far [45]. APOE, or apolipoprotein E, is a protein responsible for forming lipoproteins that can be used for purposes such as cholesterol transport. In Alzheimer's disease, the e4 version of the APOE gene can increase a person's chances of developing the disease. Two e4 alleles increase the risk of development even more. Although a link between the APOE gene and Alzheimer's has been established, the mechanism is still unknown. The allele may be linked to a higher number of amyloid plaques in the brain, contributing to the death of neurons (Fig. 2.7) [46].

Another gene, CLU, or clusterin, shares many of the same functions as APOE and plays an important part in amyloid plaques affecting brain function [47].

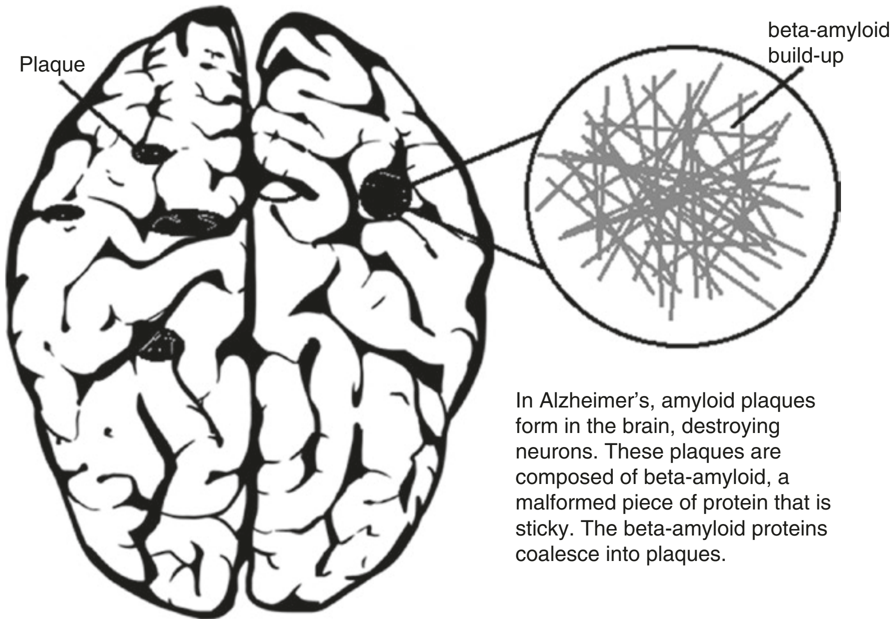

Fig. 2.7 Amyloid plaques kill neurons, which is the reason for Alzheimer's disease and symptom progression. Understanding the genetic component of plaque formation will be essential in discovering an effective treatment for the disease

PLXNA4, or plexin A4, has recently been discovered as a new receptor for CLU in the brain. In patients with Alzheimer's disease, the amount of PLXNA4 in the brain is significantly reduced compared to healthy patients. Additionally, a decrease in the normal amount of PLXNA4 by 50% was shown to have a negative effect on memory. Thus, a deficiency in PLXNA4 may be utilized as a biomarker for risk of Alzheimer's disease, and the levels of CLU and PLXNA4 in the brain may be an appropriate target for therapy in a patient with the disease [48].

2.4.4 Schizophrenia

Schizophrenia is a disease associated with hallucinations, delusions, and cognitive defects and affects 1% of the population worldwide [49]. Evidence thus far has pointed to the disease being a highly polygenic trait; 108 loci have been identified in GWAS. Each locus may contribute a small amount to the disease, but when combined, their power has strong effects [50].

Although it is difficult to find combinations of SNPs that *combined* could have an effect on disease progression, a complicated method of analysis may be used to analyze the genetic data. This method, called covariance association test, identified a set of biomarkers that may play a role in the etiology of schizophrenia. These sets

of genetic data were linked to two metabolic processes: vitamin A metabolism and immunological responses [51].

Vitamin A and other retinoic acids have a well-established role in schizophrenia. A deficiency in the hormone metabolism can exacerbate schizophrenia symptoms and is a potential target for schizophrenia treatment [52]. Immunological responses found associated with schizophrenia included dendritic responses to PAM3CSK4, an agonist to the membrane protein TLR2 [51]. A deficiency in TLR2, or toll-like receptor 2, has been shown to induce schizophrenia-like behaviors [53].

Although there is some research relating to the mental illnesses outlined above and their links to the human genome, much of the information reported has been over decades of research. This particular field is one that is underfunded but strongly needed in modern medicine. While some mental illnesses are rare on a global scale, others like Alzheimer's disease and depression affect significant portions of the population. Mental illnesses have vast impacts on the lives of those suffering with these diseases. Current treatments are not good enough. The side effects alone of depression and schizophrenia medications drive the patients to push through their illness without treatment. Alzheimer's treatments only slow down the progression of the disease and give the patient a few more years. More funding is dearly needed in this field to bring the research up to speed with other diseases, such as cancer and cardiovascular illnesses. The more research that could be done on the genome and links to mental illness, the better the health of the American, and even global, population could be.

Conclusion

The question stands: can a researcher look at a patient's genome and SNPs and determine what diseases that person is at a higher risk of developing? The answer is not simply black and white. From what has been described so far, determining risk of disease development is possible! This chapter has gone through numerous disease states: the top four types of cancer, common mental illnesses such as depression and Alzheimer's disease, and cardiovascular health issues such as coronary heart disease and stroke. Research has found genetic links to *all* of these disease states, although some links have stronger levels of evidence than others. Some of the genetic data found is already being implemented into clinical practice! The best part is the information in this chapter is only the tip of the iceberg! There are hundreds of disease states with ties to the genome, some with established research and others just waiting to be discovered.

While the genome can be used to determine risk of disease, we must take into account the effect that our environment and choices have on our health. While lung cancer can be exacerbated by genetics, the vast majority of lung cancer is a result of COPD or smoking. High cholesterol can be caused by poor dietary choices over years. To say that our genome is the sole judge of our fate is wrong. Humans are complicated, and one possible contribution cannot be pointed to as the sole reason. Disease is truly a mixture of environment, choices, and genetics. To expand on this idea, the genetic component of disease is not simply one SNP on one gene but a combination of multiple SNPs on multiple genes, the relationship of which may

be too complicated for researchers to find or understand at this time. In order to appropriately state one's risk of disease, a holistic approach is necessary.

The field of medicinal genomics has much room for growth and understanding. As previously mentioned, human diseases are multivariate and complex. Current technologies and methods only uncover the less complex interactions between genes and disease; meanwhile, some disease states may have hundreds of gene contributions. We have only barely begun to uncover the ways the genome affects our lives. Reflecting on the title of this chapter, "Where Is the Human Genome Leading Us?", it is leading us toward a future of genomic diagnoses and personalized preventative medicine. While this field is in its infancy now, it will be a major game changer for medicine as it comes of age, uncovering more genetic mysteries.

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3 Pharmacogenomics: Setting Newer Paradigms of Genetics in Therapy and Medicine

Sanjay Dey, Kumud Joshi, and Bhaskar Mazumder

Abstract

Pharmacogenomics is receiving a lot of attention for its potential clinical applications in preventive as well as personalized medicine. Pharmacogenomics provides a tool to determine the genetic makeup of individuals and help in establishing relevant genotype-phenotype correlation. This knowledge may uncover the predisposition of patients toward specific disease conditions like diabetes and cardiovascular diseases. It can also be used to gauge the possibility of toxicities of specific drugs in patients and, consequently, assist in the modification of therapy for such patients to improve clinical outcome. In this chapter, we study the current status of pharmacogenomics and its future prospects of how it will impact the current clinical practices for better therapy.

3.1 Introduction

Conventional therapy uses standardized drug dosage regimens (usually based on body weight); however, this approach of fixed drug dose therapy is not suitable for all patients, as individuals differ significantly in their drug metabolism as well as drug disposition. The body undergoes different physiological changes during

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_3

different ages, and there are simultaneous age-associated changes in body composition and organ function. Clinical parameters must also be considered while deciding drug dose for different age groups [1]. Children have significantly different metabolisms of drugs and response to different medications [2]. With recognition of the importance of pharmacological variability of different age groups, nowadays the use of age-dependent adjustments in dose is a common clinical practice.

The pharmacological therapeutic response of a patient to a drug often depends on various factors including organ functions, blood flow, age, body weight, body fat(s), etc. [3]. However, a key factor that makes a patient unique is genetic makeup, and this is often overlooked. Earliest observations of the effect of genetics on drug metabolism were reported by Kalow in 1950 who noted that some patients respond differently to succinylcholine than most of the population and that this was attributed to variation in activity of plasma cholinesterase enzyme [4]. Similarly, Carson observed excess hemolysis on primaquine administration, which is caused by deficiency of glucose-6-phosphate dehydrogenase enzyme [5]. These studies essentially established the link of variable drug responses with variation in individual genetic makeup.

Eldritch Vogel coined the term pharmacogenomics in 1959 [6]. Although pharmacogenomics was evolving during the 1970s and 1980s, through advancements of biotechnological and genomic techniques, it also began taking shape as a clinical science. Significant developments in the field of human genetics were made after the human genome project. This gave huge impetus to pharmacogenomics and provided tools for establishing correlations with drug therapy and genetics. Today pharmacogenomics is helping us to better understand how genetic variability brings about change in therapeutic responses to drugs and how to use this knowledge to achieve better therapeutic outcomes by proper dose and drug selection for individual patients.

Human genome contains more than 1.4 million single-nucleotide polymorphisms [7], out of which more than 60,000 are present in coding regions of genes. Among these single-nucleotide polymorphisms, some are associated with control of important metabolic processes and enzyme functions. These differential activities are responsible for differential drug response in various individuals within a population. Genetic linkage of interindividual variation is rooted mainly in differences in gene sequences encoding various drug targets like drug receptors and drug carriers, as well as the enzymes associated with their metabolism [1, 8–10]. Pharmacological response to a drug is an outcome of interplay between different metabolic processes which are governed by the action of different genes associated with those processes. Genetic factors have a significant effect on drug-receptor interactions and drug metabolism; thus, the study of these polygenic determinants on drug effects becomes important.

Pharmacogenomics sought to express genomic differences in populations and elucidate the difference in drug responses among different individual on the basis of variability in genetic makeup. Human genome contains an estimated 30,000–40,000 distinct genes. Though genetic makeup remains highly preserved for any species, in such huge array of genes, polymorphisms do arise producing interindividual

Table 3.1 Examples of impact of pharmacogenomics on drug pharmacokinetics

Gene product	Affected drug	Clinical impact
CYP2CP	Warfarin	Altered anticoagulant effect
CYP2C19	Omeprazole	Altered efficiency in treating *H. pylori*
CYP2D6	Antipsychotics	Increased incidence of serious side effects
CYP3A4	Tacrolimus	Altered efficiency and toxicity of tacrolimus
N-acetyltransferase	Isoniazid	Altered drug efficiency and toxicity
P-glycoprotein	Digoxin	Altered plasma concentration of digoxin

variability. Single-nucleotide polymorphisms (SNPs) are the most common and occur once in every 300 nucleotides on average.

3.2 Pharmacogenomics: Genetic Variability and Pharmacokinetics

Pharmacokinetics of drug depends on a number of factors including its absorption from the site of administration, its distribution in body compartments, the metabolic processes it undergoes, and the excretion of drug out of the body. Metabolic enzymes involved in so-called "phase I" biotransformation reactions include microsomal enzymes of the CYP450 system, while "phase II" reactions are carried out by conjugation reaction systems.

Various clinically important polymorphisms of major phase I and phase II enzymes [11] have been identified (Table 3.1).

Pharmacokinetics can importantly affect drug side effects as well as therapeutic outcome. As it is now clear that genotype is an important factor in predicting the drug pharmacokinetics, many studies are underway to improve understanding and establish relevant correlations between genotype markers and drug pharmacokinetics. This will provide valuable data to clinicians in predetermining and preventing side effects in genetically predisposed patients. This will have direct clinical implications in therapy with drugs that have severe side effects like anticancer drugs or others which have very narrow therapeutic index like warfarin. Similarly, these study may also uncover why some patients have enzymes that have higher activity than normal, as in the case of C*YP3A4*22*, *CYP3A5*3*, *CYP2C19*2*, *CYP2C19*17*, *UGT1A1*28*, and*UGT1A4*2* enzymes. Determination of relevant genetic markers will help in suitably titrating the drug dose to prevent under- or overdosing of drugs and reduce the likelihood of development of resistance [12].

Recent advancements, like development of the genotyping testing kit AmpliChip CYP450 microarray systems, allow us to check for genotypic variation in patients for genes associated with expression of different important biomolecules, including receptors and metabolic enzymes [13]. AmpliChip, recently being approved by FDA for determination of polymorphisms in enzymes including 2D6 (CYP2D6) and 2C19 (CYP2C19), is a successful example of clinical application of pharmacogenomics. These enzymes are involved in metabolism of many important drugs including tricyclic antidepressants (TCAs), venlafaxine, typical antipsychotics, and

risperidone, and determination of activity levels of these enzymes can help clinicians in prescribing suitable drugs or modifying doses of drugs to prevent side effects [14]. Similarly, tests like *HLA-B*1502* allele are important indicator of patient's predisposition to carbamazepine-induced Stevens-Johnson syndrome [15].

3.3 Pharmacogenetics and Drug Pharmacodynamics

Pharmacodynamic variability due to genetic differences is also responsible for variation of many drug responses. For example, a single-nucleotide polymorphism in the β_2 receptor gene has been found to alter receptor expression and downregulate drug binding to the receptor, reducing activity of asthma medication [16]. Likewise, many other polymorphisms in receptor expression are responsible for altered activity of drugs such as ACE inhibitors and HMG-CoA reductase inhibitors [17].

Cell receptors play an important role in a drug's access into cells. Polymorphism in genes responsible for synthesis of transmembrane cell proteins present in cell wall, such as P-glycoprotein (PGP), can significantly alter drug metabolism. PGP is involved in multidrug efflux [18] and is an important driver of anticancer and antibiotic resistance. For instance, polymorphism in the MDR-1 gene, which codes for PGP, has been reported to significantly alter absorption of drugs like digoxin and protease inhibitors. Similar genetic polymorphism of receptors like 5-HT2C receptors has been found to affect therapeutic response to antipsychotic drugs like clozapine and determine the preservation or severity of their side effects, including tardive dyskinesia and weight gain [19].

3.4 Different Types of Genetic Polymorphisms and Their Impact on Pharmacotherapy

3.4.1 Single-Nucleotide Polymorphisms (SNPs)

A SNP is a mutation that occurs in more than 1% of population. SNPs are responsible for more than 90% of variability found at genetic levels in humans. These are vastly studied in pharmacogenomics. There exist exhaustive databases like the SNP database of the National Center for Biotechnology Information (NCBI) maintaining information of different reported SNPs and their associated phenotypic outcomes. SNPs are found both in coding and noncoding regions of genome, and their effect on gene expression depends on their position in genome [20, 21].

Coding Region Polymorphism Coding regions of genes are those that are transcribed to *m*RNA and translated to proteins, and any change in coding region of DNA sequence that affects protein synthesis may cause formation of underactive or faulty proteins. These polymorphisms are also known as non-synonymous SNPs/polymorphisms. Polymorphs of thiopurine methyltransferase (TPMT) with three variants TPMT*2, TPMT*3A, and TPMT*3C are non-synonymous polymorphs,

causing allelic imbalance and altered gene expression, generating proteins with altered secondary structures [22, 23]. One such polymorphism affecting drug action involves alteration of gene expression in DNA excision repair gene (ERCC1), causing altered metabolism and drug response of cancer patients to 5-fluorouracil/oxaliplatin therapy [24].

3.4.2 Noncoding Region Polymorphisms

A major portion of the human genome constitutes the noncoding region. While polymorphisms in the coding region directly affect gene expression, polymorphisms in noncoding regions can affect splicing pattern in *m*RNA synthesis resulting in altered gene expression [25].

Different types of noncoding region polymorphisms are discussed below:

3.4.2.1 Promoter Polymorphism

Genetic differences in sequences of promoter region can bring variability in *m*RNA expression, for example, gene (UGTAI1) is involved in the bilirubin clearance pathway. Polymorphism of one dinucleotide TA can result in a variant gene (UGTAI1*28) which causes impaired bilirubin clearance leading to Gilbert syndrome [26].

3.4.2.2 5′ and 3′ UTR Polymorphism

5′ UTR genes are important regulators for important biomolecules like tumor necrosis factor alpha (TNFα); polymorphism in 5′ UTR can result in overexpression of TNFα which has been found to be responsible for increased susceptibility of African population for cerebral malaria [27].

3.4.2.3 Splice Site Polymorphism

This polymorphism results in alternative splicing of nucleotides. One classical example of this polymorphism is T to A polymorphism in the *β*-globin gene resulting in alternative splicing, producing defective polypeptides and causing thalassemia [28].

3.4.2.4 Short Tandem Repeat Polymorphism

Also called variable tandem repeat polymorphism/microsatellites, these are short sequences of DNA generally 2–5 base pair long. Variability in such short sequences is reported in the 5-lipoxigenase (LOX-5) gene, which is an important biomarker of inflammatory disease. Variability of this gene is responsible for reduced effectiveness of many antiasthmatic drugs [29].

3.4.2.5 Insertion/Deletion Polymorphism (Indels)

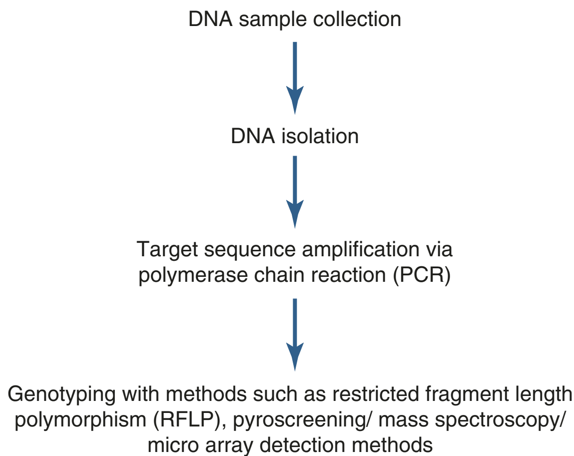
Insertion/deletion polymorphisms have an extra or a missing nucleotide in DNA. Indel polymorphism of angiotensin-1-converting enzyme (ACE1) and NOD-1 (CARD4) increases the predisposition of individual to inflammatory bowel disease (IBD) [30].

Polymorphism affects different populations and individuals in different manners, depending upon occurrence of any of the above stated phenomenon. This is a pure coincidental and random occurrence. Still, proper statistical analysis can help to predict the frequencies of some important polymorphisms. Knowledge of prevalence of clinically significant polymorphisms and their effects on drug therapies can help us to tailor pharmacogenomics-guided therapeutic regimes of side effect-causing drugs for affected populations and individuals so as to improve safety.

3.5 Technologies of Pharmacogenomics

Bringing pharmacogenomics from lab to clinic requires technologies that can detect genomic aberrations in patients and correlate them with effects on clinical outcomes in drug therapy. If such knowledge is available to the medical practitioner beforehand, they can tailor personalized drug therapy for patients and can also possibly prevent various side effects and toxicities. Historically, a lack of reliable and cost-effective technologies for testing patients' genetic profiles was the biggest hurdle in utilizing pharmacogenomics. With the advent of more accurate and reliable genotyping techniques and reduction in cost of the same, the evaluation of genetic variability of individuals has become possible at a clinical scale, which has hugely expanded the scope of pharmacogenomics.

These techniques used for genotyping are each based on common principles, discussed below:



1. *DNA sample collection*: DNA sample can be collected by different methods. The most commonly used method is collection of peripheral whole blood [31] as it can provide us with ample amount of DNA for DNA sequencing.

   Other notable methods include collection of sample from buccal cells or saliva [32] by rubbing a cryobrush or foam-tipped swab inside the mouth or using saliva as source of DNA as it contains buccal epithelial cells.

2. *DNA isolation*: After DNA sample collection, the next step is DNA isolation, extraction, and purification. Generally this process requires disruption and lysis of cells to release the genetic material. This is followed by removal of cellular debris and proteins, followed by further purification of DNA using various protocols and solvents developed for the purpose. The DNA thus obtained is stored until further use [33].
3. *DNA amplification*: The human genome contains approximately three billion base pairs, but only specific gene sequences need to be studied for specific pharmacogenomics testing. Target sequences are those which contain genes with potential for polymorphs involved in specific phenotypic responses. These target sequences first need to be amplified for subsequent genotyping studies. PCR is the method for such amplification [34, 35].

After amplification of the DNA sequence of interest, the process of pharmacogenetic testing and determination of specific polymorphism starts. Specific alleles of genes are determined, and their response on phenotype determined by genome-wide association studies (GWAS) and polymorphisms are confirmed (Table 3.2).

Genome-wide association studies (GWAS) are special statistical studies for correlating genetic polymorphism to phenotype responses, and they can determine different polymorphisms that affect drug toxicity and efficacy and help in finding out potential risk of diseases.

Table 3.2 Various methods which can be used for pharmacogenetic testing and main features of them [36]

Genotyping method	Allele discrimination principle	Allele detection method	Throughput	Advantages	Disadvantages
PCR-RFLP	Restriction endonuclease	Gel electro-phoresis	Low	• Low equipment cost	• Low throughput • Lengthy procedure • High operating cost
TAQMAN	Allele-specific hybridization	Fluores-cence	Medium to high	• Fast process • Software based • Computational genotyping	• Less amenable to multiplexing • Florescence labeling is costly
Mass spectra	Allele-specific hybridization/ primer extension	Fluores-cence and capillary gel electro-phoresis	Medium to high	• High sensitivity • High throughput • High orders • Multiplexing	• High equipment cost • Need technical expertise
DNA microarray	Allele-specific hybridization	Fluores-cence	High	• Capable of genotyping • Polymorphisms	• High cost per chip

Table 3.3 Some important tests developed for pharmacogenetic purpose [39–41]

Drug	Pharmacogenomics test	Test name
Azathiopurine-6-mercaptopurine	TPMT	Prometheus TPMT genetics
Warfarin	CYP2C9 and VKORC1	Cytochrome P450 2C9 and VKORC1—warfarin genotype
Irinotecan	UGT1A1*28	Invader UGT1A1 molecular assay
Carbamazepine phenytoin	HLAB*1502	HLAB*1502 carbamazepine sensitivity
Tamoxifen	CYP2D6	Tamoxifen response
Abacavir	HLAB*5701	HLAB*5701
5-flurouracil	DPYD	Theraguide-5-FU

Advancements in the field of genetics have made it possible to use genetic technology in diagnosis and therapy. With the introduction of portable devices, as well as quicker nucleic acid detection by advanced kits, pharmacogenomics techniques are becoming more user-friendly. Successful genetic diagnostics require four major elements, including rapid reaction systems, low cost, low energy consumption, and simple analysis.

Simple and robust methods like the SmartAmp method have been developed for drug receptor gene detection. The SmartAmp method utilizes a specialized DNA polymerase (*Aac* polymerase) with strand displacement activity and excellent capability for DNA amplification and can provide reliable results even in the presence of cellular contaminants. SmartAmp can detect SNPs in drug transporter genes (e.g., ABCB1, ABCG2, and ABCC11), as well as in genes associated with drug metabolism, like cytochrome P450s, and UDP glucuronosyltransferase UGT1A1 for predicting adverse reactions in screened patients carrying specific polymorphic genes [37, 38].

Different clinical kits have been developed for this genetic testing for pharmacogenomics purpose; some of them are discussed in Table 3.3.

3.6 Clinical Relevance of Pharmacogenomics

Pharmacogenomics is mainly focused on genes that modulate drug disposition, thereby affecting response to a particular drug. Indeed, most of the clinical pharmacogenomics studies are designed to screen subjects to determine if they are carrying specific genetic aberrations, usually a SNP, responsible for abnormal drug disposition and affecting therapeutic outcome. Different facets of clinical outcomes affected by genetic makeup are studied, and pharmacological approach is adjusted so as to achieve better clinical outcomes. Those different facets are discussed below:

3.6.1 Side Effects and Toxicity

Polymorphic genes serve a multitude of biological processes, and their polymorphisms may produce proteins with altered activities, which affect and alter pharmacological response of drugs. This causes variation in drug response in certain situations and class of patients.

Genome-wide association studies (GWAS) are important tool for determining gene-phenotype relationship between drug toxicities and genetic polymorphisms. It involves comparison of the genetic makeup and determination of specific genetic differences between the control population and the one exhibiting drug toxicities. Such studies let us know specific genetic polymorphs associated with drug toxicities. Several toxicities have been identified using this pharmacogenomics approach.

One example is myopathy associated with statins. GWAS have proved that polymorph rs4363657 present in *SLCO1B1* is associated with simvastatin-induced myopathy [42]; similarly the presence of the IL28B polymorphism is found to be an important predictor for therapeutic activity of pegylated IFN-α and ribavirin in treatment of chronic hepatitis C. Prior genomic testing may preclude the nonresponders thereby allowing medical practitioners to choose effective alternative medicines and avoid adverse drug reactions associated with noneffective therapy [43].

Similar genetic associations have been observed in flucloxacillin therapy-related drug-induced liver toxicity, where patients with genetic polymorphism of *HLA-B57:01* are at higher risk of toxicity, and pre-prescription testing is employed to avoid this [44].

Pharmacogenomics testing is found to be beneficial in determining potential ADRs of many drugs, especially those with low therapeutic indices like warfarin, and prevent them. *CYP2C9* and *VKORC1* genotypes are the main modulators of oral coumarin metabolism, and prior testing and genotype-guided warfarin therapy have been found to be more safe and effective than the empirical dose approach [45].

Similar utility of pharmacogenomics is achieved in antiretroviral drug therapy like abacavir where pharmacogenomics-guided therapy is found to reduce toxicities [46, 47].

The US FDA has approved labeling for many drugs instructing modified dose regimens in populations with specific indicated genotypes, in order to reduce adverse effects. For example, patients known to be poor CYP2C9 metabolizers are indicated to be prescribed with a reduced dose of celecoxib (https://www.fda.gov/downloads/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/UCM545881.pdf). The pre-prescription screening of abacavir to the patients of *HLA-B*5701* allele prior to initiating therapy carriers of the allele are at greater risk of hypersensitivity (https://www.fda.gov/downloads/Drugs/ScienceResearch/ResearchAreas/Pharmacogentics/UCM545881.pdf).

3.6.2 Therapeutic Efficacy

Pharmacogenomics is proving to be a useful tool for improving the therapeutic efficacy of drugs, especially when a drug has variable dynamics and kinetics in different set of individuals. This also becomes more crucial if the drug has narrow therapeutic window, as any dose variation can have important clinical implications. The effect of certain therapeutic agents may be blunted in patient populations carrying specific genetic polymorphs leading to adverse drug reactions, while some polymorphs hasten the metabolism giving subtherapeutic effects. In some cases, drugs are found to be therapeutically effective only in patients with a specific genetic

makeup. Therefore, proper drug selection and dosage are very much essential for such polymorphic individuals. Patients with specific polymorphisms of apolipoprotein E (*APOE*) gene can exhibit significantly different therapeutic responses to Alzheimer's treatment, as well as to the effect of lipid-lowering medications in a certain section of patients [48–51].

Similarly different variants of alleles of *CYP450* enzyme are found to significantly affect the therapeutic efficacy of many drugs metabolized by them including warfarin, losartan, and phenytoin [52]. One example is *CYP2C19* enzyme, for which there exists more than 27 polymorphic forms. Significant among these are *CYP2C19*2* and *CYP2C19*3*. The *CYP2C19*2* allele is responsible for slower metabolism of clopidogrel, leading to higher platelet aggregation in certain clopidogrel-treated cardiovascular patients [53].

Voriconazole, an antifungal drug, is metabolized more slowly in Asian and black populations but more quickly in white patients owing to genetic polymorphism. In each case, the drug dose can be reduced in poor metabolizers or increased in fast metabolizers (https://www.fda.gov/downloads/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/UCM545881.pdf).

The effect of pharmacogenomic status on therapeutic efficacy of anticancer drugs is also crucial. It has been noticed, for example, that the status of FcγR polymorphism is found to affect therapeutic response of anti-CD20 drug rituximab in treatment of non-Hodgkin's lymphoma. The drug has better clinical therapeutic efficacy in patients with 158VV genotype in comparison with those 158F genotype carriers. Similarly, trastuzumab is indicated for breast cancer but only in patients with cancer expressing the specific HER2 gene, as the HER2 receptor is the target for this antibody. As such, prior testing for the HER2 gene is indicated before therapy of trastuzumab. Another example of pharmacogenomics-guided medication is of venetoclax, which is indicated for targeted treatment of chronic lymphocytic leukemia in patients who have a 17p chromosomal deletion [54, 55]. These stated studies demonstrate how individual genetic makeup plays a crucial role in the therapeutic efficacy of drugs. In the future, we will see considerable more research and newer medicines prescribed specifically to patients depending on their genetic makeup.

3.7 Applications of Pharmacogenomics

Pharmacogenomics has provided new dimension to clinical medicine. The US FDA has stated pharmacogenomics as "one of the technologies that will lead to innovation in the pharmaceutical industry" [56]. Recent guidelines have formally introduced pharmacogenomics into the drug development process [57]. In the future, these studies may become a mandatory part of the FDA drug evaluation process, although complete utilization of pharmacogenomics is yet to be achieved in clinical practice. Herein, we will discuss how pharmacogenomics will improve the drug development processes and their clinical use.

3.7.1 Pharmacogenomics: Improving Productivity of Drug Development Process

Pharmacogenomics approach can enhance the selection of target compound. By identifying and selecting suitable molecular and genetic markers during the pre-clinical stage, we can determine the compounds having specificity to target and how they modulate particular pathways which can help us to narrow down our potential therapeutic candidates. This kind of application of genomic technologies will improve selection of target compounds. Genetic polymorphic markers can also be used in human trials for predicting the drug response in clinical trials, especially in phase 1 studies on volunteers who are identified to have genetic variants involved in drug's metabolism. This can help in early determination of possible side effects of the drug in specific subsets of a genetically varied population, and requisite modification in dose for such individuals can be determined by incorporating genetic technologies in phase 2 and phase 3 clinical studies. This will also be beneficial in later phases of testing as it can help in normalizing the heterogeneity of clinical outcomes of studies and streamlining the results scientifically.

Pharmacogenomics technologies are still evolving, and many of their uses and applications are yet to mature, but, in time to come, these genetic marker-assisted technologies can serve as important tools for drug development and better target selection. In the future, it can be expected to reduce the compounds needed to be tested by 20% and reduce the patients by 50% in phase 2 trials and by 10% in phase 3 trials [58].

A smarter approach to drug testing will help in reducing compound failures in clinical trials which could in turn bring down the research cost for pharmaceutical companies by up to $500 million for each drug launched [59].

3.7.2 Pharmacogenomics: Establishing Drug Safety

Adverse drug reaction monitoring is an important part in post-marketing surveillance. In the past, several drugs have been withdrawn from market because of the severe adverse drug reactions associated with their use in some patients [60–62]. Possibly some of these adverse reactions might be idiosyncratic. Genetic factors can possibly be responsible for these reactions, and, with the help of pharmacogenomics, the responsible genes can be identified beforehand, which could help in preventing side effects in predisposed populations while still keeping the drug useful for the rest of the patients. Pharmacogenomics can help us to identify the population subgroups that are at risk for adverse events upon intake of specific drugs. Drug therapy can be redesigned for these genetically susceptible subgroups of population so as to prevent drug side effects. Identifying genetically susceptible groups can help manufacturers in prior determination of drug adverse reactions, particularly if dose-related toxicity is governed by genetically controlled drug-metabolizing enzymes in individuals. Additionally, pre-prescription diagnostic

screening tests could be developed to identify patients who are at risk of suffering adverse reactions. Moreover, the drug manufacturer can ensure safety by following guidelines for specific groups of at-risk patients, precluding the use or altering the dose as appropriate [63].

Postdrug launch, pharmacogenomics can further help in assessing and addressing drug safety issues in post-marketing surveillance studies. Many research companies and organizations have already created biobanks to store DNA and other biologic specimens, from which useful information could be generated to help in predicting drug safety and therapeutic efficacy.

Postdrug exposure clinical data can also be correlated to genetic marker studies with banked samples. Together, these correlations can help in predicting the genetic polymorphisms associated with adverse drug reactions in different genetically diverse populations. These genomic associations of adverse drug reactions can be made part of the post-approval studies. Their results can be confirmed by further tests, and additional labeling can be provided with the products for therapy adjustments for risk groups.

3.7.3 Pharmacogenomics: Development of Molecular Diagnostics

Molecular diagnostic techniques are an important part of biological investigations. Pharmacogenomics holds enormous potential for the development of suitable molecular diagnostic methods, with which clinicians will be able to develop medication plans and drug doses for individual patients. Firstly, the genotype of patients is assessed for mutations of any genes responsible for particular drug intragenicity. Presently, current genotyping methods can rapidly determine thousands of single-nucleotide polymorphisms in a single assay, using small blood samples, for determination of a large number of polymorphisms (about 20,000 single-nucleotide polymorphisms in 5000 genes in a test). The important determinants of drug deposition can be identified by proper investigation. Genotyping will be most beneficial if its clinical implications can be determined according to the patient's diagnosis, as this will help clinicians in recommending suitable treatment options. With further improvements in current genotyping methodologies, it will soon be possible to perform these tests in straightforward high-throughput, automated systems, screening for thousands of SNPs in one test, which will analyze the genome exhaustively, and the treatment decisions can then be based on results of a panel of SNPs. These genotyping results will not be reported as a list of SNPs, rather they will be interpreted by the clinician for patient's disease diagnosis and designing suitable treatment options [64]. However, these new tests will not be an alternative to conventional biochemical tests that are currently used to access organ function and disease status; rather they will provide additional tools for personalizing drug therapy for individual patients. Genotyping will not eradicate the need of following up assessment of response, compliance with treatment, and other variables that influence treatment outcome.

Currently several kits and microarrays have been developed, including AmpliChip: a microarray system approved by the FDA for determination of CYP450 enzyme status that is involved in metabolizing many important drugs like antipsychotics and antidepressants [13]. Similarly other microarrays chips like Affymetrix GeneChip™, Sentrix LD BeadChip, and the Sentrix Array Matrix can determine the status of large number of genes and help in prediction of possible toxicities and drug therapeutic efficacy in patients, thus helping in achieving better clinical outcomes [65].

3.7.4 Pharmacogenomics: Application for Personalized Medicine

Personalized medicine involves providing specifically tailored therapy to patients by keeping their specific conditions, history, and genetic factors in consideration for enhanced effectiveness of medication and reducing risk of adverse effects. Pharmacogenomics can play a vital role in achieving this goal. Screening patients for specific genetic markers can help to predict predisposition toward serious drug toxicities or disease conditions, which then can be proactively prevented or avoided. Pharmacogenomics can also act as an important tool for accelerating and improving drug discovery processes by identifying genes and their associated molecular pathways involved in drug targeting, which can then be used to tailor drugs with desired specificities. It can be said pharmacogenomics will help in achieving personalized medicine and developing and selecting the right drug for the right patient.

The challenge lies in establishing correct, accurate, dependable, and medically relevant correlation between genotype of individual and phenotype outcome. Polymorphism in genes encoding drug targets (e.g., receptors) can affect the extent of drug binding and thus the drug response in different individuals [3–5].

Personalized medicine plays an important role in cancer medicine as well, for achieving better therapeutic outcomes and reduced side effects. For example, the methylguanine methyltransferase (MGMT) gene promoter controls methylation in biotransformation reactions. Variability in the said enzymes also alters the therapeutic response to drugs metabolized by these enzymes, for instance, response to therapy with carmustine for certain gliomas is associated with toxicities in some patients due to their genetic predisposition that reduces the efficiency of repair of alkylated DNA in patients carrying methylated MGMT [66]. Similarly, purine methyltransferase polymorphism is associated with the hematopoietic toxicity of mercaptopurine in certain patients [67, 68]. Identifying the targets and mechanisms of these genetic polymorphisms is critical in identification of how these heterogeneities at genetic levels are affecting the activities of drug-metabolizing enzymes, which ultimately alter the drug response and result in susceptibility of a section of population to toxicities [69]. Another example is the role of the *ADRB2* gene, which affects the signal transduction in β_2-adrenoreceptor affecting the clinical outcome in therapy with β_2-agonists. A significant correlation has been established between Arg16Gly polymorphism of *ADRB2* and predisposition of asthma [70]; at the same time, a SNP of rare *ADRB2* variants Ile164 and –376ins is associated with adverse events

during long-acting β-agonist therapy [71]. Thus, pre-prescription pharmacogenomics testing can help in selecting suitable therapy [72].

However, it is noteworthy that still there is substantial amount of work that needs to be done before the complete clinical utility of pharmacogenomics could be fully appreciated and realized. Currently there are numerous examples that illustrate the potential with more than 1200 different molecules approved as drugs by regulatory agencies in the USA, Europe, and Asia. Among them, 15% of EU-EMA- and US FDA- approved products have pharmacogenomics information on their label, but only about 7% of medications have actionable germline pharmacogenetic information [73, 74]. As a set of genes together are responsible for manifestation of a phenotypical outcome, it becomes essential to investigate the role of haploid structure rather than a single gene in the determination of effect of genotype on drug therapeutic outcome. This could even help to better correlate and predict the role of genotype and therapeutic outcome. For instance, studies show that among 8192 possible *ADRB2* haplotypes, only 12 distinct haplotypes were actually reported in different races [75]. Thus, determination of haplotype structure is frequently a better prognosticator of phenotypic response than individual polymorphisms. It would be more fruitful if we develop simple but robust molecular methods to determine the haplotype structure of patients to establish clearly defined and objectively measurable end points, i.e., phenotypes, and clinically relevant genotypic relations [76]. Currently several drugs have been launched which are meant for personalized treatment of patients with specific genetic makeup, especially within cancer therapy, where genetic makeup plays a very important role in risk and pathology of diseases. Examples of such drugs include dabrafenib which is prescribed specifically for the treatment of patients of metastatic melanoma with *BRAF* allele and V600E mutation as detected by an FDA-approved test [77]. Similarly, everolimus is indicated to be reserved for patients with advanced hormone receptor-positive and HER2-negative breast cancer [78]. Several other personalized medications have been developed for indications that are meant for groups of patients with specific genotypes. In the future, it seems that pharmacogenomics will advance further in its understandings of gene-phenotype relations and will play an important role in drug response studies. If done properly, ongoing research will bring fundamental changes in how medicine and pharmacy are practiced. The current empirical knowledge-based prescription system will shift to evidence-based prescription system, and using genotype to individualize drug therapy will become the norm.

3.8 Pharmacogenomics and Its Impact on Pharmaceutical Market

3.8.1 Market Segmentation

Traditionally, medical practice depends on clinical symptoms to recognize a disease followed by therapy. This classical practice has been in use since antiquity, but with advancements of science, more accurate and sophisticated technologies are evolving

which can more specifically and accurately find underlying disease processes and determine causative biological mechanisms.

For example, currently, hypertension is treated using different classes of drugs that can resolve the clinical symptom of rise in blood pressure, but there are many numerous underlying pathophysiological mechanisms (e.g., renin-angiotensin system, sodium reabsorption, and endothelial nitric oxide synthase). Determining specificity of underlying disease mechanism can help us in achieving better therapeutic outcomes. Applying pharmacogenomics to determine genetic markers of underlying disease mechanisms can play a vital role in this regard. Further appropriate products could be developed to target the underlying processes rather than clinical symptoms, enabling personalized medicine with greater safety and effectiveness.

With increasing awareness of the benefits of pharmacogenomics, there is a demand of more specialized personalized drug products. There exists challenges of correctly identifying genetic markers for drug selection purposes, but with newer technological advancements, it has become possible. While pharmacogenomics could usher in an era of personalized medicine, this would be accompanied by market segmentation and reduce the size of market available for a product. Moreover, revenues from pharmacogenomics testing will also be a part of earnings for pharmaceutical companies. There are companies (e.g., Genentech, Inc., San Francisco, California) that have already adopted pharmacogenomics principles for their product development and marketing strategies [79, 80]. This strategy of providing tailored therapeutic products holds special significance in critical diseases like cancers and where pharmacogenomics profiling may help in selecting suitable tailored therapy which will increase effectiveness of chemotherapy and reduce side effects.

While the current model of blockbuster drugs may get revamped, the degree of market stratification will depend on many different parameters like economic costs of drug development, the disease profile, and scope of profits [81, 82].

3.8.2 Market Expansion

Many drugs have been withdrawn from markets in the past due to serious side effects and toxicities. With the availability of advanced pharmacogenomics tools, such drugs may find their way back for use in suitable patients. It may become possible to determine the genotypical polymorphisms involved in drug toxicities, and patients carrying them could be precluded from potential therapy groups for those drugs. The testing protocols of drugs will become an important part of clinical practice for achieving proper utility of drugs in different patient populations. This in turn will help in developing marketing strategies.

Pharmacogenomics will also help in expanding the market of existing drugs, in that a greater understanding of disease pathways and pathogenesis could elucidate new ways in which a drug could act. One example of such drug is the tyrosine-kinase inhibitor imatinib, which was initially prescribed for patients with chronic myeloid leukemia. Later its therapeutic utility expanded into other forms of cancer as well like gastrointestinal stromal tumors [83] and treatment of other carcinomas [84].

Similarly, trastuzumab was also found to be effective in multiple diseases including the treatment of breast cancer and prostate cancer, owing to modulation of common disease pathways [85]. Genetic technologies could also help in identifying newer target sites for action of an existing drug molecule, expanding their clinical utility. Genetic tools may also help to unravel the causes of drug resistance [86, 87]. Proper understanding of pathological mechanism of diseases and cell processes across disease categories will help to design molecules that will cause less adverse effects on nontarget cells.

3.9 Challenges of Pharmacogenomics

Pharmacogenomics has come a long way from being a concept in the 1960s to being a science at the crossroads of genetics and medicine and has evolved into a specialized clinical field that primarily seeks to provide safe and efficacious individualized therapy to patients. It has brought the sophistication of lab-based genetic analysis onto simple ready-to-use clinical kits. Still, wide-scale and commonplace clinical utilization of this science has many challenges including scientific, financial, and ethical; some of them are discussed as follows.

3.9.1 Financing Personalized Healthcare

Prior pharmacogenomics testing for personalized healthcare offers scope for adoption of risk stratification techniques and preventive health strategies for reducing adverse clinical events [88, 89]. However, it is not clear whether this technology is going to reduce healthcare costs [90]. With personalized medicine, the size of the market for products will become smaller to cater to the needs of specific subsets of patients. While pharmacogenomics will help in tailoring therapies and providing personalized medicine that will likely to have greater efficacy and safety, this will also increase cost of medication as companies will pass on drug development costs to a smaller subset of end patients. Developing a suitable system for financing preventive and personalized medication services will be important for the success of personalized healthcare. Commitment of healthcare providers toward the goal of preventive medicine and optimization of resources for reducing long-term costs is necessary.

3.9.2 Ethical and Legal Issues

There are also apprehensions that the existing system of insurance-based healthcare may be misused by insurance companies. Companies may predetermine the genetic disposition of patients and then stratify the patient population to different disease classes and may disallow cover on certain diseases. Therefore, newer therapeutic systems need to be worked on for a proper legal framework that should safeguard

patients against any such misappropriations. Moreover costs involved in preemptive testing, preventive strategies, medication, or surgery for patients in high-risk categories will likely rise [91]. The higher cost of premier personalized medicine will need to be covered by individuals and insurance companies in a way that it will not make personalized medicine cost prohibitive. An alternative financing system may be required for preventive as well as mitigative treatment of diseases.

3.9.3 Scientific Uncertainty

While basic principles of pharmacogenomics can be applied in clinical medicine, validating the information and designing the proper course of action are yet to be mastered. Developing suitable tests for screening and preemptively diagnosing health risks is an unfamiliar domain, and developing such tests that accurately and precisely foretell such risks poses a challenge in itself.

The development of such highly specific and standardized tests that have high reproducibility is expensive, although some techniques (e.g., SNP genotyping) have been developed. RNA-based microarray techniques have been used to predict chemotherapeutic response in cancer; however, clinical validation of this technique under the Clinical Laboratory Improvement Amendments framework is still under development and needs further refinement.

Furthermore, achieving commercial viability of such high-end research has high risk. To ensure faster transition of research from laboratory to clinical practice, clinicians and patients may have to go through many uncertain instances. Clinicians have access to a large variety of risk stratification information, but how to clinically put it in use will need trials. *BRCA1*/*BRCA2* gene test kit is one such example when clinicians have to go through a lot of initial uncertainties, in achieving clinical utility of tests for drug prescription [92]. This also presents an enormous intellectual challenge. These stated challenges can be overcome with adoption of better healthcare models and education systems and obviously with newer better reliable technologies.

3.10 Future Prospects of Pharmacogenomics

A lot has been achieved in the understanding of pharmacogenomics, and with progress of techniques of genotyping, finally pharmacogenomics is making its way from lab to clinic, and newer products are coming up with pharmacogenomics-associated labeling. A lot still needs to be done to ensure proper utility of pharmacogenomics is achieved.

It is a subject of interest how pharmacogenomics will impact the future of therapeutic practices. Currently there are several aspects of pharmacogenomics which are of potential clinical relevance. The significance of genetic polymorphisms is being realized, and in the future, we are expecting a lot of application of the knowledge being generated in the field of clinical practice and drug development.

Table 3.4 Pharmacogenomics: benefits and challenges [36]

Potential benefits of pharmacogenomics	Challenges to growth and expansion of pharmacogenomics
Optimization of drug choice and dose improved drug efficacy and reduced drug side effects Development of highly efficacious for specific target population Reduced failures of drugs in clinical trials Identification of numerous potential drug targets More rapid drug development Identification of polygenic drug effects Overall reduction of healthcare costs in long term Adverse disease screening	Proper education of various healthcares provides regarding utilization of pharmacogenomics Potentially smaller and more specialized drug markets may increase drug costs for patients Complexity of polygenic drug response Resistance to genetic testing Ethical and legal issue Expensive testing may increase drug costs

A summary of potential benefits and challenges for application of pharmacogenomics in clinical field is presented below (Table 3.4).

In the future, the field of drug development pharmacogenomics will help in selection of highly specific and efficacious targets modulating activities of specific enzymes and cellular targets. Pharmacogenomics will help us to study multiple gene pathway involved and their effect on drug response. It will usher in an era of individualized therapy and will help us to design therapeutic strategies and develop suitable drug classes for genetically diverse individuals in a population.

In conclusion, it can be said with the help of pharmacogenomics in drug development process that the probability of failure of drug at clinical trials will reduce and speed of drug development will increase, while the cost of drug development will come down, and drug efficacy will improve along with a reduction of adverse effects. Ultimately, pharmacogenomics will take active role in the future to reduce healthcare costs.

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4 Bioinformatics and Pharmacogenomics: Tools to Understand and Accelerate Infectious Disease Control

Ashwani Sharma, Subrata Sinha, Surabhi Johari, and Bhaskar Mazumder

Abstract

Population science provides a helpful hand to determine the impact of deadly infectious communicable diseases such as tuberculosis, among other viral and bacterial infections, on the population [1–3]. Timely information on these diseases aids in reducing risk, incidence and deaths associated with these diseases. It also helps to improve the quality of life for survivors. These research projects provided a common platform for clinical, basic and population scientists to work collectively to further improve individual and population health. Recent trends in genetic, epidemiology, [4–6] applied and surveillance researches provided useful clues to reduce the impact of spread of infectious diseases worldwide. Many studies, associated with population science in disease control, help in many ways to control the infectious diseases, such as:

1. It improves understanding of the influence of pathogenic deadly diseases on the population, including hereditary (genetic) and environmental factors that may influence a person's risk of getting infected [7–10].
2. It may help in elucidating and understanding health problems among the population due to the influence of diseases and their pharmacological treatment or other preventive measures.

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_4

3. These studies facilitate the discovery of new treatments and the most effective ways to prevent diseases.
4. The study of population science enables rapid detection of infection among the population and also prospective cost-effectiveness analysis for treatment.

4.1 Introduction

Population science provides a helpful hand to determine the impact of deadly infectious communicable diseases such as tuberculosis, among other viral and bacterial infections, on the population [1–3]. Timely information on these diseases aids in reducing risk, incidence and deaths associated with these diseases. It also helps to improve the quality of life for survivors. These research projects provided a common platform for clinical, basic and population scientists to work collectively to further improve individual and population health. Recent trends in genetic, epidemiology, [4–6] applied and surveillance researches provided useful clues to reduce the impact of spread of infectious diseases worldwide. Many studies, associated with population science in disease control, help in many ways to control the infectious diseases, such as:

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3. These studies facilitate the discovery of new treatments and the most effective ways to prevent diseases.
4. The study of population science enables rapid detection of infection among the population and also prospective cost-effectiveness analysis for treatment.

Studies have been carried out to understand the spreading of the major diseases among the population and to control their impact on the population. One of the best examples is the development of the polio vaccine [11], which has successfully helped to eradicate polio globally.

4.2 Infectious Diseases Scenario in Developing Countries

Almost, all developing countries suffer from unhygienic conditions, poor healthcare systems and environmental pollution. These factors affect the population of these countries severely. The most prevalent disease is rhinitis, which affects 40% of the population of the developing countries. Rhinitis or coryza [12, 13] is the irritation

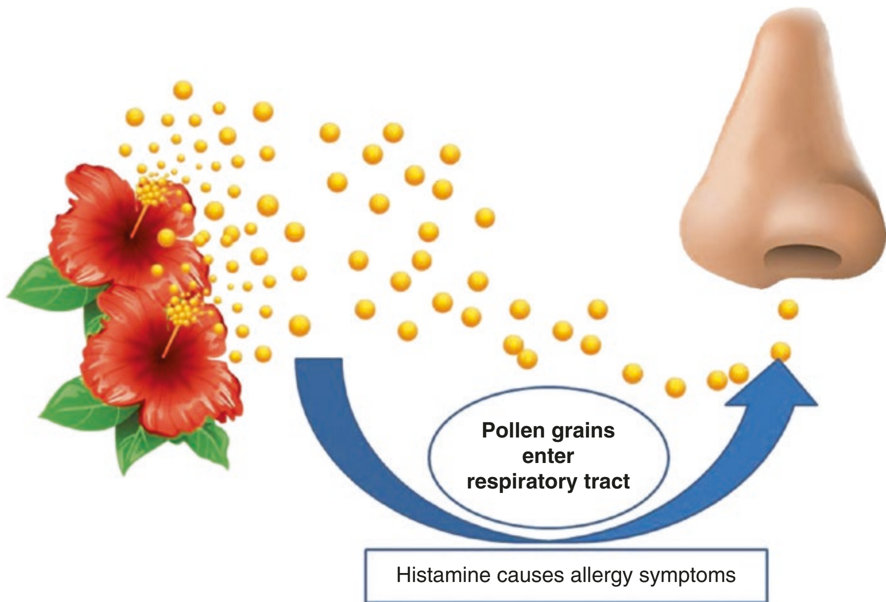

Fig. 4.1 Pathway for infection of acute rhinitis or coryza

and inflammation of the mucous membrane inside the nose. Its common symptoms include a runny or stuffy nose, sneezing, nasal itching, coughing, headache, fatigue, malaise and cognitive impairment. It can also cause watery, reddened or itchy eyes, puffiness around the eyes and postnasal drip [12, 13]. This disease can be caused by infection with a pathogenic virus or bacteria or by airborne allergen particles, such as pollen grains [14] and dander (Fig. 4.1) [15–18].

Asthma is another disease that has a major effect on the population of the world. It affects more than 40% of the [19, 20] population from developing countries due to huge air pollution and unhygienic conditions [21, 22]. Asthma is a chronic lung disease that affects the air passage of the lungs. It involves inflammation of the air passage and narrowing of the airways, which results in difficulty breathing [19, 20]. The main symptoms of asthma include recurring periods of wheezing (a whistling sound during breathing), chest tightness, shortness of breath and coughing, which often occurs at night or early in the morning [23–25]. Children are most commonly affected by asthma; however, it affects people of all ages. Developed nations are not immune from asthma. Industrial growth is accompanied by air pollution globally, which can especially impact the health of the elderly. Reports show that more than 25 million people have asthma in the United States and seven million are children (http://www.nhlbi.nih.gov) [19].

4.3 Need for Developing Bioinformatics Tools

Other diseases, such as viral infections (HIV), bacterial infections (tuberculosis) and parasitic infections (malaria), are more common in the adult population. Therefore, there is a great need to develop faster tools to detect these diseases at early stages and to prevent their symptoms before they spread further among the population. Development of bioinformatics tools and their applications in different fields have accelerated our understanding of disease outbreaks. Clinical bioinformatics represents an interesting application of these tools. Bioinformatics elucidates vast amounts of information on the biology and chemistry of medicine and can improve the efficiency of healthcare delivery by facilitating individualisation. Its development as a science was aided in no small part by the Human Genome Sequence Project, which provided a genomic source from which clinical bioinformatics experts could extract information. Bioinformatics has been applied to create numerous online databases, from which biological information has been utilised in medical practice. For example, information extracted from microarray data has been applied in medical decision-making. The US Food and Drug Administration (FDA) encourages the development of new technologies, such as microarrays, which may improve and streamline assessments of safety and the effectiveness of medical products for the benefit of public health. The FDA anticipates that these new technologies may offer the potential for more effective approaches to medical treatment and disease prevention and management (Tezek et al. 2006).

4.4 Clinical Bioinformatics

Several proteomics methods, such as mass spectrometry, liquid chromatography-mass spectrometry, protein profiling, protein chips and reverse-phased protein microarrays, have been implemented for similarity searches, structure prediction and protein modelling. Clinical bioinformatics was further revolutionised with the advent of proteomics. These data can be correlated with clinical data and analysed with relevance to diseases. Another bioinformatics application is in the field of pharmacogenomics. This field connected drug discovery with genomics studies and provided solutions to healthcare. Clinical bioinformatics includes elaborate studies of bioinformatics tools and various facets of proteomics related to drug target identification, measuring interactions between these targets and drugs, selection of the best drugs and further clinical validation. In addition, the field of clinical bioinformatics explores the use of computational and high-throughput experimental techniques in order to find new therapeutics and to provide a detailed picture of the systems biology approach for solving health-related problems. With the assistance of improving resources that capture vast amounts of biological and medical information, clinical bioinformatics has provided insights that aid in the understanding of health-related problems in large populations and may help to change practice standards in the healthcare system.

Here, we summarise different bioinformatics tools that are used to study population science related to disease control and prevention:

4.5 Bioinformatics Tools for Population Science Study

4.5.1 Google Trends

The Google company developed a server to help understand influenza outbreaks among the population [26]. It was built under the name Google Flu Trends and is used for rapid detection of regional outbreaks of influenza. It helps in the rapid detection of the spread of influenza disease in the population under study and collects and processes the data within a short interval of time.

Google Trends shows great promise as a timely, robust and sensitive surveillance system. This server is highly useful for surveillance for epidemics and diseases with high prevalence and is currently better suited to track disease activity in developed countries because, to be most effective, it requires large populations of web search users [26–34].

4.5.2 National Electronic Disease Surveillance System (NEDSS)

The online tool National Electronic Disease Surveillance System (NEDSS) [35] promotes awareness about the influence of pathogenic diseases in the population, disease patterns and outbreaks in the population under study to healthcare professionals and government agencies [35]. The working process of NEDSS incorporates bioinformational technology software, advanced computational hardware and online disease databases. All of these components help in expanding the service of the surveillance systems, such as for National Electronic Telecommunications System for Surveillance (NETSS), HIV/AIDS reporting systems, vaccination programmes and tracking systems for tuberculosis and other infectious diseases among the greater population [35]. This is very helpful to developing countries to contain the infectious diseases in their respective countries.

4.5.3 HealthiManage

The HealthiManage is an iPhone-based bioinformatics application that helps in predicting blood glucose levels in patients with type 2 diabetes. It provides relevant feedback to patients at each glucose input reading comparing the measured and predicted readings, facilitating improved self-management of the disease (Chemlal et al. 2011).

4.5.4 Oncomine

Oncomine (www.oncomine.org) is useful website for cancer profiling data across a large volume of cancer types, subtypes and experiments so that target expression can be assessed online, in seconds. This Oncomine database incorporates different

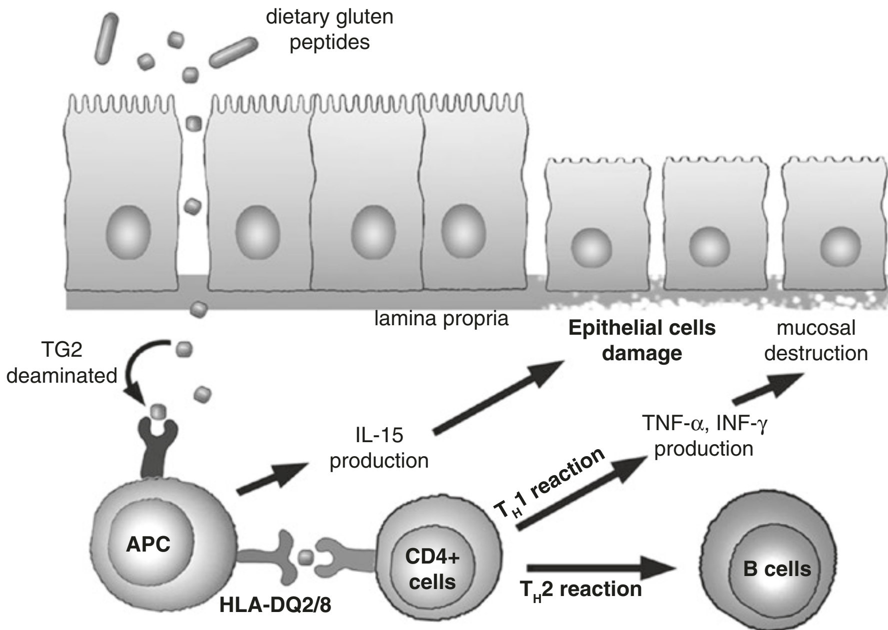

Fig. 4.2 Schematic representation of CD pathogenesis [37]

gene information for additional insights into biology, regulation, pathways, drug responses and patient populations.

4.5.5 Celiac Disease (CD) Novel Protein Risk Assessment Tool

Celiac disease (CD) [36] is an autoimmune disorder that is triggered by ingestion of gluten, whereby the immune system develops antibodies against the protein component gliadin that also recognise proteins on the intestinal epithelium (Fig. 4.2) ([38, 39]; Denham et al. 2013). At the University of Nebraska Department of Food Science and Technology, a bioinformatics tool has been developed that detects peptide matches between a query protein and a range of proteins from gluten-containing plants (barley, rye, oats). The purpose of the tool is to verify that proteins developed and introduced into food crops by genetic modification will not pose an excess risk to patients with celiac disease.

The intestinal biopsy diagnostic test has been used to detect the CD disease and well documented by the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) or the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) (Fig. 4.3) [40].

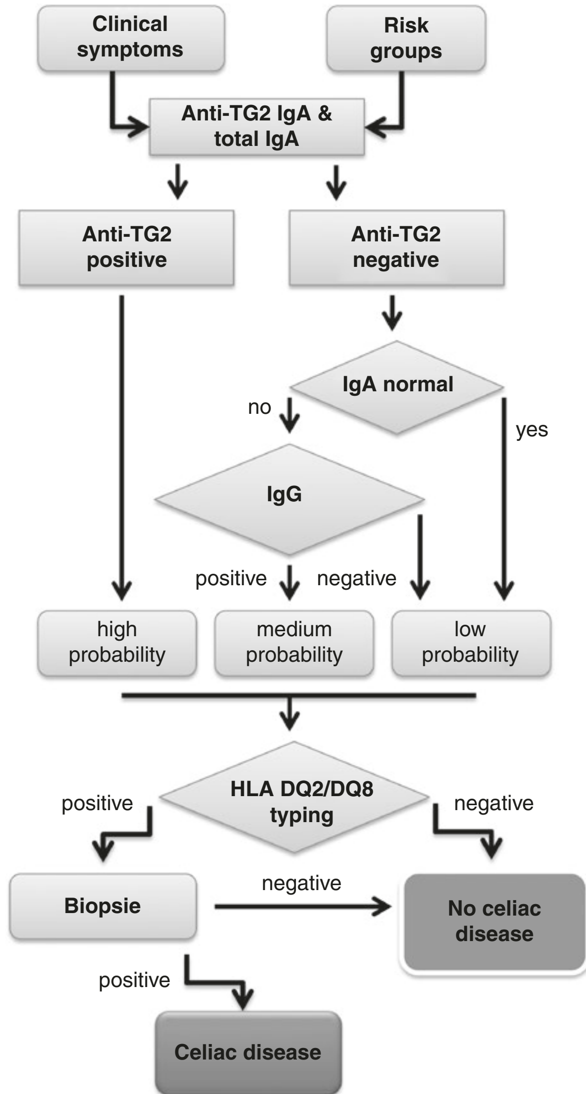

Fig. 4.3 Algorithm for the diagnosis of celiac disease (Torres et al. 2015)

4.5.6 DIVERGENOME

DIVERGENOME [41] is a bioinformatics platform that provides a powerful solution in the field of population genetics and genetic epidemiology. This is a highly efficient online tool for predicting, determining and detecting pathogenic diseases. It is highly specialised to gather, collect and analyse data from large populations. It has contributed significantly to our understanding of the spread of diseases like

tuberculosis and cholera throughout the population. The platform comprises two integrated components: (1) a relational database (DIVERGENOMEdb) and (2) a set of tools to convert data formats as required by popular software in population genetics and genetic epidemiology (DIVERGENOMEtools).

The DIVERGENOMEdb database contains information on genotypes, polymorphisms, laboratory protocols, individuals, populations and phenotypes, and this information is organised in the form of projects. It is open source and free and can be accessed online (pggenetica.icb.ufmg.br/divergenome).

4.5.7 Epi Info™

Epi Info™ is a biology field-oriented and statistical application-based tool, which is designed for the study of epidemiology. It was developed by the Centers for Disease Control and Prevention (CDCs, http://www.cdc.gov/epiinfo) in Atlanta, Georgia (USA), and made available under a public domain licence. Epi Info has been available for Microsoft Windows and for mobile devices with operating system of Android and iOS (beta). The software collects data from epidemiological studies, allowing data entry and further analysis. The software can implement statistical tests, such as t-tests, ANOVA, nonparametric statistics, cross tabulations and stratification with estimates of odds ratios, risk ratios and risk differences, logistic regression (conditional and unconditional), survival analysis (Kaplan Meier and Cox proportional hazard) and analysis of complex survey data (also from https://en.wikipedia.org/wiki/Epi_Info).

4.5.8 AnSWR: Analysis Software for Word-Based Records

AnSWR (http://www.cdc.gov/hiv/library/software/answr/) is an information technology-based software system that helps to scan and organise large data and coordinate project timelines. This application can be applied to large-scale team-based analysis projects and integrates qualitative and quantitative techniques in a short duration. The following applications are provided by AnSWR:

- It helps to coordinate team-based qualitative data analysis from a large population.
- It performs coordination and management of large, complex qualitative databases.
- It facilitates structured codebook development, which can be further arranged into hierarchical coding structures.
- It improves the assessment and text coding for gathering information.
- It provides flexible reporting options with multiple selection criteria (files, codes, coders and quantitative variables) which further enhance integration of the large number of data.
- It has easy and reliable output formats that facilitate import into both quantitative and qualitative programmes, which can be further analysed and integrated easily for user applications.

4.5.9 Lifetables

Lifetables is a very suitable and fast-working tool to aid in epidemiological studies on large populations. In addition to epidemiological studies, the software also supports mortality analysis for demography. This software can be implemented to measure variance, confidence intervals and potential gains in life expectancy and years of potential life lost and lifetime years of potential life lost.

4.5.10 Registry Plus™

Another interesting software tool is Registry Plus™ (http://www.cdc.gov/cancer/npcr/tools/registryplus/), which was developed by CDC, US Department of Health & Human Services, to facilitate implementation of the National Program of Cancer Registries (NPCR). The major roles of this software are to collect and process cancer-related data and record this in a format that adheres to US national standards. For example, TTLC PLUS is an automated tumour linkage and consolidation function for Central Registry Software (CRS) Plus. It determines multiple primary tumours and consolidates data automatically from multiple case reports into incidence records.

Conclusion

Development of several bioinformatics tools has enhanced research activities in the field of population science and disease control. The study of population science generates large amounts of data which need to be rapidly analysed to monitor disease prevalence. The spread of serious diseases, such as "swine flu" influenza and recently Zika virus infection, exposes the clear need and opportunities for such tools. Our collection and description of bioinformatics tools for the study of population science and disease control research may help the biologist to develop faster protocols in order to control disease.

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Race and Ethnicity: Understanding Difference in the Genome Era

5

Shannon Kelly and Yashwant Pathak

Abstract

The Human Genome Diversity Project and International Haplotype Map Project introduced a unique set of questions regarding race in genetics. Race and ethnicity are complex concepts, consisting of much more than a simple biological definition. Differences in drug metabolism and drug susceptibility among races seem to imply that race is biological, and thus treatment should vary. However, evidence shows that environmental factors may contribute more to health disparities than any genetic difference. Studies investigating the effects of race often use self-identification. Though this method is more efficient than individual tests, it leads to inaccuracies and gives credence to ideas of biological difference. Furthermore, the use of self-identified race encourages neglect of important environmental factors. Race-based pharmaceuticals reify biological difference while creating a niche market. Attempts to regulate the use of race in genomics research promise future scholarly debate on the roles of race and ethnicity in genetics.

5.1 Introduction

On June 26, 2000, an announcement was made from the East Room of the White House that “the entire human genome” had been sequenced [1]. The international Human Genome Project was a tremendous achievement that brought scientists from several disciplines together to strive toward a single goal: to sequence the human genome [1]. Sequencing the human genome was thought to be the first step in finding links between genes and disease [2]. Dr. Collins, director of the National Human

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_5

Genome Research Institute, later stated that the 0.1% variation discovered in human DNA sequences would provide “clues to the genetic risk for common illnesses” [3].

There are several critiques of the Human Genome Project. The completion of the DNA sequence has led to the possibility of earlier detection of the genes that cause diseases such as sickle cell or Tay–Sachs disease. Unfortunately, there is no cure to many of these genetic diseases. This burdens adults and parents with the knowledge that they or their unborn children are predisposed to an incurable disease. However, there is hope that knowledge of the human genome will lead to progress in finding treatments for abnormal or damaged genes [4].

Dr. Venter, the president and chief scientific officer of Celera Genomics Corporation, stated in the announcement of the Human Genome Project that the project sequenced the genome of five individuals regardless of race “to help illustrate that the concept of race has no genetic or scientific basis” [1]. A workshop titled “Human Genome Variation and ‘Race’: The State of Science” was held on May 15, 2003, at the National Human Genome Center at Howard University in Washington, DC, to discuss the veracity of this comment [5, 6]. Dr. Collins wrote in a commentary following this conference that “it is not strictly true that race or ethnicity has no biological connection” and referred to the terms “race” and “ethnicity” as “flawed surrogates for multiple environmental and genetic factors in disease” [5]. Ari Patrinos, the director for Biological and Environmental Research for the US Department of Energy, concluded that “oversimplified concepts of race simply don’t work in any objective realm. It’s bad medicine, and it’s bad science” [6]. Other scientists weighed in on the subject. Bonham et al. stated that the discussion of race “should be expanded to include social and behavioral scientists” [7]. This raised many questions regarding the meaning of race in terms of genetics, leading to the development of the Human Genome Diversity Project [8] and the International Haplotype Map Project.

The Human Genome Diversity Project (HGDP) was designed in response to the critique that the Human Genome Project did not “take into account genetic variation among the diverse populations that comprise our species” [9]. The HGDP was designed to “make progress in understanding the patterns of this variation and its causes” and “to provide important information for biomedical studies” [10]. The Haplotype Map (HapMap) Project was established to “determine the common patterns of DNA sequence variation in DNA samples from populations with ancestry from parts of Africa, Asia and Europe” [11]. It was hoped that the HapMap project would reveal genetic disease risk factors [11].

Many questions and concerns arose regarding the ethics of projects such as the HGDP and HapMap Project. The first of these is privacy. Research participants, scientists, and policymakers worry that insurance companies may deny coverage due to a genetic predisposition to a disease [4]. Others worry that employers may be selective in hiring individuals who have tested positive for certain disease-causing genes [12]. Larry Gostin of the Georgetown University Law Center wrote that “genomic data will enhance medical science, but may also encourage discrimination” ([13], p. 109). A survey conducted by the American Management Association estimated in 1997 that 6–10% of employers asked employees to complete genetic

testing ([14], p. 437). The Genetic Information Nondiscrimination Act of 2008 has since been enacted to protect individuals from such misuses of genetic tests.

Another concern is that of consent [15]. Henrietta Lacks died in 1951 from cervical cancer. The cancer cells, known as HeLa cells, were used to develop the first human cell line [16]. These cells were later used in scientific endeavors around the world, including the polio vaccine [17]. However, this was all conducted without her or her family's knowledge or consent. This became a problem in 2013, when the European Molecular Biology Laboratory published the HeLa genome [17]. Henrietta Lacks' family was concerned that "personal medical information about their family could be deduced by anyone who had the full genome map in their possession" [18]. An agreement was finally reached between the National Institutes of Health and the Lacks family in August, 2013, over 60 years after Henrietta's death [17].

Concerns of consent and privacy become especially relevant in the case of race-based information and studies. Study participants have reported concerns of genetic discrimination, or "stigma," following the use of race in genetic research [19]. Joseph Graves labeled any scientist who claimed biological differences could be explained by race a racist [20]. Cavalli-Sforza insisted that "there is no scientific basis for racism" and that the HGDP would "make a significant contribution to the elimination of racism" [8, 10]. Yet, many worry that progress in genetics will lead to the reification of the biological importance of race. Nikolas Rose asked if genomics would "resurrect, or finally lay to rest, the scientific racism that has played such a formative and bloody role in the history of the present" [21]. The Indigenous Peoples Council on Biocolonialism (IPCB) expressed the concern that the majority "not only determines how the information will be used, but also the kinds of stories that will be told about it and the material consequences they will generate" [8].

Ideas of racial difference have historically led to the misconception of a hierarchy among races to which science has not been immune. Psychologist Carl Brigham claimed that "Nordic" groups showed an "intellectual superiority" over other races and ethnic groups. He concluded that the intelligence of other groups could be improved by "admixture of white blood" ([14], p. 82–83). These abhorrently racist ideas led to the establishment of biased standardized tests like the SAT [14]. Popularization of social Darwinism late in the 1800s only escalated these views [22]. The First International Eugenics Congress convened on July 24, 1912 [23]. A correspondent claimed that "conscious selection must replace the blind forces of natural selection" ([14], p. 73). A multitude of publications followed and eugenics became a common theme in biology textbooks. *Modern Biology* published in 1958 by Moon et al. opens its Genetics Applied to Human Inheritance chapter with a discussion of eugenics, stating that "mental ability, scholarship, moral strength, and weakness [are] subject to heredity just as [are] physical traits" ([24], p. 638). A study conducted by Dr. Morning found that various American biology textbooks published 1952–2002 "redefined race as genetic without furnishing empirical evidence for this framing" [25]. Acceptance of "'practical' eugenics" [23] led to the adoption of sterilization laws in the United States ([26, 27], p. 114) (Fig. 5.1).

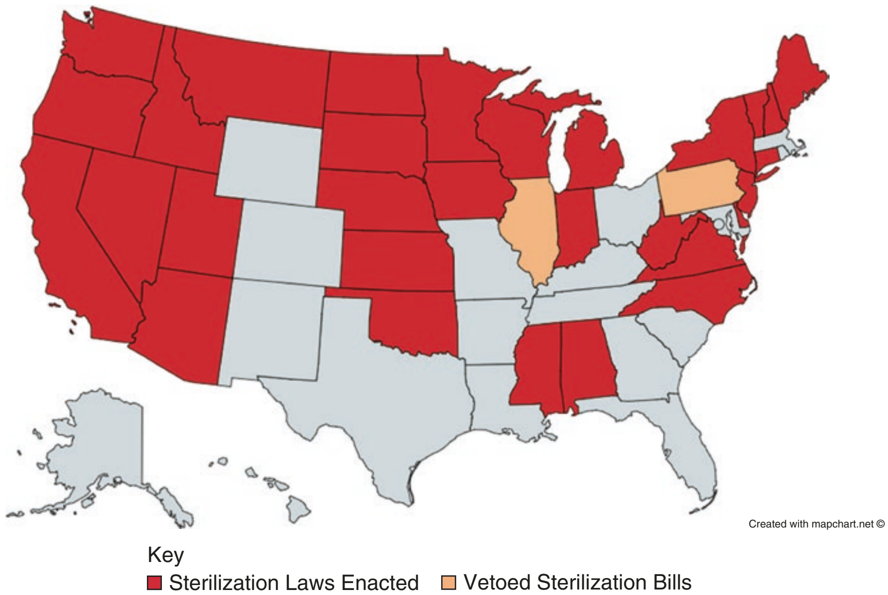

Fig. 5.1 Between 1907 and 1931, 30 states in the United States passed sterilization laws based on eugenic principles ([28], p. 135–137). Bills introduced in Illinois and Pennsylvania were not passed ([14], p. 66–67)

There is no genetic evidence for any claims of racial superiority. Mark Haller suggests that many supporters of eugenics were "already predisposed to nativism," resulting in a convenient "marriage of racism and eugenics" ([28], p. 144). Though eugenics and scientific racism are thought to be primarily historical concepts, race ethnicity reveal a multitude of ethical considerations in genetics. Collins and McKusick suggest that scientists ensure "that the advancement of the social agenda of genetics is equally as vigorous as the medical agenda" [3]. This chapter will investigate the definitions of race and ethnicity, the arguments regarding the biological significance of race, concerns behind the use of self-identified race in research, and the development of race-based pharmaceuticals.

5.2 Defining Race and Ethnicity

It is generally agreed that human life began approximately 200,000 years ago in Africa [29]. Since then, human life has spread across the globe, resulting in a multitude of genetic variations (Fig. 5.2). The terms *race* and *ethnicity* are often used to describe differences among groups of people. There is no one gene that describes race [30]. Instead, *race* refers to the physical differences often used to distinguish groups of people [29, 30]. It is these physical differences that are genetic, including eye color, body shape, and skin color [31, 32]. *Ethnicity,* on the other hand, is independent of genetic factors. People from the same ethnic group can share a language,

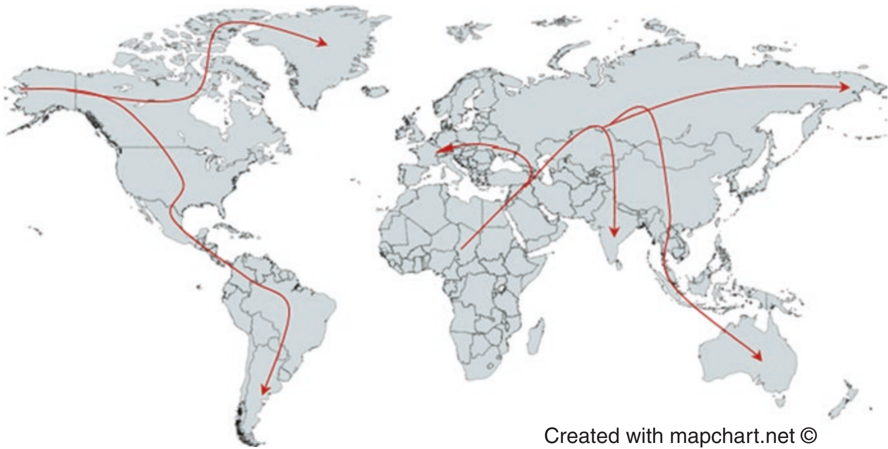

Fig. 5.2 Human life began in Africa approximately 200,000 years ago and has since spread across the globe [37]

culture, traditions, customs, nationality, or beliefs [29, 33]. Race and ethnicity are often used synonymously, but they are in fact two distinct terms. For example, German and Spanish people each have a distinct culture and language. Yet, both Germans and Spaniards belong to the Caucasian race.

Race and ethnicity alone cannot capture the complexity of human diversity [6]. Newman referred to races as "the evolutionary units of life" and are "slowly changing as a whole" ([34], pp. 190). However, most people alive today are descended from members of not one but many races and ethnicities around the world. That is, there is no such thing as a "pure" race. Because common traits are sometimes found in clusters, race has been used to imply origins from a particular geographic region [8, 35]. However, studies show that there is a more genetic variation within races than among them [36] (Fig. 5.2).

5.2.1 Race and Ethnicity Versus Ancestry

Race and ethnicity are often used to estimate an individual's geographic origin due to patterns in genetic traits. However, the social notions of race and ethnicity rarely correlate with genetic history [38]. For example, an individual who has the physical characteristics commonly associated with a particular race may in fact have ancestors belonging to multiple races. In some cases, social constrictions have interfered with the identifications of one's race. For instance, the "one-drop rule" in the United States dictated that any person with at least one ancestor from Africa would be legally considered a member of the Black race [39]. The actual genetic history of those declared Black under this law varied drastically. This variation would not be reflected in the definition of race under these circumstances. An individual's ancestry, however, would reflect this genetic difference.

Ancestry can be valuable in the determination of the inheritance of certain genetic factors. For instance, the genetic history of an individual can reveal a predisposition for a number of diseases, such as diabetes and rheumatoid arthritis [40]. This pattern of inheritance can span across several generations. It is unlikely, however, that entire races or ethnic groups would share the same patterns of heredity.

5.3 Is Race Biological?

Is race a biological reality or merely a social construct? According to Dr. Ann Morning, there are three main arguments contributing to this debate: essentialism, constructionism, and anti-essentialism [41]. Essentialists hold to the belief that race is biologically significant. In the genetic age, essentialists say that differences in drug metabolism and disease susceptibility among races exemplify this significance. Therefore, patients of different races should receive different treatments. For instance, African-American patients are more likely to die from heart failure than Caucasian patients [42]. However, this may not be caused by race directly. Other disease-causing factors, such as poor diet and access to healthcare, for example, are statistically more common among minority populations [38]. It is possible that diseases that are more prevalent in some races may be due to factors other than race itself.

The opposing constructionist and anti-essentialist views are complementary. Anti-essentialists believe that the arbitrary nature of race, as well as evidence of racial and ethnic blending due to migration and intermarriage, give credence to the idea that race is a social construct [43]. Constructionists hold that race is a social idea that has been promoted historically through practices such as slavery [43]. The following sections will analyze these views by discussing racial differences in drug metabolism and disease susceptibility as well as the anti-essentialist and constructionist explanations of race as a social construct.

5.3.1 Disease Susceptibility

It is well known that many diseases are hereditary. Huntington's disease, for example, is caused by a gene-denoted Chromosome 4, which can be inherited from either parent [44]. It is clear that sex is an important factor in disease inheritance. The defective gene that causes hemophilia is caused by the X chromosome inherited from the mother. Because men have only one X chromosome, any male who inherits the defective X chromosome will have hemophilia. Females, on the other hand, have two X chromosomes. Thus, a female who has inherited one X chromosome containing the defective gene will be a carrier of the disease but will not be a hemophiliac [45].

Table 5.1 Occurrence of sickle cell trait in 13 states—2010 Adapted from [49]

Race	Number tested for sickle cell trait	Number positive for sickle cell trait	Percent positive for sickle cell trait (%)	Percent of total sickle cell trait presence by race (%)
Asian/Native Hawaiian/Pacific Islander	73,250	164	0.22	1.14
African-American	156,732	11,461	7.31	79.85
White	899,686	2729	0.33	19.01
Total	1,129,668	14,354	1.27	100

There is much debate as to whether or not race is also an important factor in disease inheritance. The alleles associated with some diseases are unequally distributed among different populations [32]. Tay–Sachs disease, for example, is found most often in Jewish children descended from Northern Europe [46]. Likewise, sickle cell anemia occurs mostly in African-Americans. Approximately 1 in 10 African-Americans in the United States have a sickle cell trait and 1 in 600 African-Americans has the disease [47]. However, sickle cell anemia is not found solely in African-Americans. People from Central Africa, the Mediterranean, the Middle East, and India can also inherit the sickle cell trait [47]. The sickle cell trait is not associated with race, as it was once believed. Recent developments show that the sickle cell trait results in malaria resistance [48]. Thus, the sickle cell trait is often found in regions where malaria is prevalent as well as descendants from these regions. According to Table 5.1, of those in the United States who tested positive for the sickle cell trait, 1.14% identify as Asian, Native Hawaiian, or Pacific Islander, 79.85% identify as African-American, and 19.01% identify as White [49].

Environmental factors can play an important role in disease [4]. For example, African-American women in Chicago, Illinois, are more likely to die from breast cancer than Caucasian women [50]. This is likely due to disparities in access to healthcare rather than any genetic explanation [50]. It is clear that although there may be a correlation between race and the occurrence of some diseases, race is not necessarily the direct cause.

5.3.2 Drug Metabolism

Adverse drug reactions are among the most prevalent causes of death in the United States [37]. Yet, few genes have been identified that explain different drug responses [51]. Genetic variability in drug metabolizing enzymes (DMEs) is one promising explanation for varied drug response.

The varied presence of DMEs among populations means that different populations will show different average drug responses [52]. For example, reports indicate that White patients require higher doses of warfarin for anticoagulation than Asian patients. The AA genotype of VKORC1 is associated with increased sensitivity to warfarin. This genotype is found in 82% of Chinese patients and only 14% of White patients [53]. While there seems to be a correlation between race and the presence of some DMEs, this does not imply that race is a direct cause of this variation. The contribution of other factors, such as diet and healthcare, has not been evaluated [53]. Variation in drug response and in the presence of DMEs occurs within races as well as among them [52]. Thus, trends in race and DMEs may be deceiving. According to Wilson et al., "ethnic labels are insufficient and inaccurate descriptions of human genetic structure" ([52], p. 265). It may instead be beneficial to analyze drug responses individually.

5.3.3 Race as a Social Construct

We have shown that the only genetic indicators of race are those that determine superficial physical traits. But even these so-called racial definitions are arbitrary. Individuals from different parts of the world can exhibit similar outward traits. People from Kuwait and Chad, for example, often share a similar skin color [32]. Additionally, there are no explicit boundaries between races. For example, one might assume an individual has African ancestry based on physical characteristics. The African nation or tribe from which this individual is a descendent is a much more difficult matter due to the ambiguity in racial distinctions. Furthermore, generations of migration and intermarriage has led to a "gradual blending" of races and ethnicities [42]. This can especially be seen in "melting pot" societies like the United States. Moreover, research has shown that 85.4% of human genetic diversity lies within races [36].

It has long been misconstrued that because a person or a group looks different, they must be different biologically as well. History has reinforced this idea of biological difference time and time again. European settlers justified their bloody war against the American Indians by insisting that the American Indians were an inferior race [32]. American slavery was especially brutal, due to its "reduction of the slave to less than human status by the use of racial hatred" ([54], p. 28). African-Americans were not legally granted civil rights until 1964, nearly a century after slavery was finally abolished in the United States [26].

There is no genetic or scientific explanation behind these claims of superiority. According to Swedlund, "most distinctions that people make between themselves and others have much more to do with culture than with biology" ([32], p. 60). It has become clear that assumptions regarding race were not the only reason for these bloody actions. Slavery turned an enormous profit. The New World's "Indian removal" allowed the young nation to expand and build cities on the west coast ([54], p. 125). Race was a convenient way to explain the atrocities that were

committed for land and coin. These actions and ideas have fueled years of discrimination and racism that can still be seen today.

5.4 Use of Self-Identified Race in Research

It has become clear that more information is needed to determine the importance of race in the field of medicine. Social barriers may cause different genes to appear in only some populations. Collecting the race of a patient or study participant can help ascertain if there are any such correlations. Many studies utilize a patient's self-identified race. Racial identity is complex and can differ based on geography and personal experience [55]. Furthermore, it is common for people to change their racial identity throughout their lives [55]. The use of race in research can lead to ignorance of other factors leading to disparity, such as diet and access to healthcare. The use of race as a proxy for these factors can lead to a racialization of certain diseases, among other harmful social implications [56]. The following sections will analyze arguments for and against the use of self-identified race in biomedical research.

5.4.1 Advantages

Researchers are attempting to understand the nature and reasons behind the "unequal distribution" among different races of the alleles responsible for diseases such as sickle cell anemia and Tay–Sachs disease [5]. Obtaining self-identified race and ethnicity is the most efficient and economic method to study this occurrence [51]. Tang et al., for instance, conducted a genetic cluster analysis that resulted in clusters that "showed near-perfect correspondence with the four self-reported race/ethnicity categories" [35]. It is believed that shared racial identity is an indicator of shared ancestral history as well as "a useful proxy" for nongenetic factors [5, 56].

Social categories can result in barriers to interaction which can account the appearance of alleles almost exclusively in some populations [56]. The occurrence of Tay–Sachs disease in European Jewish children, for example, may be due to the founder effect [57]. The limited gene pool of small populations makes the inheritance of some genes, such as the Tay–Sachs trait, more likely. Over generations, the gene pool would only become smaller, causing the inheritance of such traits increasingly likely [32].

Some groups will only marry within the community by tradition. The Amish, for example, force those who marry outsiders to leave the community ([58], p. 183). Additionally, marriages of two people of the same race are much more common than interracial marriages. In 2010, interracial marriages accounted for only 9.5% of all marriages in the United States [59]. This, too, results in a recurrence of genes within the same population.

5.4.2 Concerns

The use of self-identified race in research has several flaws. Many studies will use racial categories (White, Hispanic, Black, Asian, or other) in questionnaires without the ability to choose multiple options [29]. This ignores any contributions of admixture, mutation, or genetic drift. Destro-Bisol et al. estimated that European admixture in African-Americans is approximately 26% [60]. Moreover, some individuals may not be aware of their full racial and ethnic history [29]. Thus, studies utilizing the above categories are likely to report inaccurate data.

Racial identity depends on many social variables and few biological factors. Racial definitions vary based on environment and personal experience [55]. For example, Eschbach, Supple, and Snipp found a correlation between racial identification and the educational attainment of American Indians [61]. Furthermore, it is common for individuals to change their racial identity throughout their lives [62]. This complexity of racial identity makes it difficult to use standard categories for research studies.

There are several concerns regarding the social implications of race in research. The inclusion of race in research can lend more credence to the biological importance of race than it deserves [56]. Such reinforcement of racial categories can harm the "reputation, integrity, or social status" of the members of any given population [19]. Participants of a study conducted by de Vries et al. termed this concern "stigma" [19]. This racialization has rekindled fear of the ideas of superiority that fueled eugenics. Risch et al. stated that "superiority is not scientific, only political" ([51], p. 11). However, science can be warped to further political agendas. The United States, for example, used limited phenotyping to legally determine African-American and Native American identities [63]. It is important that these implications are considered.

5.4.3 Alternatives

The importance of race in biomedical research cannot be denied. Nor can the concerns regarding the use of self-identified race be ignored. Some studies have tried to circumvent this problem by using geographic ancestry [35]. The use of ancestry informative markers (AIMs) shows promise as a proxy for self-identified race by avoiding the use of self-reporting [29, 55]. However, both of these methods ignore the impact of other factors on health disparities between populations. Dr. Obasogie suggested that racial categories can be used in research as long as their use is placed under "strict scrutiny" [64].

An article in Genome Medicine in 2009 outlined several recommendations regarding the responsible use of reported race in research [55]. Race should only be reported when relevant and should not be used in place of genetic, biological, social, economic, or environmental factors. All other relevant factors (diet, socioeconomic status, age, nutrition, health beliefs, etc.) should be reported and should be used

instead of race whenever possible. The variables and terms used in the study and their relevance should be clearly defined. The study should outline how the data was collected (i.e., self-reported or assigned by the researcher). Most importantly, any researcher should consider "the social and ethical implications of the study results" ([55], p. 4).

5.5 Race-Based Medicine

Race-based medicine has introduced unique social issues. In New Zealand, for example, debate has focused around who should and should not conduct research concerning the Māori people who are indigenous to New Zealand [65]. Historically, the results of Māori studies conducted by non-Māori resulted in a mischaracterization of the group on the whole, by reinforcing stereotypes and invalidating Māori knowledge [65]. This has led to the emergence of "by Māori for Māori" healthcare in New Zealand [43]. In the United States, debates have centralized around the commercial gains and science of race-based medicine. It has even been suggested that medicine produced for minorities is being held to a lower research standard than medications produced for the general public [66]. The following sections will analyze these arguments through a case study of BiDil, a heart failure preventative approved only for African-American cardiac patients.

5.5.1 Commercial Gain

Genetic research introduces an entirely new territory for pharmaceutical companies. Because personalized medicine is a new market, the chance of profit is markedly higher than other pharmaceuticals. Even rebranding an existing drug as race-specific can elicit new profit opportunities [66]. Dr. Jay Cohn took advantage of this fact when he created BiDil.

Dr. Cohn received a patent in 1989 for developing a method utilizing hydralazine and isosorbide dinitrate as a preventative for congestive heart failure (US Patent No. 4,868,179 [67]). Cohn was denied a renewal of his patent in 1999 [66]. In 2002, Cohn, sponsored by NitroMed, obtained a patent for the same combined therapy. The vital difference, however, was that this final accepted patent made the drug race-specific for African-American patients (US Patent No. 6,465,463 [68]). The controversial drug BiDil was approved by the FDA in 2005 for use administration to African-American heart patients [69]. The use of race in patenting has increasingly become a fast and cheap way to gain FDA approval [70].

The pharmaceutical industry is far from the first to use race to sell and market goods; race has been used to market products to consumers for decades [71]. Patents have cited race as the determining factor for the invention of a variety of products—toys, cosmetics, hair products, etc. [72]. The problem is not that NitroMed has made money from marketing BiDil for African-American patients. The problem is that

Table 5.2 Price comparison of BiDil to hydralazine and isosorbide dinitrate

Drug	Price per unit
Hydralazine (25 mg)	\$0.17–\$0.58
Isosorbide dinitrate (20 mg)	\$0.90–\$1.06
BiDil (37.5 mg/20 mg)	\$3.16

NitroMed was allowed to produce a drug for African-American patients that was previously declared unfit for administration to the general population due to flawed scientific claims [66] (Table 5.2).

5.5.2 Race-Based Medicine is Scientifically Flawed

As mentioned in the previous section, Dr. Jay Cohn submitted patents in 1989 and 2002 for the use of hydralazine and isosorbide dinitrate to prevent congestive heart failure. The vital difference between the two patents was that the 2002 patent specified use in African-American patients (US Patent No. 6,465,463 [68]), while the previous patent had no mention of race (US Patent No. 4,868,179 [67]). Why, 10 years later, have the same drugs become effective only in patients who identify as one specific race?

The studies that supposedly show that BiDil is most effective in African-American patients were only conducted on self-identifying African-American people [73]. No comparison studies were conducted on heart patients of other races [66]. This gives the impression that BiDil is effective only in African-American patients, when there is in fact no evidence that BiDil is any more effective in African-American patients than it is in patients of other races [74]. In fact, isosorbide dinitrate and hydralazine are frequently administered concurrently to heart patients regardless of race [66]. Yet, because BiDil was only tested on African-Americans, the FDA has approved it for African-American patients only [69].

A significant motivator for the approval of race-specific heart medication is the claim that African-American heart patients are two times more likely to die from heart failure than Caucasian heart patients [42]. This claim was based on outdated data. As of 2003, the mortality rate of African-American patients versus Caucasian patients due to heart failure was 1.1:1 [75]. Furthermore, it is yet to be confirmed that the high mortality rate of African-American heart patients is solely due to race [38]. Hypertension is also common in African-American populations and can be a precursor to heart disease [38]. However, it is more likely that diet and racial disparities in access to healthcare [76] have caused the prevalence of these conditions than any biological factor due to race.

5.5.3 Lowered Research Standards

The previous section illustrated that more research regarding heart disease among minorities was necessary before declaring BiDil a race-specific medication. Yet, the FDA approved the drug in 2005 for prescription to African-American patients [69].

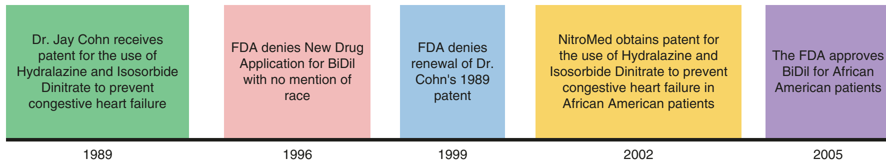

Fig. 5.3 A timeline of the events leading to the approval of BiDil

Generally, minorities have a much lower representation in clinical trials than Caucasians [38]. Nonetheless, the FDA has continually granted approval for all races based on these disproportionate trials [66]. It is interesting then, that the FDA chose to approve BiDil for administration only to African-Americans. As Dorothy Roberts boldly states, this implies that "black people cannot represent all of humanity as well as white people can" [66].

A New Drug Application for BiDil was submitted in 1996 to the FDA by the company to which Dr. Cohn licensed the rights to his 1989 patent [66]. Neither the patent nor this application mentioned race. The FDA denied the application, stating that the claims were based on data gathered in the 1980s [77, 78], and therefore did not meet FDA standards after the National Institute of Health Revitalization Act of 1993 [79]. Instead of gathering new data to substantiate the previous claims, the study focused on only African-Americans. The FDA approved BiDil on the logic that heart disease in African-American patients was similar to a rare disease, as opposed to generic heart failure [66]. The same data that had previously caused BiDil to be denied were overlooked because the drug had become race-specific [66].

The quandary surrounding BiDil is not whether or not it should be made available to those who need it, but rather, should its availability be determined by race based on the data collected. Due to the commercial gains of creating a race-based medication, the lack of definitive evidence that biology is the cause of health disparities among races, and the lower standards held by the FDA for race-based medication, it is abundantly clear that more research is necessary in the field of race-based medicine before such a conclusion can be reached (Fig. 5.3).

5.6 Future Trends

Attempts have been made to regulate the use of race in genomics research. The Genomics and Personalized Medicine Act of 2006 outlined the establishment of a Genetics and Personalized Medicine Interagency Working Group that would determine "an appropriate definition of race and ethnicity for use in genomic research" as well as "guiding ethics, principles, and protocols for the inclusion and designation of racial and ethnic populations in genomics research" (S. 3822, [80]). The 2006 act was not passed but was reintroduced in 2010. The Genomics and Personalized Medicine Act of 2010 called on the FDA to "to conduct additional post market studies to identify genetic and other biological, social, behavioral, and environmental factors that may underlie the differential drug effects when drugs are

shown to be more or less effective in certain racial and ethnic subpopulations" (H.R. 5440 [81]). The 2010 act was not passed; however, the FDA released "Regulatory Science Initiative" in 2010 [82] and recently published its intent to improve the oversight of next-generation sequencing [83]. These initiatives are encouraging signs of future discussions concerning the role of race and ethnicity in genomics research.

Conclusion

The Human Genome Diversity Project and International Haplotype Map Project introduced a unique set of questions regarding race in genetics. Concerns arose that the inclusion of race in these endeavors would reify race as a biological difference and rekindle scientific racism. Using race in research has encouraged its use as a proxy for other factors, including geographic origin and environmental factors. Self-identified race is the most economical and efficient way to gather information [51]. However, it can lead to racialization of disease or "stigma" [19, 56].

Race is becoming an increasingly important factor in medicine and research. Various studies have focused on finding a correlation between the efficacy of certain drugs and race [84]. However, any genetic differences among races may not fully explain any differences found in these studies [38]. For example, social stresses or lack of medical care among minorities could explain the frequent occurrence of certain conditions in some races, such as heart disease in African-Americans [42]. Considering that there is no single gene that is indicative of race [30], it is highly likely that nongenetic factors are equally responsible for disease.

It has been suggested that race-based research include the work of social and behavioral scientists as well as psychologists [7]. This way, the idea of biological difference among races would not be exaggerated, and social differences would be included in racial studies. The FDA recently published a "Regulatory Science Initiative" and announced its intent to improve the oversight of next-generation sequencing [82, 83]. These are promising signs of future discussions of the role of race and ethnicity in research.

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6 The Food Metabolome and Novel Dietary Biomarkers Associated with Diseases

Orhan E. Arslan and Philip Palmon

Abstract

The food metabolome is an area of research that is of rising interest, particularly the association of chronic diseases to dietary biomarkers. This chapter will review and assess the current literature of the novel biomarkers used to track dietary intake of carbohydrates, fats, proteins, vitamin C, and carotenoids, as well as to explore the relationship between dietary patterns and biomarkers associated with disease processes. There is good evidence for the use of certain novel biomarkers for each of the aforementioned dietary components. A review of the literature also reveals a relationship between a "Western" dietary pattern, characterized by higher intakes of red meat, processed meat, French fries, eggs, high-fat dairy products, sweets, and refined grains and that of increased risk for systemic inflammation, endothelial dysfunction, cardiovascular disease, metabolic syndrome, hypertension, and diabetes. Contrasting this is the "prudent" dietary pattern characterized by increased fruit, vegetables, poultry, fish, whole grains, and legumes which had improved biomarker profiles of the aforementioned diseases. Similar to the "Western" pattern is a diet with *high* saturated fat/high simple carbohydrate that correlates with increased biomarkers of Alzheimer's disease. There appears to be a large volume of literature investigating biomarkers of dietary intake and the relationship between dietary patterns and chronic diseases. However there is still need for literature looking into the relationship of novel biomarkers of fats, carbohydrates, protein, vitamin C, and carotenoids and the biomarkers of chronic diseases.

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_6

6.1 Introduction

The food metabolome is an area of research that is becoming increasingly intriguing. In recent years there has been a dramatic rise in the publications pertaining to dietary biomarkers and their application to both measuring the dietary intake and the biomarkers associated with chronic diseases. The term biomarker refers to any molecule or substance that is "a biochemical indicator of dietary intake/nutritional status (recent or long term)." It is also defined as an index of nutrient metabolism or a marker of the biological consequences of dietary intake [1]. There have been attempts linking individual molecules with certain disease processes, for example, saturated fats with cardiovascular disease or with those at risk of developing Alzheimer's disease, although the strength of this notion has not been supported [2, 3].

As indicated earlier the main purpose of this article is to assess the current literature and examine the novel biomarkers that are employed to track dietary intake of carbohydrates, fats, proteins, vitamin C, and carotenoids. As we proceed further, the validity and reliability of each of the novel biomarker and the physiology of digestion and absorption of carbohydrates, protein, and fats will also be discussed. The external factors that render a molecule to be a biomarker including genetic variability, physiological and dietary factors, type of biological sample, and the analytic methodology used to measure the biomarker [1]. Further, the significance of the relationship between dietary patterns and biomarkers of some chronic diseases including cardiovascular disorders and Alzheimer's disease will also be explored.

The dietary patterns contrasted here are typically concentrated on the intake of more fruits, vegetable, lean proteins, and low saturated fats compared to those with more refined sugars, red meats, and higher saturated fats. Lifestyle and body mass indices also play a role in determining the subjects' biomarker profile.

6.2 Carbohydrates

6.2.1 Physiology of Carbohydrate Absorption

When examining the carbohydrate, we encounter three major sources, the sucrose, lactose, and starches. The breakdown of carbohydrates starts in the oral cavity by ptyalin, an enzyme secreted by the parotid glands. Ptyalin hydrolyzes approximately 5% of all starches before the food bolus travels to the stomach to encounter additional hydrolization by the gastric secretions, and as a result, approximately 30–40% of the starches will convert into maltose. Digestion and conversion to mostly maltose continue in the small intestine with the help of pancreatic amylase, or other small glucose polymers. The breakdown of disaccharides into monosaccharides occurs by the lactase, sucrose, maltase, and alpha-dextrinase contained in the enterocytes lining of the intestinal villi. Absorption is then achieved by the sodium co-transport mechanism where the gradient of Na absorption allows for the absorption of glucose and other monosaccharides [4].

Sugars are the major contributors to the consumed energy products either as monosaccharides (i.e., glucose or fructose). In fact, research suggests that up to 22% of energy may be provided to certain adult populations, specifically in the European Community, by sugars [5]. The source of the sugars remains wide-ranging as many sugars are incorporated within the processing of our foods or within the sugar-sweetened beverages. In fact studies have postulated the possible role of sugar contained in the sweetened beverages in the epidemic of obesity as they have positively contributed to increased sugar consumption. However, a definitive link between increased sugar-sweetened beverages and obesity-associated diseases such as diabetes, hypertension, and cardiovascular disease has not been identified [6]. One of the perplexing issues that consumers face is the hidden nature of the sugars and the inability to make accurate estimations of their sugar consumption. One study in particular by Bingham et al. proposed that obese individuals are susceptible to underestimating their consumption [7], and biomarkers of carbohydrate consumption may alleviate the need for individuals to properly estimate their sugar or carbohydrate consumption.

6.2.2 Novel Sugar Biomarkers

There are several novel biomarkers that have been suggested in the literature. Tasevska et al. found that sucrose and fructose measured in 24 h urine samples correlate with the amount consumed. It has been demonstrated in this study that for every 100 mg of sucrose and fructose detected in the urinary samples, approximately 200 g of total sugars were consumed. It is theorized that the measured sucrose is a small fraction that was not hydrolyzed in the small intestine, and that the fructose is a combination of the fructose ingested as well as the hepatic breakdown product of ingested sucrose [8]. Joosen et al. showed that urinary sugars are not affected by patient's body mass index (BMI) [9]. Two methods of measuring urinary sucrose, gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry, were compared by Kuhnle et al. The results of this work demonstrated that gas chromatography was superior at identifying more compounds but is limited by the time needed for preparation and overall length of time used for measurement when compared to liquid chromatography [10].

Another novel biomarker found in cane sugar and high-fructose corn syrup is carbon stable isotope abundance of ^{13}C. Cook et al. measured the fasting ^{13}C levels in the glucose as a potential biomarker; however, these levels were proven unreliable indicators as gluconeogenesis causes ^{13}C dilution. This study, however, showed a correlation between random plasma ^{13}C glucose readings and the amount of consumed cane sugar and high-fructose corn syrup ($R2 = 0.90$) [11]. The work of Davy et al. validated the finger-stick sampling when compared head to head with venipuncture samples, along with good reproducibility ($r = 0.87$) [12]. Since measurements of finger-stick readings may only represent recent intake and not long-term consumption, more research is warranted to address this limitation and to determine the length of time reflected with

the ^{13}C readings. Additionally, ^{13}C is also limited, in that not all sugars consumed by those in these studies are a C4 derivative. For example, beet and maple sugars are C3 derivatives, while ^{13}C does not reflect C3 derivatives [6].

Plasma alkylresorcinol concentrations are another potential biomarker of whole grain wheat and rye. The plasma level of alkylresorcinol is achieved 1 week after ingestion of bread [13], and that high reproducibility can be achieved using plasma alkylresorcinol [6]. A significant correlation of r = 0.50 ($p < 0.05$) has been found between the fasting and non-fasting levels [6]. Corollary to this, an association between alkylresorcinol level in red blood cell before and after ingestion of whole wheats has been shown. Urine level of alkylresorcinol can be verified through two of its metabolites, 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-propanoic acid (DHPPA) [6]. Aubertin-Leheudre et al. found a substantial correlation between plasma alkylresorcinol levels and the urinary levels of DHBA and DHPPA, which provides less invasive means of measuring whole wheat and rye consumption. Research data suggest that alkylresorcinol may be a short to medium range marker, spanning a time period between 4 h and 2 or 3 months. Reproducibility has been shown in the 2–3 month period. A potential limitation of AR is linked to its relatively low clearance over time, permitting overestimation in periods of high intake and underestimation in periods of low intake [14].

6.2.3 Fat

6.2.3.1 Physiology of Fat Metabolism and Absorption

Fat digestion occurs most significantly in the small intestine compared to less than 10% in the mouth and the stomach. The main mechanism by which fat digestion occurs is through the emulsification of bile acids and lecithin or through the enzymatic process of pancreatic lipase. This process allows the large globules of fat to be fragmented down to smaller particles in the water-rich environment, facilitating the further disintegration of the triglycerides by the digestive enzymes of the pancreas. The breakdown of triglycerides then allows for the formation of micelles, compounds that have hydrophobic core of fat with a hydrophilic outer layer of bile salts that can be absorbed by the epithelial layer of the small intestine [4].

6.2.3.2 Novel Biomarkers of Fat

Recent literature suggests that blood lipids have the potential to be biomarkers. Bingham et al. indicated that the low-density plasma levels of cholesterol positively correlate with the dietary saturated fats and that a negative correlation between high-density cholesterol plasma levels and carbohydrates and also between the plasma levels of triglycerides and dietary fiber does exist. Bingham et al. demonstrated that the relationship may also be influenced by the type of measurement used [15]. Hegele's work showed that the relationship between dietary fiber and plasma lipid levels may be influenced by the variation of fatty acid-binding protein 2 gene [16]. Mazda et al. suggested that this might mean that both genetic variability and type of measurement used may influence any relationship found between plasma lipids and dietary fibers [1].

Studies conducted by Hegsted et al. showed that plasma lipid levels are primarily dependent on saturated fat intake [17]. The work of Wu et al. advocated the notion that the plasma low-density cholesterol levels are well correlated with the total saturated fat intake and total caloric intake of saturated fat [18]. Burnett and Hooper demonstrated that diet, lifestyle, and genetic variation will ultimately influence plasma low-density lipoprotein (LDL) levels. Population with genetic variations that include polymorphism in the APOA1, hepatic lipase, TNF alpha, NFKB1 genes, and TG levels by polymorphisms in PPAR alpha gene affect the LDL levels by polymorphisms in the APOE locus [1]. While the difference in genetic populations is not fully explored, it is clear at this point that genetics may have effect on the validity of HDL, LDL, and TG as potential biomarkers [1].

6.2.3.3 Fatty Acids

There have been multiple studies looking into the use of fatty acids as biomarkers of fat. These biomarkers are influenced by lifestyle factors such as diet and exercise, as well as fatty acid synthesis within the body [1]. Some examples of variations within the fatty acid synthesis were demonstrated by the work of Baylin et al. who demonstrated that the enzymes in the elongase/desaturase pathway of n-3 and n-6 fatty acid metabolism are greatly influenced by the genetic variations [19]. The work of Weiss et al. established a relationship between the amount of consumed fat, obesity, and fatty acid-binding protein 2 gene and the changes in the endogenous insulin metabolism [20]. In the research studies conducted by Mutch et al., it was revealed that fatty acid metabolism can be influenced by the hepatic P450 system. Disruption of this system causes changes in gene expression and ultimately changes in fatty acid metabolism [21]. The genetic variants of FADS1 and LIPC have been shown to create different metabolites, confirming the notion that increased variation in plasma fatty acid levels is closely linked to individual genotyping [22].

According to Baylin et al., an association between polyunsaturated fatty acids measured in adipose tissues and the dietary linoleic and alpha-linolenic acids does exist [23]. Similar correlation has been revealed by the author between alpha-linoleic acid in the fasting blood and that of the linoleic acid in the fasting plasma levels [24]. However, no significant differences have been noted between the fasting blood levels when compared to plasma and adipose tissue levels.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are omega-3 fatty acids derived from fish, have been studied as potential biomarkers. One study demonstrated significant correlation between plasma levels of EPA and DHA and dietary intake in both males and females [24]. Dietary EPA and DHA can also be measured through the use of a surrogate, 15N [25], and a positive correlation between levels of 15N and dietary EPA, and DHA has been drawn [26]. Although other sources of 15N are present as fortified food products and non-fish protein, the validation and ability to draw conclusions about the levels of fish consumption become difficult [6]. Despite the availability of evidence suggesting that plasma EPA and DHA are more reliable indicators of their intake, conclusive evidence that verifies the time period in which a single measurement has been obtained remains inconclusive [6].

A study conducted by Harris and research team examined the omega-3 fatty acid intake and its measurement using the total amount of EPA and DHA in RBC membranes as an index (omega-3 index). This study showed with $p < 0.001$ that correlates between omega-3 index, plasma phospholipid, and whole blood EPA and whole blood DHA do exist [27].

The biomarkers of olive oil were also explored in view of the relationship between reduction of cardiovascular diseases and the high olive oil intake. Two potential biomarkers, tyrosol and hydroxytyrosol, were studied. Tyrosol correlated positively with the amount ingested and with the urine sample levels in a dose-dependent manner at a rate of 16.9% for a single dose and 19.4% for a week long of doses. Hydroxytyrosol showed a measurement of 78.5% for a single dose and 121.5% after a week of doses. This study indicates that tyrosol is a promising marker when compared with hydroxytyrosol, a possible biomarker, which is likely derived from other sources and synthesized within the body [28].

The work of Riboli et al. demonstrated that plasma fatty acid levels correlate to the medium-term intake, on the order of weeks to months [29]. A better association has been established with fish oil-derived fatty acids that are not synthesized by the body [1].

6.2.3.4 Protein

Proteins are broken down by different mechanisms depending on the location within the digestive tract and the enzymes that comes in contact with the consumed protein molecule. Three major enzymes are involved in protein digestion have been identified including pepsin in the stomach, pancreatic proteases in the duodenum and jejunum, and by the peptidases in the small intestine. Within the stomach pepsin digests proteins most optimally at a pH of approximately 2–3 and is inactivated at a pH above 5. It facilitates the cleavage of peptide bonds using hydrolysis to separate adjacent amino acids and provides approximately 10–20% of total protein digestion. Pancreatic secretions, which consist of enzymes trypsin, chymotrypsin, carboxypolypeptidase, and proelastase, play a role in digestion in the duodenum and jejunum. Their protein-degradative function is limited to the level of single amino acids; most are left as di- and tripeptides. The final major component of protein digestion occurs within the small intestine in the microvilli along the brush border of the enterocytes, which contain peptidases capable of breaking down the di- and tripeptides into single amino acids. The absorption of amino acids through the luminal membranes of intestinal epithelial cells occurs via the sodium co-transport molecules, much in the same manner as glucose transport. There are at minimum five different transport proteins in the small intestinal epithelial cells, accounting for the large diversity of amino acids that must be absorbed [4].

Available data have identified multiple potential biomarkers of protein consumption. The work of Stella et al. demonstrated that creatine, carnitine, and trimethylamine-N-oxide are good biomarkers of meat-derived protein [30]. The role of creatinine, taurine, 1-methylhistidine, and 3-methylhistidine as potential biomarkers of animal protein has also been explored as these molecules linked

specifically to meat consumption and are excreted in the urine, and that all four components have a dose-dependent response to red meat protein consumption, with a p value <0.0001 [31]. This study looked at three levels of red meat consumption and one vegetarian protein rich diet. An interesting fact is that 1-methylhistidine and 3-methylhisitidine exhibited characteristics that enable distinguishing consumption of protein between the low level of meat and the vegetarian protein, whereas the creatinine and taurine were less sensitive to the changes in diets. Between 1-methylhistidine and the 3-methylhistidine, the 3-methylhistidine, unlike the 1-methylhistidine, appears to have greater potential as a biomarker for red meat consumption as it is not endogenously produced [31].

The work of Bingham et al. affirmed that consumption of red and processed meats leads to the formation of N-nitroso compounds within the gastrointestinal tract [32]. However, determining the level of these compounds remains a challenge to this date largely due to the fact that it requires fecal sampling of an epidemiological scale [1].

A biomarker that has demonstrated good validity is the urinary nitrogen as a way of assessing total protein intake [6]. In one study multiple 24 h urine nitrogen outputs were assessed and found to have correlation of 0.99 over a 28 day period of time. The same study demonstrated that a correlation of 0.95 can be achieved with a time span of 18 days. However, other studies concluded that urinary nitrogen may not be the most accurate in states of high or low intake, as it raises the possibility of underestimating or overestimating the results [6].

6.2.3.5 Vitamin C

Vitamin C (ascorbic acid) is an essential vitamin found mainly in the fruit and vegetables. The feasibility of use of biomarkers to determine the level of vitamin C has been explored by several studies including Benzie et al. which assessed the suitability of sampling urine and saliva for this purpose. This investigation confirmed the fact that both urine and saliva are not appropriate fluids for determining vitamin C status, as the saliva has a very low vitamin C content, while urine will only have detectable levels in those individuals who consume excess of this vitamin [33]. It appears, as the data obtained from the study conducted by Dehghan et al. that the best determinant of vitamin C levels is through direct measurement of vitamin C in plasma concentrations. In this study an average correlation between plasma concentration and estimated intake was $R = 0.4$ [34]. Several other studies indicated that the correlation appears to be population dependent, ranging from 0.12 in India and 0.53 in Spain, with a better correlation in male subjects when compared to females. It appears that additional factors came into play in the determination of the moderate correlation documented in the literatures, including errors in dietary amounts of vitamin C, differences in food processing and preparation, the volatile nature of vitamin C when exposed to high heat, lifestyle factors, and genetic variability of the individual consumer [1]. The volatility of vitamin C as a degradable molecule remains a challenge when examining the obtained samples that are stored for years without treatment with preserving agent, such as metaphosphoric acid, before analysis was conducted [1].

6.2.3.6 Carotenoids

Carotenoids are another compound that has been exhaustively studied in the literature for their potential as biomarkers of fruit and vegetable intake. This study revealed that the same limitations which are encountered with vitamin C in terms of measurement errors remain valid when considering the use of carotenoids for biomarkers, and subsequently the measured amounts may not accurately reflect the bioavailability. Additionally, food labels typically list lycopene and beta-carotene but not all carotenoids found in the product as only a limited number of these ingredients (e.g., alpha-carotene, beta-carotene, beta-cryptoxanthin, canthaxanthin, lycopene, lutein) are measureable in a significant fashion in the blood [1]. When contrasted with vitamin C, carotenoids are not as degradable during long-term frozen state storage [35]. The lipid solubility of the carotenoids affects absorption and thus influences their bioavailability. In fact intestinal absorption appears to range from 10 to 50% [1]. A moderate correlation between dietary consumption and blood concentrations ranging from 0.2 to 0.5 has been determined [36].

The fact that metabolites of carotenoid metabolism are not measured, the bioavailability of the fruits and vegetables that are being tracked is not properly assessed. An example of this has been reported by Fraser and Bramley group who demonstrated that alpha-carotene, beta-carotene, and beta-cryptoxanthin eventually break down to retinol [37]. The study conducted by Nagao proposed that the metabolism and presence of metabolites do not affect bioavailability and remain inconsequential particularly in individuals with sufficient reserves of Vitamin A; however, this metabolic pathway may be significant in determining bioavailability of carotenoids in those who are vitamin A deficient [38]. The impact of oxidative stress caused by smoking or higher alcohol consumption is perceptible and can't be ignored as it tends to reduce the measured levels of carotenoids [1]. This finding may have paved the way to examine the role of carotenoids as an antioxidant, although the physiologic basis of this role has not been fully elucidated in the literature and appears to be more observational conclusion [39]. Questions remain as to which carotenoid is the best candidate for tracking bioavailability, although some suggest that the total sum of all measureable carotenoids may be more appropriate standard than determining the levels of individual carotenoids [40]. This may prove to be challenging to ascertain as most epidemiologic studies utilize select carotenoids rather than measuring total blood carotenoids.

There is limited literature exploring the interaction between genetic variability and carotenoid levels in subjects. The work of He et al. noted some differences between the risk of breast cancer in those who have polymorphisms in MPO and COMT and carotenoid intake [41]. Goodman et al. illustrated interactions between XRCC1 polymorphisms and lycopene consumption with regard to prostate cancer risk [42]. The precise mechanism of interaction involving carotenoid metabolism, and the potential role of carotenoid as antioxidant, and the overall effect on the validity of carotenoids as biomarkers have not yet been established [1].

One of the earlier studies examined the use of carotenoids as a measurements of fruit and vegetable consumption in patients who had previously treated for stage I or II squamous cancer of the lung or the head and neck. These patients were

compared and contrasted before and after a 3 month period of robust fruit and vegetable consumption [43], and the baseline levels of fruits, vegetables and plasma carotenoids were recorded. They were then instructed, with the assistance of a dietician, to increase the intake of fruits and vegetables to eight servings a day. Subsequently, the blood samples were drawn at 2 and 3 month time intervals and then stored frozen until analysis could be conducted on all the samples simultaneously [43].

The above study demonstrated that there was statistically significant change both in the mean number of fruits and vegetables consumed ($p < 0.001$) and in the mean levels of total carotenoids consumed ($p < 0.001$) as well as for certain individual carotenoids. It also showed a significant increase in the mean total carotenoids in the subject's blood samples ($p = 0.02$). The 3 month correlations between the increase in intake of fruits and vegetables and the increase in carotenoids in the blood samples were moderately strong with a range of ($0.4 < r < 0.7$), a clear demonstration of the viability of carotenoids as a biomarker of fruit and vegetable consumption [43].

6.2.4 Cardiovascular Disease and Biomarkers

Cardiovascular disease has long been associated with hyperlipidemia and is in fact a major modifiable risk factor that is tracked clinically for treatment decision-making. Per the AHA/ACC 2012 guidelines, any individuals with a low-density lipoprotein level of >190 is recommended with starting high-intensity statin treatment. In the past clinicians tracked LDL levels and made adjustments in accordance with LDL goals. However, the current trend is to move away from these standardized rules when treating patients. A notable exception to this tenet are those who have endured ischemic stroke [44].

Literature survey also indicates the presence of patterns of diet and biomarkers of cardiovascular diseases. The research team of Fung et al. identified two distinct dietary patterns: a "prudent" and "Western" dietary patterns. According to this research work, the "prudent" pattern is based on a "high intake of fruit, vegetables, fish, whole grains, and legumes," while the "Western" pattern revolves around "higher intake of red and processed meat, high-fat dietary products, sugar-containing beverages, sweets, and desserts." This study examined the correlation between known biomarkers of cardiovascular disease such as "plasma lipids, thrombogenic factors, glycemic indicators, inflammatory markers, and leptin, folate, and homocysteine factors" in individuals with both dietary patterns [2]. In order to properly assess the development of cardiovascular disease, only individuals with no known diseases, such as stroke, diabetes, or cancer, were allowed to participate in the investigation. The dietary patterns of participants were assessed by asking the participants to fill out a food-frequency questionnaire containing approximately 130 items. The questionnaire had the frequency of each food item in nine increments ranging from "almost never" to ">6 times/day." These questionnaires were filled out every 4 years between 1986, the beginning year of study, and until 1994, the last year of

study. To assess variables other than food choices, the participants were asked every 2 years to provide information about their BMI, smoking habits, and exercise routine. The exercises were reported as metabolic equivalent hours (METs). In 1994, the last year of this assessment blood samples were drawn from individuals to determine the levels of the previously discussed biomarkers of cardiovascular diseases. The diet patterns were analyzed and were similarly characterized at all time points. There was a correlation of 0.65 for the "prudent" pattern and 0.70 for the "Western" pattern for the years between 1986 and 1990. A correlation of 0.67 for the prudent pattern and 0.69 for the "Western" pattern was noted for the years of 1990 through1994. A last correlation of 0.58 for both the "prudent" and "Western diets" was drawn between the years of 1986 and 1994 [2].

A correlation between dietary patterns of the individuals and their lifestyle choices was also apparent. For example, those who followed "prudent" diet pattern were more likely to exercise, consume vitamin supplements, watch less television, and less likely to smoke. Similarly those who adopted more of a "Western" pattern of diet were more likely to "exercise less, watch television, consume fewer vitamin supplements, and smoke [45].

As illustrated in Table 6.1, which is derived from the same published paper and shown below, higher Western pattern scores correlated with lower plasma lipoprotein concentration, higher triacylglycerol, higher C-peptide, higher plasma leptin, higher homocysteine concentrations, and lower plasma folate concentrations. On the other scale, high "prudent" pattern scores correlated with lower triacylglycerol concentrations, lower insulin concentration, and higher plasma folate concentrations [45].

It was also noted in the study that both patterns of eating were not mutually exclusive; eating a diet scoring highly on the "Western" pattern did not necessarily correlate to consuming a diet that scored low on the "prudent" pattern. The research group noted that the association between diet patterns and profiled biomarkers followed general expectations. For the most part, those diets that score highly along the "prudent diet" tend to have a more favorable biomarker profile for cardiovascular disease; in contrast, those who had diets that scored highly with the "Western" pattern had biomarker profiles that placed individuals more at risk for cardiovascular disease. Since these diet patterns were neither optimized nor mutually exclusive, the authors were unable to make specific recommendations other than stating that increasing a "prudent" score of an individual's diet most likely to lower cardiovascular disease risk [2].

Another area of interest in the literature was the role of dietary patterns in endothelial disruption as the pathophysiologic basis of cardiovascular disease eminently correlates with the dysfunction of the endothelial lining before progression to atherosclerosis.

One of the studies examined the relationship between dietary patterning and known markers of inflammation and endothelial dysfunction, including C-reactive protein, interleukin 6, E-selectin, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule [46]. As indicated in this paper, the C-reactive protein and interleukin 6 are markers of systemic inflammation and

Table 6.1 Age- and energy-adjusted mean biomarker values by quintile of prudent and Western diet scores in 1994[a]

	Prudent diet (factor 1)				Western diet (factor 2)			
Biomarker	Q1: −1.17[b]	Q3: −0.15	Q5: 156	*P* for trend	Q1: −1.16	Q3: −1.14	Q5: 1.49	*P* for trend
Cholesterol (mmoL/L)	6.3 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	0.64	6.1 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	0.12
HDL cholesterol (mmoL/L)	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.81	15 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.74
LDL cholesterol (mmoL/L)	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	0.63	3.7 ± 0.1	4.0 ± 0.1	3.8 ± 0.1	0.29
Total cholesterol:HDL cholesterol	4.3 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	0.63	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	0.94
Triacylglycerols (mmoL/L)[c]	1.7 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	0.56	2.0 ± 0.2	2.3 ± 0.2	1.5 ± 0.2	0.14
Apolipoprotein A-I (g/L)	1.5 ± 0.03	1.5 ± 0.03	1.5 ± 0.03	0.65	1.5 ± 0.03	1.6 ± 0.03	1.6 ± 0.03	0.03
Lipoprotein(a) (μmol/L)	1.0 ± 0.2	1.1 ± 0.1	1.4 ± 0.2	0.31	1.4 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	0.03
Fibrinogen (μmol/L)	5.5 ± 0.01	5.5 ± 0.01	5.4 ± 0.01	0.61	5.4 ± 0.01	5.5 ± 0.01	5.5 ± 0.02	0.06
von Willebrand factor	1.51 ± 0.07	1.48 ± 0.06	1.52 ± 0.07	0.48	1.45 ± 0.07	1.54 ± 0.06	1.54 ± 0.07	0.44
Factor VII antigen	1.01 ± 0.012	0.99 ± 0.011	0.98 ± 0.012	0.20	0.98 ± 0.01.	1.02 ± 0.01	0.99 ± 0.01	0.07
tPA antigen (ng/L)	129 ± 5	120 ± 5	110 ± 5	0.13	99 ± 5	120 ± 5	140 ± 5	<0.0001
Insulin (pmol/L)[c]	62.9 ± 7.3	70.7 ± 7.0	90.7 ± 7.9	0.54	82.8 ± 7.7	70.8 ± 7.0	63.5 ± 8.1	0.21
C-peptide (nmol/L)[c]	0.52 ± 0.05	0.65 ± 0.05	0.82 ± 0.06	0.65	0.78 ± 0.05	0.65 ± 0.05	054 ± 0.06	0.29
Glycated hemoglobin	0.057 ± 0.001	0.057 ± 0.001	0.058 ± 0.001	0.57	0.057 ± 0.001	0.058 ± 0.001	0.058 ± 0.001	0.76
Leptin (ng/L)	6741 ± 504	6310 ± 482	6431 509	0.75	5277 ± 487	6331 ± 472	8941 ± 525	<0.0001
C-reactive protein (mg/L)	2.4 ± 0.3	1.6 ± 0.3	1.8 ± 0.3	0.17	1.7 ± 0.3	1.9 ± 0.3	2.5 ± 0.3	0.04
Homocysteine (μmol/L)	16.5 ± 0.6	14.7 ± 0.5	14.0 ± 0.6	0.03	13.9 ± 0.6	15.4 ± 0.5	16.8 ± 0.6	0.004
Folate (nmol/L)	18.4 ± 0.8	19.3 ± 0.8	20.3 ± 0.8	0.34	21.4 ± 0.8	18.3 ± 0.7	17.2 ± 0.8	0.007

[a] $\overline{x} \pm SE$. *tPA*, tissue-type plasminogen activator
[b] Quintile of diet score (1, 3, or 5) and median score
[c] Values for those who had fasted <6 h were excluded

E-selectin, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule are systemic markers of endothelial dysfunction. This study utilized a sample of 121,700 female registered nurses who participated in questionnaire every 2 years beginning with the year 1976, with a control group of 732 women who, at the time of blood collection, had no known cardiovascular disease, cancer, or diabetes. Blood was collected in 1989 and 1990. A food frequency questionnaire was sent in 1986 and 1990 with 116 food items and a frequency ranging from "almost never" to greater than six times a day. Similarly cigarette smoking and body weight were assessed in 1990 along with physical energy expenditure [46].

Using the same "prudent" and "Western" dietary patterns as discussed earlier, correlations were drawn between the biomarkers of endothelial dysfunction. The "prudent" dietary pattern had an inverse relationship with C-reactive protein and E-selectin plasma concentrations. The "Western" diet followers had a positive correlation with C-reactive protein, interleukin 6, E-selectin, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule [46]. While the Western pattern BMI is a confounding variable for interleukin 6, the rest of the biomarkers remain positively correlated but independent of BMI. Given the positive correlation between the Western dietary pattern and the increase in known biomarkers associated with inflammation and endothelial dysfunction, it appears reasonable to make an association between the Western dietary pattern and increased cardiovascular disease. Corollary to this, the decreased marks of inflammation and endothelial dysfunction point to the "prudent" dietary pattern as potentially protective from cardiovascular disease. Thus, it was theorized that the "prudent" diet is high in factors that decrease endothelial activation and improve endothelium-dependent vasodilation. While this sample population is not representative of the general population, it does provide more support of dietary contributions to cardiovascular disease and potential biomarkers to follow [46].

6.2.5 Biomarkers and Amnestic Mild Cognitive Impairment

Another area of interest is the role of dietary patterns in patients with amnestic mild cognitive impairment, a prelude to Alzheimer's disease. It has been long theorized that increased saturated fat would increase the risk for cognitive impairment. Conversely, it has been theorized that a diet rich in polyunsaturated fats would similarly decrease the chances of developing cognitive impairment and possibly reverse it. Studies looking into the results of increasing intake of polyunsaturated fats have not achieved the desired expectations. The prevailing theory is that these studies do not take into account the dietary patterns as a whole and that the combination of all nutrients has a greater effect than any single component [3].

A research work conducted in 2011 by the same team demonstrated that diets high in fruit and vegetables, unsaturated fatty acids, and fish and low intake of saturated fats may reduce the risk for amnestic mild cognitive impairment and Alzheimer's disease. This research project examined the effects of a 4 week diet full of high saturated fat/high simple carbohydrate foods compared to a low saturated

fat/low simple carbohydrate meal in subjects with amnestic mild cognitive impairment. It explored the effects of this diet on known cerebrospinal fluid markers of Alzheimer's disease, namely, Aβ42, Aβ40, tau protein, and phosphorylated tau. Insulin, blood glucose, and cholesterol levels were also measured. A total of 49 individuals were recruited for the study, 29 adults with amnestic mild cognitive impairment while 20 healthy adults served as controls. The high saturated fat/high simple carbohydrate meal, or *high* diet, was composed of 45% fat (saturated fat 25%), 35–40% carbohydrates, and 15–20% protein. The low saturated fat/low simple carbohydrate meal, or *low* diet, was 25% fat (saturated fat <7%), 55–60% carbohydrates, and 15–20% protein [3].

The results showed that there was a statistically significant varied effect of the CSF Aβ42 concentrations when the amnestic mild cognitive impairment group was compared to the control group ($p < 0.001$). The *low* diet increased CSF Aβ42 for the amnestic mild cognitive impairment group but decreased CSF Aβ42 in the control groups ($p < 0.001$) [3]. The *high* diet increased CSF Aβ42 for healthy adults but did not produce any noticeable changes in those with amnestic mild cognitive impairment. There were no changes noted in the CSF levels of Aβ40, tau protein, or phosphorylated tau. The *high* diet increased insulin, whereas the *low* diet lowered insulin in both the healthy and the amnestic groups. Total cholesterol was likewise increased in the *high* diet, and the total cholesterol was lowered in the *low* diet; however, the amnestic subjects experienced twofold changes compared to healthy adults. The *low* diet had a statistically significant effect on delayed memory, and that both the healthy control group and the amnestic group had improved in the *delayed visual recall* ($p = 0.04$). No changes were noted in the immediate memory, executive, or motor speed domains [3].

The study concluded that a relationship between a *high* dietary pattern and increased risk of Alzheimer's disease exists, and a similar correlation between a *low* dietary pattern and decreased risk of Alzheimer's disease has been noted. Although the exact mechanism has not been completely elucidated at this time, the study demonstrated an improvement of the symptoms of amnestic mild cognitive impairment with a *low* dietary pattern.

6.2.6 Biomarkers Associated with Diabetes Mellitus, Metabolic Syndrome, and Hypertension

Metabolic syndrome is a constellation of physical findings and laboratory abnormalities associated with increased risk for development of diabetes mellitus and cardiovascular disease. Abnormal levels in three or more of the following: elevated waist circumference (waist circumference >102 cm in men or >88 cm in women), elevated triglycerides (TG ≥150 mg/dL), reduced HDL cholesterol (HDL cholesterol <40 mg/dL in men or <50 mg/dL in women), elevated blood pressure (systolic blood pressure ≥130 *or* diastolic blood pressure ≥85 mmHg), or elevated fasting glucose (fasting glucose ≥100 mg/dL) were considered diagnostic criteria consistent with metabolic syndrome [3].

The healthy aging in neighborhoods of diversity across the life span (HANDLS) study was utilized to examine the effects of race and socioeconomic status on the risk for developing cerebrovascular and cardiovascular disease using biomarkers as objective measurement [47]. In this study individuals of African-American and White ethnic background between ages 30 and 64 were recruited. The sample consisted of 1260 African-Americans (553 men, 707 women) and 916 Whites (392 men, 524 women) [47]. Questionnaires about subject's diet, health status, health service utilization, psychosocial factors, neighborhood characteristics, and demographics were provided. Clinical measures of the study were blood pressure, symptoms of depression, waist circumference, serums, triglycerides, serum cholesterol, high-sensitivity C-reactive protein, hemoglobin A1C, homeostasis model assessment insulin resistance, and serum ferritin. In accordance with the questionnaires, the subjects were grouped into different food cluster patterns. The following is a Table 6.2 acquired from the published paper with the cluster patterns listed.

A mean adequacy ratio (MAR), a measure of the quality of a diet, was calculated by the amount of micronutrients present in different types of food along with the quantity. In order to conduct a comparison, average MAR scores of each of the previously mentioned clusters had been calculated. It was noted that those with the "sweet drink pattern," the cluster with the lowest MAR tended to have the poorest health outcomes; the highest percentage of individuals with metabolic syndrome (55.6%), risk for depression (47.8%), and individuals have likelihood of smoking (61.5%). The MAR score for the pasta/rice dish cluster was significantly greater than the sweet drink, poultry, alcoholic drink, and sandwich clusters ($p < 0.05$). This cluster pattern was consumed equally by the African-Americans and Whites and almost equally by the economic distribution of <125% PIR (42.4%) and >125% PIR (~57.6%). It was also acknowledged that African-Americans cluster consumption was primarily in poultry, starchy vegetables, and alcoholic drink clusters, whereas Whites were predominately in the pizza cluster [47]. The mean serum triglycerides and cholesterol were within normal limits for all clusters, suggesting that dyslipidemia was not a result of the dietary patterns. The mean hemoglobin A1C was above 5.7 in all clusters, consistent with prediabetes. C-reactive protein and insulin resistance were elevated in all clusters as well. It also demonstrated that African-Americans and White consume

Table 6.2 Top food groups associated with each cluster pattern

Cluster pattern	Next 5 food groups in cluster pattern
Pasta/rice	Cereals, fruit, sweet drink, salty snacks, sandwich
Sandwich	Sweet drink, dessert, salty snacks, starchy vegetable, eggs
Starchy vegetable	Sandwich, eggs, red meat, sweet drink, dessert
Sweet drink	Sandwich, dessert, starchy vegetable, pasta/rice, salty snacks
Dessert	Sandwich, sweet drink, cereal, pasta/rice, poultry
Bread	Sweet drink, processed meat, sandwich, dessert, eggs
Poultry	Sweet drink, sandwich, dessert, pasta/rice, bread
Frozen meal	Sandwich, sweet drink, dessert, bread, pasta/rice
Alcoholic drink	Sandwich, sweet drink, pasta/rice, poultry, green/orange vegetable
Pizza	Sandwich, sweet drink, bread, dessert, salty snacks

similar dietary clusters. The overall biomarker profiles revealed increased risk for the development of metabolic syndrome, inflammation, hypertension, and prediabetes in both African-Americans and Whites in similar socioeconomic status. The literature recommends that the HANDLS participants follow the dietary approaches to stop hypertension (DASH) because the evidence that shows that greater adherence for a DASH diet has shown to reduce risk for metabolic syndrome, cardiometabolic abnormalities, and mortality [47]. Despite the differences in the clusters as distinct dietary patterns, all of these clusters fall under the umbrella of a "Western" dietary pattern as discussed above.

Conclusion

At this time there is a wealth of information pertaining to the subject of biomarkers and how dietary patterns influence biomarkers of chronic diseases. For carbohydrates the total urinary sucrose and fructose, urine samples of ^{13}C levels, and plasma alkylresorcinol appear to have good validity and reproducibility as biomarkers of dietary intake [8, 10, 11]. Fatty acids, triglycerides, lipoprotein levels, as well as biomarkers for the consumption of olive oil have been explored [1, 6]. The literature is suggestive that these biomarkers have a moderate correlation with dietary consumption. Available data validate the role of proteins, creatine, carnitine, trimethylamine-N-oxide, creatinine, taurine, 1-methylhistidine, 3-methylhistidine, and urinary nitrogen role as potential biomarkers for dietary consumption [30, 31].

There is good evidence that direct measurement of plasma vitamin C is the best biomarker for the correlation of dietary consumption. While vitamin C is present in urinary and salivary samples, it is not reflective of the amounts consumed [33, 34]. Given the volatile nature of vitamin C, it is difficult to get an accurate direct measurement if the sample is not immediately analyzed, leading to errors and difficulty correlating measured levels with dietary consumption [1].

Carotenoids are potential biomarkers of fruit and vegetable consumption. Carotenoids can be measured directly in the plasma, typically α-carotene, β-carotene, β-cryptoxanthin, canthaxanthin, lycopene, and lutein [1]. Similar to vitamin C, issues of degradation prior to measurement remain challenging. Despite the noted limitations, the use of carotenoids as biomarkers for fruit and vegetable consumption including subjects who have been previously treated for cancer has been corroborated [43].

In cardiovascular diseases a possible link between a "prudent" dietary pattern of increased fruit, vegetables, poultry, fish, whole grains, and legumes and improved cardiovascular biomarker profiles has been proposed [2]. The "Western" dietary pattern, which is characterized by higher intake of red meat, processed meat, French fries, eggs, high-fat dairy products, sweets, and refined grains, noted to have association with biomarker profiles that placed subjects at higher risk for cardiovascular disease. Additionally, certain lifestyle choices also correlated with both dietary patterns. Increased exercise and less smoking were noted in the subjects who exhibited a dietary habit more consistent with the "prudent" dietary pattern, whereas subjects who follow "Western" dietary pattern

were inclined to spend less time exercising and more likely to smoke [2]. It has also been demonstrated that not only biomarkers for cardiovascular disease were influenced by the "prudent" or "Western" dietary patterns but also the biomarkers for inflammation and endothelial dysfunction [46]. Similarly, the "prudent" dietary pattern correlated with a more favorable biomarker profile for systemic inflammation and endothelial dysfunction. The "Western" dietary profile appeared to correlate with a less favorable biomarker profile in regards to systematic inflammation and endothelial dysfunction. While endothelial dysfunction is an extension of the cardiovascular disease spectrum, it reinforces the concept that a "Western" dietary pattern places individuals to more at risk category for cardiovascular disease [46].

As discussed earlier a link between a *high* dietary pattern, which is similar to "Western" diet, and the risk of Alzheimer's disease has been proposed. It has also been demonstrated that in healthy subjects, the *high* dietary pattern increased CSF Aβ42 levels, a known biomarker for amnestic mild cognitive impairment, which as previously discussed is a precursor to Alzheimer's disease [3]. It was also demonstrated that both healthy and amnestic subjects consuming the *low* dietary pattern had shown improvement in their delayed visual recall ($p < 0.04$), though the exact mechanism for this improvement has not been elucidated at this time.

One last area of discussion is the effects of socioeconomic status and race on biomarkers of metabolic syndrome, diabetes mellitus, and hypertension, and it appears to correlate with the dietary clusters noted by the researchers. The dietary clusters in general followed the same "Western" dietary pattern as previously mentioned, revealing that African-Americans and Whites of similar socioeconomic status also follow similar dietary patterns, and that their dietary clusters and patterns in general demonstrated serum triglycerides and cholesterol, suggesting that dyslipidemia was not a result of the dietary patterns. The mean hemoglobin A1C was above 5.7 in all clusters, indicative of prediabetes. Elevation of C-reactive protein and insulin resistance were noted in all clusters as well [47].

In summary a large volume of literature were devoted to investigate the biomarkers of dietary intake and the relationship between dietary patterns and chronic diseases. However, validation of the biomarkers is warranted in large epidemiological studies to assess the accuracy and reproducibility of these biomarkers. Despite the presence of evidence correlating the "Western" dietary pattern of increased saturated fats and simple carbohydrate with an increase in the risk of diseases, no studies appear to have examined the correlation between the biomarkers of fats as discussed above and the biomarkers of chronic diseases such as metabolic syndrome or Alzheimer's disease. Similarly the relationship between biomarkers of carbohydrate intake and biomarkers assessing risk of cardiovascular disease has not been investigated. This information would be invaluable in helping clinicians demonstrate exactly how much of a macronutrient or micronutrient is affecting an individual's risk of disease onset or progression. This is an area undoubtedly warrants further research.

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7 Impact of Genomics on Drug Discovery and Clinical Medicine

Deepak Gupta

Abstract

Variability in a drug response, whether it is a safety or an efficacy concern, can likely be addressed by genetic differences among individuals. Incorporating human or relevant microbial genetic alterations during drug discovery phases has vast implications for a drug to be successful in the long run. Pharmacogenomic studies help in identification of superior target and better lead molecules with much higher chances of success; therefore, minimizing risk of drug failure in early preclinical studies. Applications of these principles continue during preclinical animal model selection to address variability in dose-response studies. Use of pharmacogenomic principles comes with unique challenges; however, it also brings on tremendous opportunities to abate drug attrition at later stages of drug development as illustrated by numerous drug examples. Lessons learned from these drug development studies are then applied to product labeling and the post-marketing comprehension of drug behavior.

Keywords

Pharmacogenomic principles · Drug discovery · Animal models · Lead · Development · Preclinical trials · Clinical trials · Dose-response · Regulatory consideration

7.1 Introduction

Publication of the human genome sequence in 2003 and its subsequent decoding has provided a plethora of information linking genes to different disease states. Further exploration through biological and *in silico* approaches supplied a wealth of

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Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_7

information, thus systemically identifying diseases, random curated non-diseases, as well as druggable genes from a total of approximately 25,000 human genes [1, 2]. This database incorporates up to 1000 disease-related genes with druggable domains, thus pointing to a vast number of possible drug targets. However, existing drugs account for less than 50% of these genes targeted. This leaves us with hundreds of recognized targets, and thus, a tremendous opportunity that needs to be explored within pharmaceutical and academic settings. It has been estimated that the probability of established drug target reaching preclinical development is 17% compared with only 3% for unestablished or new drug targets [3]. These well-characterized targets significantly increase chances of drugs reaching the market and will have substantial effect in the reduction of drug attrition rates.

After the publication of the genome sequence, association of genes with disease states is evolving fast, and this type of crucial information is responsible for paradigm shift in drug discovery programs. Several databases like The Human Gene Mutation Database [4], GeneCards [5], OMIM [6], LocusLink [7], and COSMIC [8, 9] have information about gene mutations and their link to the diseases. Sakharkar et al. used *in silico* approaches to explain druggable human genome as shown in Fig. 7.1. These types of studies further enhance the application of pharmacogenomic (Pgx) principles into drug discovery and development programs [1].

Pgx principles can be helpful from the very start of drug discovery processes. Their application continues during preclinical and clinical drug development to address important issues like safety, efficacy, adverse drug reactions (ADRs), and pharmacokinetic (PK) variability—all playing a pivotal role in drug failures. Genetic

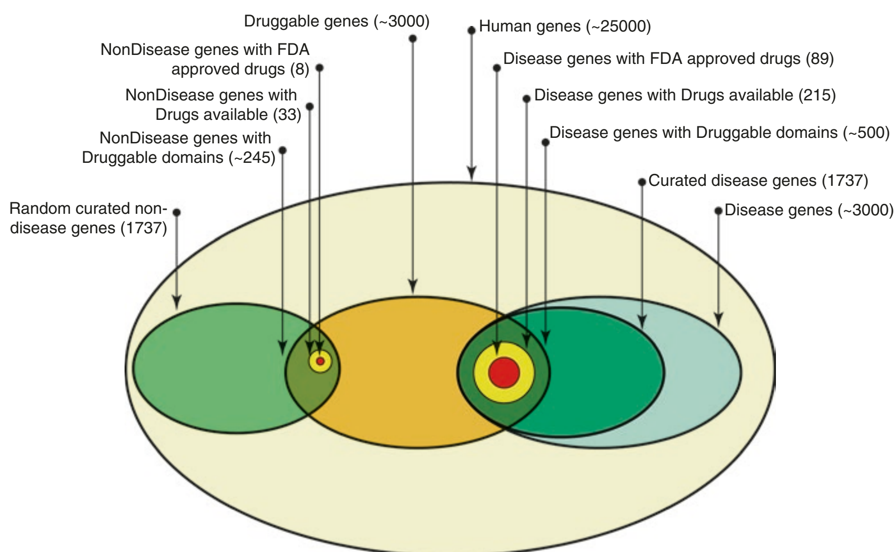

Fig. 7.1 In silico approach toward druggability of human disease genes (Reprinted with permission from Meena Kishore Sakharkar, *The International Journal of Biochemistry & Cell Biology* (Elsevier Ltd., 2007), 1156–1164

alterations can be helpful in understanding differences in pharmacokinetics (PK), pharmacodynamics (PD), as well as dose-response profiles of a drug. Even if response is seen with a drug in a patient with genotype variability, Pgx principles can be helpful in understanding outliers. For example, if the shape of dose-response curve is altered because either maximal effect is not reached or the shape does not correlate with the dose given, genotype of that patient may be supportive of this altered response in those cases.

Pgx based approaches for safer and better drugs has been steadily increasing in diseases like HIV, cancer, thrombosis, and epilepsy, as drug exposure can be influenced by polymorphism in transporters, metabolizing enzymes, as well as targets. Chemotherapy is particularly influenced by interindividual variability, and the use of genetic profiling to discover better drugs can be an answer to individualized drug therapies.

7.2 Population Stratification

The human genome sequence has identified genes that can be used as biomarkers for patient stratification to be useful during various drug discovery and development phases. There are more than 350 known genes designated as biomarkers that affect absorption, distribution, metabolism, or elimination (ADME) of a drug. Out of all these genes, more than half account for transporters alone (Fig. 7.2). Besides, phase I and phase II metabolism genes also play a significant role in patient stratification. During early phases of drug development, when a drug's PK is well elucidated, ADME properties of a new molecule can be cross-checked with these biomarkers. If the new chemical entity (NCE) utilizes one of these transport or metabolism mechanisms, then all protein variants should be taken into account to see PK as well as PD variability. ADME data can be helpful in patient stratification, and future studies can be fine-tuned based on the results from these studies [10]. Understanding these common variations in genes mainly encoding for drug metabolism and transporters help in understanding ADRs, safety, and efficacy of NCEs quite early in preclinical studies, thus, increasing chances for a successful personalized medicine to the market. This translates into significant economic benefits with superior

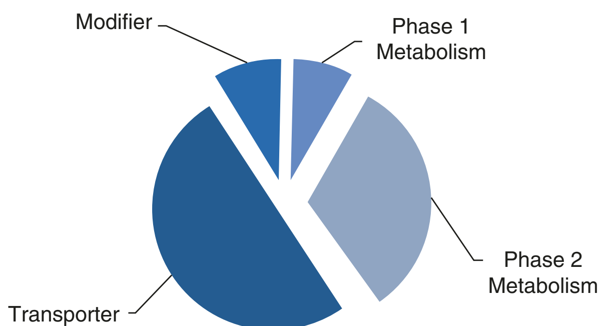

Fig. 7.2 Approximate distribution of ~350 genes useful as pharmacokinetic biomarkers for patient stratification during drug development phases

health-care outcomes. ADME chips are now available for fast and global profiling of crucial metabolic pathways in drug metabolism. For example, DMET™ Plus Solution kits encompasses 1936 SNPs, copy number, and other biomarkers across 231 genes that includes structural variants in transporter genes like ABCG2_c.421C > A(Q141K) as well as biomarkers associated with safety like CYP3A4_-329A > G. Besides extensive coverage of core ADME genes, these kits also include population-specific markers like VKORC1_c,-1639G > A. Besides, test kit Dako HercepTest™ (to assess overexpression of HER2 protein) [11], AmpliChip™ (for genotyping 27 alleles in CYP2D6 and three alleles in CYP2C19, FDA approved in 2005) [12], PhyzioType™ (for assessing ADRs especially in antipsychotic pharmacotherapies) [13, 14], and PGxPredict:CLOZAPINE® (to assess clozapine-induced agranulocytosis) [15] to name a few help in identifying certain pharmacogenetic polymorphisms and genetic mutations. This represents a sound and validated analytical platform helpful in (a) pharmacology research, (b) translational clinical research, (c) preclinical research and development, and (d) clinical research trials to develop personalized medicine and reduce attrition rate in pharmaceutical industry [16, 17].

7.3 Understanding Differences in Genetic Makeup

7.3.1 Differences in Human Genome

Understanding genetic differences in human metabolic pathways, enzymes, receptors, and transporters and further utilizing these differences to select in vitro and in vivo study models can have a significant impact in reducing drug attrition rate. For example, if CYP2D6 is significantly involved in drug metabolism, then phenotype due to CYP2D6 variant may be an important predictor of the treatment outcome. CYP2D6 variability is well documented among Caucasians, Asians, African-Americans, as well as Middle Eastern populations [18, 19]. Within Caucasians, it has been found that approximately 80% of the population can be categorized as extensive metabolizer, while the rest can be categorized as 2% ultrarapid, 10% intermediate, and 8% poor metabolizer.

One of the important examples is the metabolism of tamoxifen, where CYP2D6 plays a central role to form active metabolite endoxifen. It was then postulated that due to well-known variability in CYP2D6, metabolism status of a patient can have significant effect on response rate. In a recent study, effect of phenotype regulated by CYP2D6 was studied in women using tamoxifen for metastatic breast cancer [20]. Pharmacogenetic analysis (*CYP2D6*3*, **4*, **5*, **6*, **10*, and **41*) was done on patient's blood samples, and it was found that overall survival was much shorter in poor metabolizers. Thus, CYP2D6 phenotype is an important predictor of treatment outcome in patients taking tamoxifen for metastatic breast cancer. These post-marketing studies can have huge impact on drug discovery and development approaches. For example, if CYP2D6 plays a crucial role in the metabolism of a

NCE, evaluation of the association between CYP2D6 genetic polymorphisms and the treatment effect is warranted and can be very useful in understanding safety as well as efficacy issues seen with the drug.

7.3.2 Differences in Microbial Genome

Understanding genetic differences as well as genetic evolution of microbes can give clues to an effectiveness of a drug, which can be helpful during antimicrobial drug development programs. Ever evolving resistance to existing drugs is one of the important concerns, and new therapies are continuously needed to keep up with the mutation rate of microbes. Genetic changes in microbes must be studied to overcome resistance issues seen with existing drugs. For example, mutations in malarial parasite *Plasmodium falciparum* led to alteration of chloroquine influx pump leading to drug resistance problems with chloroquine. Genetic studies revealed that *Plasmodium falciparum chloroquine resistant transporter* (*pfcrt*) gene was responsible for effluxing the drug out of the parasite. Recent advances in the genomic and proteomic sciences and particularly the most recent advances in the membrane transporters have enabled new mechanistic approaches to make better therapeutic agents. In-depth study of altered genotype of *Plasmodium falciparum* will drive drug discovery approaches toward novel analogues similar to chloroquine that will not be recognized by *pfcrt*. In such types of approaches, newer analogues can either overcome resistance mechanism(s) or bypass these altogether [21, 22]. As shown in Fig. 7.3, less entry of chloroquine into the mutated parasite can lead to reduced therapeutic response and in addition can cause other problems like toxicity associated with more than normal levels of drug in plasma. On the other hand, designing similar analogues that bypass resistance mechanisms can be an effective approach in delivering novel analogues or prodrugs at the site of action, thus producing desired therapeutic response.

7.4 Target Identification

Identifying a suitable target is the single most important decision for a successful drug discovery program. Principles of Pgx can be applied from the very start of the process, and validated targets can be explored. Choice of targets becomes much more critical as all the genes may not have disease relevance or these genes may not be safer targets. So, a systematic genetic approach can help in choosing ideal targets with much higher chances of success.

For example, chemokine receptor-5 (CCR-5) was found to be the major co-receptor, in addition to CD4, for primary HIV-1 strains, and it has been observed that individuals homozygous for the mutant allele (Δ32/Δ32) were highly resistant to macrophage-tropic (M-tropic) HIV-1 viruses without any health consequences [23]. In addition, individuals that are heterozygous for this mutation (WT/Δ32) generally show slower disease progression [24].

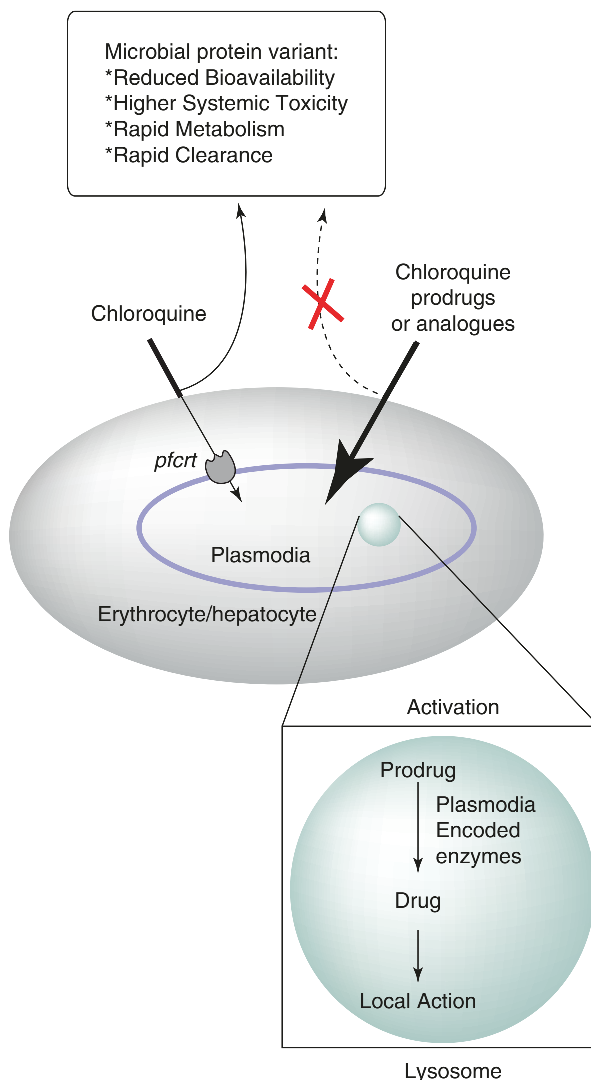

Fig. 7.3 Pharmacogenomic principles applied to the discovery of antimicrobials

Based on the observations about the importance of CCR-5 co-receptor mediating infection by M-tropic viruses, it was postulated that variants of CCR-5 (homozygous or heterozygous) may have relative or absolute resistance to HIV-1 tropic infection. Further pharmacogenomic studies helped in validating CCR-5 as an excellent target for anti-HIV drug discovery, and it was later hypothesized that antagonists at this receptor will protect individuals from M-tropic HIV infection. Maraviroc (Selzentry®), an oral CCR-5 antagonist, was later approved by US Food and Drug Administration, FDA, in September 2007 [25]. This drug simulates the homozygous mutant allele that protects individuals from M-tropic HIV infection.

Pgx based approaches can also be applied retrospectively on existing drug molecules, and the significance of target can be correlated with the genome sequence. For example, enzyme renin is a well-documented target, and in the last four decades, drug discovery approaches were targeted to find renin inhibitor as potential therapy for the treatment of hypertension. Publication of the genome sequence validated this target and supported the findings that decrease in plasma renin activity by renin inhibitors may clinically be advantageous without much genotype/phenotype variation. Aliskiren hemifumarate (Tekturna®) was the first renin inhibitor approved by FDA in March 2007, and genomic analysis validated that Pgx-based dosing guidelines are not required for this drug [26].

Thus, renin served as an excellent target, and drug discovery approaches are focused on finding more efficacious or safer renin inhibitors. One such molecule, SPP635, targeting renin has completed phase IIa clinical studies; however, its current status is not known [27].

Another important example of the use of these principles for target identification is the discovery of Philadelphia chromosome mutation and hyperactive *bcr-abl* protein. This hyperactive protein served as a very specific target for chronic myelogenous leukemia (CML). Identification of this crucial target, present in 95% of patients with CML, was followed by high-throughput screening (HTS), and further modification of chemical lead paved the way to the approval of imatinib (Gleevec) [28]. Thus *Bcr-Abl* tyrosine kinase corroborated the clinical usefulness of protein kinase inhibitors, and the first drug was FDA approved in 2001 [29].

Thus, choosing a target based on the genome sequence or retrospective analysis to access validity and risk associated with existing targets is the single most important decision in a drug discovery program.

7.5 Lead Identification

An ideal lead candidate should possess limited PK variability, limited mechanism-based toxicity, and similar affinity to target variants, thus leading to similar efficacy. PK variability can be accessed preclinically in vitro involving human and animal tissues followed by in situ and in vivo studies in the animal models. The compounds with high-variability risk can be identified well before clinical studies. Similarly, mechanism-based toxicity studies can be helpful in eliminating undesirable compounds at early stages of drug discovery. Chemical leads can be thoroughly probed using advanced genetic tools like gene knockouts, RNA interference, transcription profiling, and molecular signature algorithms. Understanding mechanisms of toxicity can be very crucial during preclinical development as it can help to eliminate risky compounds at very early stages of the drug discovery. Increasing availability of gene-knockout cell lines and animal models can be very helpful in choosing the right candidate for further development. Similarly, specific gene-signature readouts predictive of toxicities can be obtained by transcriptional profiling and can play a pivotal role in selecting good chemical leads. For example, Speedel's lead product SPP100 (aliskiren), with limited Pgx variability, served an excellent lead molecule

for the development of next generation of renin inhibitors. Based on that finding, similar molecules were further explored and as mentioned before, SPP635 served as one of the important compounds in the SPP600 series. This compound has already completed phase IIa study testing its safety and efficacy in mild to moderate hypertension [27]. Further clinical trials on efficacy and safety of SPP635 in diabetic and hypertensive patients with albuminuria support rational drug design approach based on first approved drug aliskiren [30]. If this drug makes it to the market, chances of this drug having efficacy or safety problems (at least due to Pgx variability) are minimal. This increases the chances of drug's success rate as some of the potentially risky Pgx factors were already explored during the drug discovery process.

7.6 Preclinical Pharmacogenomics Studies

7.6.1 Selection of Appropriate In Vitro Models

Although it may not be practical all the time, using human tissues in early phases of drug discovery clearly has an advantage towards promising in vitro-in vivo correlation (IVIVC). Genetic variations are routinely seen in animal and human tissues, and an ideal in vitro model should be able to assess and reproduce variability seen in PK or PD response when drug binds to the target protein variants.

7.6.2 Selection of Appropriate In Vivo Models

Mice model is unarguably the most common animal model during in vivo studies. However, PK and efficacy studies can be correlated with humans only after using appropriate animal models that reflect true human pathophysiology. For example, one of the main reasons of anticancer drug failures is the lack of good animal models, which further point to the genetic variability in humans. Knockout animal models sometimes can be helpful in understanding common variation among different groups and can address some polymorphism concerns. Based on the results of these studies, patient inclusion/exclusion criteria can be applied during clinical testing, and the dose can be projected in early human studies [31].

7.7 Proof-of-Concept Clinical Trials in Early Human Studies

Lack of desired response or lower efficacy is the leading cause of drug failure in clinic. Almost 30% of drugs fail in clinical trials mainly due to this reason. Proof-of-concept studies can be useful in understanding issues a drug may face at later stages of drug development. These types of studies will give clues if drug is able to reach site of action and bind to appropriate target eliciting desired response. This will allow failing undesired drugs early in the development process and will reduce much expensive drug attrition occurring at later stages of drug development. Besides

that, lessons learned in early phases can be applied to other molecules, and exclusion/inclusion criteria can be applied during clinical trials as needed.

7.8 Dose-Response Studies

7.8.1 Dose Projection

Relationship of drug dose to blood concentration is an important factor in understanding PK properties of a drug. This dose-PK relationship has successfully been applied in various demographic subgroups. Relationship between blood concentration and response can be another critical factor in dose-projection studies. This PK-PD relationship should be well established among different population subsets, and dose should be projected accordingly. These PK-PD studies are critical not only to correlate accurate dose to a subgroup but also to understand safety and efficacy issues commonly seen with the drugs during late development phases. For example African-American and Caucasian populations show different response to angiotensin-converting enzyme (ACE) inhibitors in terms of efficacy as well as safety [32]. So, it is imperative that these dose-projection studies should be based not only on the response desired but should also be correlated with different population subsets. In a subset of population, different doses can be projected to match both C_{max} and AUC. Genetic tools can be very helpful in identifying specific biomarkers that can correlate well with the dose given for a particular condition. These genetic tools should also be able to find population subgroups, where simply adjusting the dose may not produce desired response, and those subsets must be excluded from the studies. Thus, dose selection is an important criterion as lower than required doses may not show desired response and higher than required may lead to enhanced adverse reactions or toxicity.

7.8.2 Interpreting Observed Genetic Differences During Drug Development to Transform Dosing Recommendations

Four important factors that affect clinical recommendations are (1) effect of PK and drug exposure on PD, (2) magnitude of effect, (3) magnitude of increased adverse effects, and (4) loss of response.

Treatment recommendations are based on the fact that a drug should be efficacious and safe enough to justify its clinical use. Dose of the drug should be adjusted accordingly. Dose can be titrated based on monitoring plasma levels while keeping drug levels within therapeutic window and evaluating the response desired. If dose needs to be altered, there should be sufficient evidence about its efficacy and safety at the altered dose. If there is no need for dose adjustment, it should be consistently shown that changes in drug levels across patient population do not have clinical implications. Further, variation in drug response should be tied to intrinsic and extrinsic factors like age, gender, weight, and altered physiological state like renal/

hepatic impairment. PK Variability in healthy volunteers must be correlated with variability in patients and if there is any significant change in response or safety of the drug, clinical consequences of this alteration should be evaluated.

Active metabolites produced by prodrugs or some other drugs should also be monitored to correlate their levels with efficacy or drug response. Dose can be adjusted according to active metabolites desired to elicit desired response. Further, some metabolites can be toxic, and their levels need to be monitored, and the dose should be adjusted accordingly.

Thus, drug exposure levels across patient population should be carefully monitored to maintain safety without compromising efficacy. Therapeutic drug monitoring can give an idea about plasma drug levels. If the dose needs to be titrated based on genetic differences, it needs to be shown clearly that different doses for general versus special population are suitable to achieve desired response while limiting adverse effects.

7.8.3 Selective Indication

Not all humans are the same, and even if two humans have the same type of disease. For example, the pathophysiology of breast cancer can be very different. So, the same drug cannot be indicated if genetic makeup of the disease is different. In fact, such type of indication will not be able to show therapeutic effect and may even lead to toxic response that may be dangerous to the patient. One important example is the approval of trastuzumab for certain breast cancer cells that overexpress epidermal growth factor (EGF) receptors due to amplification of human epidermal growth factor receptor 2 (HER2) gene. Trastuzumab inhibits EGF receptors due to overexpression of HER2 leading to regression in breast cancer. If the breast cancer is not related with overexpression of HER2, trastuzumab will not show any beneficial effect and may even cause toxicity. For this reason, trastuzumab is approved for the treatment of HER2 overexpressing breast cancer or metastatic gastric or gastroesophageal junction adenocarcinoma only. For patients who do not overexpress HER2, this drug will not have therapeutic advantage [33]. Patient's HER2 gene expression must be analyzed before starting treatment with this drug. FDA has approved two kits, namely, Dako HercepTest® and Ventana Pathway®, to assess patient's HER2 gene expression [34]. In addition to that, fluorescent in situ hybridization (FISH) assay can be performed if HER2 gene expression assay does not give definite answer about treatment decision. These are important genetic tools to predict response to therapy and selective indication of a drug whose response is determined by genetic makeup of the disease. Similarly, EGFR/KRAS expression status was used to treat cancer patients with panitumumab or cetuximab.

Furthermore, Pgx principles can also be applied to find a subset of population that will experience greatest benefit from the drug. For example, it has been shown that patients with polymorphism in some apolipoproteins may have maximal benefit with HMG-CoA inhibitors as compared to those without these polymorphisms. On

the similar lines, patients more prone to toxicity can be omitted from using a certain drug. For example, before using rasburicase or pegloticase for the treatment of gout, patient's G6PD status should be determined to avoid possible hemolysis [35]. Therefore, understanding a drug's response and correlating it with altered genotype can help in selective indication for that drug. This approach minimizes drug failures at advanced stages of development. Besides, clinical trials can be customized based on the specific genotype needs, thus, decreasing risk factors that may lead to drug failure in clinic [36, 37].

7.9 Clinical Pharmacogenomics Studies

Preclinical studies play a critical role in identifying gene variants that can affect drug's transport, metabolism, and its action on the receptor. Early studies to assess drug's PK properties and desired response can be anticipated based on preclinical studies. For example, if it is observed that a drug is a substrate of a well-established polymorphic gene like OAT1B1, it would be prudent to determine extent of PK variability to adjust dose in future studies. If such type of studies shows clinically significant effect of protein variant, then future clinical studies need to consider this variability to define inclusion or exclusion criteria. These studies can determine if human genomics' studies are required and if needed can also help in the study design and analyze results accordingly. For example, if an outlier is present in a data set, previous studies based on Pgx differences can help in supporting the results as this type of outlier can easily be correlated with preclinical studies.

7.9.1 Phase 1

PK and PD studies in healthy volunteers provide important information that can be useful for later clinical trials. Phase I studies can be designed based on preclinical studies keeping in mind inclusion/exclusion criteria related to Pgx differences. In vitro data can be useful in understanding genetic influence on drug's PK properties. When a drug is metabolized by polymorphic enzyme or transporter and in vitro data predict that polymorphic enzyme or transporter can be responsible for a significant effect on drug's response, a decision can be made if trial should be continued. If enough information is available from preclinical studies, trial can be customized, and phase I population can be genotyped for the relevant gene variants.

Single- and multiple-dose PK studies during phase I can give information about area under curve (AUC) as well as information about C_{max}, T_{max}, clearance, and other relevant PK parameters. This type of data can support previous findings and/or project possible interindividual variability in subsequent trials. DNA samples from volunteers can be collected, and variability in PK can be correlated with specific ADME genes. Thus, patients with normal metabolizing enzymes or transporters can be stratified from polymorphic population. These inclusion/exclusion criteria can be

helpful in understanding results affected by polymorphism in enzymes or transporters. Similarly, Pgx principles can be very helpful in dose-range selection in phase 1 trial. For example, if poor metabolizer can lead to increased exposure of the active drug molecule, trial should only be continued in that type of patient population with doses lower than expected to be safe in a normal metabolizer. Pgx data in early phases can be useful in later phases of the trial for similar inclusion/exclusion or dose-selection criteria.

Generally, DNA samples can be collected from all the volunteers, and common gene variants affecting phase 1 and 2 metabolism and transport can be identified. Population PK studies can be correlated with their genetic makeup, and possible PK parameters can be addressed. When toxicity is a concern, especially for some drugs with narrow therapeutic index, DNA samples can be useful to genotype so as to identify subjects that may be at risk, and thus either those patients may be excluded from trial or they may be given different dose to account for genetic variability in question. In some cases, PK studies can be correlated with genotype retrospectively to understand observed variability, and further clinical trials can be modified accordingly. When in vitro or initial clinical data suggests the involvement of polymorphism affecting safety of a drug, clinically relevant genotype studies for genes of interest must be performed so that sufficient data can be generated to support labeling recommendations for the use of drug in a genetically defined population.

7.9.2 Phase 2

Since phase 2 trials are based on phase 1 results, variability in the dose of drug administered with blood levels across healthy volunteers needs to be taken into account. When this variability can be characterized, chances of a drug failing due to lack of efficacy are minimized. For example, during clinical trials of trastuzumab, it was found that this drug will be effective only in tissues overexpressing HER2 receptor. So during phase 2 trials, only patients overexpressing HER2 receptors were selected. The drug was finally approved with this special Pgx indication. This example demonstrates how patient inclusion/exclusion criteria can be applied based on prior studies. Thus, when genotype variability is significant enough to alter a drug's response, trials can be (1) enriched with possible responders, (2) improved with eliminating possible nonresponders, (3) amended with elimination of patients likely to show toxicity, (4) stratified into various groups like poor metabolizers or extensive metabolizers, (5) adjusted for dose-range selection, and (6) modified to change the dose in a subset of population.

Phase 2 studies generally provide proof of concept and establish therapeutic dose and adverse effects. Further, phase 2 studies can be adjusted based on genetic variability to optimize dose selection and study design for phase 3 trials. Figure 7.4 illustrates that pharmacogenomics can affect PK or PD properties during drug development phases, which can have effect on therapeutic response as well as on adverse effects or toxicity of a drug.

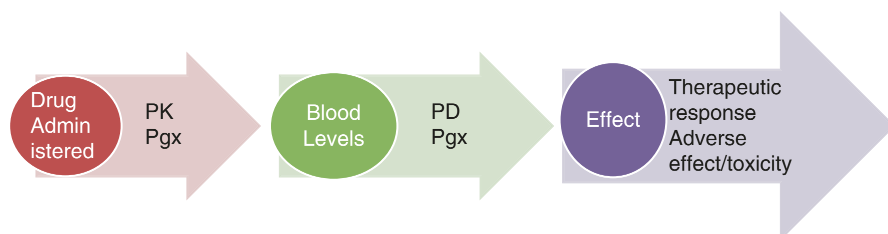

Fig. 7.4 Involvement of pharmacogenomics in PK and PD

7.9.3 Phase 3

Data accumulated from preclinical, phase 1 and 2 studies is important to understand the magnitude of polymorphism in safety and efficacy and clinical relevance of PK and PD studies in different population. In phase 3 trials, patient inclusion and exclusion criteria can be applied based on the following scenarios:

1. If data in prior studies suggests that differences in PK among different population groups are not significant enough to be clinically relevant, but certain subset of patients does not show therapeutic response, then patients with specific genotype/phenotype should be excluded from the study.
2. If data in prior studies suggests that differences in PK and PD among different population groups are not significant enough to be clinically relevant, then studies should be focused on confirming findings from earlier studies. In addition, phase 3 studies should be enriched by including various genetic subpopulations to confirm existing data on comparable efficacy and safety in a diverse population.
3. If data in prior studies suggest that differences in PK and PD among different populations are significant enough to be clinically relevant, then genotype/phenotype-based dosing is recommended for specific subset of population. For this type of scenario, those patients with variability in specific genotype/phenotype should be included and closely monitored for PK and PD effects in relation to normalized dose.
4. If data in prior studies suggests that differences in PK and PD among different population are significant enough to be clinically relevant, but dose is normalized based on biomarkers present and not based on differences in genotype, then dose should be titrated based on biomarkers, and those patients should be included and closely monitored for PK and PD effects in relation to biomarker-based dosing.
5. If data in prior studies suggests that differences in PK and PD among different populations are significant enough to be clinically relevant but dose cannot be titrated due to marketing or some other reasons, then patients with specific genotype/phenotype should be excluded from phase 3 studies.

7.9.4 Phase 4

Phase 4 studies are very important for analysis of reported adverse events and correlating these post-marketing results with Pgx tests. There are many examples of drugs that were approved without much Pgx consideration, but changes in labeling for drugs like clopidogrel, abacavir, and warfarin after they were approved reflect the importance of post-marketing studies to improve safety and effectiveness of drugs. This also helps in benefit/risk refinement and improvement in pharmacovigilance. Although Pgx link might be discovered post-market, it provides valuable information for discovery of similar molecules and also supports the use of genomic tools early in drug discovery so that these tools can be linked to clinical findings to support drug's efficacy and safety. Figure 7.5 provides an overview of the use of preclinical and clinical findings and their relation with genotype of a patient.

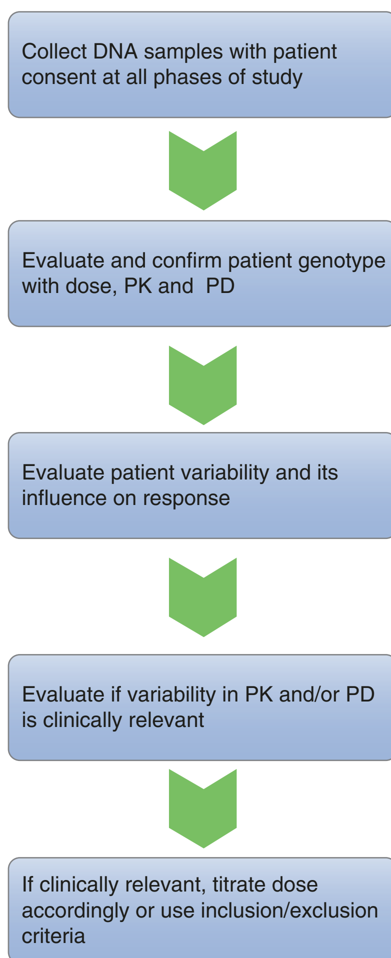

Fig. 7.5 Relation of preclinical and clinical findings with genotype of a patient

7.10 Impact of Using Genomics During Drug Discovery and Development

7.10.1 Challenges and Opportunities

While understanding genomic variability very early in the drug discovery processes creates opportunity for customized preclinical and clinical studies, it also can lead to increase in time spent to understand effects of possible variations and how clinical outcome can be affected based on those findings. It can certainly drive up the cost if specialized tissues or animal models are needed for testing particular hypothesis. Thus, incorporating genomics early in drug discovery may create many challenges. However, this would minimize attrition of new chemical entities due to safety and efficacy problems; the two important parameters leading to drug failure. Thus in the long run, applying Pgx principles during drug development phases is critical to minimize drug failure and to support some of the post-marketing findings.

7.10.2 Advancing Research

Pgx can be helpful in advancing research to find better drugs by the following approaches:

(a) Identifying genes responsible for genetic diseases, e.g., uterine cancer
(b) Mapping, sequencing, and decoding human genomes by developing new protocols
(c) Advancing technology to analyze gene expression on DNA microarray chips
(d) Advancement in computational tools to interpret and analyze genome data
(e) Developing animal models mimicking genetic disorders, e.g., gene knockout mice
(f) Designing specific genetic testing kits and methodologies
(g) Use of technology to reduce cost of genetic testing

7.10.3 Use of Genomics Information on Product Labeling

More than 150 drugs have been approved by FDA with pharmacogenomic information in their labeling. Drugs have been associated with relevant biomarkers (gene variants, functional deficiencies, protein variants, and polymorphic changes) and include specific actions that need to be taken [38]. Based on preclinical and clinical studies during drug discovery and development, important information can be generated for special labeling, which may be useful to update some of the following product label categories.

1. Indications and usage: When there is a need for proper patient selection and testing is recommended in those patients
2. Dosage and administration: When there is a need for genotype-based dosing

3. Clinical pharmacology: When dose of a drug shows differences in PK or PD
4. Drug-drug interactions (DDIs): When genetic variation can contribute toward significant DDIs. When genetic polymorphism affecting a drug's metabolism or transport can lead to alteration in that drug's plasma levels due to a drug or group of drugs
5. Drug-food interactions (DFIs): When genetic variation can contribute toward significant DFIs. When genetic polymorphism affecting a drug's metabolism or transport can lead to alteration in that drug's plasma levels due to food or other supplements taken up by patients
6. FDA black box warning, contraindications, warnings and precautions, and/or adverse reactions: When genetic variability affects safety of a drug
7. Warnings and precautions and use in specific population: When genetic variability affects a specific population subset
8. Clinical studies: When genetic variability affects efficacy of a drug.

7.10.4 Regulatory Considerations and Recommendations

Differences in clinical outcomes due to variability in intrinsic/extrinsic factors are well-known issues faced during drug development. Individualized drug therapies are geared toward understanding this variability and to estimate safety and efficacy with some certainty. Adequate understanding of variability is desirable (sometimes required) for an efficient drug approval process. Some of the important factors that can positively influence regulatory decision include the use of adequate biomarkers, studies incorporating genetic variability, patient inclusion/exclusion criteria, and dose optimization. Population-specific studies are becoming increasingly important in areas like oncology, cardiovascular disorders, and antivirals. For example, abacavir, a nucleoside reverse transcriptase inhibitor (NRTI), has potent antiviral activity; however, ~5% patients on abacavir develop potentially fatal abacavir-induced hypersensitivity reaction. Post-marketing exploratory studies found that patients who carry the HLA-B*5701 allele were at high risk of experiencing a hypersensitivity reaction to abacavir. Drug label of abacavir was later updated to include recommendations for HLA-B*5701 allele screening prior to initiating therapy. Based on these types of experiences, FDA now recognizes the growing significance of intrinsic or extrinsic factors in altered drug response. In the last decade, FDA has significantly increased its efforts to access genomics data for newer drugs. FDA now recommends and encourages companies to submit genomics data into voluntary exploratory data submission (VXDS) program. It has been found that in the last few years, IND (investigational new drug), BLA (biologic license application), and NDA (new drug application) submissions with genomic data have increased significantly [39]. Although submission of genomic data may not be a requirement, this still ensures better clinical outcome. The Genomics Group at Office of Clinical Pharmacology at FDA is involved in developing regulatory policies and procedures for efficient use of genomics during drug development. Regulatory advice can be given and, if applicable, labeling information can include Pgx information for individualized therapy. Further regulation by FDA is expected in coming years as new

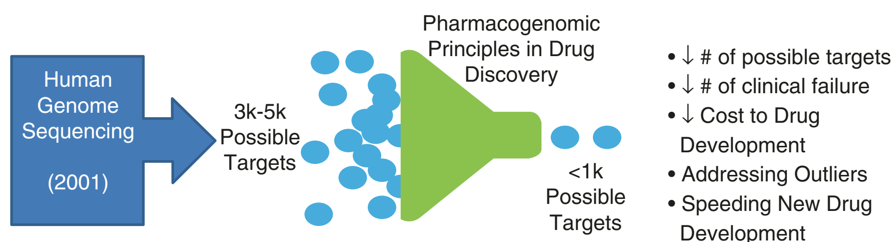

Fig. 7.6 Application of pharmacogenomics principles at different stages of drug development

genetic details about metabolism, transporters, and post-marketing adverse effects come to surface.

7.10.5 Application of Pharmacogenomics Principles at Various Stages of Drug Development

Figure 7.6 illustrates how incorporation of human genome sequencing early into drug discovery approaches can help in reducing possible number of targets, and this can translate into numerous advantages during drug development phases. In fact, advantages continue beyond associating a drug with genetic information or biomarker. This genetic data can be translated to make a clinical decision for a particular drug or dose. Collaborative efforts of clinical pharmacogenomics implementation consortium (CPIC) and pharmacogenomics research network (PGRN) define dosing recommendations based on genetic information available. These recommendations/guidelines are mainly designed to help clinicians make informed decision and choose the best available drug and dose combination based on the specific genetic requirement of a patient [40].

7.11 Advantages of Pharmacogenomic Studies

Application of Pgx studies in a drug discovery and development setting can be very helpful in minimizing drug failures in clinics and can also improve clinical outcome. Traditionally, pharmaceutical companies face high drug failure rates before filing IND application. Advancement in genomic tools can be very helpful in increasing certainty of a clinical outcome especially for new molecular entities (NMEs). Some of the important advantages are:

1. Increased understanding of interindividual differences in efficacy and safety
2. Use of genetic data to
   (a) Identify possible reasons for PK variability in clinical response
   (b) Understand clinical relevance of significant DDIs
   (c) Understand molecular mechanisms of loss of drug response in a patient population
   (d) Understand molecular basis of adverse effect

   (e) Feedback future studies based on the results from earlier studies
   (f) Adjust dose according to PK/PD variability
3. Understanding biomarkers and diagnostic genomic tests can be helpful in identifying special patient population in respect to the following:
   (a) If therapeutic response to a drug is seen
   (b) If there are more chances of adverse effects and even hypersensitivity
   (c) If pharmacological response shown is extended or diminished
   (d) If treatment is beneficial or alternative options should be considered
   (e) If drug exposure levels can be adjusted by adjusting drug dose
4. Other significant advantages include decrease in overall cost and time saved when existing studies can be helpful to support some trial results. It will eventually lead to better drugs with well-defined safety and response, two most important criteria to minimize drug failures in clinic.

7.12 Post-marketing Lessons Useful in New Drug Development

Genomics information gathered during drug development and post-marketing evaluation have been useful to improve safety and efficacy of some drugs. These post-marketing experiences support the idea that Pgx principles should be applied in early drug discovery. Even when Pgx does not have much effect on clinical outcome, information gained can be useful to confirm that potential pathways leading to variation are not responsible for altered response.

One classical example is the post-marketing studies on clopidogrel (Plavix®) to identify patients with reduced response. Clopidogrel belongs to thienopyridine class of antiplatelets that antagonizes P2Y12 ADP platelet receptors. For patients with non-ST-elevation myocardial infarction (NSTEMI), clopidogrel has been shown to decrease the rate of a combined endpoint of cardiovascular (CV) death, myocardial infarction (MI), or stroke, as well as the rate of a combined endpoint of CV death, MI, stroke, or refractory ischemia. For patients with ST-elevation myocardial infarction (STEMI), clopidogrel has been shown to reduce the rate of death from any cause and the rate of a combined endpoint of death, reinfarction, or stroke. Clopidogrel is a prodrug which must be metabolized to elicit response. As shown in Fig. 7.7, clopidogrel is extensively metabolized to active and inactive metabolites. Approximately 85% of the drug is hydrolyzed by esterase enzyme to produce inactive carboxylic acid metabolite. Activation pathway leads to 2-oxo-metabolite as an intermediate, which is then followed by second activation step to produce thiol metabolite responsible for binding to the receptor. CYP2C19 is involved in the formation of both 2-oxo-metabolite and terminal active metabolite. Although CYP2C19 phenotypes can lead to ultrarapid, extensive, intermediate, or poor metabolizers, CYP2C19*2 and *3 loss-of-function alleles are responsible for about 85% White and 99% Asian poor metabolizers. Approximately, 2% Whites, 4% Blacks, and 14% Chinese are regarded as poor metabolizers and may not show desired response to the drug [41].

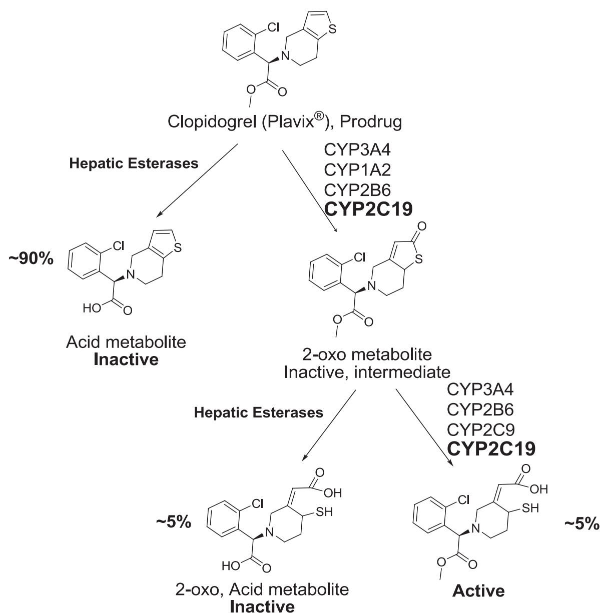

Fig. 7.7 Understanding metabolism of clopidogrel for discovering newer thienopyridines

Based on these post-marketing studies, drug label of clopidogrel was updated on March 2010 to include Pgx information related to diminished drug response and increase in CV events in poor metabolizers. Genetic tests are now available to check patient's metabolism status. In addition, FDA advised health-care professionals to consider the use of other antiplatelet medications or alternative dosing strategies for Plavix in patients identified as poor metabolizers.

DNA samples from phase 3 trials of clopidogrel were used to substantiate post-marketing experiences, and lessons learned from these studies supported the development of prasugrel in July 2009, another thienopyridine prodrug which bypasses requirement of CYP2C19 for activation (Fig. 7.8). Primary aim of this drug development project was to develop another thienopyridine antagonist with improved pharmacological properties (less effect of protein variants) and at least equivalent therapeutic benefits. As shown, CYP enzymes used in activation of prasugrel have less protein variants, and metabolizer status of a patient does not have significant effect on therapeutic outcomes. In fact, so far there are no known reports of polymorphism in 3A4 or 2B6 affecting response to prasugrel. Significant metabolism issues with clopidogrel also created need for more antiplatelet drugs, and ticagrelor was approved in July 2011. Significantly, this is not a prodrug and does not require metabolic activation in vivo thus eliminating the need for checking patient's metabolism status. Thus, understanding genomic pathway in drug discovery can help in discovering better drugs to answer genomic questions.

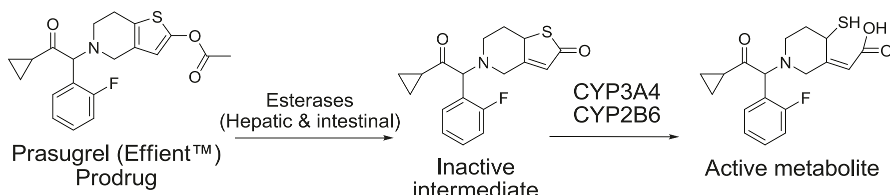

Fig. 7.8 Prasugrel metabolism highlighting uninvolvement of CYP2C19 during activation

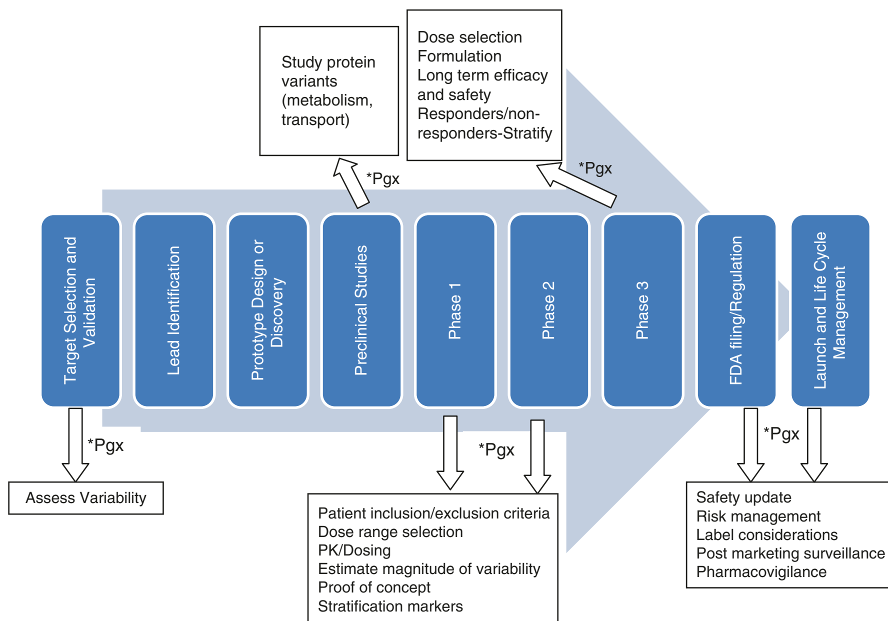

Fig. 7.9 Application of pharmacogenomics principles in drug discovery and development

Conclusion

It has been estimated that more than three million prescriptions written annually in the USA alone are either incorrect or ineffective. The cost of adverse drug reactions is expected to be >$177 billion annually. Potential considerations in variability in metabolism, transporters, clearance to understand PK, and PD results, both in preclinical and clinical studies, are needed to minimize drug attrition rates. Pgx principles can be applied at various stages of drug development and continued even after drug is approved and launched in the market, as depicted in Fig. 7.9. Advances in sequencing tools and newer technologies for collecting and analyzing data have created a gigantic database, and it has been estimated that every year, 40 petabytes (40 million gigabytes) of data has been created by genome sequencing [42]. While information is power and serves as

indispensable tool to solve scientific puzzles at different drug development stages, this tsunami size data comes with substantial challenges, and pharmaceutical companies face significant barriers to the effective utilization of available information while maintaining their profit margins. Challenges will remain along the way. Nevertheless, future is already here, and this current era of "precision medicine" is delivering promises starting from drug discovery to its effective utilization.

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8 Genomics and Drug Transporters and Application in Drug Discovery, Delivery, and Development

Robert Gharavi and Hazem E. Hassan

Abstract

Drug transporters are membrane-bound proteins known to regulate the entry (influx) and exit (efflux) of both exogenous (drugs) and endogenous molecules in various tissues throughout the body [1, 2]. They have emerged as key determinants in the absorption, distribution, metabolism, excretion, and toxicity of a number of drug molecules [2, 3]. Furthermore, they have been identified as sources of harmful inherited diseases, drug-drug interactions (DDIs), and increased resistance and sensitivity to various chemotherapy, antiviral, and antibiotic treatments [4–9]. Regulatory health agencies from across the globe have released official guidance in recent years highlighting the emerging importance of transporters in the disposition of drugs and the need to thoroughly investigate their drug interactions [10–12]. Most genes encoding drug transporters are polymorphic, resulting in phenotypes that can vary greatly in their levels of expression, protein folding, membrane localization, and transporter efficiencies [3, 13]. These phenotypic differences can ultimately result in major interindividual variabilities in response to identical drug molecules (i.e., pharmacokinetics (PK), pharmacodynamics (PD), safety, and efficacy). To date, there are a myriad of reports of how a single nucleic acid base pair change in a transporter gene can result in markedly different patient responses to the same molecules [3]. As a result, the US Food and Drug Administration (FDA)

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_8

currently requires pharmacogenomic biomarker information included in the drug labeling of a list of approved therapies [14]. Therefore, there is an evolving interest in the role that drug transporter polymorphisms may play in predicting individual responses and understanding interethnic differences in drug therapies.

8.1 Introduction

Drug transporters are membrane-bound proteins known to regulate the entry (influx) and exit (efflux) of both exogenous (drugs) and endogenous molecules in various tissues throughout the body [1, 2]. They have emerged as key determinants in the absorption, distribution, metabolism, excretion, and toxicity of a number of drug molecules [2, 3]. Furthermore, they have been identified as sources of harmful inherited diseases, drug-drug interactions (DDIs), and increased resistance and sensitivity to various chemotherapy, antiviral, and antibiotic treatments [4–9]. Regulatory health agencies from across the globe have released official guidance in recent years highlighting the emerging importance of transporters in the disposition of drugs and the need to thoroughly investigate their drug interactions [10–12]. Most genes encoding drug transporters are polymorphic, resulting in phenotypes that can vary greatly in their levels of expression, protein folding, membrane localization, and transporter efficiencies [3, 13]. These phenotypic differences can ultimately result in major interindividual variabilities in response to identical drug molecules (i.e., pharmacokinetics (PK), pharmacodynamics (PD), safety, and efficacy). To date, there are a myriad of reports of how a single nucleic acid base pair change in a transporter gene can result in markedly different patient responses to the same molecules [3]. As a result, the US Food and Drug Administration (FDA) currently requires pharmacogenomic biomarker information included in the drug labeling of a list of approved therapies [14]. Therefore, there is an evolving interest in the role that drug transporter polymorphisms may play in predicting individual responses and understanding interethnic differences in drug therapies.

With advances in genotyping technologies and creation of vast human genetic databases that are publically available, drug transporter pharmacogenomics has become an increasingly important area within drug development and safety [3]. Transporter genotypes and expression patterns may serve as informative biomarkers to assist in optimizing patient pharmacotherapy and moving toward more personalized medicine. For example, in 2014 the FDA recommended that carriers of certain transporter gene alleles be prescribed lower doses of simvastatin due to an increased risk of statin-induced myopathy. It is likely that further dosing recommendations based on drug transporter pharmacogenomics will follow in the coming years. This chapter will discuss the pharmacogenomics of drug transporters and their implications for drug discovery and delivery.

8.1.1 Transporters

Following administration (oral, subcutaneous, intravenous, etc.), almost all drug molecules must cross cellular membranes to exert their pharmacological effects.

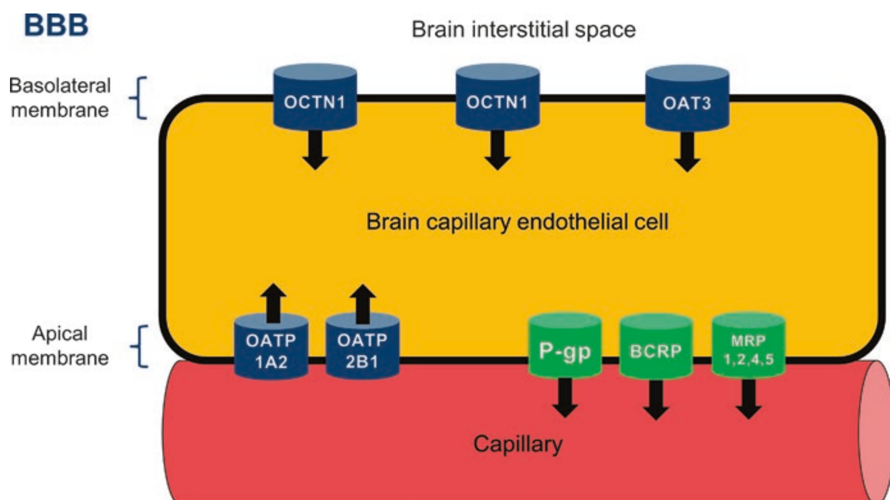

Fig. 8.1 Drug transporters at blood-brain barrier (BBB) interface. Arrows indicate the transporter's primary direction of transport

Transport across a cellular membrane depends on a number of factors including the molecule's physicochemical properties (size, charge, polarity) and the composition of the cellular membrane it must cross. Transport may be a passive process, such as diffusion, or it may be active, requiring energy and the assistance of membrane-bound proteins known as membrane drug transporters [15].

Drug transporters are located within the cellular membranes of many different cell types where they may actively transport drug molecules into (influx or uptake) or out of (efflux) cells [15]. Drug transporters vary greatly in their substrate specificities and tissue distributions. There are publicly available databases that summarize the tissue distribution and various substrates and inhibitors of transporters, based on in vitro, in vivo, and clinical data, such as the UCSF-FDA TransPortal [16].

Since the advent of human genome and exome sequencing projects, over 400 drug transporter proteins have been identified [3]. This information, along with new technologies such as genome-wide screenings, have allowed researchers to perturb the functions of thousands of genes [17]. Despite their seemingly large number and the extensive research in this area, only around 20 transporter genes have been functionally identified so far, highlighting that our current understanding of drug transporters is still evolving [2, 18].

Drug transporters are broadly categorized into the two superfamilies: the ATP-binding cassette (ABC) and solute carrier (SLC) families. At major physiological interfaces such as the blood-brain barrier (BBB) (Fig. 8.1), kidney (Fig. 8.2), liver (Fig. 8.3), and intestine (Fig. 8.4) drug transporters have been shown to be key determinants in the disposition of drug molecules, regulating their tissue-specific concentrations, and ultimately influencing their PK, PD, and toxicity [2]. Loperamide is a prominent example of the clinical impact that drug transporters may have on the

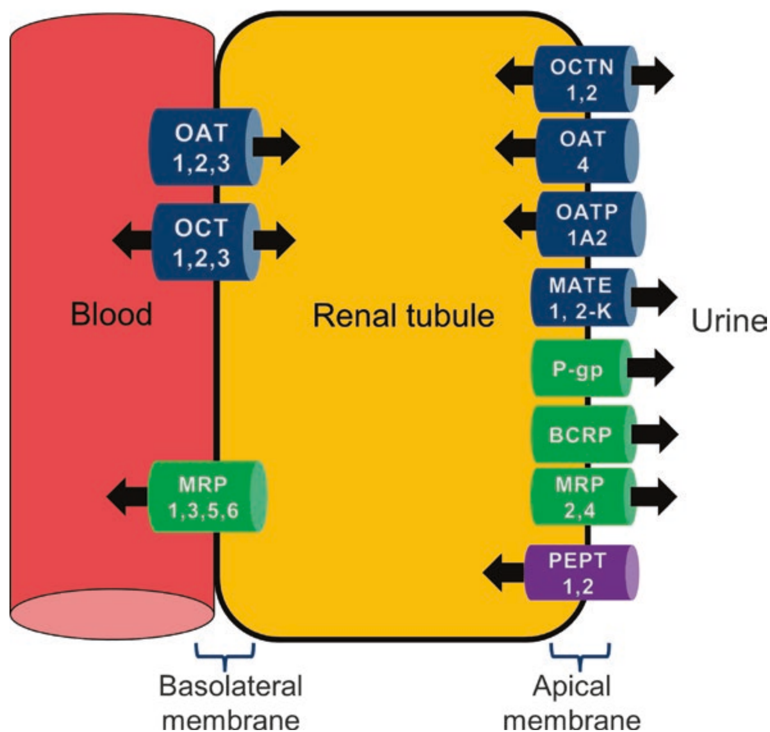

Fig. 8.2 Drug transporters at kidney interface. Arrows indicate the transporter's primary direction of transport

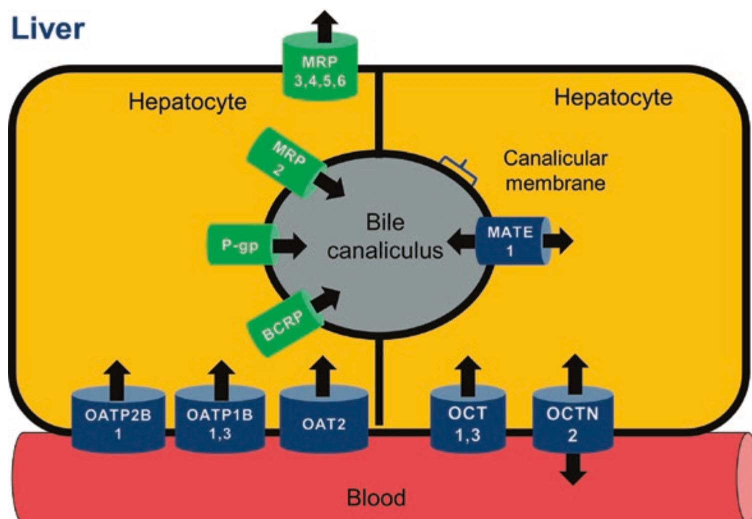

Fig. 8.3 Drug transporters at liver interface. Arrows indicate the transporter's primary direction of transport

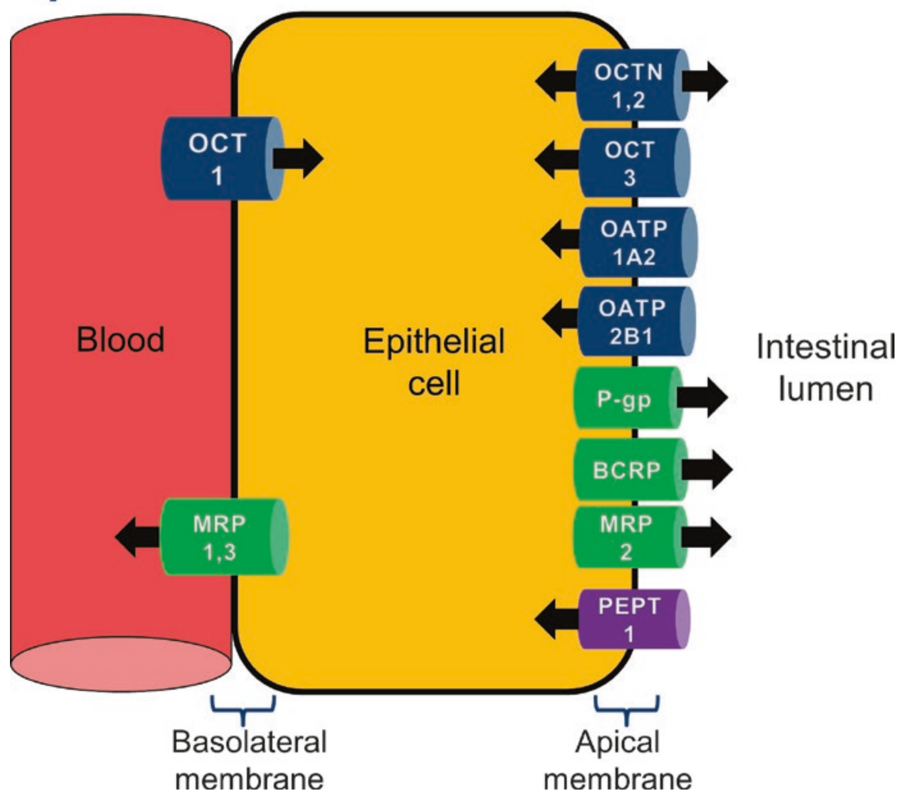

Fig. 8.4 Drug transporters at intestinal epithelia. Arrows indicate the transporter's primary direction of transport

PK/PD of a drug molecule. Despite being an opioid agonist with a high affinity for the μ-opioid receptor, it is used as an antidiarrheal agent rather than a potent analgesic. It is believed that this is due to loperamide being a strong P-glycoprotein (P-gp) substrate which restricts its BBB penetration, limiting its interaction with opioid receptors located in the brain [19].

The expression of drug transporters is regulated in large part by xenobiotic receptors, most notably the pregnane X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AhR) [20–22]. Activation or inhibition of these receptors by small molecules has been shown to significantly alter the expression and function of important drug transporting proteins leading to potentially significant drug-drug interactions or other clinical ramifications [23]. Interestingly, it has been shown that the expression of certain drug transporters can be altered without direct receptor activation by opioids such as oxycodone [24]. Further, the expression and function of drug transporters can be significantly altered by genetic polymorphisms [25].

Table 8.1 Known genetic polymorphisms of drug transporters

Type	Example	Description
Genomic reference: Numbering begins at the first nucleotide in the coding region and ends at the last	49T > C	At the 49th nucleotide of the gene, a thymine mutated to a cytosine
	−1789G > A	The negative number means this SNP is located at 5′ of the ATG initiation codon
Amino acid reference: The number corresponds to the codon number. The letters correspond to the single-letter amino acid code	F17L	At the 17th codon, phenylalanine (F) mutated to leucine (L)
Reference SNP cluster (rs#)	rs622342	This is the reference number stored by the Single Nucleotide Polymorphism Database (dbSNP)
Haplotype reference	*SLCO*1A*	This haplotype includes the two SNPs 388G > A and 521T > C

8.2 Pharmacogenomics of Drug Transporters

Given their significant influence on the disposition of many drug molecules, and the advances in pharmacogenomics as a field of study, there is increasing interest and investigation into the pharmacogenomics of drug transporters. Single-nucleotide polymorphisms (SNPs) in the coding regions of transporter genes may result in the same (synonymous; sSNP) or altered (non-synonymous; nsSNP) protein being expressed. Synonymous polymorphisms may result in different mRNA sequences which potentially result in altered mRNA, stability, transport, and translation. They may exert functional effects by influencing the tertiary structure of proteins through changing ribosomal kinetics during translation [26, 27]. Non-synonymous polymorphisms generally affect protein stability, intracellular trafficking, and membrane localization of transporters thereby impacting transporter substrate specificity and activity [3]. Polymorphisms in the noncoding regions of transporter genes including in introns and gene promoter regions have been shown to have important implications on mRNA regulation, generation, integrity, and processing [3, 6]. In many cases, SNPs appear to be linked and inherited together and as a result are grouped together in haplotypes (specific groups of genes or alleles inherited from the same parent). A number of transporter haplotypes have been associated with drug response phenotypes in the clinic. Several known transporter polymorphisms are shown in Table 8.1, while their clinical and preclinical impacts are illustrated in Table 8.2.

8.3 ABC Family of Transporters

The ABC superfamily of transporters harness the energy released due to ATP hydrolysis to actively pump substrates across lipid bilayers, often against steep concentration gradients [197, 198]. Many are efflux transporters, moving substrates out of tissues and back into the systemic circulation for clearance through the liver and

Table 8.2 Clinical and preclinical impact of transporter polymorphisms

ABCB1 (P-gp)	
554G > T G185 V rs1128501	*In vitro*: Increased resistance to colchicine [28]
F335	*In vitro*: Altered substrate specificity Decreased resistance to vinca alkaloids and cyclosporine D [29]
1199G > A S400N rs2229109	*In vivo*: Associated with increased trans-epithelial transport of five HIV protease inhibitors (amprenavir, indinavir, lopinavir, ritonavir, and saquinavir) [30]
1236C > T G412G rs1128503	*Clinical*: Associated with decreased clearance of docetaxel in patients with solid tumors [31] ABCB1 polymorphisms have no association with the clearance of docetaxel [32] 1236CC genotype associated with significantly shorter overall survival (OS) compared to carriers of 1236T in NK-AML [33] Associated with higher digoxin plasma concentrations [34]
2677G > T/A S893A/T rs2032582	*In vitro*: Altered P-gp efflux activity [35] Altered substrate specificity and efflux activity [36] *In vivo*: Associated with increased P-gp efflux of fexofenadine [37] *Clinical*: TT genotype associated with decreased intestinal P-gp expression and elevated digoxin plasma concentrations [35, 38] Carriers associated with elevated ABCB1 mRNA but not protein expression in cardiac tissue [39] Carriers associated with decreased placental P-gp expression [40] Haplotypes containing the G2677T/A allele associated with improved responses to fluvastatin [41] Associated with higher digoxin plasma concentrations [34] Associated with lower digoxin plasma concentrations [42] Not associated with responses to atorvastatin [43] 2677GG associated with improved response to docetaxel-cisplatin chemotherapy in Han Chinese NSCLC patients [44] Associated with grade 4 irinotecan-induced neutropenia [45] 2677TT or AA Swedish ovarian cancer patients found to respond better to paclitaxel treatment [46] Associated with improved PFS from paclitaxel and carboplatin combination therapy in Australian ovarian cancer patients [47] No association with outcome or toxicity following docetaxel or paclitaxel treatment in Scottish ovarian cancer patients [48] Associated with improved OS in de novo NK-AML patients [49]

(continued)

kidneys [13]. The physiological functions of ABC transporters emerged following studies of multidrug resistance where a number of ABC transporters were found to contribute toward the development of cellular resistance to various cytotoxic molecules by inhibiting their intracellular accumulation via active efflux [137]. These

Table 8.2 (continued)

3435C > T I1145I rs1045642	*In vitro*: Associated with decreased mRNA stability, expression levels, and decreased efflux ability [13] Altered substrate specificity due to changes in tertiary structure [26] Functional impacts on mRNA and protein expression unclear [50] No effect on leukemic blasts P-gp function [51] *Clinical*: No influence on ABCB1 mRNA or protein expression in cardiac tissue [39] Associated with decreased duodenal P-gp expression [38] Associated with elevated exposures to oral digoxin [38, 50] Haplotypes containing 3435C > T associated with improved response to atorvastatin [52] 3435TT associated with improved CD4 cell counts after 6 months of HIV antiretroviral therapy, while 3435CC associated with higher treatment failure [53] 3435CT associated with higher nelfinavir plasma levels and antiviral response than 3435CC in HIV-1 infected children [54] Associated with lower fexofenadine plasma concentrations in subjects with 2677AA/3435CC haplotypes [55] 3435CC associated with treatment-resistant epilepsy [56] 3435CC is not associated with treatment-resistant epilepsy [50, 57, 58] No influence on the PK of P-gp substrates tacrolimus, fexofenadine, cyclosporine A, and many others [50] Carriers associated with elevated risk of colon cancer in under 50 years old population [59] and non-clear cell RCC [60] Carriers associated with increased paclitaxel AUC and paclitaxel-induced neutropenia in Japanese ovarian cancer patients [61] 3435TT associated with higher risk of docetaxel-induced grade 3 neutropenia in patients with solid tumors [62] and higher frequency of grade 3 irinotecan-induced diarrhea in NSCLC patients [45] Higher frequency of 3455CC in sub-Saharan African populations may explain higher incidence of chemotherapy resistance [63–65] No effect on clinical outcomes of Korean AML patients receiving standard induction treatment [51]
rs2032583	*Clinical*: Associated with improved response to antidepressant treatment [66]
ABCC1 (MRP1)	
C43S	*In vitro*: Loss of membrane localization and decreased resistance to vincristine [67]
218C > T T73I	*In vitro*: No change in protein expression or function [68]
257C > T S92F	
350C > T T117M	
G128C	*In vitro*: No change in protein expression or function [68] Changes in membrane localization [69]

Table 8.2 (continued)

689G > A R230Q	*In vitro*: No change in protein expression or function [68]
R433S	*In vitro*: Decreased transport or conjugated organic anions but increased resistance to doxorubicin [70]
1898G > A R633Q	*In vitro*: No change in protein expression or function [68]
2168G > A R723Q	
2965G > A A989T	*In vitro*: No change in protein expression, decreased estradiol-17beta-glucuronide transport [68]
3140G > C C1047S	*In vitro*: No change in protein expression or function [68]
3173G > A R1058Q	
4535C > T S1512L	*In vitro*: No change in protein expression [68]
ABCC2 (MRP2)	
−24C > T	*In vitro*: Reduced kidney tissue mRNA expression [71] *Clinical*: Associated with higher response to irinotecan/cisplatin chemotherapy in NSCLC patients [45]
R412G	*In vitro*: Resulted in loss of function [72] *Clinical*: Associated with DJS and impaired elimination of methotrexate [72]
2302C > T A768W	*Clinical*: Associated with DJS [73, 74]
2439T > C	*Clinical*: Associated with DJS [73]
3972C > T I1324I	Associated with an increased risk of cholangiocarcinoma and HCC [75] Associated with higher response to irinotecan/cisplatin chemotherapy in NSCLC patients [45, 76]
rs717620	*Clinical*: Associated with shortened PFS and OS [77]
ABCC3 (MRP3)	
211C > T	*Clinical*: Carriers associated with decreased mRNA expression in liver samples of Caucasian patients [78] Not associated with decreased mRNA expression [79] Associated with a poor prognosis in Israeli AML patients [80] and significantly worse PFS in primary lung cancer patients receiving first-line chemotherapy [81]
R1381S	*In vitro*: Intracellular accumulation of immature MRP3 [82]

(continued)

Table 8.2 (continued)

S346F S607N	*In vitro*: Loss of transporter function [82]
ABCC4 (MRP4)	
rs3765534	*In vitro*: Impairs transporter function by disrupting membrane localization, may predict increased thiopurine sensitivity [83]
3463A > G	*Clinical*: Associated with impaired clearance of tenofovir [84]
G187W	*In vitro*: Decreased expression and function [85]
G487E	*In vitro*: Decreased function [85]
ABCG2 (BCRP)	
−15622C > T	*Clinical*: Carriers associated with lower BCRP mRNA expression in multiple tissues [86, 87] Carriers associated with higher exposure to erlotinib [87] and greater risk of gefitinib-induced diarrhea [88]
34G > A V12M rs2231137	*In vitro*: Not shown to alter BCRP expression or function [89–92] Decreased transporter activity and disturbed membrane localization [93] *Clinical*: Carriers significantly associated with ALL [94] No impact on the risk of ALL [95] Carriers associated with gefitinib-induced skin rash [96] Carriers associated with irinotecan-induced grade 3 and 4 neutropenia, and 34AA carriers associated with cisplatin combination therapy-induced grade 3 and 4 neutropenia in Japanese cancer patients [76] Carriers with AML demonstrated increased OS as well as toxicity [97] 34AA DLBCL patients displayed worse survival compared to 34GG or 34GA patients [98] 34GG Chinese leukemia patients displayed longer disease-free survival and OS compared to carriers of the polymorphism [99] 34GG carriers associated with poor responses to imatinib in advanced stage CML patients [100] No impact on OS in primary lung cancer patients receiving platinum-based chemotherapy [81] No effect on recurrence-free survival in Japanese breast cancer patients receiving tamoxifen treatment [101] No significant effect on the PK or toxicity of irinotecan in advanced NSCLC patients [45]
151G > T G51C	*In vitro*: Decreased transporter activity [92]
376C > T Q126Stop rs7255271	*In vitro*: Loss of expression and activity [90–93] Encodes premature stop codon [102] *Clinical*: Dramatically increased risk of gout in Japanese patients [103]

Table 8.2 (continued)

421C > A Q141K rs2231142	*In vitro*: Increased intracellular degradation [104, 105] Impaired cellular trafficking leading to intracellular retention [106] Instability in the NBD leading to decreased BCRP expression [107] Decreased protein expression and altered substrate specificity [90, 91] Decreased ATPase activity [93] Increased intracellular accumulation of gefitinib and erlotinib [108] *In vivo*: Increased systemic exposure to sunitinib and sunitinib-induced toxicity [109] *Clinical*: Decreased BCRP expression in erythrocytes [110] Carriers associated with increased exposure to sulfasalazine [111], atorvastatin [112], rosuvastatin [113], simvastatin lactone, fluvastatin [114], diflomotecan [115], topotecan [116], gefitinib [108] and imatinib [100] Carriers displayed impaired clearance of imatinib [117, 118] and erlotinib [119] Carriers associated with increased risk of gout [106, 107, 120], Alzheimer's disease [121], DLBCL [98], non-papillary RCC [122] No impact on the risk of CRC [123, 124], prostate cancer [125] or AML [81, 95] and a decreased risk of CLL [77] Associated with a 6-month increase in PFS in carriers with advanced ovarian cancer treated with a platinum- and taxane-based chemotherapy [125] 421A associated with increased survival beyond 15 months in docetaxel-treated hormone-refractory prostate cancer patients [126] 421A carriers associated with a decrease in OS in primary lung cancer patients receiving platinum-based chemotherapy [81] Potential biomarker of poor prognosis for adult AML patients receiving idarubicin-based chemotherapy [127] No effect on recurrence-free survival in Japanese breast cancer patients receiving tamoxifen treatment [101] or OS in American patients with potentially resectable pancreatic adenocarcinoma who were treated with preoperative gemcitabine-based chemoradiotherapy [128] Carriers associated with increased risk of chemotherapy-induced diarrhea in DLBCL patients [129]), sunitinib-induced toxicity in metastatic RCC patients [130], gefitinib-induced diarrhea in NSCLC patients [131], and docetaxel-induced febrile neutropenia in breast cancer patients [132]
458C > T T153M	*In vitro*: No change in transporter activity [92]
496C > G Q166E rs1061017	*In vitro*: Increased protein expression with minimal change in transporter activity [102] Minimal change in protein expression [91] Decreased transporter activity [133]
616A > C I206L rs12721643	*In vitro*: Decreased protein expression, increased transporter activity [134]
623T > C F208S rs1061018	*In vitro*: Complete loss of protein expression and transporter activity [92] Encodes a nonfunctional BCRP [102]

(continued)

Table 8.2 (continued)

742T > C S248P rs3116448	*In vitro*: Decreased protein expression [134] Decreased transporter activity [92]
1000G > T, E334Stop rs3201997	*In vitro*: Loss of transporter expression and activity [92, 135]
1143C > T rs2622604	*Clinical*: Carriers associated with decreased BCRP mRNA expression [86, 87] No significant impact on imatinib PK parameters [86] Carriers associated with higher erlotinib AUC and C_{max} [87] Associated with irinotecan-induced severe myelosuppression [136]
1291T > C F431L	*In vitro*: Decreased transporter activity [92, 102] Altered substrate specificity [92]
1322G > A S441N	*In vitro*: Results in intracellular localization and decreased protein expression [91] Loss of transporter activity [92]
1465T > C F489L	*In vitro*: Decreased transporter activity [92]
1711T > A F571I rs9282571	*In vitro*: Altered substrate specificity [92]
1723C > T R575stop	*In vitro*: Not determined [137] Encodes premature stop codon [76]
rs6857600	*Clinical*: Carriers of at least one A allele associated with decreased risk of B-NHL [77]
1768A > T N590Y rs34264773	*In vitro*: Increased protein expression [134] Decreased transporter activity [92, 134]
1858G > A D620N rs34783571	*In vitro*: Increased protein expression, decreased transporter activity [134]
SLCO1B1 (OATP1B1)	
−11187G > A	*Clinical*: Associated with increased AUC of pravastatin [138]
217T > C F73L rs56101265	*In vitro*: Decreased transporter activity [139]
245T > C V82A rs56061388	*In vitro*: Decreased transporter activity [139]
388A > G N130D rs2306283	*In vitro*: Decreased or no change in transporter activity [139] *In vivo*: No change in transporter function [140]

Table 8.2 (continued)

<table>
<tr><td>463C > A
P155T
rs11045819</td><td>In vitro:
No change in transporter function [139]
Clinical:
Associated with enhanced lipid-lowering efficacy of fluvastatin [141]
463CA associated with decreased AUC of rifampin [142]</td></tr>
<tr><td>467A > G
E156G
rs72559745</td><td>In vitro:
Decreased transporter activity [139]
Clinical:
Associated with reduced AUC of rifampin [142]</td></tr>
<tr><td>521T > C
V174A
rs4149056</td><td>In vitro:
Decreased expression and transporter activity [139, 143–146]
Clinical:
Associated with increased risk of statin-induced myopathy [140, 147, 148]
521CC associated with increased in AUC of the antidiabetic repaglinide and fexofenadine [149]
Associated with increased AUC of antidiabetic nateglinide [113]
Associated with impaired clearance of methotrexate in children ALL patients [150]
Generally associated with impaired hepatic uptake and clearance of substrates [140]
Associated with elevated serum bilirubin levels [151]
Associated with increased AUC of irinotecan [76, 152, 153]
Associated with decreased clearance of lopinavir [154]</td></tr>
<tr><td>578T > G
L193R
rs72559746</td><td>In vitro:
Loss of transporter function and membrane expression [155]</td></tr>
<tr><td>1058T > C
I353T
rs55901008</td><td rowspan="2">In vitro:
Decreased transporter activity [139]</td></tr>
<tr><td>1294A > G
N432D
rs56387224</td></tr>
<tr><td>1385A > G
D462G
rs72559748</td><td>In vitro:
Decreased transporter activity [139]
No change in transporter function [140]</td></tr>
<tr><td>1463G > C
G488A
rs59502379</td><td rowspan="2">In vitro:
Decreased transporter activity [139]</td></tr>
<tr><td>1964A > G
D655G
rs56199088</td></tr>
<tr><td>rs11045879</td><td rowspan="2">Associated with the increased clearance of methotrexate in a study of children with ALL. These SNPs were found to be in complete linkage disequilibrium with each other, but both appear to enhance the hepatic uptake of methotrexate [150]</td></tr>
<tr><td>rs4149081</td></tr>
</table>

(continued)

Table 8.2 (continued)

*SLCO*1A* 388A, 521T	*Clinical*: *1A/*1A genotype associated with higher AUC of pravastatin and antidiabetic repaglinide than *1B/*1B [156] *1A/*1A genotype associated with increased plasma levels of various bile acids [157]
*SLCO*1B* 388G, 521T	Associated with increased transporter activity [140] *Clinical*: Associated with decreased AUC of pravastatin [140, 156, 158] *1B/*15 genotype associated with reduced non-renal clearance of pravastatin compared to *1B/*1B genotype [159] *1B/*1B associated with decreased AUC of ezetimibe [146] *1B/*1B associated with increased AUC and decreased clearance of torsemide [160, 161]
*SLCO*5* 388A, 521C	Low-activity haplotype [140] *Clinical*: Associated with increased risk of statin-induced myopathy [162] Associated with reduced lipid-lowering effects of multiple statins [163]
*SLCO*15* 388G, 521C	Low-activity haplotype [140] Associated with increased risk of statin-induced myopathy [162, 164] *15/*15 associated with increased AUC and decreased clearance of irinotecan [165] and olmesartan [166]
SLCO1B3 (OATP1B3)	
344T > G	*In vitro*: No alteration in expression or transport activity [167] Reduced transport of testosterone and mycophenolic acid [168–170] *Clinical*: Associated with increased AUC of mycophenolic acid [171] Associated with decreased plasma concentration of mycophenolic acid in renal transplant patients [169] Associated with increased clearance of imatinib in Japanese AML patients [172]
699G > A	*In vitro*: No alteration in expression or transport activity [167] Reduced transport of testosterone and mycophenolic acid [168–170] *Clinic*: Associated with increased AUC of mycophenolic acid [171] One of four SLCO1B3 variations in a haplotype tagging SNP associated with a decreased clearance and increased AUC of docetaxel [173]
1559A > C	*In vitro*: Decreased membrane expression [174]
1679T > C	*In vitro*: Decreased membrane expression [174]
IVS12- 5676A > G rs11045585	*Clinical*: Increased AUC and decreased clearance of docetaxel [175] One of four SLCO1B3 variations in a haplotype tagging SNP associated with a decreased clearance and increased AUC of docetaxel [173] Increased AUC of telmisartan [176]
SLCO2B1 (OATP2B1)	
935G > A R312Q rs12422149	*Clinical*: Decreased plasma concentrations of montelukast and lower improvement of asthmatic symptoms [177]

Table 8.2 (continued)

1457C > T S486F rs2306168	*Clinical*: Decreased AUC of fexofenadine [178] Decreased AUC of celiprolol [179]
SLCO1A2(OATP1A2)	
−1105G > A	*Clinical*: Decreased clearance of imatinib [176]
−1032G > A	
−361G > A	
SLC22A1 (OCT1)	
rs622342	*Clinical*: Increased blood-glucose lowering effects of metformin [180, 181] Associated with patients requiring higher doses of levodopa [182]
rs683369	The GG genotype along with advanced stage correlated with a high rate of treatment failure to imatinib in CML patients [100]
286C > T R61C rs12208357	Low-function allele [183–185] *In vitro*: Decreased metformin uptake [186] *Clinical*: Increased Cmax and AUC and decreased VD of metformin [187] Increased renal elimination of metformin with multiple copies [185] No effect on imatinib response in CML patients [184]
P160L	
S189 L rs34104736	Low-function allele [185, 187, 188] *In vitro*: Decreased metformin uptake [186]
P341L	*In vitro*: Increased uptake of MPP^+ [183]
G401S rs34130495	*In vitro*: Decreased metformin uptake [186] *Clinical*: Increased Cmax and AUC and decreased VD of metformin [187] Increased renal elimination of metformin with multiple copies [185] Decreased metformin efficacy on HbA1c [188]
M420del rs72552763	Low-function allele [185, 187, 188] *In vitro*: Decreased metformin uptake [186] *Clinical*: Increased Cmax and AUC and decreased VD of metformin [187] Increased renal elimination of metformin with multiple copies [150] Decreased metformin plasma concentrations with increasing copy number [188]
G465R rs34059508	Low-function allele [185, 187, 188] *In vitro*: Reduced membrane expression of OCT1 [183] Decreased metformin uptake [186] *Clinical*: Increased Cmax and AUC and decreased VD of metformin [187] Increased renal elimination of metformin with multiple copies [185]

(continued)

Table 8.2 (continued)

SLC22A2 (OCT2)	
−130G > A	Homozygous carriers had significantly poorer responses to metformin-induced HbA1C lowering [189]
158G > A rs2289669	*Clinical*: Associated with reduced HbA1C in subjects receiving metformin [190] Possibly interacts with OCT1 SNP rs622342 in contributing to metformin clearance [180]
808G > T rs316019	*In vitro*: Increased metformin transport capacity [191] Altered uptake of norepinephrine, dopamine, and propranolol [114] *Clinical*: Increased renal clearance of metformin [191] Decreased renal clearance of metformin [192, 193] No effect on metformin PK [114, 185, 188] Associated with reduced risk of cisplatin-induced nephrotoxicity [194], though not confirmed [195]
SLC47A1 (MATE1)	
158G > A rs2289669	*Clinical*: Associated with reduced HbA1C in subjects receiving metformin [180] May interact with the OCT1 SNP rs622342 in contributing to metformin clearance [180]
SLC47A2 (MATE2)	
−130G > A	*Clinical*: Homozygous carriers had significantly poorer responses to metformin-induced HbA1C lowering [189]. No effect on the disposition of metformin in heterozygous carriers of SLC47A variations [196]

studies also demonstrated that resistance was inducible where it was evident that the expression of ABC transporters, such as P-gp and breast cancer resistance protein (BCRP), increased following initial exposures and eventually resulted in complete resistance.

Presently, there are 51 genes known to encode transporters in the ABC superfamily, which are divided into seven subfamilies (A–G) (http://www.genenames.org/genefamilies/ABC). They are expressed in various tissues throughout the body, particularly in epithelial and endothelial cell layers at physiological barriers, such as the small intestines, kidney, liver, and BBB [137]. Various polymorphisms in several ABC transporters have been linked with inherited diseases or predisposition to multigenic diseases, as well as increased risk of toxicity resulting from certain drug therapies [197, 199, 200]. Based on crystal structure studies and homology models, ABC transporters appear to share the structural characteristic of a large central cavity formed by multiple transmembrane domains [190, 201–203]. This feature allows for multiple drug binding regions and perhaps explains the broad range of substrates transported by these proteins [137].

8.3.1 ABCB1 (P-glycoprotein, P-gp)

ABCB1 is located on chromosome 7 in humans and encodes P-gp or the multidrug resistance 1 (MDR1) protein [204]. Hundreds of structurally diverse therapies and endogenous molecules have been found to interact with P-gp [205, 206]. ABCB1 is expressed in the kidney, liver, intestine, testes, heart, placenta, endothelial cells, hematopoietic stem cells, and at the BBB and endothelial cells [16, 207–210]. P-gp generally functions as a unidirectional efflux transporter, pumping its substrates out of tissues back into the lumen or systemic circulation for metabolism and excretion [211]. P-gp has been linked with treatment resistance in a number of disease states including cancer, HIV/AIDS, epilepsy, depression, and heart diseases [6, 56].

Structurally, P-gp consists of a substrate-binding domain (SBD), which is a large open cavity made of 12 transmembrane domains that span the lipid bilayer, and cytoplasmic nucleotide-binding domains (NBD) where ATP binding and hydrolysis occurs. The structure contains a number of highly conserved motifs that are believed to be involved in forming ATP-binding pockets [6, 202, 212]. Hence, mutations in the SBD may result in altered substrate specificity, while mutations in the NBD may result in functional impairment.

Genetic variations within the ABCB1 gene have been of particular interest in the field of pharmacogenomics for years [6]. Although extensively studied, the functional impacts of the pharmacogenomics of P-gp remain unclear and even controversial in some cases [211]. Over 60 coding SNPs and several insertions, deletions, and promoter region alterations have been identified within ABCB1 to date [4, 13].

Despite the large number of coding SNPs identified, only 14 (12 nsSNPs and 2 sSNPs) have so far been associated with functional impacts on ABCB1 expression or P-gp activity [6]. Three of these SNPs have been extensively studied in multiple populations. They are the sSNPs 1236C > T and 3435C > T and the nsSNP 2677G > T/A (A893S/T) [6, 37]. The sSNP 1236C > T is present in exon 12 and occurs at relatively high allele frequencies across multiple ethnic groups. This SNP appears to be most prevalent in populations of East Asian origin with an allele frequency from 56 to 69%, followed by Caucasians (38–48%) and individuals of sub-Saharan African ancestry (12–25%) [6, 61]. Clinical findings are conflicting regarding this SNP with some suggesting this polymorphism is associated with a decreased clearance of docetaxel, and others suggesting there is no association [31, 32]. Another study concluded that normal karyotype acute myeloid leukemia (NK-AML) patients with the 1236CC genotype were associated with significantly shorter overall survival (OS) compared to carriers of 1236T [33]. 1236C > T has also been associated with higher digoxin plasma levels [34].

The sSNP 3435C > T is by far the most well-studied polymorphism of ABCB1. This polymorphism occurs most frequently in East Asians (>50%) and Caucasians (53–62%) and at a lower frequency in individuals of sub-Saharan African ancestry (11–20%) [6, 61]. In vitro studies have suggested this SNP results in decreased mRNA stability and protein expression, as well as altered substrate specificity [13, 26].

However, others have also suggested this SNP has no functional consequences or that they are unclear [50, 51]. Clinical studies are also somewhat inconsistent on this matter, with some suggesting that the 3435C > T mutation results in decreased duodenal P-gp expression, while other suggest the SNP has no impact on mRNA or cardiac P-gp expression [38, 39].

Other clinical studies have suggested that the 3435C > T polymorphism is associated with higher plasma levels and improved responses to HIV antiretroviral therapies [53, 54]. This SNP has also been implicated in increased risk of colon cancer in patients under 50 years of age and non-clear renal cell carcinoma [59, 60], as well as increased risk of paclitaxel-, docetaxel-, and irinotecan-induced toxicities [45, 61, 62]. The higher prevalence of the wild-type 3435CC genotype in individuals of sub-Saharan African ancestry has been proposed as a possible explanation for the higher incidence of chemotherapy resistance in this population [63–65]. However, another study in Korean acute myeloid lymphoma (AML) patients concluded this SNP has no impact on clinical outcomes [51]. In relation to treatment-resistant epilepsy, one study suggested the reference genotype 3435CC is associated with treatment resistance [56], while others have not confirmed this finding [50, 57, 58]. It is worth mentioning that some clinical studies have suggested that the 3435C > T mutation impacts the PK and/or PD of digoxin, atorvastatin, fexofenadine, and tramadol, while others have challenged these findings [38, 50, 52, 213].

The nsSNP 2677G > T/A (A893S/T) occurs in exon 21, a region which encodes an intracellular loop of P-gp. The amino acid change from an alanine to a serine or threonine is believed to have structurally important ramifications, though the functional effects remain yet to be definitively elucidated [13, 39]. The 2677G > T polymorphism appears to occur much more frequently than the 2677G > A mutation across all populations. Further, the 2677G > T SNP has a greater allelic frequency in populations of East Asian origin (39–57%) than in Caucasian (38–44%) or sub-Saharan African populations (0–12.5%) [6, 40, 61]. In vitro studies have suggested this SNP results in altered P-gp substrate specificity and transporter activity [35, 36]. Some clinical studies have indicated that this SNP alters the PK or PD of a number of commonly used therapeutics, including fluvastatin, digoxin, irinotecan, paclitaxel, and chemotherapy combinations such as docetaxel with cisplatin and paclitaxel with carboplatin [34, 38, 41, 42, 44, 45], while others have contended that there are no significant associations, such as in responses to atorvastatin, docetaxel, and paclitaxel [43, 48]. The 2677G > T mutation has also been associated with improved overall survival (OS) in de novo NK-AML patients [49].

The 1236C > T, 2677G > T/A, and 3435C > T mutations have also been extensively investigated in regard to their impact on the disposition and clinical responses to various opioids. There are conflicting reports on the role of these polymorphisms on the PK/PD of morphine [214–216], methadone [217–221], oxycodone [222–224], and fentanyl [225, 226].

As with individual SNPs, the frequency patterns of haplotypes often reflect different ethnicities. For example, the genotypes 1236CT, 2677GT, and 3435CT are found in high frequencies in White Americans, while the 1236CC, 2677GT, and

3435CC genotypes are found more commonly among African-Americans [37]. Various ABCB1 haplotypes have been shown to impact the PK or PD of a number of therapeutic molecules such as digoxin, fluvastatin, atorvastatin, fexofenadine, irinotecan, and cisplatin [34, 41, 44, 45, 52, 55, 227, 228].

In addition to highlighting ethnic differences, the expression patterns of P-gp may also vary by gender, where studies have shown that women express significantly lower hepatic P-gp than men [229]. This could confer a higher exposure to P-gp substrates in women. For chemotherapy substrates this may suggest a greater efficacy but also a greater risk of side effects [230, 231].

8.3.2 ABCG2 (BCRP)

ABCG2 is a well-characterized gene which encodes the breast cancer resistance protein (BCRP). BCRP is a member of the G subfamily of ABC transporters and demonstrates an exceptionally broad substrate specificity, which includes hydrophobic and hydrophilic compounds, cations, anions, and even large phase II drug conjugates [232, 233]. BCRP functions primarily as an efflux transporter, and, like all members of the G subfamily, BCRP is a half-transporter, forming dimers or oligomers to function [234]. In human physiology, BCRP is most strongly associated with chemotherapy resistance. In a number of solid tumor and hematological cancers, BCRP has even been proposed as a prognostic biomarker, where elevated expression of BCRP is associated with a poorer prognosis [235].

BCRP is expressed in a variety of tissues including the colon, small intestine, liver bile canaliculi, gall bladder, kidney proximal tubules, adrenal gland, prostate, testes, uterus, lung, pancreas, prostate placenta, and capillary endothelial cells of the BBB [16, 137, 209, 236]. Structurally, BCRP consists of a NBD and transmembrane domain. BCRP has been shown to have multiple distinct and overlapping substrate-binding sites [137, 234], and various site-directed mutagenesis studies have demonstrated that certain polymorphisms can result in altered substrate specificity and transporter activity [92, 93, 134].

The pharmacogenomics of the ABCG2 gene have been extensively studied. To date, over 50 ABCG2 SNPs have been identified, including a number of nsSNPS [237–239], though functional consequences of these SNPs are still emerging. Certain SNPs are associated with increased risk for various types of cancer and increased chemotherapy toxicity [94, 240]. One of the most investigated ABCG2 SNPs is the 34G > A mutation. This nsSNP occurs in a region encoding the N-terminus of BCRP and results in the amino acid change V12M. The 34G > A SNP has been found at the greatest allele frequencies in East Asians (19–30%) and is much less common in Caucasians and African-Americans [90, 93, 133, 135, 199, 241]. One study concluded that carriers of this SNP are associated with an increased risk of acute lymphoblastic leukemia (ALL), though this report has since been refuted [94, 95]. The 34G > A polymorphism has been associated with an increased risk of toxicity induced by chemotherapy agents such as gefitinib, irinotecan, and

cisplatin [94, 228, 240]. One study suggested that this SNP is associated with increased OS in AML patients [97]. However, this finding is countered by others that suggest the 34AA genotype is associated with decreased survival rates in diffuse large B-cell lymphoma (DLBCL) patients and a decreased OS in Chinese leukemia patients [98, 99]. Still, other studies have suggested that this SNP has no impact on survival in lung or breast cancer patients [45, 81, 101].

The most well-characterized ABCG2 variant is Q141K (421C > A, rs2231137) in exon 5. This SNP occurs at allele frequencies of approximately 30% in Asians and 10% in Caucasians [90, 93, 133, 135, 199, 241]. The Q141K amino acid mutation results in increased intracellular degradation of BCRP, shortening its half-life and ultimately reducing its efflux capacity [104]. In vitro studies have demonstrated that this SNP decreases the protein expression of BCRP by approximately 50% relative to wild-type cells [13]. This mutation has been found to be associated with the development of gout [106, 107, 120], Alzheimer's disease [121]), DLBCL [98], and non-papillary RCC [122]. However, other studies have suggested that 421C > A is associated with a decreased risk of chronic lymphocytic leukemia (CLL) [77]. In addition, some reports suggested that this SNP has no impact on the risk of AML [95] and colorectal [123] and prostate [242] cancers.

Clinical studies have found that carriers of the Q141K mutation are associated with increased exposure to a number of therapeutic agents, such as sulfasalazine [111], atorvastatin [112], rosuvastatin [243], simvastatin lactone, fluvastatin [112], diflomotecan [115], topotecan [116], gefitinib [108], and imatinib [100]. Other studies have found that carriers are associated with increased PFS in ovarian cancer [125] and increased survival following docetaxel treatment in hormone-refractory prostate cancer [126]. However, carriers have also been found to be associated with decreased OS in primary lung cancer patients receiving platinum-based chemotherapy [81]. Further, the 421C > A SNP has been proposed as a biomarker of poor prognosis in AML patients receiving idarubicin-based chemotherapy [127]. Still, other studies have concluded that this SNP has no impact on survival in Japanese breast cancer patients receiving tamoxifen treatment [101] or in American pancreatic adenocarcinoma patients treated with gemcitabine-based chemotherapy [128]. In addition, the 421C > A mutation has been found to be associated with an increased risk of chemotherapy-induced toxicities to agents such as sunitinib in metastatic RCC [130], gefitinib in NSCLC [131], and docetaxel in breast cancer [132].

There are many other BCRP SNPs that have been identified, but only a few of those have been investigated clinically [91, 244]. The intronic variant 1143C > T has an allele frequency of approximately 25% in Caucasians and occurs at lower frequencies in East Asians and Africans. Previous studies of this SNP concluded that carriers are associated with decreased ABCG2 mRNA levels [86, 87]. One study concluded that the 1143C > T mutation is associated with increased plasma exposure to erlotinib [87], while another demonstrated that it is associated with irinotecan-induced myelosuppression [136]. However, another report suggested this SNP has no impact on the PK of imatinib [86].

8.3.3 ABCC1 (MRP1)

ABCC1 encodes the multidrug resistance protein-1 (MRP1). MRP1 was first discovered in multidrug resistance studies in cells that demonstrated chemotherapy resistance without expressing P-gp [245]. This transporter is expressed in many tissues such as lung, brain, kidney, heart, pancreas, testes, and skeletal muscle, where it is generally localized at the basolateral membrane of cells for the efflux of substrates back into the bloodstream [246–248]. MRP1 demonstrates a broad substrate specificity of structurally diverse compounds, including uncharged or anionic hydrophobic compounds as well as glutathione and glutathione-conjugated phase II metabolites [249]. The expression of MRP1 has also been associated with resistance to anthracyclines and vinca alkaloids [4]. Previous in vitro investigations appear inconsistent on the potential functional consequences of a number of SNPs within ABCC1 [67–69]. However, clinical studies are yet to be conducted to support or refute these findings.

8.3.4 ABCC2 (MRP2)

ABCC2 encodes the MRP2 transporter, also known as the canalicular multispecific organic anion transporter (cMOAT). ABCC2 is primarily expressed in the liver, kidney, and intestine where it is localized at the apical membrane of cells and is involved in the transport of a broad range of glutathione-conjugated phase II metabolites, including conjugated bilirubin. In the liver, MRP2 plays a prominent role in exporting these metabolites from hepatocytes to the canaliculi and ultimately to the bile for elimination [4].

The pharmacogenomics of ABCC2 is of particular interest due to the transporter's association with the rare autosomal recessive disease Dubin-Johnson syndrome (DJS). DJS is highlighted by an inability to excrete conjugates of bilirubin into the bile. Certain polymorphisms of ABCC2 result in a decreased functional capacity of MRP2, leading to excessive buildup of conjugated bilirubin where it is eventually deposited in hepatocytes and leads to toxicity. The polymorphisms appear to exert these consequences by impairing the function of MRP2's nucleotide-binding domain or impairing its transcription and membrane localization [4]. The 2302C > T and 2439T > C SNPs have been associated with DJS [72–74].

In vitro studies in pancreatic cancer cell lines have suggested that the expression of MRP2 is associated with resistance to gemcitabine and cisplatin combination chemotherapy [250]. One study in non-small cell lung cancer (NSCLC) found that carriers of the −24C > T SNP were associated with a greater response to combination chemotherapy of irinotecan with cisplatin [45]. In another NSCLC study, the ABCC2 of this SNP was associated with shortened progression-free survival (PFS) and OS [251]. This mutation of ABCC2 was found to be associated with a decreased risk of irinotecan-induced diarrhea, possibly due to reduced hepatobiliary secretion of irinotecan [252]. The R412G amino acid replacement was found to be associated

with impaired methotrexate clearance [72], while the sSNP 3972C > T was found to be associated with an increased risk of cholangiocarcinoma and hepatocellular cancer (HCC) [75].

8.3.5 ABCC3 (MRP3)

ABCC3 encodes the multidrug resistance protein-3 (MRP3) transporter. MRP3 is primarily expressed in the liver, pancreas, kidney, intestine, and lung where it is localized to the apical membrane of cells [4, 247, 248]. Although a close relative of MRP1, MRP3 does not appear to transport glutathione and only weakly interacts with glutathione-conjugated phase II metabolites. It does, however, transport the glucuronidated metabolites of acetaminophen, morphine, estradiol, and bilirubin, among others [249, 253, 254].

ABCC3 mRNA was found to be upregulated and correlated with tumor grading in pancreatic carcinoma samples [247]. The promoter region SNP 211C > T has been associated with a poorer prognosis in Israeli AML patients and a significantly worse progression-free survival (PFS) in primary lung cancer patients receiving first-line chemotherapy [80, 81]. The impact of this SNP on mRNA expression remains unclear [78, 79].

8.3.6 ABCC4, ABCC5, and ABCC6 (MRP4, 5, 6)

ABCC4, ABCC5, and ABCC6 encode their corresponding MRP4, MRP5, and MRP6 drug transporting proteins, respectively. These transporters are much less studied than the other three MRPs mentioned above. MRP4 appears to be primarily expressed in the kidneys, brain, pancreas, prostate, platelets, and T cells [246, 247, 255–258], while MRP5 is expressed in the heart, brain, and pancreas [246, 247, 259]. As with MRP3, MRP5 mRNA was found to be significantly increased in pancreatic carcinoma samples and is suggested to play a role in chemotherapy resistance [247]. MRP6, which is also known as anthracycline resistance-associated (ARA) protein or the multispecific organic anion transporter-E (MOAT-E), is primarily expressed in the liver and kidney. Mutations within the ABCC6 gene leading to transporter deficiencies have been linked to the connective tissue disorder pseudoxanthoma elasticum [4, 260, 261].

8.4 SLC Family of Transporters

Solute-linked carrier (SLC) transporters represent another superfamily of membrane transporters which interact with a broad range of endogenous and exogenous molecules and are expressed in various tissues throughout the body [262]. The SLC superfamily encompasses more than 40 diverse subfamilies of transporters, including the organic anion transporters (OAT) and organic cation transporters (OCT)

[262]. Transporters within the same subfamily generally share over 60% of their amino acid sequence, though there may be little to no homology between transporters of different subfamilies [263]. Inclusion into the SLC superfamily is therefore based on functional characteristics rather than evolutionary relationships [4, 264].

8.4.1 SLCO1B1 (OATP1B1)

SLCO1B1 encodes the transporter protein OATP1B1. Also known as OATP2, OATP-C, and liver-specific transporter 1 (LST1), this transporter is uniformly expressed throughout the liver on the basolateral membrane of hepatocytes where it plays a crucial role in the hepatic uptake and elimination of its various substrates [264, 265]. Substrates of OATP1B1 include endogenous substances such as various bile acids, eicosanoids, bilirubin metabolites, and thyroid hormones as well as xenobiotics including various statins, antibacterials, HIV protease inhibitors, and chemotherapy agents such as irinotecan and methotrexate [140, 264, 266].

The pharmacogenomics of SLCO1B1 have been extensively investigated with over 40 nsSNPs identified to date [140]. The combined dysfunction of OATP1B1 and OATP1B3 (the two share 80% amino acid identity) is associated with Rotor syndrome, a rare autosomal recessive disorder similar to DJS that results from the impaired ability to excrete conjugated bilirubin [267]. Polymorphisms within the SLCO1B1 gene have been associated with altered OATP1B1 expression, substrate specificity, and transporter function [266]. A number of studies have demonstrated that SLCO1B1 variants can alter the PK and/or drug response of molecules that interact with OATP1B1, including many statins, bile acids, ezetimibe, irinotecan, lopinavir, fexofenadine, olmesartan, atrasentan, methotrexate, mycophenolic acid, rifampin, torasemide, and others [76, 138, 145, 146, 154, 157, 161, 166, 268, 269]. Genetic variations within SLCO1B1 have been of particular interest with regard to patient responses to statin therapy, as they exert their pharmacological effects by inhibiting the HMG-CoA reductase enzymes in the liver [265].

The SLCO1B1 SNP 521T > C confers decreased transporter activity, resulting in decreased hepatic uptake and elimination of substrates from the body [138]. This SNP has received particular attention due to its association with an increased risk of statin-induced myopathy during treatment with simvastatin, atorvastatin, lovastatin, pravastatin, rosuvastatin, and pitavastatin [140, 147, 148]. Therefore, the 521C allele is associated with a decreased therapeutic index of most statins, particularly simvastatin [140]. 521T > C has also been associated with an impaired clearance of methotrexate [150] and lopinavir [154], elevated AUCs of nateglinide [243] and irinotecan [76, 152, 153], and elevated serum levels of bilirubin [151]. Despite their increased safety concerns, SLCO1B1 polymorphisms do not appear to affect the lipid-lowering efficacy of these agents, as their total hepatic exposure is likely not changed by impaired OATP1B1 function [140, 270–274].

Two SLCO1B1 SNPs, rs11045879 and rs4149081, were found to be significantly associated with the increased clearance of methotrexate in a study of children with acute lymphocytic leukemia (ALL). These SNPs were found to be in complete

linkage disequilibrium with each other, but both appear to enhance the hepatic uptake of methotrexate [150].

The various combinations of the 388A > G and 521T > C SNPs form four distinct haplotypes: *1A (388A with 521T), *1B (388G with 521T), *5 (388A with 521C), and *15 (388G with 521C) [159, 275]. Patients homozygous for *1A have been associated with increased exposures to pravastatin, repaglinide [156], and various bile acids [165]. Studies investigating the functional consequences of *1B have demonstrated mixed and even controversial results, with various reports suggesting decreased transporter activity, increased transporter activity, or no change in activity [143–146, 276]. However, the *5 and *15 haplotypes have consistently been reported to confer decreased transporter activity [144, 145, 276–279]. Both of these haplotypes have been associated with increased risk of statin-induced myopathies, while patients homozygous for *15 have also been associated with increased area under plasma concentration curve (AUC) and decreased clearance of irinotecan [165] and olmesartan [166].

A number of other haplotypes have also been identified including *17 (11187G > A, 388A > G, 521T > C) and *14 (463C > A, 1628T > G, 11187G > A) [265]. Both *17 and *14 have been associated with an elevated AUC of the irinotecan metabolite SN-38 as well pravastatin [138, 152]. In pediatric patients, *14 was found to be associated with a lower AUC and maximum plasma concentration (Cmax) of pravastatin [280].

8.4.2 SLCO1B3 (OATP1B3)

SLCO1B3 encodes the transporter OATP1B3 and shares 80% of its amino acid sequence with OATP1B1. OATP1B3 is exclusively expressed on the basolateral membrane of hepatocytes where it is known to modulate the hepatic uptake of endogenous and xenobiotic substrates from the portal vein [281]. OATP1B1 plays a critical role in bile acid and bilirubin transport, and like SLCO1B1, non-functioning variations in SLCO1B3 are linked to the development of Rotor syndrome [144, 282]. There is limited data available on the functional consequences of genetic variations with OATP1B3 [282].

A number of SNPs have been identified within the SLCO1B3 gene. The most investigated polymorphisms in humans are the nsSNPs 344T > G and 699G > A and the intronic variant IVS12-5676A > G (rs11045585). The allelic frequency of these SNPs is very heterogeneous with wide ranges across multiple ethnic groups [173]. The 344T > G SNP was found to be associated with an increased AUC of mycophenolic acid [169] but also decreased plasma concentrations of mycophenolic acid in Japanese renal transplant patients [171]. This same study in renal transplant patients found the 699G > A polymorphism to be associated with an elevated AUC of mycophenolic acid. In addition, this SNP was associated with increased clearance of imatinib in Japanese AML patients [283]. IVS12-5676A > G has been associated with increased AUCs of docetaxel and telmisartan [175, 176]. The 699A > G and IVS12-5676A > G mutations, along with two other SLCO1B3 variants, were found to be part of a haplotype tagging SNP that significantly influenced the disposition of docetaxel, resulting in a decreased clearance and elevated AUC [173].

8.4.3 SLCO2B1 (OATP2B1)

SLCO2B1 encodes the transporter protein OATP2B1. Like OATP1B1 and OATP1B3, OATP2B1 is expressed on the basolateral membrane of hepatocytes where it is involved in the hepatic uptake and clearance of substrates from circulation [284, 285]. However, OATP2B1 is also expressed in other cell types, including enterocytes, as well as in the lungs and placenta [286]. OATP2B1 exhibits pH-dependent transport and shares similar substrates with OATP1B1 and OATP1B3. The SLCO2B1 nsSNP 935G > A was found to be associated with decreased circulating plasma levels of montelukast, leading to impaired treatment of asthmatic symptoms [177]. Another SLCO2B1 SNP, 1457C > T, was found to be associated with decreased AUCs of S-fexofenadine [178] and celiprolol [179].

8.4.4 SLCO1A2 (OATP1A2)

SLCO1A2 encodes the transporter OATP1A2. OATP1A2 has been found to be expressed in the liver, at the BBB, and the intestine [285, 287]. There are three intronic variants of SLCO1A2: −1105G > A, −1032G > A, and −361G > A, which have been associated with a decreased clearance of imatinib [172].

8.4.5 SLC22A1 (OCT1)

SLC22A1 encodes the transporter OCT1. OCT1 is a Na^+-dependent transporter that is primarily expressed in sinusoidal cells of the liver [288]. OCT1 has also been found to be expressed at the basolateral membranes of renal tubular cells and enterocytes [185, 188]. This transporter plays a pivotal role in the hepatic elimination of its organic cation substrates, which includes commonly used therapeutic agents such as acyclovir, imatinib, metformin, famotidine, ranitidine, and many others [289, 290].

SLC22A1 has been found to be highly polymorphic [186, 290]. At least 15 OCT1 variant proteins have been identified across diverse population samples [291]. Certain OCT1 alleles have been identified as “low-function” alleles with decreased transporter activity. These include R61C, S189L, G220V, P341L, G401S, M420del, and G465R [183, 185].

OCT1 polymorphisms have focused primarily on metformin PK and PD. The SNPs R61C, S189L, M420del, G401S, and G465R have all been identified as decreasing cellular metformin uptake [186]. One clinical study suggested that subjects carrying one of the four low-function alleles R61C, G401S, M420del, or G465R demonstrated an increased AUC and Cmax and decreased volume of distribution (Vd) of metformin [187]. However, another clinical study suggested that carriers of multiple copies of these same four low-function alleles, R61C, G401S, M420del, or G465R, demonstrated an increased renal clearance of metformin [185]. Increasing copies of M420del was found to correlate with decreasing steady-state concentrations of metformin. The same study concluded that the long-term decrease in HbA1C was clearly associated with the number of low-function OCT1 alleles a

patient carried [188]. Other clinical investigations have suggested that homozygous carriers of the intronic variant rs622342 demonstrate improved HbA1C lowering by metformin [181, 190]. These findings may be explained by the impaired hepatic elimination of metformin providing an extended exposure period for the molecule to enter hepatocytes and exert its pharmacological effects.

The same intronic SNP rs622342 mentioned above was also found to be associated with patients requiring higher doses of the anti-Parkinsonian drug levodopa [182]. The GG genotype of rs683369 along with advanced stage was found to correlate with a high rate of treatment failure with imatinib in chronic myelogenous leukemia (CML) patients [100]. Variations within OCT1 have also been associated with altered PK of other therapeutic agents such as tropisetron, ondansetron, O-desmethyltramadol (metabolite of tramadol), and morphine [185, 292–294].

8.4.6 SLC22A2 (OCT2)

SLC22A2 encodes the OCT2 transporter. OCT2 is a kidney-specific transporter, expressed on the basolateral membrane of renal proximal tubule cells and involved in the renal secretion of organic cations. Some of the substrates include commonly used therapeutic agents such as metformin and procainamide [295, 296].

Pharmacogenetic investigations of SLC22A2 have revealed at least nine nsSNPs, though only 808G > T appears to have an allele frequency of greater than 5% [2, 297]. Similar to SLC22A1, there have been a number of investigations into the pharmacogenomics of SLC22A2 with respect to metformin PK and PD. OCT2 is considered the primary transporter involved in the renal elimination of metformin. Unfortunately, the functional impacts of variations within SLC22A2 on metformin appear unclear, with conflicting reports.

Clinical investigations have found the SNP 808G > T (rs316019) to be associated with both increased [191] and decreased [192, 193] renal elimination of metformin. Further, other clinical studies have suggested that the SNP has no impact on metformin's renal elimination [185, 188]. 808G > T has also been associated with increased cisplatin-induced nephrotoxicity [194], though this was inconsistent with another study [195].

8.4.7 SLC22A6 (OAT1) and SLC22A8 (OAT3)

SLC22A6 and SLC22A8 encode the OAT1 and OAT3 transporters, respectively. Both of these transporters are primarily expressed at the basolateral membrane of renal proximal tubule cells, though they are also expressed to a lesser extent in the kidney, brain, and placenta [295]. The OAT transporters are key players in the renal excretion of their substrates, which are generally small hydrophilic organic anions including a number of commonly used medications such as methotrexate, certain beta-lactams, diuretics, and anti-inflammatory drugs [2, 18]. There has been relatively limited clinical investigation on the pharmacogenetics of SLC22A6 and

SLC22A8. Of the studies that have been conducted, polymorphisms within these genes do not appear to have significant impacts on the PK of their substrates [2].

8.4.8 SLC47A1 (MATE1) and SLC47A2 (MATE2)

SLC47A1 and SLC47A2 encode the multidrug and toxin extrusion transporters 1 (MATE1) and 2 (MATE2). They are proton-coupled transporters that are primarily expressed at the apical membranes of renal tubular cells where they play important roles in the renal secretion of their cationic substrates. MATE1 is also expressed at the canalicular membrane of hepatocytes where it is involved in the hepatic elimination of substrates. Substrates include some commonly used therapeutic agents such as metformin [2, 188, 298].

Over 30 SNPs have been identified within SLC47A1 [16]. One of which, the sSNP 158G > A (rs2289669), was found to be associated with reduced HbA1C in subjects receiving metformin [190]. Further investigation suggested that this SNP may interact with the OCT1 SNP rs622342 in metformin clearance [180]. However, another study in Japanese diabetic patients who were heterozygous for SLC47A variations concluded that these polymorphisms do not affect the disposition of metformin [196]. Lastly, another study concluded that homozygous carriers of the SLC47A2 SNP −130G > A had significantly poorer responses to metformin-induced HbA1C lowering, compared to reference gene carriers [189].

8.4.9 Other SLC Transporters

In addition to the abovementioned transporters, other transporters that belong to the SLC superfamily and may have polymorphisms include SLC21A6 (OATP-C), SLC6A4/serotonin transporter (SERT), SLC6A3/dopamine transporter (DAT1), SLC6A2 gene/norepinephrine transporter (NET), SLC15A1/PEPT1, SLC15A2 PEPT2, SLC19A1 reduced folate carrier-1 (RFC-1), SLC28A1, SLC28A2, SLC28A3, and SLC29A1 to SLC29A4 (ENT1 to ENT4) [2].

8.4.10 Epigenetic-Dependent Regulation of Drug Transporters

There has been a recent increase in investigating the regulation of drug transporters and metabolizing enzymes by epigenetic influences. Epigenetic regulation of genes refers specifically to heritable factors resulting in modifications of the genome which do not result in alterations in the gene's DNA sequence. Specifically, these regulatory mechanisms include DNA methylation, histone modifications, and non-coding RNAs (ncRNA) [299]. Studies have illustrated that in addition to drug interactions and genetic polymorphisms, DNA methylation plays an important role in ADME gene expression and can elicit potentially clinically relevant effects on drug action and resistance, particularly in cancer therapies [300]. Recently, substantial

effort has been focused on elucidating the role of noncoding RNA (ncRNA) in the regulation of ADME genes and the resulting pharmacological consequences [300]. Importantly, it is likely that more investigations in this area would unfold in the next few years that would help shape our understanding of epigenetic-dependent regulations of drug transporters.

Conclusion

Despite the advances in pharmacogenomics and the wealth of reports regarding transporters, there are still much needed investigations to rectify our understanding of the pharmacogenomics of membrane drug transporters. A number of drug transporters have not yet been fully characterized in vitro or in vivo. Indeed, there are many reports of genetic variations within transporter genes impacting the PK and/or PD of therapeutic molecules, especially chemotherapeutics; however, the precise functional impacts of these variations remain, in many cases, unclear or even controversial. The allele frequencies of many SNPs often demonstrate clear differences among different ethnic or racial groups. They may provide at least a partial explanation for interethnic differences in drug responses. Further, looking at individual SNPs in isolation is likely too limited in scope as many SNPs and other variations appear to interact with one another. Moving forward, haplotype investigations may provide a more comprehensive description of the impact genetic variations within transporter genes may have on the disposition and clinical responses of drug therapy.

There are a number of ways in which the pharmacogenomics of drug transporters may contribute to drug discovery and development (Table 8.2). Harmful diseases linked to dysfunctional drug transporters such as Dublin-Johnson syndrome, Rotor syndrome, pseudoxanthoma elasticum, systemic primary carnitine deficiency, Tangier disease, Stargardt's disease, cystic fibrosis, sitosterolemia, various inflammatory diseases, and possibly gout and even Alzheimer's disease may potentially be treated with gene therapies encoding fully functional transporters [5, 301]. This information will at least assist in identifying potential targets for future therapies. Drug transporters are especially linked to chemotherapy resistance. As such, anticancer agents should be thoroughly screened for their interactions with a panel of drug transporters during development. Similarly, in the development of any new CNS-acting drugs that must cross the BBB, a thorough screening of transporter interactions (especially those expressed at the BBB) would be required to during their development process. These transporter studies early in drug development processes will not only point to potential drug resistance, BBB penetration, PK, or PD issues but also will help in avoiding any potential drug-drug interactions had the newly developed drug co-administered with other transporters substrates [18].

In terms of drug delivery, drug transporter pharmacogenomics along with more readily available genotyping technologies may provide key individual information to assist in optimizing drug therapies. For example, carriers of certain SLCO1B1 alleles have been associated with an increased risk of statin-induced myopathies, requiring decreased doses. Similarly, a number of other

frequently used medications such as metformin, levodopa, and a host of others may also have revised dosing recommendations based on drug transporter pharmacogenomics information.

Drug transporter pharmacogenomics and expression patterns in tumor cells may be of particular assistance in chemotherapy delivery. For example, chemotherapy resistance due to drug transporter activity may be attenuated with concomitant administration of an inhibitor of that transporter. In addition, for solid cutaneous tumors expressing transporters that may decrease the anticancer efficacy of substrate chemotherapies, intra-tumoral administration may be an alternate administration option.

Taken together, there is unmet need to significantly incorporate individual patient genotyping prior to prescribing certain medications which are drug transporter substrates (and/or substrates for drug-metabolizing enzymes). This practice is slowly becoming implemented for polymorphisms of drug transporters and metabolizing enzymes due to high frequencies of adverse events arising from altered protein expression and function. Clinically, personal genotyping tests are readily available, efficient, reliable, and not overly costly. Implementing them into routine medical care will ensure best therapeutic outcomes and will certainly have socioeconomic impact.

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9 Genomics and Drug-Metabolizing Enzymes and Its Application in Drug Delivery: Evaluating the Influence of the Microbiome

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Abstract

Biotransformation or metabolism is responsible for elimination of 70% of drugs available in the market today [1]. Drug-metabolizing enzymes (DMEs) are an assorted group of enzymes responsible for metabolizing xenobiotics such as drugs, carcinogens, pesticides, and food toxicants as well as endogenous compounds such as prostaglandins, steroids, and bile acids [2, 3]. R.T. Williams coined the concept of two-phase elimination of xenobiotics; reactions such as oxidation, reduction, and hydrolysis are categorized as phase I or activating reactions, while conjugation reactions constitutes phase II reactions and are generally detoxifying in nature [4]. Cytochrome P450 (CYP) families of enzymes are responsible for catalyzing majority of phase I reactions. Phase I reactions convert lipophilic molecules to their water-soluble counterparts [4]. Phase II reactions are catalyzed by enzymes such as uridine diphosphate glucuronosyltransferase (UGT), glutathione transferases (GSTs), *N*-acetyltransferase (NAT), and sulfotransferases (SULTs) [4]. Phase II enzymes catalyze conjugation of water-soluble molecules to intermediates of phase I reactions for the purpose of improving water solubility. In most cases, the net outcome of both phase I and phase II types of reactions is to impart hydrophilicity to xenobiotics and facilitate their elimination from the body. However, phase I and phase II reactions can also activate inert compounds to pharmacologically active entities [5], toxic end products, and procarcinogens into carcinogenic compounds [4, 6, 7]. CYPs and phase II metabolizing enzymes are known to exhibit polymorphism and have been associated with interindividual variability in drug response and toxicity.

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_9

9.1 Introduction

Biotransformation or metabolism is responsible for elimination of 70% of drugs available in the market today [1]. Drug-metabolizing enzymes (DMEs) are an assorted group of enzymes responsible for metabolizing xenobiotics such as drugs, carcinogens, pesticides, and food toxicants as well as endogenous compounds such as prostaglandins, steroids, and bile acids [2, 3]. R.T. Williams coined the concept of two-phase elimination of xenobiotics; reactions such as oxidation, reduction, and hydrolysis are categorized as phase I or activating reactions, while conjugation reactions constitutes phase II reactions and are generally detoxifying in nature [4]. Cytochrome P450 (CYP) families of enzymes are responsible for catalyzing majority of phase I reactions. Phase I reactions convert lipophilic molecules to their water-soluble counterparts [4]. Phase II reactions are catalyzed by enzymes such as uridine diphosphate glucuronosyltransferase (UGT), glutathione transferases (GSTs), *N*-acetyltransferase (NAT), and sulfotransferases (SULTs) [4]. Phase II enzymes catalyze conjugation of water-soluble molecules to intermediates of phase I reactions for the purpose of improving water solubility. In most cases, the net outcome of both phase I and phase II types of reactions is to impart hydrophilicity to xenobiotics and facilitate their elimination from the body. However, phase I and phase II reactions can also activate inert compounds to pharmacologically active entities [5], toxic end products, and procarcinogens into carcinogenic compounds [4, 6, 7]. CYPs and phase II metabolizing enzymes are known to exhibit polymorphism and have been associated with interindividual variability in drug response and toxicity.

Interpatient variation of drug metabolism has paramount role in determining the safety and efficacy of drugs in various populations. Identification of acetylation polymorphism in the early 1960s was the very first evidence of polymorphism of drug-metabolizing enzymes [8]. The next crucial discovery in the area of pharmacogenetic variation in drug metabolism was the discovery of polymorphism in CYP 450 family of drug-metabolizing enzymes [1]. Since then, we have come a long way accepting the role of genetics in determining the fate of drug metabolism in individuals, as evidenced by the development of PCR-based tests enabling testing of common genetic polymorphisms of drug-metabolizing enzymes [1]. Although, the application of pharmacogenomics in regular clinical practice is not increasingly prevalent, constant efforts are made to advance toward having wider application of pharmacogenomics in clinical practice. The recent impetus from FDA to include pharmacogenetic information on approximately 10% of currently marketed drugs also attests to the fact that pharmacogenomics of drug metabolism is the need of the hour [1]. The number of drugs requiring pharmacogenetic tests is expected to grow in the future as more and more data is obtained on interpatient variations in the form of standardized clinical trials and post-marketing studies. The FDA is making constant efforts in providing detailed recommendations about the use of pharmacogenetic tests in clinical practice. The recommendations are updated on a quarterly basis [1].

The following sections will address the functional polymorphism of both phase I and phase II enzymes and their influence on safety and efficacy of drugs.

9.2 Phase I Metabolic Enzymes

Majority of phase I metabolism is catalyzed by Cytochrome P450 family of enzymes.

Cytochrome P450 are a large group of DME belonging to hemoproteins superfamily of enzymes. They are found mainly in hepatocytes but are also present in the small intestine, lungs, kidneys and brain. As shown in Fig. 9.1, CYP1, CYP2, and CYP3 are three major families responsible for the oxidative metabolism of drugs [9]. The human CYP genes are greatly polymorphic. The peer-reviewed information of various alleles is summarized at the human CYP allele nomenclature home page (www.cypalleles.ki.se) present on the server at Karolinska Institutet [10]. The website hosts information about more than 350 functionally different alleles [10]. According to the website, the highest numbers of alleles described are *CYP2D6* (63 alleles), *CYP 2B6* (28 alleles), *CYP1B1* (26 alleles), and *CYP2A6* (22 alleles) [10]. The functional *CYP* polymorphisms consist of gene deletions, gene duplications, and deleterious mutations creating inactive gene products [10]. Additionally, changes in amino acid sequence due to mutation can change the substrate specificity. The polymorphism in CYP enzymes can result in copy number variation where multiple functional gene copies of one allele can result in increased enzyme activity and increase in drug metabolism [11, 12]. The most important polymorphic CYP enzymes with respect to drug metabolism are CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9,CYP2C19, CYP2D6, and the CYP3As (Fig. 9.1) [10]. The following sections will address the polymorphism of above listed enzymes with special emphasis on clinical significance.

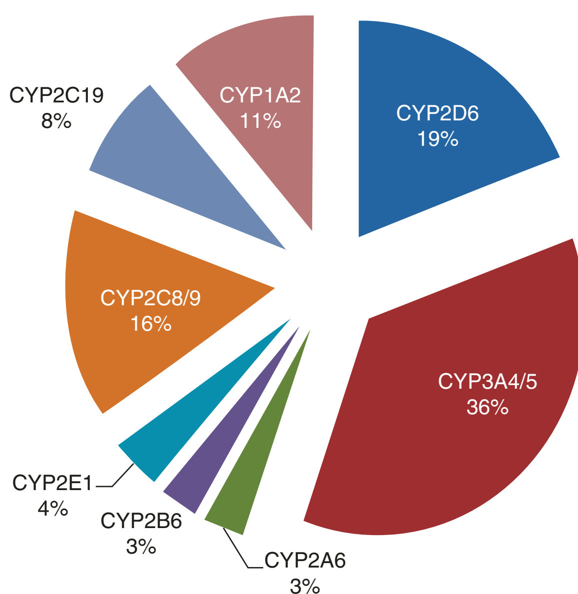

Fig. 9.1 Distribution of important polymorphic CYP enzymes with respect to drug metabolism

9.2.1 CYP 1A2

CYP1A2 is one of the major CYPs in the human liver amounting to be approximately 13–15% of total CYP enzymes and is responsible for metabolizing 15% of clinically available drugs such as clozapine, theophylline, tacrine, zolmitriptan, duloxetine, and verapamil to name few [13]. Environmental factors and genetic mutations are responsible for the large interindividual variability in the elimination of drugs metabolized by CYP1A2 [14, 15].

Various modeling studies have suggested that the majority of CYP1A2 substrates are hydrophobic with high logP values suggesting the role of hydrophobic interactions in the binding to CYP1A2 [15]. CYP1A2 along with CYP1A1 and 1B1 also play a crucial role in the bioactivation of procarcinogens such as aromatic amines and polycyclic aromatic hydrocarbons [16]. There are wide interindividual differences (10–200-fold) in CYP1A2 expression and activity [15]. There have been reports of approximately 15- and 40-fold interindividual variations in CYP1A2 mRNA and protein levels in human livers [17]. The frequencies of poor metabolizers who are nonsmokers were reported to be 5% in Australians, 14% in Japanese, and 5% in Chinese [18]. There is clear difference in increased CYP1A2 activity in different races; Swedes have 1.5-fold higher activity as compared to Koreans [19]. Asian and African populations have been reported to have lower CYP1A2 activity as compared to Caucasians [20]. The NCBI dbSNP database (www.ncbi.nlm.nih.gov) reports that more than 200 polymorphisms exist in *CYP1A2* gene. Polymorphisms in *CYP1A2*1C*, *CYP1A2*1D*, *CYP1A2*1E*, and *CYP1A2*1F* were reported in Japanese population. Of the polymorphic alleles, *CYP1A2*1C*, **1D*, **1F*, and **1K* have been associated with altered enzyme activity [13]. Concerning mutations in the regulatory regions, alleles *CYP1A2*1F* and *CYP1A2*1K* have received substantial attention [12]. The *CYP1A2*1F* allele contains 163C>T mutation in intron 1, and this has been shown to influence the inducibility of the gene and affect the intensity of increase of in vivo caffeine metabolism after both smoking [21, 22] and omeprazole treatment [23]. A variant of this allele is found in African populations [24]; in addition to 163C>T, it also contains −729C>T which abolishes binding site of an Ets nuclear factor resulting in significant decrease in CYP1A2 expression and caffeine metabolism.

A number of clinical studies have been conducted to study the impact of *CYP1A2* polymorphisms on drug metabolism, clearance, and response. Resistance to clozapine therapy due to increased metabolism and lower plasma levels in smoking schizophrenic patients possessing *CYP1A2*1F* allele was reported [25, 26]. Higher concentrations of clozapine and its metabolite *N*-desmethylclozapine was detected in patient carrying the two *CYP1A* variants associated with reduced enzyme activity [27]. In spite of the absence of any functional gene variants, many epidemiological association studies have been published to link particular polymorphic sites to disease susceptibility [14, 15]. Numerous reports have surfaced suggesting the role of CYP1A2 polymorphism and susceptibility to cancers such as bladder cancer [28], adenocarcinoma, lung cancer [29], and head and neck squamous cell carcinoma [30]. Additionally, the relationship between polymorphism and disease susceptibility for conditions such

as myocardial infarction, tardive dyskinesia, and schizophrenia as well as recurrent pregnancy loss was reported without much consensus information [14, 15].

9.2.2 CYP2A6

CYP2A6 is mainly expressed in hepatic tissue and is responsible for metabolizing several important therapeutic agents, toxins, and procarcinogens [31]. It is also known to be responsible for the metabolism of nicotine and its metabolite cotinine and is touted to be the responsible factor in interindividual variability in nicotine metabolism, smoking behavior, and the risk of tobacco-related cancer [32–34]. A large amount of interindividual variability in CYP2A6 activity is reported by the in vitro and in vivo estimation of CYP2A6 activity by measuring coumarin oxidation [35]. As with any polymorphic enzyme, CYP2A6 shows ethnic variation in interindividual variability with 1% Caucasians and 20% of Asians being PM. *CYP2A6* expression can only be slightly induced suggesting that the environmental factors contribute very little to the CYP2A6 variability. Single amino acid deletion in *CYP2A6*2* makes the enzyme inactive, whereas *CYP2A6*4* has gene deletion causing decrease in enzyme activity and is seen in majority of PM in the Asian population. Additionally, the variants *CYP2A6*5* and *CYP2A6*20* result in abolished activity of the enzyme [10]. Eight additional *CYP2A6* alleles (**6*, **7*, **10*, **11*, **12*, **17*, **18*, and **19*) lead to enzymes with limited activity [10]. As CYP2A6 is greatly involved in the metabolism of nicotine and cotinine, the genetic variations and interindividual difference play a role in smoking and tobacco-related cancer risks [32, 34].

9.2.3 CYP2B6

CYP2B6 is relevant for the metabolism of anticancer drugs such as cyclophosphamide and ifosfamide and HIV drugs like efavirenz and nevirapine [36–38]. The human *CYP2B6* gene is highly polymorphic and has 29 allelic variants many of which are responsible for increased, decreased, or complete lack of activity [39]. The alleles with lower expression or activity includes *CYP2B6*6*, *CYP2B6*16*, and *CYP2B6*18* [40, 41]. *CYP2B6*6* variants are observed in 20–30% frequency in different populations; *CYP2B6*16* and *CYP2B6*18* occur commonly in Black population with the frequency of 7–9% [40, 41]. In various clinical studies, it has been proven that the subjects homozygous for combinations of the alleles including *CYP2B6*6*, *CYP2B6*16*, and *CYP2B6*18* show lower rate of metabolism of CYP2B6 substrates [40, 41]. The 516G>T and 785A>G mutations resulting in amino acid substitutions and Q172H and K262R in *CYP2B6*6* allele give rise to haplotypes with high or low activity of CYP2B6. The clinical relevance of CYP2B6-mediated interindividual variability still needs more investigation and development of efficient tools to predict the specific capacity for metabolism of CYP2B6 substrates.

9.2.4 CYP2C8

CYP2C8 is crucial for the metabolism of drugs such as repaglinide, rosiglitazone, pioglitazone, paclitaxel, chloroquine, amiodarone, and dapsone among others. The withdrawal of cerivastatin due to severe or sometimes fatal rhabdomyolysis was a strong evidence of relevance of CYP2C8-mediated drug interactions. Most of the cerivastatin adverse effects were observed when gemfibrozil, a CYP2C8 inhibitor, was coadministered [42, 43].

In vitro variation in the metabolism of CYP2C8 substrates and CYP2C8 expression is very large [44]; however, lack of standardized test to the phenotype activity makes the estimation of in vivo variation rather difficult.

Several coding region SNPs have been reported in the *CYP2C8* gene with crucial interracial variations; *CYP2C8*2* is observed in Africans, while *CYP2C8*3* and *CYP2C8*4* are mainly found in Caucasians [45, 46]. There are additional two SNPs, **1B* and **1C*, described in the promoter region of *CYP2C8* [44]. In vitro studies using heterologous proteins revealed that CYP2C8.3 had reduced activity using paclitaxel as substrate; however, there was no difference in amiodarone metabolism. In vivo studies are inconclusive as mentioned above due to lack of proper evaluation tools. Two recently identified haplotypes of *CYP2C8* have both lower and higher activity of the enzyme when tested with paclitaxel and repaglinide as substrates [47]. It was shown that high-activity allele carried *CYP2C8*1B* which binds nuclear factor, while Ile26Met present in *CYP2C8*4* was part of the low-activity haplotype [10]. Therefore, CYP2C8 is a highly conversed gene that has no important functional allele or null alleles distributed among individuals. The clinical relevance of *CYP2C8* needs further investigation and validation.

9.2.5 CYP2C9

CYP2C9 is mainly expressed in the liver forming 20% of the total CYP content and is the highest expressed among the CYP2C enzymes. CYP2C9 is responsible for metabolizing 10% of all drugs including nonsteroidal anti-inflammatory drugs (NSAIDs), antidiabetics, anti-infectives, hypnotics, antiepileptics, oral coagulants, sulfonylureas, psychotropics, and angiotensin receptor blockers [10]. A large interindividual variability in CYP2C9 activity is reported which is also responsible for interindividual variations in drug response and adverse effects. The variations could be the result of environmental factors, such as induction by prototypical CAR, GR, and PXR ligands through different elements in the promoter gene [48]. Multiple single nucleotide polymorphisms within *CYP2C9* are reported, and at least 13 of these SNPs encode for CYP2C9 alleles [49, 50]. The polymorphic behavior of CYP2C9 is predominantly determined by two common coding variants, *CYP2C9*2* (R144C) and *CYP2C9*3* (I359L), both of which produces enzyme with decreased activity. *CYP2C9*2* and *CYP2C9*3* are present in Caucasians with 11 and 7% frequency, respectively. The frequency is lower in Africans and Asians [51]. It has been shown that the substrate affinity of CYP2C9.2 is unaffected for some substrates, whereas it is drastically reduced for the others [52]. However, CYP29.3 shows significant reduction in the catalytic activity as compared to the wild type [52]. The drugs metabolized by CYP2CP include S-warfarin, glimepiride, glyburide, tolbutamide, angiotensin receptor blockers, fluvastatin, and NSAIDs such as celecoxib, diclofenac,

flurbiprofen, and ibuprofen [53]. *CYP2C9*2* has significant impact on the clearance of S-warfarin, tolbutamide, and celecoxib and does not significantly impact others [53]. In case of *CYP2C9*3* heterozygous individuals, the clearance for most substrates was 50% as compared to the wild type, while in case of *CYP2C9*3* homozygous individuals, the reduction was five- to tenfold. All in all, CYP2C9 polymorphism is of great clinical significance and should be taken into account for effective therapeutic outcome.

9.2.6 CYP2C19

CYP2C19 is an important CYP enzyme located on chromosome 10. A range of drugs have been found to be substrates, inhibitors of inducers of this enzyme. Some of the important substrates of CYP2C19 are TCA and SSRI antidepressants, PPIs, antiplatelet drug clopidogrel, antiepileptics like diazepam, mephenytoin, phenobarbital, and anticancer drug cyclophosphamide. Polymorphism in *CYP2C19* (mainly **2*, **3*) is responsible for poor metabolizer (PM) genotype in ~3–5% Caucasians and ~15–20% Asians [54]. The safety and/or efficacy of CYP2C19 substrates can be compromised in these individuals, and major clinical outcome is affected in PM. Sagar et al. studied the effect of CYP2C19 genotype affecting omeprazole metabolism and subsequently its effect on acid inhibition. They found that analysis of CYP2C19 genotype may be important to avoid negative effects on therapy especially for patients on long-term treatment [55]. Similarly, PMs can show reduced effectiveness to clopidogrel as it is not effectively converted to the active metabolite. Out of the total dose of clopidogrel administered, only 15% of prodrug is converted to the active metabolite, while the remaining 85% is converted to inactive derivatives by the action of esterases (Fig. 9.2). CYP2C9 contributes to 21% of the active metabolite generation [56].

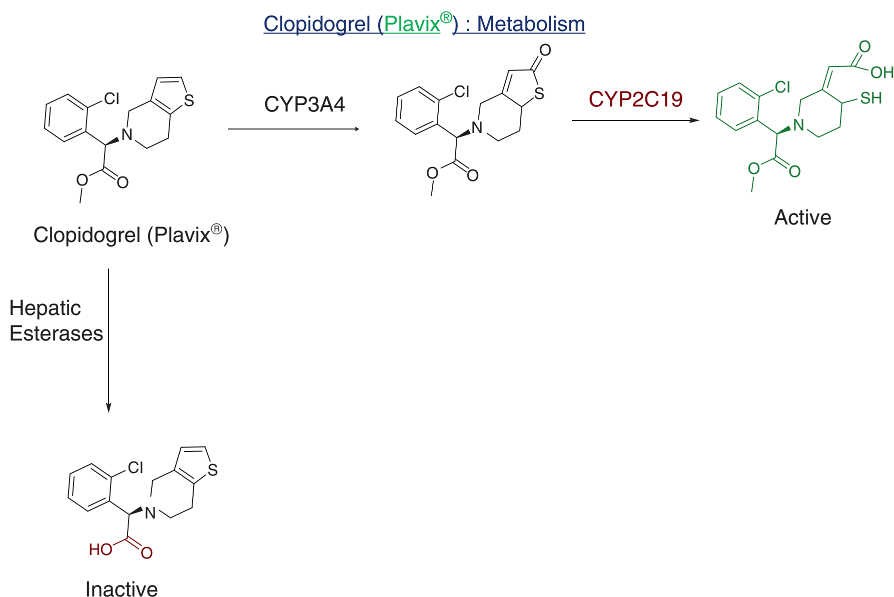

Fig. 9.2 Activation and inactivation pathway of clopidogrel

*CYP2C19*2* carriers have been reported to have diminished antiplatelet activity of clopidogrel due to reduction in generation of active metabolite [57, 58]. Omeprazole and clopidogrel which can be prescribed together as PPIs may reduce the risk of gastrointestinal bleeding in patients receiving clopidogrel after acute coronary syndrome or percutaneous coronary intervention. In this pharmacogenomic-based DDI, effects can be more pronounced. Lately, US FDA has issued a warning that PPI omeprazole reduces the antiplatelet effect of clopidogrel by about 50% by inhibiting CYP2C19 isoenzyme. Although the clinical outcome of this DDI is still not clear, caution must be exercised when using clopidogrel and omeprazole together. Polymorphism in *CYP2C19* (**17*) has been found to be responsible for extensive metabolizer (EM) status of a patient. Approximately 18–20% Caucasian and Ethiopians are found to be EMs and can affect metabolism of a variety of drugs [59]. Study shows that increased metabolism of omeprazole in carriers of CYP2C19*17 allele may be responsible for subtherapeutic drug expossure [60].

9.2.7 CYP2D6

CYP2D6 encompasses the largest number of protein variants among all the CYPs. CYP2D6 is responsible for metabolizing a large number of drugs (~50%) in the market today, and polymorphism in CYP2D6 can significantly affect metabolism of these drugs, thus altering therapeutic outcome. Some of the important CYP2D6 substrates are TCAs, SSRIs, typical antipsychotics, opioid analgesics, antiemetics, β-blockers, and some antiarrhythmics [61]. CYP2D6 is the only CYP enzyme which is not inducible, and so the interindividual variation is a result of genetic variations. The polymorphism of CYP2D6 is of great clinical significance due to its role in metabolizing the large number of currently used drugs. More than 63 different functional *CYP2D6* have been reported, and they are classified into alleles causing abolished, decreased, normal, and ultrarapid enzyme activity. Different protein variants account for normal (**1*, **2*), extensive (**1xN*, **2xN*, **33*, **35x2*), intermediate (**9*, **10*, **17*, **29*, **41*, **49*, **50*, **54*, **55*, **59*, **72*), and poor metabolizers (**3–*8*, **11–*16*, **18–*21*, **31*, **36*, **38*, **40*, **42 *44*, **47*, **51*, **56*, **62*) of CYP2D6 [10, 61]. *CYP2D6*4* allele is found to be higher in Caucasians, **10* in Asians, and **17* in Africans. PM 7–10% in Caucasians [62–66] is mainly due to *CYP2D6*3* and**4*. Only up to 1% Asians are PMs. *CYP2D6*10* is the most frequent allele in Asians, and this variation accounts for 50–70% variation due to CYP2D6 protein variants [67]. *CYP2D6*17* is most frequently seen in Africans, and it has been estimated that Zimbabweans account for 34% CYP2D6 variation due to *CYP2D6*17* alone [68]. Although the PM phenotypes are at higher risk for adverse drug reactions, the UM phenotype areas are also vulnerable to adverse reactions as a result of high concentration of metabolite. A tragic example of UM experiencing adverse effects is incidence of death of a healthy newborn as a result of breastfeeding by a UM mother taking high dose of codeine for the mitigation of postpartum pain; codeine was metabolized into morphine (Fig. 9.3) which was transmitted to the infant via breast milk in toxic quantities leading to severe respiratory depression and death [69]. It is reported that in UMs, the concentration of metabolites can go up to

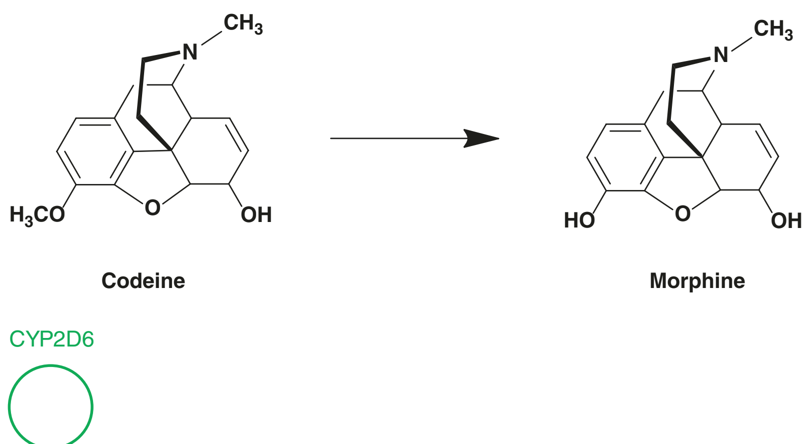

Fig. 9.3 Metabolism of codeine

10–30-fold as compared to normal metabolizers. The other side of the coin is that UMs can be unresponsive to drugs such as antidepressant due to extensive inactivation of the drugs [70].

CYP2D6 is involved in metabolism of tamoxifen to yield active and much potent metabolite endoxifen. CYP2D6 PMs will show reduced response when tamoxifen is used for treatment of breast cancer. Besides that, inhibitors of CYP2D6 also led to lower than normal levels of plasma endoxifen [71–73]. Some studies show that PM with breast cancer demonstrated a shorter time to recurrence or shorter survival time [74–76]. However, some retrospective studies do not agree with this. Still PM of tamoxifen can affect clinical outcome, and caution should be exercised in prescribing tamoxifen to these patients. Drugs such as antidepressants that are known to decrease the activity of CYP2D6 will have decrease in therapeutic activity of tamoxifen.

CYP2D6 polymorphism has significant effect on tramadol drug therapy. Tramadol is metabolized to an active metabolite, *O*-demethyl tramadol, by CYP2D6. The *CYP2D6* genotype has shown to be the determinant factor in dictating concentration of *O*-demethyl tramadol thereby affecting the efficacy of tramadol [77]. PMs for CYP2D6 have less response to postoperative tramadol analgesia as compared to the EMs [78].

All in all, the polymorphism of *CYP2D6* is the most clinically relevant polymorphism affecting drug therapy [79]. Genotyping will help in explaining the non-responsiveness or susceptibility to adverse reactions of CYP2D6 substrates in various individuals.

9.2.8 CYP3A4/5/7

The clinically relevant members of CYP3A isoform include CYP3A4, 3A5, and 3A7. All three enzyme subfamilies have wide substrate specificity due to which they are the most important drug-metabolizing subfamily. CYP3As are expressed

primarily in liver and intestine and is responsible for metabolizing 45–60% of currently marketed drugs. CYP3A isoforms are also responsible for first-pass metabolism of its substrates. CYP3A expression and activity are affected by a combination of genetic, nongenetic, and environmental factors resulting in vast interindividual variability [80]. Up to 40-fold interindividual variations are seen for substrates such as triazolam, midazolam, and ciclosporin [81]. CYP3A4 has the highest activity toward common CYP3A substrates as compared to other isoforms [82]. In case of CYP3A5 and CYP3A7, clinically significant variations includes *CYP3A5*3* and *7* and *CYP3A7*1B* and **1C*, respectively [80]. In spite of having the large contribution of CYP3A4 toward drug metabolism and also associated variability, the polymorphisms affecting CYP3A4 are not widely reported except the most recent report of **22* allele. The effect of polymorphisms in CYP3A5 and CYP3A7 on drug metabolism is dependent on the concomitant expression status of CYP3A4 [81]. The following subsections will briefly address the polymorphisms in CYP3A4, 5, and 7.

Extensive studies of allelic variants of *CYP3A4* have revealed variant proteins of CYP3A4 with diminished activity (**6*, **17*, and **20*). These alleles are not considered as the reason for interindividual variations due to their low frequency of occurrence. It has been shown that the difference in transcription rate of *CYP3A4* is the main cause of interindividual variability [83]. Despite multiple efforts, the exact mechanism of CYP3A4 expression variability is still unknown.

CYP3A5 is highly polymorphic as a result of mutations that drastically reduce the enzyme activity. The common variations include, *CYP3A5*3*, *6*, and *7* out of which *CYP3A5*3* is the most common defective allele with an allele frequency of about 90, 75, and 20% in Caucasians, Asians, and Africans, respectively [84, 85]. However, *CYP3A5*6* and *CYP3A5*7* are not present in Caucasians and Asians and are 17 and 8% in Africans, respectively [86]. As mentioned above, many drugs metabolized by CYP3A5 are also substrates for CYP3A4, so distinguishing the effect of CYP3A5 polymorphism on drug metabolism is rather difficult.

CYP3A7 is mainly expressed in fetus, with its expression starting after 50–60 days of gestation and continuing up to 6 months of postnatal age [87]. The interindividual variation of CYP3A7 is important because the enzyme is responsible for metabolizing endogenous compounds and xenobiotics reaching the fetus through maternal circulation; the degree and extent of metabolism will have an effect on embryotoxicity and teratogenicity. CYP3A7 shows one frameshift mutation (*CYP3A7*2*) [88] and one coding polymorphism (*CYP3A7*2*). The *CYP3A7*2* SNP codes for enzyme have slightly higher activity than CYP3A7*1 and have an allele frequency of 8, 28, and 62% in Caucasians, Asians, and Africans, respectively [89]. In vitro studies using fetal liver microsomes did not report any significant differences in the metabolism of dehydroepiandrosterone (DHEAS) by the liver carrying *CYP3A7*2* and *CYP3A7*1* [90]. In addition to polymorphisms resulting in alternative CYP3A7 proteins, a genetic promoter *CYP3A7*1C* is reported to be pertinent for the expression of CYP3A7 [91]. The carriers of *CYP3A7*1C* alleles have high expression of CYP3A7 which showed decreased DHEAS levels in a small clinical study conducted in 208 elderly women and 345 elderly men [92]. There were some reports of correlating *CYP3A7*1C* expression and bone density in elders, but the findings were inconclusive. The effect of CYP3A7 polymorphism on drug metabolism and disease pathology needs further research.

9.3 Phase II Metabolic Enzymes

Phase II reactions involve conjugation of functional groups with endogenous molecules generally aimed at increasing water solubility of xenobiotics. Some of the important phase II reactions are sulfonylation/sulfation, glucuronidation, acylation, methylation, and amino acid and glutathione conjugations as shown in Fig. 9.4. Different types of enzymes are involved in these conjugation reactions, and polymorphism in these enzymes can significantly affect drug's safety and efficacy, two important criteria due to which drug fails in clinic. Given below are some of the important reactions and enzymes involved in phase II metabolism.

9.3.1 Glucuronidation

UDP (uridine diphosphate)-glucuronosyltransferases commonly known as UGTs are the most common group of glucuronidation enzymes. Some of the common functional groups susceptible to glucuronidation are hydroxyl (–OH), amine

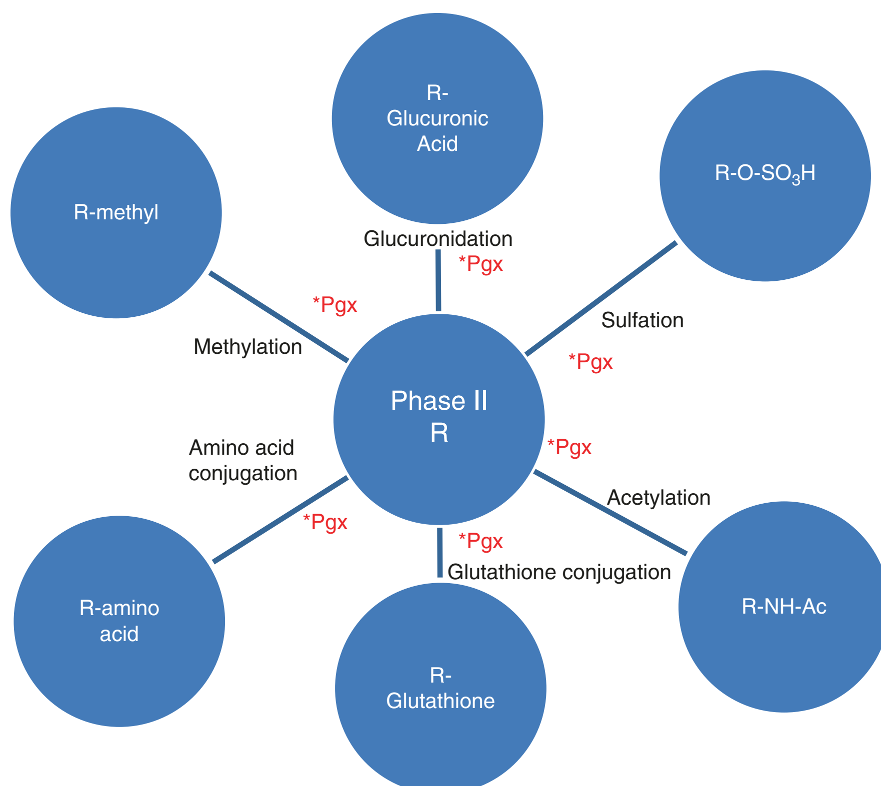

Fig. 9.4 Important phase II conjugation reactions and involvement of pharmacogenomics (Pgx) in these reactions

(–NH_2), and carboxyl (–COOH) groups. Generally, glucuronide conjugates are much less active than the parent molecule and result in terminal metabolite that can be readily eliminated by kidneys. Some of the common drug substrates for UGTs are NSAIDs, acetaminophen, hydroxysteroids, benzodiazepines, irinotecan, indacaterol, and nilotinib. UGTs are mainly represented by three gene subfamilies: UGT1A, UGT2A, and UGT2B. Protein variants of diverse UGTs have been reported that affects biotransformation and clearance of drugs. Some of these variants have clinically significant effect on the efficacy and/or safety of drugs. One important example is the metabolism of irinotecan, a topoisomerase II inhibitor indicated for metastatic colorectal cancer. As shown in Fig. 9.5, UGT1A1, UGT1A6, and UGT 1A7 are mainly involved in glucuronidation of active metabolite of irinotecan. Gene variant UGT1A1*28 has been found to be mainly responsible in diminishing metabolism of active metabolite leading to clinically relevant toxicities like neutropenia and diarrhea [93, 94]. FDA has updated irinotecan label recommending dose reduction for individuals having homozygous alleles of UGT1A1*28. Studies have shown that gene variant UGT1A1*6 can also affect glucuronidation during irinotecan metabolism [95].

Similarly, drugs can inhibit protein variants of UGTs and may result in toxicity arising due to altered metabolism of endogenous molecules. One such example is the use of HIV protease inhibitor – atazanavir; this drug inhibits UGT1A1, UGT1A3, and UGT1A4. It has been found that individuals homozygous for UGT1A1*28 receiving atazanavir showed higher incidence of jaundice due to altered metabolism of bilirubin, a well-known substrate for UGTs [96].

Similarly, there are studies on significance of role of the UGT1A8*2 variant allele on mycophenolate mofetil-induced diarrhea [97] and diclofenac-induced hepatotoxicity due to genetic variation in UGT2B7, CYP2C8, and ABCC2 [98].

9.3.2 Methylation

Methylation is a very important phase II reaction mainly targeting endogenous molecules containing hydroxyl (–OH), amine (–NH_2), or thiol (–SH) functional groups. Catecholamines like epinephrine and norepinephrine are deactivated by catechol-*O*-methyltransferases (COMT) that catalyze the transfer of a methyl group to one of the catechol hydroxyls. Similarly, *N*-methylation of norepinephrine to epinephrine is mediated by phenylethanolamine *N*-methyltransferase in kidneys. *S*-adenosyl methionine (SAM) was found to be a common cofactor mediating methylation. Some of the important methylating enzymes exhibiting clinically significant polymorphism are thiopurine methyltransferases (TMPTs) and COMT.

9.3.2.1 Thiopurine Methyltransferase (TMPT)

Thiopurines represent three important drugs useful as immunosuppressant or as anticancer. Azathioprine (Imuran) is useful to prevent organ rejection and other autoimmune diseases like Crohn’s diseases and ulcerative colitis (UC). 6-mercaptopurine or 6-MP (Purinethol) is generally useful for treating certain types of leukemia and

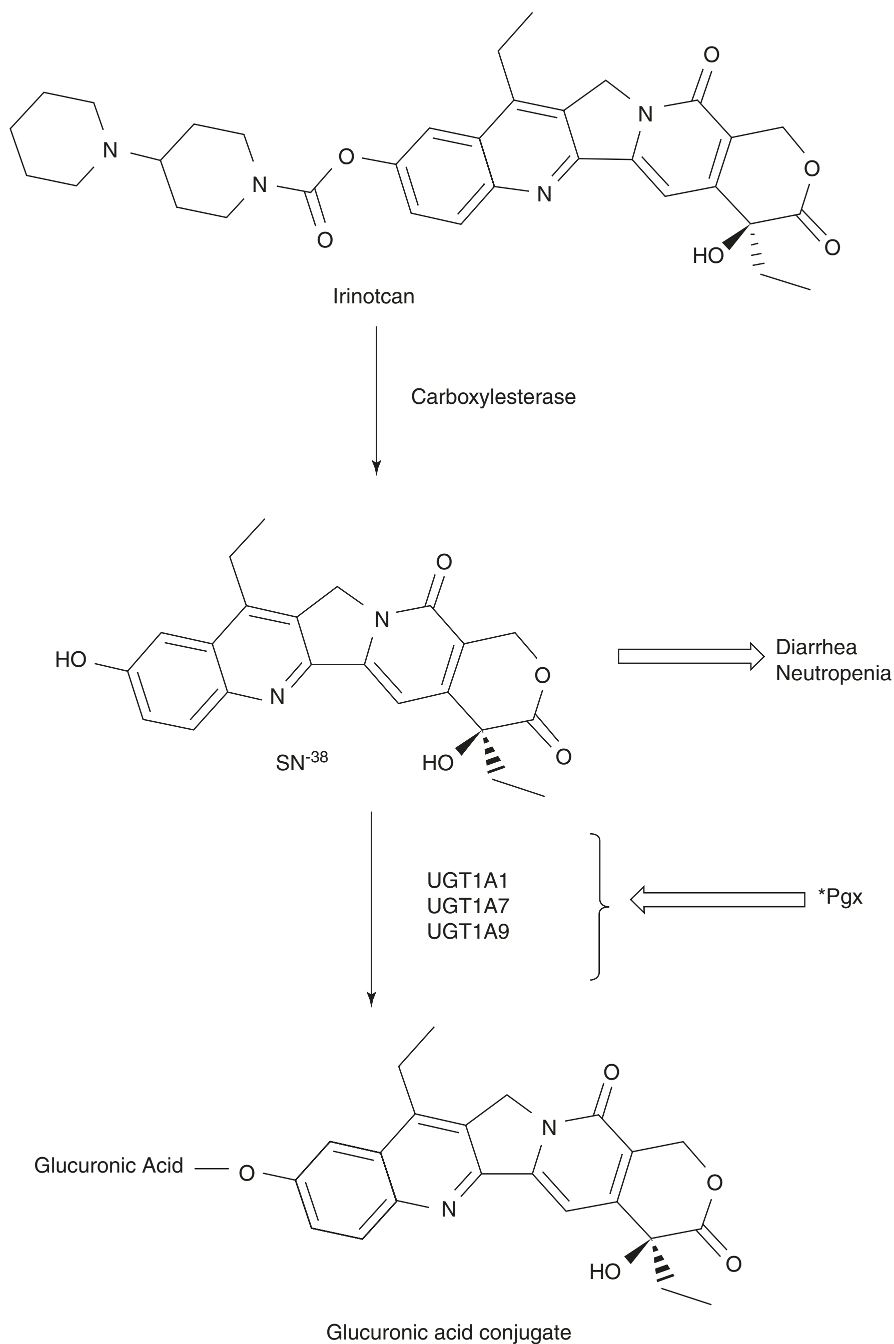

Fig. 9.5 Glucuronidation of active metabolite SN-38 of irinotecan and effect of UGT protein variants on increased side effects like diarrhea and neutropenia

also acts as immunosuppressive agent for Crohn's diseases and UC. Thioguanine or 6-TG (Tabloid) is another purine antagonist that acts as an antimetabolite and interferes with the synthesis and metabolism of endogenous purine nucleotides. As shown in Fig. 9.6, 6-MP and 6-TG must be converted to active thiopurine ribonucleotide by HGPRT (hypoxanthine-guanine phosphoribosyltransferase). This intermediate is then methylated by TMPT to form active *S*-methylthiopurine ribonucleotide to exert cytotoxic action. TMPT is also an important enzyme to terminate the effects on 6-MP or 6-TG by methylation. Individuals with polymorphism in TMPT have less capacity to deactivate these thiopurines which leads to overproduction to cytotoxic thiopurine nucleotides. These excessive activated nucleotides can result in life-threatening toxicities like myelosuppression. Further, clinical efficacy will be compromised due to altered metabolism. Similarly, as shown in Fig. 9.7, azathioprine first gets converted to 6-MP nonenzymatically, but the next activation step requires HGPRT similar to 6-MP and 6-TG metabolism. Subsequently TMPT is required to terminate this drug's effect. So, protein variants of TMPT can alter azathioprine's clinical outcome as well.

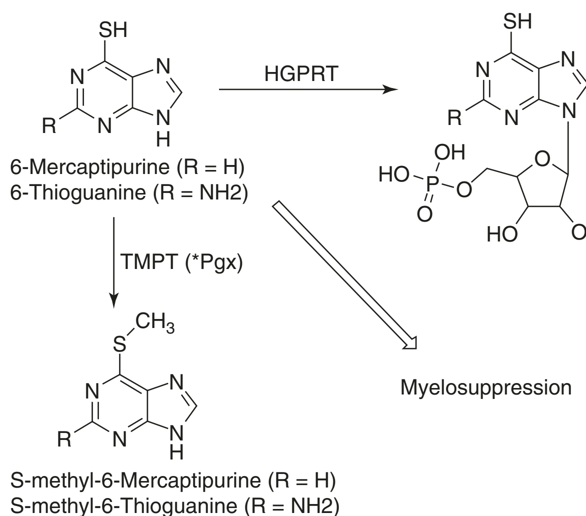

Fig. 9.6 Bioactivation of 6-MP and 6-TG and their inactivation to methylated analogue. Protein variants of TMPT can be responsible for increased myelosuppression

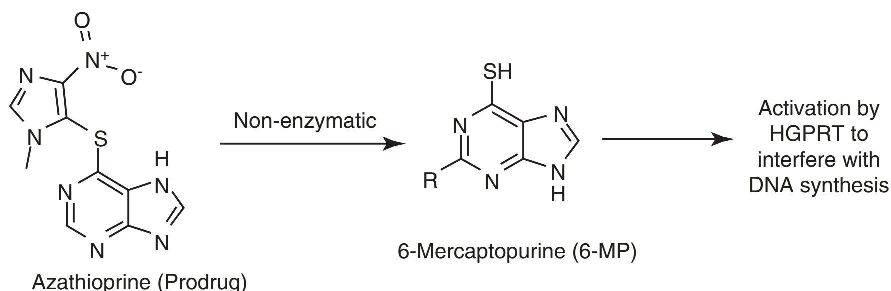

Fig. 9.7 Conversion of azathioprine to 6-MP which further requires HGPRT for activation and TMPT for forming inactive methylated metabolite

At the molecular level, TMPT*3 is the most common protein variant that accounts for three fourth defective alleles in TMPT. TMPT variants *2, *3A, and *3B are common polymorphisms seen. Allele frequency varies by ethnic population, and it has been found that *3A is the most common variant in Caucasians, while *3C is the most common variant in Asians and African-Americans [99].

FDA updated labels of azathioprine and thiopurines to include information about TMPT polymorphism and recommends testing of TMPT genotype of a patient before starting therapy with these drugs. Depending on TMPT genotype, either dose can be modified to achieve similar therapeutic outcomes or alternative therapies can be prescribed in patients found to be homozygous. For example, ~10% Caucasians are found to be poor TMPT metabolizers and dose of azathioprine and thiopurine may be decreased by 10–15- fold to avoid myelosuppression and to keep drug plasma levels within therapeutic window.

9.3.2.2 Catechol *O*-Methyltransferase

COMT has been involved in metabolism of a number of drugs as well as endogenous molecules like central neurotransmitters. Polymorphism in COMT has been reported among various ethnic groups, and the frequency of homozygous and heterozygous alleles varies. For example, it has been found that 50% Caucasian are heterozygous and 25% are homozygous for COMT allele [100]. However COMT variation on drug levels has not found to be much clinically significant so far.

9.3.3 Acetylation

N-acetyltransferases (NATs) catalyzes the acetylation reactions generally for amine ($-NH_2$) groups and less commonly hydroxyl (–OH) and thiol (–SH) groups. There are two isoforms of NAT commonly known as NAT1 and NAT2. Protein variants NAT1*10 and *11 alleles are generally referred to as *fast acetylators*. Genetic variation in NAT2 is much more common, and a number of variants like *5, *6, *7, *10, *14, and *167 are responsible for altered enzyme activity. Patients are mainly categorized as *fast acetylators*, *normal acetylators*, or *slow acetylators* depending upon their NAT genotype variations. Allele frequency varies among different ethnic groups as well as within same group [101].

NAT protein variants can affect levels of antituberculosis drug regimen (rifampin, isoniazid, and pyrazinamide) as well as antihypertensive combination (hydralazine-isosorbide). One well-known example of polymorphism in NAT2 is seen with metabolism of hydralazine. As shown in Fig. 9.8, hydralazine undergoes acetylation

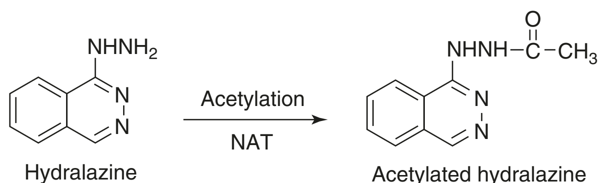

Fig. 9.8 Acetylation of hydralazine and protein variants of NAT can affect plasma levels of hydralazine

to form inactive acetylated metabolite. In *slow acetylators*, drug stays in plasma for much longer time (plasma $t_{1/2}$ can be increased from 2 to 4 h to up to 8 h) and increases incidence of systemic lupus erythematosus (SLE). In *fast acetylators*, a subtherapeutic response is achieved which is most commonly seen in 50% African-Americans and Caucasians and the majority of American Indians, Eskimos, and Chinese population.

9.3.4 Sulfation/Sulfonylation

Sulfotransferases (SULTs) catalyze the transfer of sulfonyl ($–SO_3H$) group to various drug molecules containing hydroxyl (–OH), thiol (–SH), and amine ($–NH_2$) functional groups. For example, sulfonylation is the major route of acetaminophen metabolism in children where –OH group is conjugated with sulfonyl group to inactive the drug. Two important genes that exhibit polymorphism are SULT1A1 and SULT1A2. One important example of influence of protein variants of SULTs is seen with endoxifen, an active metabolite of tamoxifen. It undergoes sulfonylation at hydroxyl (–OH) group, and protein variants in SULT1A1*2 and *3 may be responsible for decreased therapeutic response. It has been found that SULT1A1*1 leads to rapid sulfonylation of endoxifen which may lead to apoptosis in a breast cancer [102]. This suggests that SULTs can play a role in improving survival in cancer patients and also in decreased therapeutic response. However, so far no recommendations have been made by the FDA. So, polymorphism can sometimes be helpful in understanding a drug's efficacy and individualize drugs based on patient's genotype.

9.3.5 Glutathione (GSH) Conjugation

Glutathione S-transferases (GSTs) catalyze the transfer of tripeptide molecule glutathione (GSH) to electrophilic centers in a drug molecule. Resulting S-bridge between glutathione and the drug results from covalent bond formation, and this drug-glutathione S-conjugate can be eliminated as such or can further degrade tripeptide for elimination. Anticancer alkylating agents and platinum compounds are major targets for metabolism by GSTs. There are different types of GST like GSTP, GSTA, GSTT, GSTO, GSTZ, and GSTS located on different chromosomes. These GSTs can have protein variants affecting metabolism of drugs or elimination of their metabolites. For example, GSTP1 is involved in metabolism in platinum compounds (cisplatin, oxaliplatin) and may metabolize these drugs faster to diminish their therapeutic effects. It has been reported that patients with ovarian cancer showing GSTP1*B polymorphism may have better progression-free survival than patients with GSTP1*A [103]. On the other hand, decreased activity of GSTP1 with protein variant can decrease metabolism of platinum compounds and can lead to toxicity. Similarly, GSTA1*B lead to increased therapeutic effects of cyclophosphamide and increased survival in breast cancer patients [104]. Another anticancer drug busulfan undergoes significant

metabolism by GSH, and significant differences in plasma levels of drug in different patient populations have been reported [105]. Further studies are needed to really understand the role of protein variants of GST on the safety and efficacy of these anticancer compounds.

9.3.6 Amino Acid Conjugation

Various amino acids like glycine glutamine, arginine, and taurine can conjugate with carboxyl (–COOH) functional group present in drug molecules. This is an important metabolic pathway for some drugs like valproic acid and salicylic acid. However, so far there are not many reports on significantly altered drug levels due to polymorphism in enzymes catalyzing amino acid conjugations.

9.3.7 Impact of Polymorphism on Phase II Enzymes

The FDA periodically updates drug labels as critical information related to pharmacogenomics of existing drugs become available, and this can potentially affect clinical outcome. This section provides information about some important phase II enzymes and their effect on product labeling that the US FDA has updated based on pharmacogenomics information that came to surface after the drug was approved in the market. Labeling information pertaining to pharmacogenomics of drugs can be found at the FDA website, (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/).

9.3.8 Role of Microbiome in Drug Metabolism

The human intestine is home to a complex community of microorganisms known as the gut microbiota which has undergone coevolution with its host [106]. The microbes function as an organ within themselves with metabolic, immunologic, and endocrine-like actions that can affect human health [107]. Advances in molecular techniques have made it possible for a reliable assessment of gut bacteria. The three types of bacteria that dominate human gut include *Firmicutes* (Gram-positive), *Bacteroidetes* (Gram-negative), and *Actinobacteria* (Gram-positive) [108]. Changes in the structure and diversity of the microbiome can affect the overall health of the host and pathological states such as inflammatory bowel diseases (IBD), obesity, and diabetes [109–111]. Another important area where microbiome has a role is in drug metabolism. Microbiome expresses a wide range of enzymes which have the ability to metabolize drugs efficiently and extensively [112]. More than 30 drugs and other bioactive molecules are reported to undergo modification/metabolism by gut microbiome [113]. The number of the drugs continues to grow as more and more evidence becomes available from in vitro and clinical studies. Deducing the exact mechanism of action and the type of microorganisms involved remains unclear due to the complexity of the microbiome. The types of reaction catalyzed by gut microbiome include azoreduction, nitroreduction, sulfoxide reduction, N-oxide reduction, hydrolysis, acetylation, and deacetylation [114].

Multiple studies have shown that the gut microbiome can affect the pharmacokinetics of orally administered drugs having significant impact on the bioavailability [115]. There is interindividual variability in the population and structure of gut microbiome; therefore, it can be assumed that the variability will be reflected in the degree of metabolic reactions catalyzed by these microorganisms. Microbiome-catalyzed drug metabolism complicates the interindividual variability of drug metabolism, which varies from individual to individual as a result of pharmacogenomics.

Conclusion

Drug metabolism is a very complex phenomenon which varies across the patient population due to pharmacogenomics of the drug-metabolizing enzymes and the host microbiome. Pharmacogenomics of the drug-metabolizing enzymes has been studied in detail; there is plenty of data supporting the variability. However, the variability due to metabolism by microbiome is an evolving field, which should become clearer as we gather evidence and understand the whole process with time. Therefore, it is the need of the hour to look at the interpatient variability from both microbiome and pharmacogenomics of DME point of view.

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10 Essentials of Genomics in the Continuum of Translational Research

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Abstract

New developments in the genomics field have led to increasing expectations for its impact on clinical practice and disease prevention. There is a need to develop comprehensive research guidelines so that human genome discoveries can be translated into health practice. This should be done in a way that maximizes health benefits and minimizes harm to individuals and populations.

Genomic testing is used in predicting the risk of certain diseases. It helps us in defining the pathogenesis of certain diseases, and with pharmacogenomics, we can identify new drug targets and the beneficial effects of new drug agents on specialized populations. Our health-care systems and policy makers are expected to provide sufficient information as to what exactly genomic testing is and why new discoveries need to be translated into clinical practice. The clinicians should be able to understand the processes by which new research discoveries are translated into clinical and useful genomic testing so that a framework for personalized medicine can be developed. This chapter focuses mainly on the continuum of an evidence-based framework for multidisciplinary translational research in genomics along with several principles that must be taken into consideration. In order to correctly use and interpret genomics and understanding its significance in the real world, a brief overview of the whole process, current challenges, and approaches to overcome them are presented in this chapter. Different processes that are used to evaluate the clinical utility of newly developed tests will also be described here.

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_10

10.1 Impact of Genomics in Health and Disease

The field of genomics has an incredible relevance starting from biomedical research to health care and then personalized health care to the patient and their families. Over the last two decades, there has been great advances in the field of genomics, a discipline in medicine that involves a person's genetic information and its use in the clinical practice for treatment and prevention of a disease. With the advancement in the technological field and addressing limitations of use in certain complex fields, genomic testing seems to be increasingly acceptable for detecting many disorders and diseases. For disorders with a suspected genetic origin, it has the potential to eventually become a universal first-line diagnostic testing.

The NIH roadmap presented in 2003 was designed to enhance the translation of research findings to patient care by developing a new domain of clinical and translational science. This new domain is broader than the two current domains of translational research and clinical investigation and included genomic medicine [1]. However, in its current form, genomics still seems like it is in the initial phase of translational research. The continuum of translational research that has been studied previously in medicine and public health can be applied to genomic medicine provided there is a framework based on the available evidence. There is still an uncertainty about the validity and reliability of available genetic tests and how successfully they predict outcomes. It is very basic to the field of genomics that we investigate the benefits and harms associated with the clinical use of testing and what actions should be taken based on their results. We still need to involve public health officials, policy makers, and the medical community in order to further our understanding of genomics. In order to collect appropriate information and its application toward genomic testing, we need to generate a positive response.

Currently, many DNA-based approaches are used to quantify predisposition and susceptibility to different chronic and complex diseases. However, these genomes do not tend to change in one's lifetime—and this results in a lack of insight into DNA variations.

The Human Genome Project (HGP) that was started in 1990 is an example of an effort geared toward better understanding of DNA and its role. It was based on the recognition of DNA as the hereditary material, identification of its structure and genetic code, the development of recombinant DNA technologies, and DNA sequencing methods. The success of this project predicted an evolving area of personalized medicine and disease prevention based on genomic testing for genetic susceptibilities. During the past two decades, so much has happened in the genomics field, from the primary sequencing of genes to new technologies where genomic codes are studied and cataloged to make interpretation of genes easier. Therefore, in order to advance human health particularly in the understanding of the biology of genes, biology of disease, and translation of science into medicine and health-care practice, we have come to a stage to take more evidence-based decisions. With more information

and understanding of this field, the experts also hoped that pharmacogenomics would allow us to develop safe drugs and find ways for their effective use.

Initially, genomic testing was offered to a patient for diagnostic purposes—for those who had some types of characteristics suggestive of specific genetic susceptibility or for predictive purposes for those individuals with a family history of any known genetic condition. Since then, for the diagnosis, treatment, prognosis, and disease prevention, several types of genomic testing have emerged as potential tools. Some of the genomic testing with clinical utility can detect the risk-increasing alleles that have high penetrance; however, most of these tests lack evidence basis of clinical validity and its use in clinical practice and disease prevention. We know that the vast majority of the genetic variants that affect the risk of complex common diseases—such as diabetes, asthma, and cancer—have low penetrance. Even though each variant plays a small role in an individual's risk of developing a disease, it becomes important to combine results and information from hundreds of low-penetrance alleles to determine the exact risk. Genomic researchers are trying to identify more of these low-penetrance variants to determine the best way to use these tests in personalized medicine. At present time, many genomic testing companies are offering tests that use low penetrance variants for determining the risk of disease. Conversely, it can cause overestimation of the significance of these testing for the consumers.

There is the debate on whether new techniques in genomic testing and their appropriate utility are sufficient to extract balanced and complete information. A test should be considered valuable when the information gained leads to beneficial impact outweighing the harmful effects. It becomes very important for health-care providers to inform patients that not all testing provides medically or clinically actionable information. Even if no changes are made in the treatment and preventive strategies, genomic testing has its value in diagnostic testing, avoiding invasive testing and informing future life planning. Genomic testing is done to optimize preventive health-care strategies and drug therapies while people are still healthy or at the earliest stages of a disease. The findings or results should be translated into specific diagnostic tests and targeted therapies so that genomic testing can be easily used by health-care providers and their patients. Since the overarching goal is to optimize medical care and outcomes for each individual, the treatment, medication type and dosage, and disease prevention plans differ for each individual, and this results in the customization of patient care or use of personalized medicine. At this time assessment of the added value of personalized genomics is multifaceted. Several factors including criteria for the diagnostic testing or treatment, availability and use of biomarkers, patient management, and comparative effectiveness research of treatment versus standard of care, all are involved in this process of genomics discovery. As with any emerging technology, identifying areas of uncertainty and moving toward standard regulatory and reimbursement practices have facilitated the acceptance of genomics into clinical practice [2].

10.2 Translational Research Phases in Genomic Research

Khoury et al. proposed the following phases in genomics translational research. All the phases are detailed below [3].

10.2.1 Gene Discovery: T1 Research

The first phase, T1, starts with gene discovery. The main goal of this phase is to develop candidate applications and a clinical evaluation of effective therapeutic options. T1 research builds genomic profile and its testing combinations to predict the increased risk of certain diseases. In this phase, increased genetic testing is performed mainly for the diagnosis and management that is based on both observational studies and clinical trials. Two main approaches, (1) human genome epidemiology and (2) framework for evaluating the genomic testing, were used by Khoury et al. when they systematically reviewed the evidence gained from these studies. HuGE (Human Genome Epidemiology) is an observational population-based research that measures the frequency distribution of genotypes and how it correlates with phenotypes. Additionally, it estimates the risk of diseases that are associated with genetic variants. This type of research is very crucial in assessing the clinical validity of genomic testing. A major challenge in this phase is the evaluation of several smaller studies with inconsistent results. HuGE network, in collaboration with the other journals, has published systematic reviews and meta-analysis to improve the evaluation processes for estimating disease risks. However, the main focus of this phase is on a small portion of human genome instead of across the genome making this a major limitation. There is a possibility that an emphasis placed on meta-analysis might have missed the inclusion of some important data from individual well-powered studies of genome-wide associations [3].

10.2.2 From Health Application to Evidence-Based Guidelines: T2 Research

The second phase, T2, is based on the validity and utility of a developed genomic application for health practice. This assessment eventually leads to the development of evidence-based guidelines so that genomic testing can be used in practice. However, the development and evaluation of genomic applications are very challenging and highly unregulated [3]. The ACCE (analytical validity, clinical validity, clinical utility, and ethical and social implications) model by the CDC (Center for Disease Control and Prevention) was an analytical framework that was created to guide the evidence review [4].

Analytical validity shows whether or not a test is reliable, accurate, and reproducible. Significant evidence gaps exist in assessing the analytic validity due to tests that are developed in the lab, interests related to patents and copyrights, and inadequate regulation.

Clinical validity examines the significance of the test to determine if the results would translate to something with clinical importance or not, for example, the risk of developing a disease, metabolism of a drug, or response to the drug. Clinical utility shows the use of the test in clinical decision-making and its translation to an important health outcome; it also assesses if any harms are outweighed by the benefits.

On the other hand, in clinical utility, there are very few randomized controlled trials on the efficacy of the testing in clinical practice. Therefore, the benefit of genomic testing and its possible harms need to be further investigated.

10.2.3 Evidence-Based Guidelines in Health Practice: T3 Research

The third phase, T3, attempts to use evidence-based guidelines into health practice through dissemination, implementation, and diffusion research. Even though there is extensive work in public health research on developing effective strategies for disease prevention and health promotion, there is still a huge gap in evaluating these methods.

10.2.4 From Practice to Its Impact on Population Health: T4 Research

As proposed by Khoury et al., the fourth phase, also known as T4 is regarding the evaluation of health outcomes of a genomic application in clinical practice. In the field of biomedical translational research, we know that the evidence obtained is valid when based on systematic review and meta-analysis of clinical trials and observational studies. Conversely, in genomic medicine, the evidence-based knowledge is less clear because most genetic testing is being conducted in the laboratory, and in general, there are no clear regulations and guidelines from regulatory agencies.

10.2.5 Synthesis of Available Information

A systematic approach to appraising the available evidence on certainty and uncertainty is referred to as knowledge synthesis, and as presented by Khoury et al., knowledge synthesis has an essential role in all phases of translational research related to genomic medicine [5]. Systematic reviews and meta-analysis are becoming common in developing evidence-based recommendations in clinical practice. The Cochrane Collaboration publishes systematic reviews to facilitate the search for evidence from clinical trials and observational studies and these reviews and meta-analysis are then used to develop clinical practice guidelines.

10.3 Advisory and Working Groups in Genomic Medicine

For the evaluation of genomic testing along with a variety of other health-care services, several groups and committees in the USA have been actively involved in bringing evidence-based knowledge and information. For example, the advisory committee on Heritable Disorders in Newborns and Children evaluates the use of genetic testing for newborn screening panels. Similarly another working group, Evaluation of Genomic Applications in Practice and Prevention (EGAPP), offers an evidence-based assessment of genomic tests and other applications used in genomic medicine.

The EGAPP Working Group is an independent group that has a focus primarily on the ACCE framework. This working group has been known for its use of more traditional methods for evidence-based practice in medicine and public health. The EGAPP initiative was established by CDC, Office of Public Health Genomics and its efforts are mainly focused on the synthesis, grading, and identifying knowledge gaps of the research conducted in multidisciplinary areas. However, there is a scarcity of evidence in published peer-reviewed literature for this type of research and its synthesis. Since 2005, EGAPP has developed new approaches, included evidence-based reviews, and provided recommendations on genomic tests, and several more recommendations are underway. To further improve health-care practices, the EGAPP Working Group has combined methods and models of other advisory groups such as the US Preventive Services Task Force (USPSTF). The USPSTF task force has been a key resource in evaluating and making recommendations on clinical preventive services in the USA for over two decades. The USPSTF was the first proposed and recognized national process that focused on developing practice guidelines for genetic testing and recommended evidence-based approaches. For example, the testing of BRCA1 and 2 was focused on evaluating the risk for heritable breast cancer and on HFE testing for establishing the diagnosis of hereditary hemochromatosis [6, 7]. Although the overall process for evaluating the genetic testing has been quite slow as compared to the testing of other new and developing technologies and its applications in clinical practice, EGAPP has been successful in establishing a key methodological foundation for the evaluation of genomic testing based on evidence-based practice guidelines.

The EGAPP has used the following approaches for the evaluation of genetic testing and provides recommendation such as (1) the knowledge and evidence from existing processes should be incorporated into the new process; (2) the framework provided by the ACCE on genetic testing should be integrated and then assessed; (3) methods for assessing the quality of each study, evidence for each component of the framework, and certainty of net benefit should be evaluated; (4) the synthesis process provided by the Evidence-based Practice Center of Agency for Healthcare Research and Quality (AHRQ) and a thorough systematic evidence-based review should be conducted; and (5) optimizing novel modeling methods to report evidence gaps and clinical recommendations with clear linking to the evidence should be developed [8].

10.3.1 Work Process of EGAPP

1. First, there is identification of the topic or genomic application that is under consideration; this process is undertaken by EGAPP staff, EGAPP Working Group (EWG), and EGAPP stakeholders group.
2. A clinical scenario for using genomic test is then defined, for example, diagnosis of the disease, risk assessment, and prognosis which is then followed by preparing brief summaries. This process involves using a format that includes disorder or test or clinical scenario, and this is conducted by CDC-based EGAPP staff.
3. To guide the evidence-based review, an analytical framework of key questions is created followed by the search for evidence with evaluating the quality and adequacy of studies and synthesizing the existing literature.
4. The net benefit of the clinical testing is determined and that generates recommendations based on the certainty of the net benefit.

An evidence-based review comprises of many steps and is meant to synthesize available evidence on a particular disorder, test, and clinical scenario. The CDC uses comprehensive reviews conducted in partnership with the Agency for Healthcare Research and Quality (AHRQ) Evidence-based Practice Centers (EPCs). EPCs use comprehensive literature search methods and evaluations and provide detailed documentation of methods and results.

Based on the information provided by Teutsch et al., the CDC-based EGAPP staff coordinates these reviews with technical contractors and expert consultants. Evidence reports and the products of these reviews are quite detailed and systematic. They provide objective assessments of the evidence on a specific topic. Evidence reports are the basis for further consideration by the EGAPP Working Group as they develop their Recommendation Statements. In this process, the CDC assigns the review, the EWG develops the key questions that need to be addressed, and the selected review team conducts the review and produces a report. A Technical Expert Panel, designated by the review team, provides guidance usually including topic experts and three EWG members. Evidence reports undergo external expert review [8].

The EGAPP Working Group reviews the evidence report and examines related issues and other sources of evidence. The group prepares a draft recommendation statement which is then peer-reviewed and submitted for publication. The recommendation statements created are based on CDC-commissioned evidence reports, other review of evidence, potential clinical and social impact of using the test in practice, and quality of available data. In this step, EWG, with support from CDC-based EGAPP staff and consultants are involved. Review of comments from the industry and a range of stakeholders including from consumer groups, professional organizations, health plans, and public health programs are conducted. All these processes lead to peer-reviewed, published EWG recommendation statements [8].

10.3.2 Existing Evidence on Validated Approaches

10.3.2.1 Three-Tier Classification of Recommendations on Genomic Applications

On the basis of available evidence, Khoury et al. proposed the classification of genomic testing into three tiers.

1. Implementation of genomic testing in routine practice using evidence-based recommendations
2. If there is insufficient evidence, then do not use testing, and consider adequate information on analytic and clinical validity as promising but not definitive information on clinical utility.
3. Promoting informed decision-making is a valuable recommendation when sufficient evidence is available on analytical and clinical validity and the risk and benefit analysis is favorable but not definitive. Furthermore, discourage the use of testing when there is no or little information on validity or utility and evidence of harm. These recommendations and rigorous outcomes research can provide sufficient guidance in clinical practice for assessing the impact of genomic testing on patients, their families, and population-based health outcomes.

According to Khoury et al., since clinical genomics and the field of personalized medicine are still evolving, the term insufficient evidence in Tier 2 category will always be used in the field of genomics for many years to come. In general, when there is lack of evidence in any field, informed and shared decision-making seems to be an effective measure, though too much reliance on shared decision-making at the same time might lead to a risk of not collecting sufficient evidence. It becomes important to use all the available evidence in this category to the best possible extent so as to guide the clinical practice. According to the EGAPP Working Group and the USPSTF approaches to decision-making, all inadequate evidence is not created equally. If the quality and quantity of evidence is taken into consideration, one can reach a level of low, moderate, or high certainty. As presented by the EGAPP Working Group, tier 2 category is divided into two groups, 2a, where level of certainty is low, and 2b, which seems to be very similar to tier 3 based on several characteristics; for example, the recommendation is to discourage the use of genetic testing for the genetic variant CYP450 in the cases of primary depression before it is treated with selective serotonin reuptake inhibitors. Similarly, for the group 2a, an initial risk-benefit analysis might present favorably though the level of evidence is low. For example, to use targeted chemotherapy in patients with the high risk of recurrence of developing breast cancer, even though the evidence of clinical utility may not be present, the tests should have clinical and analytical validity [8].

Khoury et al.'s classification based on EGAPP Working Group uses tier 1 example of Lynch syndrome testing. The testing for all new colorectal cancer cases is done in all first-degree relatives of all new colorectal cancer cases in order to reduce morbidity and mortality [8]. The example for tier 3 would be a population screening for HFE gene mutations to prevent morbidity and mortality from iron overload as

this promotes informed decision-making. Since evidence is available on analytical and clinical validity, risk/benefit analysis is favorable but not definitive. The examples used above demonstrate that much more investigation is needed in developing quantitative approaches so that the classification of insufficient evidence is further modified as designated by the EGAPP Working Group.

10.4 Challenges in Genomic Medicine

The complexity of genomic testing specifically with the new emerging technology and bio-informatics is certainly becoming a great challenge for the health-care providers, research centers, and academic institutions. In addition, more stringent regulatory oversight and a broad scope of genomic testing have made its transition difficult from research to practice. During the past few decades, many new technologies regarding genomic testing have been developed, but not many have reached clinical practice despite being heavily marketed to the health-care professional [9]. One can argue that the genomics field is still behind in developing standardized guidelines, and these challenges still exist in clinical practice for health-care providers and policy makers. We still need evidence-based guidance for its practice so that health benefits can be maximized while minimizing the harms.

The framework on risk and benefit given by Veenstra et al. was assessed in response to the lack of available evidence for the translation of genomic discoveries into clinical practice [9]. It's evident that evidence-based medicine (EBM) has become the accepted standard for such decisions. In the domain of EBM, certain standards have been developed including a requirement for detailed evidence reviews before making recommendations. However, in the world of genomics, the use of EBM is now becoming frequent as compared with the past when the field of medical genetics has focused on rare genetic diseases. There is a misconception that evidence-based practice is mostly used for medical- and health-related information rather than genetic and genomic information. There is a need for adequate evidence of the clinical utility of genomic testing in practice. Regulatory and reimbursement policies have slowed down the process due to lack of comparative outcomes data for genomic applications. Even though it is comparatively easier access for genomic testing in the market including its availability direct to consumer testing, still lack of evidence makes it more challenging [10]. So far, there are no evidence-based requirements for genomic test evaluation, and stakeholders continue to rely on the findings of observational studies while accepting its potential benefits, whereas some experts of the field emphasize the need for randomized controlled clinical trials [3]. In recent years some valid and quantitative tools have been made available that can be used in assessing the quality of evidence.

Major advances in our knowledge of genomic medicine and its testing are rapidly changing the application of these findings into clinical practice. The literature is scarce on studies that provide evidence not only regarding genomics knowledge and its application into clinical practice but how it will change behavior in improving population health. There is an uncertainty in making treatment decisions because of the available genomic testing and its significance to a specific treatment.

Despite high expectation, investigations, and novel technological expertise, there is still evidentiary challenge present on the validity or the utility of genetic testing [11]. The decision for genetic testing makes it even harder for the patient to decide if they would like to gain further knowledge on certain conditions and how much of a change is expected of their lifestyle based on the findings of the testing. The results can be inconclusive sometimes, and patients need to be well informed about the risks of getting the testing done. It becomes the responsibility of the health-care providers to disseminate the information that sometimes genomic testing can have a little or no effect on their short-term or long-term health [12, 13]. To help patients in making complex decisions regarding the utility of genomic testing, there is an increased demand to provide information on the risk and benefit of genomic testing through education and training, counseling, and through decision-making aids. Veenstra et al. proposed a risk-benefit framework for the assessment of genomic testing [9]. It was proposed that several methods used in evidence-based medicine such as health outcomes research, decision analysis, and assessment of health technology can be combined for further assessment of uncertainty in genomics field. The main goal of the proposed framework is to use genomic tests in assessing improvement in health outcomes and presenting results using a risk-benefit tool. This will eventually facilitate the interpretation of the findings from these data vital to clinical practice.

The field of genomic testing is currently faced with challenges of expectations of improving health outcomes and resolving uncertainty in the rapidly changing regulatory and reimbursement settings. Even though we are currently in an environment where cost is increasing and health decision-makers are making difficult decisions to keep the balance of costs and benefits, genomic technologies still hold the potential to improve health outcomes. The above-stated goals can be reached when the value for the money can be demonstrated through evidence-based reviews and data uncertainties are addressed keeping in view the risk-benefit framework.

10.5 Ethical, Legal, and Social Implications

Aside from the challenges stated above, other issues such as ethical, legal, and social issues also exist. Genomic testing can identify individuals at a risk for disease long before they develop signs and symptoms. This raises concern regarding stigmatizing and discriminating an individual. One view is that information obtained reduces blame and social stigma of an individual living with mental disorder while some view it as a stigmatization problem. Some of these issues can be addressed with genetic counseling or through legislation.

Concerns about testing into routine care have also been brought up as a challenge for both patient and physician. One study found that most patients and physicians appreciated the availability of genetic tests for a disease such as asthma, but a third of patients worried about potential unfavorable test results and violation of privacy. Physician concerns were mostly related to the possibility that patients feel pressured to be tested or to be disadvantaged by health insurance [14].

Both health insurance employment companies and institutions cannot discriminate Americans based on their genetic information as they are protected by the Genetic Information Nondiscrimination Act of 2008 (GINA). GINA further specifies that health insurance issuers should not use genetic information of a person to make eligibility, health coverage, and premium decisions (GINA Title I). Furthermore, GINA prevents employers from using genetic information in making hiring, promotion, and salary decisions (Title II) and does not allow employers or any other covered entities to request genetic information as a part of the hiring process [15].

The process of incorporating genomics data into the practice of medicine has been another challenge. Some of the major barriers delaying this integration of novel genomic technology into health-care practice include the difficulty of changing the standard of care practices to account for the use of genetic testing. Even though genomic testing has been in use in clinical practice for decades, patients and health-care providers have limited knowledge and evidence-based assessment to support genomic testing along with other issues. The uncertainty about reimbursements, privacy, and data confidentiality create further delay in integrating genomic technologies in health-care practices [16]. Since genomic testing provides limited information about an inherited condition, we are not certain about the disease progression over time and if the person will ever show signs, symptoms of that disease and its severity [16].

10.6 Overcoming the Challenges in Genomic Testing

In order to move the human genomic discoveries into health-care practices, approaches that maximize health benefits and minimize harm to individuals and populations, we need to address above-listed challenges and gaps. The areas of gene discovery are relatively well-known, well-funded, and moving forward at a fast pace; however, the translational research, including both clinical trials and large, well-designed observational studies, are still lagging behind. Several steps can be taken to invest more time, funding, and resources in translation research especially in T2–T4 phase, which has received less support than T1 research in genomic medicine [3, 5, 17]. Since lack of evidence is the primary barrier to the translation of genomics into practice, conducting research to provide that evidence is important and represents the first step in addressing the challenge of evidence. Increased translational research is also a component of the NIH Roadmap and FDA Critical Path Initiative [1]. Both the NIH and the CDC have funding projects available that are related to the translation of genome discoveries in the future.

Over one million Americans are estimated to be at an increased risk of cancer due to one of the two genetic conditions, hereditary breast, ovarian, and other cancers associated with BRCA gene and hereditary colorectal, endometrial, ovarian, and other cancers due to Lynch syndrome [18]. Recent studies have shown that these conditions are more common than they were thought initially, and most

people with these conditions are unaware of this and if detected and treated early can save their lives. Evidence-based recommendations and several public health programs on genomic testing are available in reaching out to people and populations at risk. A number of agencies including the Center for Disease Control (CDC), the Division of Cancer Prevention and Control (DCPC), and the Office of Public Health Genomics (OPHG) support state genomics programs and help to implement recommendations. These recommendations include identifying people eligible for genomic services, using cancer registries, educating health-care providers and patients, facilitating payer coverage, and monitoring implementation of genomics recommendations by evaluating new data sources [18].

In 2014, OPHG published an online toolkit. The main goal is to facilitate sharing the model public health genomics approaches across states so that these approaches can be applied to other evidence-based genomic testing applications such as familial hypercholesterolemia. The OPHG is actively working to enhance the impact of these new public health approaches. With the use of cascade screening, it is now possible to reach out to at-risk family members of people with BRCA and Lynch Syndrome that are identified through these public health genomics programs. Other efforts of OPHG in partnership with CDC programs include pursuing new approaches to monitor implementation of evidence-based genomic testing at the state and national level. The OPHG continues to establish strategic partnerships with professional organizations, disease support groups, and health-care systems.

The EGAPP initiative has set high standards for the evaluation and synthesis of evidence based on systematic reviews and meta-analysis that also align with ACCE criteria. Despite significant resources provided by governmental agencies on funding genomic research, there is still a need for continuous emphasis on providing funding in translational research and evidence-based reviews. There is no question on the significance of this form of research in the hierarchy of evidence. Evidence-based reviews in genomics play a fundamental role in providing us with the knowledge on the validity and utility of genomic applications, crucial to investigators, health-care providers, patients, and policy makers. The dissemination of this information should be carried out extensively through peer-reviewed journals, scientific meetings, and community organizations [19].

To incorporate genomics application into health-care practice, the field of implementation science may be able to provide insights and efficient ways. The focus of implementation studies is to identify barriers and apply best approaches to promote the uptake of research findings. In November 2015, the National Academies of Sciences, Engineering, and Medicine held a workshop in Washington, DC, to further explore the integration of genomics into health-care practice. Participants of this workshop explored the challenges and opportunities of integrating genomic advances into the clinical practice through the lens of implementation science. The report summarizes the presentations and discussions from the workshop and is a helpful tool in understanding the impact of genomic testing and its future direction [20].

10.7 Future Prospects of Genomic Medicine

Finally, we hope genomic testing will change the landscape for inherited diseases and personalized medicine. Genomic technologies are changing at a very fast pace, and it is hard to predict how quickly new technologies will become adopted in routine clinical practice. There is a possibility that significant increase will happen within the next few years to develop and tailor treatments on the basis of a genome. A great percentage of patients will have their genome sequenced for at least one reason. In considering the future of genomic medicine, we should consider the information provided by Khoury et al. and their published work on evidence-based approaches. Evidence-based studies have already been taken into consideration to determine the degree of their impact on patient-related health outcomes. There is already so much work conducted on electronic health records and the storage of genetic data to assist busy clinicians in utilizing relevant genomic data at the proper time for treatment and diagnosis. We are at a point in the field of genomics where the analytic validity of new technologies is high. The easy access to testing, clinical interpretation, and knowledge about patient responses to genomic information has made it easier for its application in health-care practice [21]. Genomic medicine seems like being the best possible way in future toward preventing the manifestations of inherited diseases. With a great demand for genomic testing and availability of more evidence, the cost of sequencing is coming down, and it seems like within the next few years, people will be able to determine the complete genome sequence. The information provided on the impact of genomics, our knowledge of the genes that cause disease, its prevention, and pharmacogenomics; all will help patients to understand the genetic roadmap of their potential inherited diseases. This will empower the health-care providers to design specific tests to track each patient's progress along with their genetic roadmap as well as provide recommendations for disease prevention based on utilizing the evidence-based approaches.

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11 Charting a Course for Genomic Medicine from Base Pair to Bedside

Teresa Vo

Abstract

The completion of the Human Genome Project in 2003 was surrounded by lots of excitement in the scientific and lay communities because it was a milestone, along with other advancements in technology that have revolutionized our understanding of the contributions of genetic variability in shaping health and disease. One mystery the Human Genome Project helped scientists and clinicians unravel from a health perspective was why some patients responded differently to medications from the rest of the general population. Pharmacogenomics is the study of how genes influence an individual's response to medications. The term pharmacogenomics is often used interchangeably with the term pharmacogenetics, which usually refers to how polymorphisms in a single gene influence response to a single medication. For more than 150 FDA-approved drugs, pharmacogenomic information can be found in the product labeling describing risk for adverse drug events, genotype-specific dosing, and/or variations in pharmacokinetic and pharmacodynamic parameters. For a select group of medications, such as codeine and clopidogrel, pharmacogenomic information may even be highlighted in a black box warning further emphasizing the important role of our unique genetic makeup in response to medications. Inherited genome variations influence the function of gene products that determine the pharmacokinetic and pharmacodynamic properties of a particular medication. In cancer, somatically acquired genomic variations and inherited genome variations influence response to anticancer agents. In infectious diseases, genomic variations in the bacteria or virus influence antimicrobial sensitivity. Pharmacogenomic research endeavors have sought to uncover the relationship between treatment response and genomic differences since it was first characterized in the 1950s by Sir Archibald Garrod, and the term was coined in 1959 by Friedrich Vogel. Some early pharmacogenomic

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_11

examples include *NAT2* gene deficiency and isoniazid-induced neuropathy, *G6PD* gene deficiency and primaquine-induced acute hemolytic crisis, and *BChE* gene deficiency resulting in succinylcholine-induced prolonged apnea. The translation of these findings and others into clinical practice in a sustainable and scalable model is more of a recent initiative to further optimize patient care.

11.1 Introduction

The completion of the Human Genome Project in 2003 was surrounded by lots of excitement in the scientific and lay communities because it was a milestone, along with other advancements in technology that have revolutionized our understanding of the contributions of genetic variability in shaping health and disease. One mystery the Human Genome Project helped scientists and clinicians unravel from a health perspective was why some patients responded differently to medications from the rest of the general population. Pharmacogenomics is the study of how genes influence an individual's response to medications. The term pharmacogenomics is often used interchangeably with the term pharmacogenetics, which usually refers to how polymorphisms in a single gene influence response to a single medication. For more than 150 FDA-approved drugs, pharmacogenomic information can be found in the product labeling describing risk for adverse drug events, genotype-specific dosing, and/or variations in pharmacokinetic and pharmacodynamic parameters.[1] For a select group of medications, such as codeine and clopidogrel, pharmacogenomic information may even be highlighted in a black box warning further emphasizing the important role of our unique genetic makeup in response to medications. Inherited genome variations influence the function of gene products that determine the pharmacokinetic and pharmacodynamic properties of a particular medication. In cancer, somatically acquired genomic variations and inherited genome variations influence response to anticancer agents. In infectious diseases, genomic variations in the bacteria or virus influence antimicrobial sensitivity. Pharmacogenomic research endeavors have sought to uncover the relationship between treatment response and genomic differences since it was first characterized in the 1950s by Sir Archibald Garrod, and the term was coined in 1959 by Friedrich Vogel.[2] Some early pharmacogenomic examples include *NAT2* gene deficiency and isoniazid-induced neuropathy, *G6PD* gene deficiency and primaquine-induced acute hemolytic crisis, and *BChE* gene deficiency resulting in succinylcholine-induced prolonged apnea. The translation of these findings and others into clinical

[1] "US Food and Drug Administration." Table of Pharmacogenomic Biomarkers in Drug Labeling. http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm. Accessed on 10 December 2015.

[2] Vogel F. Moderne problem der humangenetik. Ergeb Inn Med U Kinderheik. 1959;(12):52–125.

practice in a sustainable and scalable model is more of a recent initiative to further optimize patient care.

Current medical practice takes this "one-size-fits-all approach" where a population with the same diagnosis is given the same medication. Most of the time, the medication prescribed is what the clinician is most familiar with. With this approach, a portion of the population will receive a benefit from therapy, another portion of the population will obtain no benefit from therapy, and a small proportion of the population will have an adverse effect. The adverse effect can range from anything as minor as rash or inconvenient muscle pain to myelosuppression and death. One approach to mitigate ADEs is to incorporate pharmacogenetics into the clinical decision-making process. For certain medications, we can tailor treatment to a patient's genetic makeup to increase the likelihood that all patients will benefit from therapy. Pharmacogenetics will not always explain every clinical scenario, but it's another piece of clinical information clinicians can take into account, along with other clinical factors such as hepatic function, renal function, drug interactions, and past family and medical history. Ultimately, the goal is maximizing therapeutic efficacy, dose optimization, patient safety, and medication adherence. Efforts with the recent 2015 Precision Medicine Initiative at the national level will continue to drive pharmacogenomic research into clinical practice [1].

11.2 Evidence-Based Practice Guidelines for Pharmacogenomics

Despite the expanding evidence supporting genetic variability on drug response, the clinical application of pharmacogenomics has been limited in part by knowledge among the lay and clinical community. A survey assessing pharmacogenomic knowledge among primary care physicians, cardiologists, and psychiatrists found that few physicians were familiar with pharmacogenomics and would not know what test to order along with uncertainty of the clinical value of pharmacogenomic testing [2]. Pharmacists as medication experts are well positioned to facilitate the use and application of pharmacogenomic information as well in partnership with physicians; however, a little over 80% would rate their current understanding of pharmacogenomics as poor to fair [3]. Given a rise in consumer interest in pharmacogenomic testing, clinicians and pharmacists will need to be educated about this evolving field. In an effort to springboard the translation of pharmacogenomic research into clinical practice, the Clinical Pharmacogenomics Implementation Consortium (CPIC) was established in 2009 to create guidelines that would serve as a resource to help clinicians interpret and apply genetic results to patient care given the absence of clear, curated, peer-reviewed guidance [4]. The Consortium is composed of more than 160 members from 16 countries, including clinicians, scientists, and observers from the National Institutes of Health (NIH) and the Food and Drug Administration (FDA). To date, there are 16 published CPIC guidelines available to personalize and optimize treatment using genetic information based on the premise that clinical high-throughput and preemptive genotyping will become common and

Table 11.1 Clinical Pharmacogenomics Implementation Consortium (CPIC) guidelines

1. Thiopurines and TPMT	9. SSRIs and CYP2D6, CYP2C19
2. Clopidogrel and CYP2C19	10. TCAs and CYP2D6, CYP2C19
3. Warfarin and CYP2C9, VKORC1	11. Carbamazepine and HLA-B 15:02-
4. Codeine and CYP2D6	12. 5FU, capecitabine, and DPYD
5. Abacavir and HLA-B 57:01	13. Pegylated interferon and IL28B
6. Atazanavir and UGT1A1	14. Ivacaftor and CFTR
7. Simvastatin and SLCO1B1	15. Rasburicase and G6PD
8. Allopurinol and HLA-B 58:01	16. Phenytoin and CYP2C9, HLA-B 15:02

Table 11.2 EGAPP Working Group recommendations

Gene	Drug	Recommendation
KRAS	Anti-EGFR therapy	2013, sufficient evidence to recommend for clinical use
UGT1A1	Irinotecan	2009, insufficient evidence to recommend for or against use
CYP2D6/ CYP2C19	SSRI	2007, insufficient evidence to recommend for or against use

SSRI selective serotonin reuptake inhibitor

readily available in the clinics before a prescription is written (Table 11.1).[3] These guidelines are updated regularly to incorporate any emerging evidence. Evidence is also reviewed for the development of new guidelines for other drug-gene pairs.

Each guideline put forth by CPIC provides (1) background information on the gene and drug, (2) relationship between the gene and drug, (3) genetic test interpretation, (4) available genetic test options, (5) incidental findings, (6) therapeutic recommendations, (7) potential benefits and risks for the patient, (8) clinical decision support (CDS) resources, and (9) supplemental information. The supplemental information, by itself, provides an in-depth list of the literature evaluated, frequency of variants in the population, special considerations, and CDS language [5]. The quality of the evidence is based on a three-tier scheme from level 1 (well-designed, well-conducted study) to level 3 (insufficient evidence), while the strength of the recommendation falls into one of three categories: strong, moderate, or optional.

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) is another independent panel established by the Centers for Disease Control and Prevention in 2005 that evaluates genetic tests for prognostic, diagnostic, predictive, and pharmacogenomic uses [6]. Of the available EGAPP Working Group recommendation statements, three pertain to pharmacogenomics (Table 11.2) [7, 8]. Of note, the recommendations regarding the clinical actionability of CYP2D6/CYP2C19 genotype-guided selective serotonin reuptake inhibitors (SSRIs) are in contrast to CPIC recommendation. However, EGAPP statements for SSRIs have not been updated since 2007. Internationally, the Dutch pharmacogenetics Working

[3] "The Pharmacogenomics Knowledge Base." PharmGKB. https://www.pharmgkb.org/. Accessed on 10 December 2015.

Group (DPWG) and the Canadian Pharmacogenomics Network for Drug Safety also function in a similar manner as CPIC in developing clinical guidance on the use and interpretation of pharmacogenomic results.

11.3 Optimizing Treatment Guided by Pharmacogenomics

Preventing adverse drug events is a national patient safety priority as they account for more than 700,000 emergency department visits and 120,000 hospitalizations annually adding up to approximately $3.5 billion spent on extra medical costs [9]. For several medications, pharmacogenomics can help clinicians predict whether a patient may experience a particular adverse drug reaction given a "typical" dose of a medication. A classic example is the TPMT gene encoding the TPMT enzyme involved in inactivating thiopurines (azathioprine, mercaptopurine, thioguanine) prescribed to patients for several indications, including acute lymphoblastic leukemia or inflammatory bowel disease [10]. The most common loss of function alleles in *TPMT* accounting for 90% of individuals with TPMT enzyme deficiency are *2, *3A, *3B, and *3C [11]. Other less common alleles include *3D, *4, *5, *6, *7, *8, *10, *11, *16, *21, and *25. An individual that inherits two inactive TPMT alleles has deficient TPMT activity (e.g., *TPMT**2/*2) leading to high concentrations of the active thioguanine nucleotide metabolite and subsequently severe myelosuppression. The FDA recommends but does not require genetic or phenotypic testing for TPMT. In the general population, 86–97% have normal TPMT function, 3–14% have intermediate TPMT activity, and 1 in 178 to 1 in 3736 have low TPMT activity. However, by capturing the few individuals with low TPMT activity and obtaining a TPMT genotype before a thiopurine is ever prescribed, we can proactively determine whether prescribing a thiopurine would be a safe therapeutic option and if a dose reduction is warranted to minimize the patient's risk of myelosuppression. In 2012, the FDA placed a boxed warning on codeine informing of death related to ultrarapid metabolism of codeine to morphine via CYP2D6. The box warning placed on codeine was prompted by three deaths, and one case of severe respiratory depression was reported in children who received codeine after undergoing tonsillectomy and/or adenoidectomy for obstructive sleep apnea syndrome further highlighting the important role of pharmacogenomics in guiding treatment to prevent adverse event [12, 13].

For certain medications, routine therapeutic drug monitoring is the standard of care and can oftentimes be a challenge to attain and maintain drug levels within a narrow therapeutic range. Organ transplant patients are among the most complex patients requiring long-term immunosuppressive therapy. Individualizing immunosuppressants in the critical days directly after transplantation is key to preventing organ rejection and prolonging graft function and survival. While therapeutic drug monitoring is helpful for adjusting doses based on trough concentrations, it is not helpful for determining the initial dose. Tacrolimus is metabolized by CYP3A5 to inactive metabolites. A patient with a CYP3A5*1/*3 genotype is predicted to be a CYP3A5 expresser; hence, CPIC guidelines recommend increasing the starting dose 1.5–2 times the recommended starting dose noting that the total starting dose

should not exceed 0.3 mg/kg/day. In addition to other clinical factors, knowing a patient's CYP3A5 genotype can help tailor the initial dose and increase the likelihood of achieving target tacrolimus concentrations [14].

Pharmacogenomics can also be utilized to select the medication that is predicted to work best for a patient when there are several options in a particular drug class to choose from, such as antiplatelet therapy (clopidogrel, prasugrel, ticagrelor) prescribed to patients after an acute coronary syndrome (ACS) or undergoing percutaneous coronary intervention (PCI) to prevent future cardiovascular events. Clopidogrel is a prodrug that is metabolized primarily (approximately 85%) by carboxylesterase 1 to an inactive metabolite but is also bioactivated via a two-step enzymatic process involving CYP2C19 to the active metabolite. Patients with the wild-type *CYP2C19**1 allele have functional CYP2C19 metabolism. Pharmacokinetic studies have demonstrated the impact of genetic variations in *CYP2C19* on the concentrations of the active metabolite of clopidogrel in both healthy volunteers and in patients with cardiovascular disease [15–17]. The key contribution of a common loss of function polymorphism (*2) for *CYP2C19* on the efficacy of clopidogrel was recognized almost a decade after initial FDA approval of clopidogrel in 1997. Among those of European and African ancestry, 25–30% carry at least one loss of function *2 allele versus up to 60% in Asians. Other less frequent losses of function alleles include *3, *4, *5, *6, *7, and *8. Carriers of *CYP2C19* loss of function alleles (e.g., *CYP2C19**2/*2) are at increased risk for major adverse cardiovascular events (MACE) (e.g., stent thrombosis, death, stroke, recurrent myocardial infarction) when prescribed clopidogrel due to reduced platelet inhibition and increased residual platelet aggregation [18–20]. In the absence of contraindications, prasugrel and ticagrelor are alternative antiplatelet treatment options for clopidogrel. The gain of function polymorphism (*17) in the promoter region of *CYP2C19* has been associated with increased CYP2C19 activity leading to higher concentrations of the active metabolite of clopidogrel and potentially increased risk of bleeding in ACS/PCI patients treated with clopidogrel [21, 22]. To date, CPIC guidelines currently recommend clopidogrel for an individual carrying two gain of function alleles (*17) or one functional allele (*1) plus one gain of function allele (*17).

While many of these pharmacogenomic examples are in reference to germline variations to guide treatment, tumor molecular profiling can also be performed to guide chemotherapy and other targeted therapies. For instance, in patients with unresectable or metastatic melanoma harboring BRAF V600E or V600K mutations, a signaling pathway (MAPK/ERK pathway) is hyperactivated leading to tumor cell growth and proliferation [23]. This hyperactivated signaling pathway can be targeted and suppressed by trametinib, a MEK inhibitor.

11.4 Implementing Pharmacogenomics into Clinical Practice

Healthcare centers implementing pharmacogenomics are currently taking one of two approaches. Pharmacogenomic testing can be performed and integrated into patient care activities either preemptively (before a drug is prescribed) or at the time a drug

is prescribed. With preemptive pharmacogenomic testing, a panel of genes are tested on a patient and stored in the electronic health record (EHR), so results are readily available for clinical decision-making at the time a medication is prescribed. The main idea behind preemptive pharmacogenomic testing is that from a single blood test or buccal swab, results are applicable over the patient's lifetime informing future treatments they may be placed on with pharmacogenomic implications. Another approach to implementing pharmacogenomics is to introduce a single gene at a time into the health system, providing immediate benefits from testing and increasing the likelihood that results are utilized to guide treatment. However, by the time a clinician determines a patient needs a medication impacted by pharmacogenomics, treatment delays can occur depending on lab turnaround times for results.

A clinical pharmacogenomic service can be implemented in a variety of settings including the hospital, outpatient clinics, and community pharmacy through different methods [24–27]. In the hospital or outpatient clinics, pharmacogenomics can be integrated into clinical decision support (CDS) tools to alert a physician that a pharmacogenomic test may be recommended for a medication that was ordered for a patient without a particular genetic test result on file. If pharmacogenomic results are available in the EHR, an alert may be built to warn the clinician of the risk for an adverse drug reaction or a patient that is unlikely to respond to a medication given a particular genotype and provide alternative recommendations compatible with the patient's genotype result. Alternatively, a separate database can be created that houses the patients' pharmacogenomic information but also serves as a drug-gene information resource with interpretations of the pharmacogenomic test results and recommendations on how to proceed with treatment, analogous to current electronic drug information resources available. In the community pharmacy setting, candidates for pharmacogenomic testing may be identified when dispensing medications to patients or through a medication therapy management service. A pharmacogenomic service can also be developed in partnership with medical geneticists and genetic counselors.

Activities involved in a clinical pharmacogenomic service include, but are not limited to:

1. Facilitating pharmacogenomic test orders
2. Interpreting genetic test results
3. Evaluating the literature to make evidence-based treatment recommendations guided by pharmacogenomics
4. Educating clinicians and counseling patients
5. Documenting interventions and patient outcomes
6. Developing the language for clinical decision support tools and consultation notes
7. Leading or serving on committees to advocate for the implementation of pharmacogenomics

The use of pharmacogenomic results in clinic is not always straightforward despite the availability of CPIC guidelines for key drug-gene pairs. When

determining whether a patient is a candidate for pharmacogenomic testing, factors to consider include necessity, availability of alternative treatments and a validated test to order, turnaround time, evidence supporting the drug-gene pair, acceptance from patients and providers, test reimbursement/payment, and documentation of care [28].

11.5 Resources for Launching a Clinical Pharmacogenomic Service

According to the ASHP Statement on the Pharmacist's Role in Clinical Pharmacogenomics released in [29], pharmacists "have a fundamental responsibility to ensure that pharmacogenomic testing is performed when needed and that results are used to optimize medication therapy." Pharmacists have a strong foundational knowledge of medication therapy management and drug-drug interactions. Recognizing drug-gene interactions and individualizing treatment to a patient's genetic makeup is a natural extension of a pharmacist's role [30].

In many of the pharmacogenomic implementation efforts occurring in the USA, pharmacists serve as the driving force for assembling a multidisciplinary team. Key team members include clinician champions, informatics, lab personnel, patients, and institutional leadership. Clinician champions provide insight on how to seamlessly incorporate pharmacogenomic testing into the current workflow processes and the development of treatment algorithms guided by pharmacogenomics. A dedicated informatics pharmacist or group assists in the design of the clinical decision support tools. Depending on the lab resources available at your disposal, pharmacogenomic testing may be performed internally or outsourced to a CAP-/CLIA-certified commercial lab, which can provide single-gene or multigene panel results. Keep in mind that reimbursement or coverage for pharmacogenomic tests from a commercial lab varies and should be discussed with the patient upfront before a genetic test is ordered. Educational outreach endeavors are essential for raising awareness among healthcare providers and the community on the benefits of pharmacogenomics. Institutional leadership provides a platform in support of implementation efforts.

A major resource for implementing pharmacogenomics into clinical practice is the CPIC guidelines. The CPIC guidelines serve as a blueprint for integrating pharmacogenomics into patient care. Along with a compilation of the literature to support a drug-gene pair, the supplement of each CPIC guideline houses workflow diagrams and example CDS alerts and consults that can be modified and tailored to the use of each institution. CPIC guidelines can be found at https://www.pharmgkb.org/.

Lastly, an important component to launching a clinical pharmacogenomic service is demonstrating the clinical utility of pharmacogenomics. Data can be

prospectively collected on how genetic results altered clinical management and capture information on drug-related outcomes. For example, a recent clinical study demonstrated that CYP2C19 genotype-guided antiplatelet therapy reduced major adverse cardiovascular events.[4]

Resource	Site
APhA DrugInfoLine, Pharmacogenomics Corner	http://www.aphadruginfoline.com/pharmacogenomics¬corner
American Society of Health-System Pharmacists	http://www.ashp.org/menu/PracticePolicy/ResourceCenters/Emerging-Sciences/Pharmacogenomics.aspx
Clinical Pharmacogenetics Implementation Consortium Guidelines	http://www.pharmgkb.org/page/cpic
Genetics/Genomics Competency Center (G2C2)	http://g-2-c-2.org/
Genetics Science Learning Center	http://learn.genetics.utah.edu/
National Human Genome Research Institute	http://www.genome.gov/
P450 Drug Interaction Table	http://medicine.iupui.edu/clinpharm/ddis/main-table/
Personalized Medicine Coalition	http://www.personalizedmedicinecoalition.org
PharmGenEd at UC, San Diego	https://pharmacogenomics.ucsd.edu
Pharmacogenomics Knowledgebase (PharmGKB)	http://www.pharmgkb.org/
SNPits	http://personalizedmedicine.ufhealth.org/tag/snpits/
WarfarinDosing	http://warfarindosing.org/Source/Home.aspx

Conclusion

Research and innovative practice models will continue to drive pharmacogenomics into clinical practice to overcome barriers for widespread adoption and optimize treatment. Treatment tailored to a patient's genetic makeup, in addition to other clinical factors, can help prevent adverse drug events, identify an initial starting dose, and predict the likelihood a patient will respond to a particular medication. Institutions across the USA and the world are working toward making pharmacogenomics a clinical reality with guidance documents (CPIC, DPWG) enabling implementation capturing the growing evidence along with the drop in the cost of sequencing.

[4] Cavallari LH, Magvanjav O, Anderson RD, et al. Clinical implementation of CYP2C19 genotype guided antiplatelet therapy reduces cardiovascular events after PCI. Circulation 2015;132:A11802.

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Pharmacogenomics and Pharmacoepigenomics: Impact on Therapeutic Strategies

12

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Abstract

Recent studies suggest that adverse drug reactions (ADRs) are a major cause of death and disability. Furthermore, when medications cause no harm, they may be ineffective leading to undesirable consequences [1]. Pharmacogenomics and personalized medicine have the potential to minimize ADRs and improve healthcare quality by tailoring pharmacotherapy to individual patients. Although pharmacogenomic testing is considered a burden on therapeutic cost, one-time pharmacogenomic testing for asymptomatic patients was shown to be cost-effective to minimize lifetime ADRs for a given age group [2]. This should stimulate and encourage preemptive genotyping especially with the recent advances and cost reduction in genomic sequencing technologies.

12.1 Introduction

Recent studies suggest that adverse drug reactions (ADRs) are a major cause of death and disability. Furthermore, when medications cause no harm, they may be ineffective leading to undesirable consequences [1]. Pharmacogenomics and personalized medicine have the potential to minimize ADRs and improve healthcare quality by tailoring pharmacotherapy to individual patients. Although pharmacogenomic testing is considered a burden on therapeutic cost, one-time pharmacogenomic testing for asymptomatic patients was shown to be cost-effective to minimize

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_12

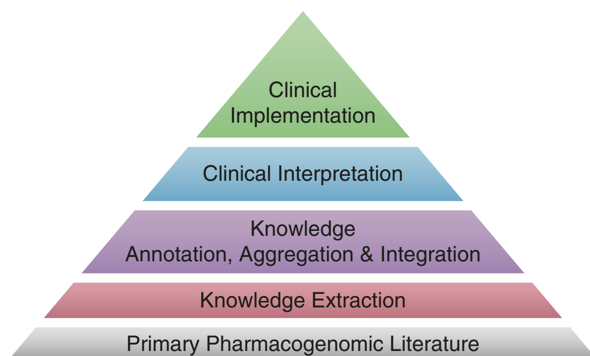

Fig. 12.1 The PharmGKB Knowledge Pyramid. The pyramid describes the sequential steps of translating knowledge from the pharmacogenomics literature into useful clinical guidelines

lifetime ADRs for a given age group [2]. This should stimulate and encourage pre-emptive genotyping especially with the recent advances and cost reduction in genomic sequencing technologies.

One barrier to the clinical implementation of pharmacogenetics is the lack of peer-reviewed, updatable, and detailed gene/drug clinical practice guidelines. The development of database resources and consortia facilitated the implementation of recent findings in pharmacogenomic research into clinical settings. The PharmGKB is an example of such database that provides a wealth of information about drug dosing and genetic variations. A visual representation of the steps involved in acquiring and integrating the information from the literature into clinical practice by the PharmGKB is depicted in Fig. 12.1. The PharmGKB is a partner of the NIH Pharmacogenomics Research Network (PGRN) research consortium. It helps clinicians understand the impact of genetic variations on ADRs and implement discoveries in pharmacogenomics into the clinical settings. The Clinical Pharmacogenetics Implementation Consortium (CPIC) is one of the projects of the PharmGKB that provides peer-reviewed dosing guidelines based on the patients' genetic profile. Additionally, the CPIC classifies and assigns a level of strength that ranges from A to D for drugs based on their need for pharmacogenetic testing. For drugs at the A level, pharmacogenomics should be used to affect medication prescribing. For B level, pharmacogenetic data may potentially be used toward that end. For levels C and D, no prescribing action is recommended because of weak or little evidence that support the need for such test, respectively.

12.2 Implications of Pharmacogenes in Therapeutics

Pharmacogenes are genes that affect drug disposition and action [3]. Genetic variations in metabolizing enzymes, transporters, and receptors are associated with variations in drug response and ADRs. Next-generation DNA and RNA sequencing are techniques that facilitate the analysis of the whole genome sequence and the

transcript level with an unprecedented resolution, respectively [4]. Advances in genomic sequencing technologies reduced the cost of sequencing a single base [5] significantly, and whole genome sequencing (WGS) is approaching the key goal of achieving the $1000 genome [6]. The NIH-supported PGRN initiated a genome-wide RNA sequencing project to report variations in gene expression and splicing of pharmacogenes across individuals in different tissues (liver, kidney, heart, and adipose tissues).

12.3 Polymorphism of Drug-Metabolizing Enzymes

Drug metabolism involves the addition of polar groups during phase I of metabolism followed by phase II conjugation reactions. Finally, the conjugated drugs are transported out of the cells by efflux transporters in phase III. Phase I reactions include oxidation, hydrolysis, reduction, hydroxylation, and addition of oxygen or removal of hydrogen, carried out by mixed function oxidases. Cytochrome P450 monooxygenases (CYP450) play an important role in phase I reactions. Genetic variations or polymorphism in CYP450 contribute to the interindividual variations observed in drug metabolism. Accordingly, it is essential to have a common nomenclature for CYP450 genetic variants and a system that allows researchers to be rapidly updated within the field. The Human Cytochrome P450 (CYP) Allele Nomenclature Committee website http://www.cypalleles.ki.se/ is responsible for cataloging the different polymorphs and updating their database with novel alleles from recent peer-reviewed literature. The CYP allele website offers a rapid online publication of new alleles, provides an overview of peer-reviewed data, and serves as a form of quality control on research on new alleles. Figure 12.2 depicts the relative contribution of the different CYP450 alleles to drug metabolism. A very useful tool to predict efficacy and/or adverse effects of drugs is the abbreviated P450 Drug Interaction Table also known as the “clinically relevant” table. The table established by the Clinical Pharmacology Research Institute at Indiana University is available at http://medicine.iupui.edu/CLINPHARM/ddis/clinical-table and consists of eight columns that list the drug names that are substrates for eight different CYP450 enzymes. The table also lists drugs that induce or inhibit CYP450 enzymes and further classify them into strong, moderate, or weak according to pharmacokinetic parameters like plasma AUC and clearance.

Cytochrome P450 enzymes are responsible for the phase I metabolism of several endogenous and exogenous substrates. CYP2C19 is a highly polymorphic enzyme of the CYP2C subfamily and is involved in the metabolism of drugs like antidepressants, proton-pump inhibitors, and warfarin and activation of the platelet aggregation inhibitor prodrug clopidogrel. Interindividual and interethnic variability in the metabolism of these drugs by poor metabolizers or ultrarapid metabolizers is common and results in variable clinical response. The impact of CYP2C19 genetic polymorphisms is not restricted to the metabolism of drugs as it could affect the equilibrium of endogenous compounds. For instance, increased CYP2C19 activity influenced the brain development and affective behavior [7] and was associated

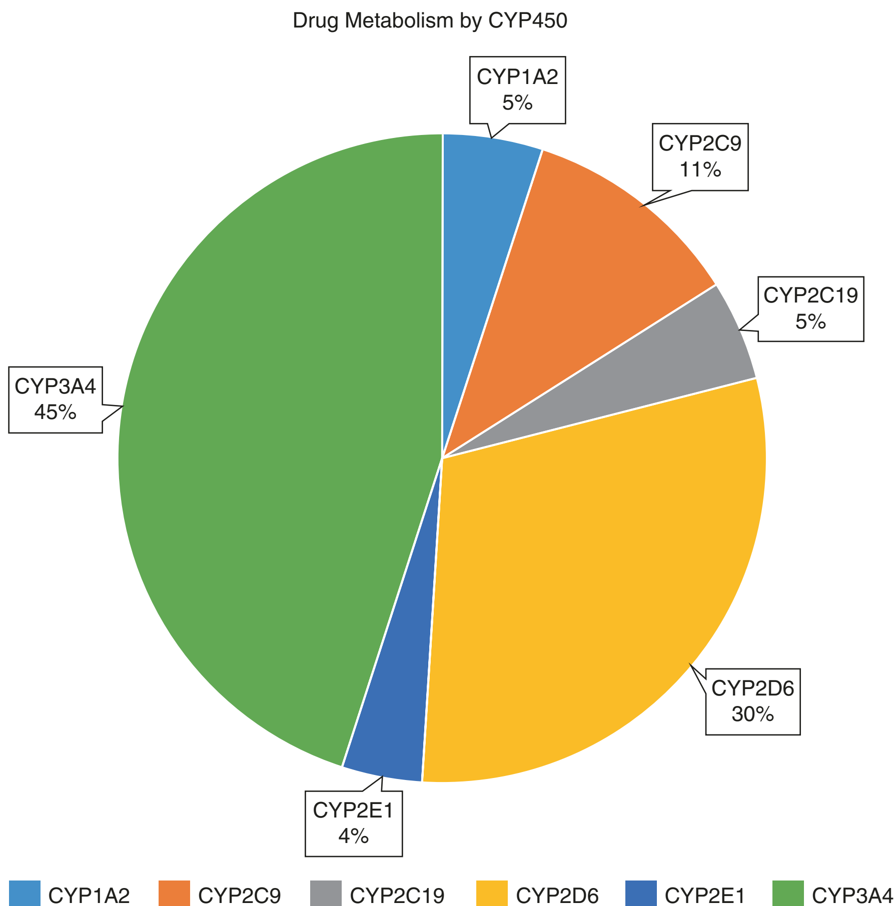

Fig. 12.2 CYP450 isoforms' relative contribution to drug metabolism. The pie chart depicts the approximate relative contribution of different CYP450 isoforms to the metabolism of xenobiotics

with depressive symptoms [8]. The most common poor metabolizer phenotypes encode for nonfunctional proteins and are known as CYP2C19*2 and CYP2C19*3. On the other hand, CYP2C19*17 was reported to be associated with ultrarapid CYP2C19 activity [9]. Two single nucleotide polymorphisms (SNPs) are specific for the CYP2C19*17 allele (−806C > T and −3402C > T), and evidence supports the involvement of the −806 site mutation in increased enzyme transcription [10]. The allele frequency of the ultrarapid enzyme varies highly among different populations, being low in Japanese and Chinese and higher in Europeans and Ethiopians.

CYP2D6 is another highly polymorphic enzyme of the CYP2D subfamily. Tamoxifen is a prodrug that binds to estrogen receptors and inhibit the transcription of estrogen-responsive genes [11]. Tamoxifen is used to treat and prevent breast cancer in both women and men. It is metabolized by several CYP isoforms to form several metabolites, including 4-hydroxy-tamoxifen, N-desmethyl-tamoxifen, and

4-OH-N-desmethyl-tamoxifen (endoxifen) [12, 13]. Both endoxifen and 4-OH-tamoxifen are active metabolites and have 100-fold higher affinity to the estrogen receptor compared to the parent drug [14, 15]. CYP2D6 plays a major role in endoxifen formation, and a strong association between CYP2D6 genotype and plasma levels of endoxifen was reported [16]. *CYP2D6* is highly polymorphic with more than 100 genetic variants leading to interindividual variability of enzyme activity [17]. CYP2D6 alleles with reduced or lost function result in the intermediate metabolizer and the poor metabolizer phenotypes, respectively. On the other hand, functional and duplicated *CYP2D6* alleles correlate with extensive and ultrarapid metabolizer phenotypes, respectively [18]. Indeed, homozygous wild-type subjects demonstrated a fourfold difference in endoxifen concentration compared with subjects homozygous for the nonfunctional CYP2D6 variants [19, 20]. Nonetheless, studies assessing the association between CYP2D6 genotype with patient outcomes to tamoxifen are not consistent. A significant association between CYP2D6 genotype and overall survival was not discovered in a retrospective study [21].

12.4 Pharmacogenetics and Anticancer Therapeutics

Pharmacogenetics and pharmacogenomics predicted survival and tumor progression in cancer patients. Additionally, the response and adverse effects related to the administration of monoclonal antibodies and chemotherapy in cancer patients were also predicted using these “omics” approaches. The cytotoxicity of monoclonal antibodies is mediated through antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity [10]. ADCC triggers tumor cell death by the binding of the Fc portion of an antibody-bound to tumor cells to Fc receptors (FcγR) on immune effector cells. The impact of FcγR polymorphism on clinical response was extensively studied for rituximab in non-Hodgkin’s lymphoma. SNP at residue 158 of the FcγRIIIA receptor determined clinical response to rituximab. This gene dimorphism results in either a phenylalanine (F) or a valine (V) at residue 158 of the receptor, and better clinical responses were observed with 158VV genotype compared to those with 158F genotype [22]. Trastuzumab is an anti-HER2 monoclonal antibody that was developed for treating specific population of breast cancer patients. The clinical use of trastuzumab is coupled to a diagnostic test that measures the expression level of HER2 receptor and consequently determines whether a patient with breast cancer would benefit from the drug. Nevertheless, the clear dependence of clinical response on the expression of the receptor does not always prevail. For instance, a correlation between epidermal growth factor receptor (EGFR) expression of and the efficacy of cetuximab is not well-established [23].

Genetic polymorphism of metabolizing enzymes is a major source of ADRs. Thiopurine methyltransferase (TPMT) metabolizes 6-mercaptopurine (6-MP) and its prodrug azathioprine into the inactive metabolite 6-methylmercaptopurine (6-MMP). Competition with TPMT for the metabolism of 6-MP by enzymes like hypoxanthine-guanine phosphoribosyltransferase (HPRT) converts 6-MP into a toxic metabolite known as thioinosine monophosphate (TIMP) that causes

myelosuppression. The therapeutic efficacy and toxicity of 6-MP can be predicted by studying TPMT polymorphism. The CPIC provided recommendations for 6-MP dosing based on TPMT phenotype [24]. The TPMT phenotype was classified into three categories: the homozygous wild type (genotype: two functional alleles), the heterozygous phenotype (genotype: one functional and one nonfunctional allele), and the homozygous variant type (genotype: two nonfunctional alleles). TPMT*3A is the most common variant in Caucasians and together with other allelic variants exhibit lower catalytic activity compared to wild-type allele due to lower stability and enhanced proteasomal degradation [25].

Measuring the enzymatic activity of TPMT in red blood cells as a phenotype test could substitute for TPMT genotype testing [26]. For instance, perfect concordance between phenotype and genotype testing was observed in adult patients using 6-MP. However, discordance was observed in leukemia patients and those receiving chemotherapy, and consequently genotyping is preferred [27]. Furthermore, TPMT genotyping demonstrated superiority over TPMT activity in predicting treatment outcome [28–30].

5-fluorouracil (5-FU) is widely used in the treatment of colorectal cancer. Dihydropyrimidine dehydrogenase (DPYD) enzyme converts 5-FU into an inactive metabolite. DPYD is the rate-limiting enzyme in pyrimidines catabolism and the synthesis of β-alanine [31]. DPYD polymorphism may reduce the metabolism of 5-FU with consequent ADRs like neutropenia, neurological symptoms, and death [32, 33]. DPYD*2A is the most common DPYD polymorphism associated with impaired DPYD activity and truncated protein [34]. Despite association of DPYD polymorphisms with severe 5-FU toxicity, about one- to two-thirds of patients who experienced treatment toxicity do not have a molecular basis for DPYD deficiency [35]. Consequently, the implementation of DPYD pharmacogenetic testing is not recommended currently.

UDP-glucuronosyltransferases belong to phase II detoxifying enzymes that catalyze the glucuronidation of lipophilic metabolites. Glycosylation converts lipophilic compounds into more soluble metabolites to enhance their renal elimination. The *UGT1* gene expresses several functional UGT1A proteins with different enzymatic activities by alternative splicing mechanism [36]. UGT1A1 is the major isoform responsible for the glucuronidation of bilirubin and SN-38, the active metabolite of the topoisomerase 1 inhibitor irinotecan [37], which is used in the treatment of colon cancer. Reduced glucuronidation of SN-38 by the genotype UGT1A*28 resulted in higher frequencies of ADRs like diarrhea and neutropenia [38, 39].

EGFR signaling plays an important role in angiogenesis and the proliferation of cancer cells. Dysregulation and overexpression of the epidermal growth factor receptor (EGFR) are frequent in a number of epithelial cancers including non-small cell lung cancer (NSCLC) and head and neck cancer [40–42]. Gefitinib and erlotinib are orally active EGFR tyrosine kinase inhibitors (TKI) approved for use as second- or third-line therapy in advanced NSCLC. The Japanese patients showed higher rates of response to gefitinib compared to Caucasians [43]. Surprisingly, the response did not correlate with EGFR expression [44]. Most patients who responded to gefitinib and erlotinib had somatic mutations clustering around the ATP-binding

site in the tyrosine kinase domain of the EGFR [45]. It is postulated that these mutations stabilize the interaction between the drugs and the tyrosine kinase domain. Other studies linked EGFR-TKI sensitivity to increased EGFR gene copy number in lung cancer where high EGFR gene copy number was associated with better response and survival when treated with gefitinib [46, 47]. Table 12.1 summarizes the different classes of drugs and the genes of interest for which the FDA requires pharmacogenetic testing.

Table 12.1 Drugs from different classes that require pharmacogenetic testing by the FDA

Drug class	Examples	Gene(s) of interest	Reason for use
	Endocrine/metabolic disorder		
Synthetic metabolite/substrate (endocrine)	Carglumic acid, cholic acid, eliglustat	*NAGS, AKR1D1, CYP27A1, CYP2D6*	Targeted therapy
Recombinant enzymes	Elosulfase alfa, rasburicase, velaglucerase alfa	*GALNS, G6PD, GBA*	Targeted therapy/ safety
Immunosuppressants	Everolimus	*ERBB2, ESR1, ESR2*	Targeted therapy
Hyperammonemia agents	Sodium phenylacetate/ sodium benzoate, sodium phenylbutyrate	*ASS1, OTC, ASL, NAGS*	Targeted therapy
	Infectious disease		
Antiretroviral agents	Abacavir, maraviroc	*HLA-B*5701, CCR5*	Safety
	Neurology		
Anticonvulsants	Carbamazepine, divalproex sodium	*HLA-B*1502, POLG*	Safety
Antipsychotics	Pimozide	*CYP2D6*	Dosing
Monoamine depletion	Tetrabenazine	*CYP2D6*	Dosing
	Oncology		
Aromatase inhibitors	Anastrozole, exemestane, letrozole	*ESR1, ESR2, PGR*	Targeted therapy
Cyclin-dependent kinase inhibitors	Palbociclib	*ERBB2, ESR1*	Targeted therapy
Estrogen receptor antagonists	Fulvestrant	*ESR1, PGR*	Targeted therapy
Interleukin-2 receptor ligands	Denileukin diftitox	*IL2RA*	Targeted therapy
Monoclonal antibodies	Cetuximab, panitumumab, pembrolizumab, pertuzumab, trastuzumab	*EGFR, KRAS, HER2*	Targeted therapy
Polyadenosine 5-diphosphoribose polymerase (PARP) inhibitors	Olaparib	*BRCA1, BRCA2*	Targeted therapy
Serine/threonine kinase inhibitors	Cobimetinib, dabrafenib, vemurafenib	*MAPK, MEK1, MEK2, BRAF*	Targeted therapy

12.5 Pharmacoepigenomics and Therapeutics

Recent evidences support the involvement of epigenetics in the variable response to drugs and ADRs [48]. DNA methylation, histone posttranslational modifications, and microRNAs (miRNA) gene expression play an important role in modulating the gene expression of drug metabolizing enzymes, receptors, and transporters. It is expected that most of the unexplained interindividual differences to drug response or toxicity may be related to epigenomics.

DNA methylation was shown to affect the expression of CYP450 enzymes. CYP1A1 methylation in the lung was shown to increase after smoke quitting, suggesting that DNA methylation regulates CYP1A1 induction after tobacco smoking [49]. Interleukin-6 (IL-6) induced CYP1B1 enzyme which promoted colorectal cancer development via activation of chemical carcinogens [50]. The mechanism of induction involved epigenetic silencing of the miR27b by DNA methylation, which targets the CYP1B1 gene. CYP24A1 is the rate-limiting catabolic enzyme for vitamin D3, which demonstrated antiproliferative effect in lung cancer. CYP24A1 was overexpressed in human lung adenocarcinoma, and its expression and enzyme activity were regulated by both DNA methylation and histone modifications [51]. Furthermore, DNA methylation was also shown to be associated with key regulatory CYP3A4 promoter regions and contributed to the commonly observed interindividual expression of CYP3A4 [52].

Histone modifications were also associated with epigenetic mechanisms involved in interindividual differences to drug metabolism or response and differential tissue expression. Chromatin immunoprecipitation assay demonstrated that the histone H3 associated with the promoters of the mouse transporters Oatp1b2, Ntcp, Bsep, and Abcg5/g8 was hyperacetylated in the liver and less acetylated in the kidney and cerebrum, suggesting the involvement of histone acetylation in the tissue-specific expression of these transporters [53]. Histone acetylation was also shown to be involved in CYP2E1 gene expression, which plays a role in the multistep process of liver carcinogenesis [54]. Histone H3 methylation at lysine 27 (H3K27) by the polycomb repressive complex (PRC2) was shown to silence the gene expression of CYP2C9 [55], which is involved in the metabolism of many exogenous and endogenous substrates.

miRNAs are small noncoding RNA molecules (about 22 nucleotides) found in plants, animals, and some viruses that function in RNA silencing and posttranscriptional regulation of gene expression [56]. miRNAs could target the mRNA of the enzymes involved in DNA methylation like DNMT3a and DNMT3b leading to their degradation and consequent DNA hypomethylation. For instance, miR-29, miR-29c, miR-370, and miR-450A target DNMT3a, while miR-29, miR-148, and miR-29b target DNMT3b [57, 58]. miRNA could also target the mRNA of the histone deacetylase (HDAC) enzymes. For instance, miR-449 and miR-874 target HDAC1, while miR-1 and miR-155 target HDAC4 [59]. miRNAs could also affect the expression of drug efflux transporters. The expression of miR-27a and miR-451 was upregulated in multidrug-resistant (MDR) cancer cell lines and upregulated the expression of MDR1 mRNA and its encoded protein P-glycoprotein [60].

In parallel to pharmacogenomics, pharmacoepigenomics can provide us with novel mechanisms that describe interindividual differences in drug response or toxicity. It can also identify novel biomarkers that can be useful for understanding drug response. However, the implementation of the recent discoveries in epigenetics and epigenomics in the clinical settings remains a challenge.

Conclusion

Recent advances in pharmacogenomics and pharmacoepigenomics explained the mechanisms of variability in drug response and toxicity. The clinical implementation of these discoveries played an important role in the development of personalized medicine. The utility of these discoveries is demonstrated in predicting the efficacy of drug treatment, predicting toxicity, and titrating an effective medication dosage. Although the therapeutic cost of pharmacogenetic testing is a burden, the cost reduction associated with proper prescribing and safety improvement associated with its use justify its clinical utility. Nonetheless, the lack of consensus on how to apply and utilize the results from pharmacogenetic testing is a drawback that needs to be addressed.

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13 Clinical Pharmacogenomics and Personalized Medicine: New Strategies to Maximize Drug Efficacy and Avoid Adverse Drug Reaction

Chonlaphat Sukasem and Sadeep Medhasi

Abstract

Genetic variability among drug-metabolizing enzymes (DMEs) and transporters influences the pharmacokinetics of the drug and is associated with marked interindividual variability in therapeutic effects and toxicity. Therapeutic drug monitoring (TDM) can facilitate the individualization of dose adjustment of the drug by measuring the plasma concentrations of drug. TDM can be incorporated with the pharmacogenomics, and the metabolic status of the patient can be characterized to optimize the dosage regimen according to the patient's needs. Several polymorphisms among *cytochrome P450 (CYP)* and phase II enzymes that contribute to the adverse drug reactions (ADRs) have been updated on a regular basis in PharmGKB. A number of pharmacogenomic markers are reported by the Food and Drug Administration and Clinical Pharmacogenetics Implementation Consortium (CPIC) among DMEs for commonly used drugs that are potentially associated with variability in drug response. This review focuses on the genetic polymorphisms of phases I and II DMEs and their associations with drug responses. The drugs discussed in this review requiring a pharmacogenomic test before being prescribed includes efavirenz, voriconazole, clopidogrel, warfarin, tamoxifen, irinotecan, tacrolimus, azathioprine, and risperidone. This chapter also presents the application of pharmacogenomics in the clinic and patient counseling. Finally, a section focuses on the future perspectives of pharmacogenomics and the translation of pharmacogenomic research into routine clinical care.

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_13

13.1 Introduction

Adverse drug reactions (ADRs) are classified as either type A, pharmacological dose dependent, or type B, idiosyncratic [1]. Interindividual genetic differences among the genes encoding for Drug-metabolizing enzymes (DMEs), drug transporters, and drug targets contribute as risk factors for ADRs due to the alterations in pharmacokinetics and pharmacodynamics of the drug [2]. Therapeutic drug monitoring (TDM) refers to the optimization of clinical outcomes in patients by measuring the drug levels in blood with appropriate medical interpretation and medication regimen [3]. The definable drug concentration-response relationships in several clinical studies explain the incorporation of pharmacogenomics information to optimize therapeutic effects while minimizing side effects. Genetic variants have prominent effects on drug responses because of the modulation in gene expression, mRNA processing and stability, and protein structure. Genetic polymorphisms in DMEs and drug transporters alter the protein structures and thereby exhibit considerable differences in plasma and target tissue concentrations [4]. Pharmacogenomic studies have provided evidence of causal relations between genotypes and plasma concentrations of drugs which account for differences in response. By incorporating pharmacogenomic data and serum concentrations of drugs, individual therapeutic regimens for optimal patient benefits with an increase in number of responders and decrease in patients affected by ADRs can be achieved.

The advent of different techniques and advancements in genotyping technology allows the identification of genetic polymorphisms contributing to the variations in pharmacokinetic parameters. There are several genotyping technologies currently in use which allow for genomic interrogation among the pharmacokinetic genes responsible for changes in plasma drug concentrations. These methods include TaqMan® Drug Metabolism Genotyping Assays, Affymetrix DMET Plus array, Prometheus TPMT genotyping, Illumina Golden Gate chip, Sequenom iplex Gold MassARRAY, Sanger sequencing, and Beckman Coulter GenomeLab SNPstream [5, 6].

The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Royal Dutch Pharmacists Association-Pharmacogenetics Working Group (DPWG) have published evidence-based guidelines for dosing and drug selection based on genetic biomarker information. The Pharmacogenomics Knowledgebase (PharmGKB) has the updated pharmacogenomic associations of genetic variants and phenotypes with dosing guidelines along with the US Food and Drug Administration (FDA) label information for selected drugs (https://www.pharmgkb.org/). Currently, pharmacogenomic-oriented TDM can predict drug concentrations for multiple drugs, including efavirenz, voriconazole, clopidogrel, warfarin, tamoxifen, irinotecan, tacrolimus, risperidone, and azathioprine. Among many, clinically important pharmacogenes associated with the plasma concentrations of drugs and explained in PharmGKB database include *cytochrome P450 2B6* (*CYP2B6*), *CYP2C19*, *CYP2D6*, and *CYP3A5*, the *UDP glucuronosyltransferase family 1*

member A1 (*UGT1A1*), and *thiopurine S-methyltransferase* (*TPMT*). This pharmacogenomic information can be used to evaluate and individualize dosage regimens. This article will review the evidence of pharmacogenomic associations for the risk of ADRs with the changes in plasma concentrations of drugs due to the altered pharmacokinetic characteristics.

13.2 Genetic Variations in DMEs and Transporters

DMEs and transporters function together in determining the plasma and tissue concentrations of drugs and their metabolites. Genetic variations in genes encoding metabolic enzymes phases I and II are major factors influencing plasma drug concentrations and drug responses. Several clinically relevant polymorphisms among phases I and II enzymes have been documented among several populations, and the effects from the polymorphisms on the pharmacokinetics of drugs along with consequences have been reported (Table 13.1) [7–13]. CYP450 enzymes are highly polymorphic, and the polymorphism includes copy number variations, small insertions and deletions, and single-nucleotide polymorphisms (SNPs) [14]. Based on DMEs activity, individuals can be classified into four phenotypes: poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultrarapid metabolizers [15].

The phase II enzyme biotransformation reaction conjugates phase I metabolites and endogenous molecules in order to enhance renal or biliary excretion. Clinically relevant phase II enzymes include uridine diphosphate glucuronosyltransferase (UGT), sulfotransferase (SULT), glutathione S-transferases (GST), N-acetyltransferase (NAT), and TPMT. Polymorphisms among phase II enzymes result in diminished drug elimination and increased toxicities [16].

Considerable evidence exists regarding the polymorphisms among two transporter superfamilies: the solute carrier (SLC) transporters or influx transporters and the ATP-binding cassette (ABC) transporters or efflux transporters which are responsible for the interindividual differences in drug responses [17]. Drug transporters with significant genetic variants have been widely studied and include ABCB1, *ABCG2*, *SLC22A6*, *SLC22A8*, *SLC22A2*, *SLCO1B1*, and *SLCO1B3* [18]. A meta-analysis regarding the role of genetic variants among efflux transporters indicated the decreased risk of irinotecan-induced neutropenia among the carriers of *ABCB1* 2677G > T/G and an increased risk of irinotecan-induced diarrhea among the patients expressing *ABCG2* 34G > A [19]. Similarly, irinotecan-induced neutropenia was found to be highly prevalent among east-Asian patients expressing *SLCO1B1* 521T > C or 1118G > A in a meta-analysis of the role of polymorphisms among influx transporters in ADRs [20]. Consideration of polymorphisms among relevant transporters will aid in predicting the inter-patient variability in drug responses (Table 13.2).

Table 13.1 Pharmacogenomics of drug metabolism: the common variations and functional and clinical relevance

CYP gene	Common variations	Functional and clinical relevance
CYP1A1	*CYP1A1*2C*_2454A > G(I462V) *CYP1A1*_134G > A(G45D)	In *2C* variant, the AA genotype has decreased progression-free survival time compared to AG and GG genotypes in women with breast cancer treated with capecitabine and docetaxel
CYP1A2	*CYP1A2*1C*_−3860G > A(promoter) *CYP1A2*1F*_−163C > A(promoter) *CYP1A2*1K*_−739T > G(promoter) *CYP1A2*_5347T > C(N516N)	Genotypes AA and AG are associated with decreased metabolism of theophylline as compared to genotype GG in asthma patients for *1C* Allele A is associated with tardive dyskinesia in patients taking antipsychotic medications compared to allele G among smokers for *1C* polymorphism Patients with *1F* variants are associated with reduced serum concentration of olanzapine and subsequent decreased response
CYP2A6	*CYP2A6*9*_−48T > G(promoter) *CYP2A6*_51G > A(V17 V) *CYP2A6*_3570C > G *CYP2A6*_5336G > A	*9* is associated with increased plasma concentration of efavirenz
CYP2B6	*CYP2B6*2*_64C > T(R22C) *CYP2B6*_14593C > G *CYP2B6*4*_18053A > G(K262R) *CYP2B6*6*_15631G > T(Q172H) *CYP2B6*_18273G > A *CYP2B6*_21563C > T	*4* is associated with increased plasma concentration of efavirenz *6* is associated with increased efavirenz plasma concentrations
CYP2C19	*CYP2C19*2*_19154G > A(P227P) *CYP2C19*3*_17948G > A(W212X)	*2* and *3* is known to affect the metabolism and/or responses of several drugs, like amitriptyline, clopidogrel, sertraline, citalopram, and escitalopram
CYP2D6	*CYP2D6*_4180G > C(S486T) *CYP2D6*_2850C > T(R296C) *CYP2D6*_1661G > C(V136V) *CYP2D6*_100C > T(P34S) *CYP2D6*_−1584C > G *CYP2D6*_−2178G > A	Amitriptyline, nortriptyline, paroxetine, codeine, and tramadol are some of the drugs whose pharmacokinetics, response, and toxicity are known to be influenced by *CYP2D6* polymorphisms
CYP3A5	*CYP3A5*3*_6986A > G(SpliceDefect)	Patients with *3/*3* genotype show higher concentration of tacrolimus as compared to *1/*1* and *1/*3* genotypes
CYP3A7	*CYP3A7*2*_26041C > G(T409R)	Presence of *2* is associated with higher concentration/dose (C/D) ratio of tacrolimus

(continued)

Table 13.1 (continued)

CYP gene	Common variations	Functional and clinical relevance
UGT1A1	*UGT1A1*6*_c.211G > A(G71R) *UGT1A1*60*_c. −3279T > G(promoter) *UGT1A1*76*_c.*211C > T(3′UTR) *UGT1A1*79*_c.*440C > G(3′UTR) *UGT1A1*80*_c. −364C > T *UGT1A1*93*_c. −3156G > A(promoter)	**6* is associated with increased risk of neutropenia when treated with irinotecan **93* is associated with increased risk of hematologic toxicity in irinotecan-treated patients
UGT1A7	*UGT1A7*12*_c. −57 T > G(5′UTR)	**12* is associated with increased risk of hyperbilirubinemia when treated with atazanavir
UGT2B7	*UGT2B7*2*_c.802C > T(H268Y) *UGT2B7*3*_c.211G > T(A71S)	Genotype TT shows better response to lorazepam and valproic acid as compared to genotype CC in **2* polymorphism [47] **3* is associated with decreased clearance of carvedilol [48]
GSTA5	*GSTA5*_c. −31 + 2057C > T *GSTA5*_−8526G > T	rs4715354 and rs7746993, both combined, are associated with decreased busulfan clearance
GSTP1	*GSTP1*B*_c.313A > G(I105V)	**B* is associated with increased response and decreased severity of toxicity among breast cancer patients treated with cyclophosphamide and epirubicin Efficacy and/or toxicity of oxaliplatin, methotrexate, and fluorouracil is associated with **B* variant
GSTZ1	*GSTZ1*_c.245C > T(T82 M) *GSTZ1*_c.94G > A(E32K)	rs7975 and rs1046428 are associated with clearance of dichloroacetic acid
NAT2	*NAT2*_c.481C > T(L161 L) *NAT2*5*_c.341 T > C(I114T) *NAT2*6*_c.590G > A(R197Q) *NAT2*7*_c.857G > A(G286E) *NAT2*13*_c.282C > T(Y94Y)	rs1799929 variant is associated with hepatotoxicity when treated with antituberculosis drugs **5*, **6*, **7*, and **13* influence the metabolism of isoniazid

13.3 Current Metabolic Enzyme Pharmacogenomic Biomarkers for Drugs

13.3.1 Efavirenz

Efavirenz is a non-nucleoside reverse transcriptase inhibitor prescribed for the treatment of human immunodeficiency virus (HIV) infection. Efavirenz is a viable candidate for TDM because of its interindividual variability in plasma concentrations. Efavirenz plasma concentrations <1 μg/mL have been linked with virological

Table 13.2 Pharmacogenomics of drug transporter genes: the common variations and functional and clinical relevance

Transporter genes	Common variations	Functional and clinical relevance
ABCB1 (MDR1)	*ABCB1*_c.1236C > T(G412G) *ABCB1*_c.3435C > T(I1145I) *ABCB1*_c.IVS9-44A > G	1236C > T is associated with overall increased survival period among oxaliplatin-treated patients with colorectal neoplasms 3435C > T is associated with increased serum concentration of digoxin and nevirapine-induced hepatotoxicity rs10276036 is associated with increased risk of death in osteosarcoma patients after chemotherapy
ABCC1 (MRP1)	*ABCC1*_c.*1512T > C	rs212091 is associated with virological failure in antiretroviral drug therapy
ABCC2 (MRP2)	*ABCC2*_c. −24C > T(5′UTR) *ABCC2*_c.1249G > A(V417I) *ABCC2*_c.3972C > T(I1324I)	rs717620 and rs3740066 affect the response to antiepileptic drugs rs717620 influences the metabolism of erythromycin and is associated with toxicity among patients treated with fluorouracil, leucovorin, and oxaliplatin rs2273697 influences the pharmacokinetics of talinol and irinotecan
ABCG2 (BCRP)	*ABCG2*_c.421C > A(Q141K)	rs2231142 is associated with the plasma concentration of rosuvastatin
SLC22A1 (OCT1)	*SLC22A1*_c.156T > C(S52S) *SLC22A1*_c.480C > G(F160L) *SLC22A1*_c.1022C > T(P341L) *SLC22A1*_c.1222G > A(V408M)	c.480C > G polymorphism is associated with pharmacokinetics of imatinib rs628031 is associated with gastrointestinal side effects when treated with metformin
SLCO1B1 (OATP1B1)	*SLCO1B1**17_c.-11187G > A(promoter) *SLCO1B1**1B_c.388A > G(N130D) *SLCO1B1**5_c.521T > C(V174A) *SLCO1B1*_c.571T > C(L191L)	**17* is associated with the efficacy and pharmacokinetics of pravastatin **5* is associated with high risk of muscular diseases when treated with simvastatin Other drugs associated with *5 variant include cerivastatin, pravastatin, and rosuvastatin

failure, whereas plasma concentrations >4 μg/mL have been associated with central nervous system-related toxicity [21]. CYP2B6 enzyme plays a major role in the metabolism of efavirenz, and the activity of CYP2B6 is highly variable among individuals. *CYP2B6* c.516G > T variant associated with reduced enzyme activity may be associated with higher plasma efavirenz concentrations among different ethnic populations. A recent cohort study conducted in Italian HIV-1 patients demonstrated that the patients with genotype TT had higher efavirenz concentrations [22]. This finding supports the results demonstrated in a study among Ghanaian patients where

patients with genotype TT showed a five times higher efavirenz concentration as compared to genotypes GG and GT [21]. In Thai populations, c.785A > G, g.21563C > T, and g.18492C > T also showed a significant association with efavirenz plasma concentrations in addition to the c.516G > T variant, as well as the haplotype containing 516G > T, 785A > G, and 21563C > T that was also a predictor of higher plasma efavirenz concentrations [23, 24].

13.3.2 Voriconazole

Voriconazole is a second-generation triazole antifungal agent used for the treatment of a wide spectrum of fungal infections, including invasive aspergillosis and candidiasis. Voriconazole is metabolized by CYP2C19 and CYP3A4, and genetic polymorphisms of *CYP2C19* have resulted in variable exposures of voriconazole across several populations [25]. The therapeutic range between 0.5 and <5.0 μg/mL for voriconazole trough plasma concentrations (C_{min}) has been considered to exhibit maximum efficacy with fewer neurological and hepatic side effects [26]. The DPWG guidelines recommend the monitoring of plasma concentrations of voriconazole for CYP2C19 in poor and intermediate metabolizers [27]. Wang et al. conducted a clinical trial in 144 individuals and assessed the effect of *CYP2C19* genotype on voriconazole C_{min} [28]. Poor metabolizers demonstrated significantly higher levels of voriconazole C_{min} as compared to extensive metabolizers and intermediate metabolizers. A study among Caucasians has reported a significant impact of *CYP2C19*2* (decreased CYP2C19 activity) and *CYP2C19*17* (increased CYP2C19 activity) on the voriconazole C_{min} and the mean voriconazole doses required to achieve the therapeutic ranges that were significantly higher in *CYP2C19*17* allele carriers as compared to *CYP2C19*1/*1* carriers [29]. A recent study among 115 Thai adults observed increased voriconazole C_{min} with the *CYP2C19*3*, the decreased CYP2C19 activity variant [30]. Nevertheless, the individuals carrying *CYP2C19*2* variant did not affect voriconazole C_{min}, and *CYP2C19*17* was not detected in this population. Pharmacogenomic testing for *CYP2C19* variants with the incorporation of TDM could help clinicians in achieving effective and nontoxic concentrations of voriconazole among the patients.

13.3.3 Clopidogrel

Clopidogrel, an oral antiplatelet agent used for the treatment of acute coronary syndrome and/or following percutaneous coronary intervention, is a prodrug requiring hepatic biotransformation by the enzyme CYP2C19 to form active metabolite, 2-oxoclopidogrel [31]. The CPIC guidelines imply that the individuals carrying two loss-of-function alleles, *2 and *8, of *CYP2C19* are likely to experience a diminished pharmacodynamic response and an increased risk of major adverse cardiovascular events compared to non-carriers [32]. A meta-analysis found *CYP2C19*17*, a gain-of-function allele, was associated with an increased risk of bleeding during

clopidogrel therapy [33]. In addition, DPWG recommends that patients who are *CYP2C19* poor metabolizers be considered for an alternative antiplatelet therapy [27]. Based on the accumulated evidence of pharmacogenomic influence on clopidogrel response, *CYP2C19* genotyping before clopidogrel therapy might provide a predictable level of drug exposure in the patient's body and ease the complications of its narrow therapeutic index.

13.3.4 Warfarin

Warfarin is a potent antithrombotic agent administered for the prevention and treatment of thromboembolic disorders. Due to the warfarin's narrow therapeutic index and high interindividual variability, there are possibilities of lethal bleeding and hemorrhage which complicates warfarin management and therapy. Dosing of warfarin is carefully monitored by maintaining the international normalized ratio (INR) of prothrombin time in blood between 2.0 and 3.0 [34]. Warfarin is a racemic mixture of *S*- and *R*-warfarin enantiomers, and *R*-warfarin, the more active isomer, is exclusively metabolized by CYP2C9. *CYP2C9*2* and *CYP2C9*3* are the two variants with clinical evidence linked with altered warfarin metabolism and an increased risk of bleeding complications [35, 36]. According to the guidelines recommended by CPIC, pharmacogenomic testing for *CYP2C9* and *VKORC1* is useful in determining optimal doses of warfarin to achieve an INR of 2.0–3.0 and avoid the risk of bleeding [37]. There is wide ethnic variation in the distribution of *CYP2C9*2* and *CYP2C9*3* alleles requiring variations in warfarin doses. Populations of African and American ancestry require larger doses of warfarin as compared to the Asians and Europeans [38].

13.3.5 Tamoxifen

Tamoxifen, a selective estrogen receptor modulator (SERM), is used in the treatment and prevention of estrogen receptor-positive (ER+) breast cancer by binding to the estrogen receptor (ER) and inhibiting the transcriptional activity of the ER [39]. Tamoxifen is a prodrug predominantly biotransformed to its major active metabolite, endoxifen, by the CYP2D6 enzyme. Variants in the *CYP2D6* allele can influence plasma concentrations of endoxifen and influence tamoxifen treatment outcome [40]. The DPWG has made recommendations to determine the optimal tamoxifen therapy for breast cancer patients based on *CYP2D6* genotypes [27]. Their recommendations include considering the use of aromatase inhibitors for postmenopausal women with poor and intermediate metabolizer genotypes because of increased risk of relapse of the breast cancer. In addition, they recommend not to use CYP2D6 inhibitor agents during tamoxifen therapy among intermediate metabolizer patients. The frequencies of *CYP2D6* variants are highly variable among different ethnic/racial groups. The variants encoding reduced function of CYP2D6 enzyme include **10*, **17*, **29*, *36, and **41*, whereas the variants producing a

nonfunctioning *CYP2D6* enzyme include **3*, **4*, **5*, **6*, and **40* [39]. *CYP2D6*10* is the most common variant among Asian populations as compared to the other populations [41–44]. *CYP2D6*17* is more prevalent in Africans and African-Americans, and *CYP2D6*4* is found in a higher frequency among European Caucasians as compared to other races [45]. In a study among the Spanish ER+ breast cancer patients, the plasma levels of endoxifen were found to be significantly lower among the poor metabolizer patients (*CYP2D6*4/*4*) as compared to the extensive metabolizers [46].

13.3.6 Irinotecan

Irinotecan is one of the most effective anticancer drugs that inhibit topoisomerase I and is a key drug used in the treatment of metastatic colorectal cancer [47]. Irinotecan is a prodrug and is biotransformed by carboxylesterases to an active metabolite SN-38. SN-38 then undergoes hepatic glucuronidation by the UGT1A1 enzyme to form an inactive compound SN-38G [48]. The side effects associated with the irinotecan treatment include severe neutropenia and severe diarrhea [49]. Studies have indicated the association of *UGT1A1* genotypes with a high risk of side effects and altered treatment response of irinotecan. *UGT1A1**1 is a wild type which has six thymine-adenine (TA) tandem repeats in the TATA box promoter region of *UHT1A1*. The polymorphism of TA repeats in the TATA box has been shown to reduce SN-38 glucuronidation and occurrence of severe adverse effects. *UGT1A1*6*, *UGT1A1*27*, and *UGT1A1*28* are associated with impaired SN-38 glucuronidation and increased irinotecan toxicity [50–52]. A retrospective study conducted on Thai metastatic colorectal cancer patients showed a significant association between irinotecan-induced neutropenia and patients with **6* and **28* variants [53]. A similar association was observed among the Japanese gynecologic cancer patients with the increased risk of neutropenia and/or diarrhea among carriers of *UGT1A1*6/*6* and *UGT1A1*6/*28* [54]. A guideline has been produced by the French joint working group comprised of the National Pharmacogenetics Network (RNPGx) and the Group of Clinical Onco-pharmacology (GPCO-Unicancer) and DPWG for dosing of irinotecan based on *UGT1A1*28* genotype [27, 50]. The groups have recommended dose reductions of irinotecan among the carriers of *UGT1A1*28/*28* receiving higher doses of irinotecan. The DPWG recommends reducing the initial dose by 30% for high-dose irinotecan (>150 mg/m^2)-receiving patients.

13.3.7 Tacrolimus

Tacrolimus, a calcineurin inhibitor, is used as an immunosuppressive medication to prevent solid organ transplant rejection [55]. Tacrolimus is extensively metabolized by CYP3A4 and CYP3A5 enzymes [56]. The CPIC guidelines have provided dosing recommendations for Tacrolimus based on the *CYP3A5* phenotype [57]. The

guidelines recommend increasing the starting dose of tacrolimus 1.5–2 times of the recommended starting dose with the total starting dose not exceeding 0.3 mg/kg/day among recipients with extensive or intermediate metabolizer phenotypes (CYP3A5 expresser). *CYP3A5*1* is associated with normal function of the enzyme and is expressed in approximately 70% among Africans, 30% among Asians, and 7% among Caucasians. *CYP3A5*3*, *CYP3A5*6*, and *CYP3A5*7* are associated with decreased CYP3A5 activity. The significant relationship between mean blood concentrations of tacrolimus ≤7 ng/mL with occurrence of acute graft-versus-host disease (GVHD) and incidences of non-relapse mortality (NRM) suggests the genotyping of patients and monitoring of tacrolimus concentrations [58]. A study among 25 South Indian adult renal transplant patients showed significant associations of blood concentrations of tacrolimus with acute rejection episodes [59]. *CYP3A5*1/*1* group (mean tacrolimus level, 5.154 ng/mL) had more acute rejection episodes as compared to *CYP3A5*1/*3* group (mean tacrolimus level, 5.348 ng/mL) and a *CYP3A5*3/*3* group (mean tacrolimus level, 9.483 ng/mL). Also, nephrotoxicity was more frequent among the poor metabolizers (CYP3A5 non-expressers) as compared to the CYP3A5 expressers. A genome-wide association study among the African-American kidney transplant recipients reported the significant influence of *CYP3A5*3*, *CYP3A5*6*, and *CYP3A5*7* in the trough blood concentrations of tacrolimus [60]. Similar results were reported in other studies with the influence of *CYP3A5* genotypes on blood concentrations of tacrolimus across several populations [61–64].

13.3.8 Azathioprine/6-Mercaptopurine

Azathioprine and 6-mercaptopurine (6-MP) are the thiopurine drugs used as anti-inflammatory, anticancer, and immunomodulating agents in solid organ transplant recipients [65]. Both azathioprine and 6-MP are prodrugs with no intrinsic biological activity. Azathioprine is converted to 6-MP, and TPMT is the enzyme responsible for the metabolism of both azathioprine and 6-MP in determining the rate and extent of metabolite formation and resulting drug response [66]. The patient with reduced functional activity of TPMT is at a high risk of severe bone marrow suppression supposedly due to the increased levels of 6-thioguanine nucleotides (TGN) [67, 68]. There are more than 40 genetic variant alleles of *TPMT*, and most of them are associated with lower TPMT enzyme activity (www.imh.liu.se/tpmtalleles). *TPMT**2, *TPMT**3A, *TPMT**3B, and *TPMT**3C are the most common alleles across several populations, including Caucasians, Asians, and Africans with lower TPMT activity [69]. Various pharmacogenomic guidelines have been devised to individualize the dosing of thiopurines to optimize the therapeutic efficacy and identify patients with increased risk for drug-induced toxicity based on TPMT activity status. The guidelines published by CPIC recommend a normal starting dose for thiopurines with dose adjustments for disease-specific guidelines among the carriers of homozygous wild-type alleles, 30–70%

of target dose and titration based on tolerance among the heterozygotes, altering the drug or reduction of the daily dose by tenfold, and dosing thrice weekly instead of daily if using thiopurine drugs among the carriers of homozygous mutant alleles [70]. DPWG has recommended selecting an alternative drug or reducing the initial dose by 50% for patients with the intermediate metabolizer phenotype and selecting an alternative drug or reducing the initial dose by 90% for patients with a poor metabolizer phenotype [27].

13.3.9 Risperidone

Risperidone, an atypical antipsychotic, has a strong binding affinity for dopamine (D_2) and serotonin ($5\text{-}HT_{2A}$) receptors and is clinically prescribed for the treatment of schizophrenia, acute manic phase of bipolar disorder, and irritability associated with autistic disorders [71, 72]. CYP2D6 is the major enzyme to catalyze the 9-hydroxylation of risperidone to its active metabolite, 9-hydroxyrisperidone. Both of them contribute to the drug's overall antipsychotic effect, and the sum of both substances represents the "active moiety" [73]. Genetic polymorphisms among *CYP2D6* contribute to the variability in the clinical response and adverse events among the patients treated with risperidone. In a population pharmacokinetic analysis of risperidone and 9-hydroxyrisperidone among healthy Koreans, *CYP2D6*10* allele significantly influenced risperidone clearance and the absorption rate constant [74]. Meanwhile, a study among a Thai autism spectrum disorder population investigating the influence of *CYP2D6* polymorphisms on risperidone plasma levels reported a significantly higher plasma level of risperidone among the carriers of *CYP2D6*5/*1*, *CYP2D6*10/**10, and *CYP2D6*10/*41* as compared to carriers of wild-type *CYP2D6*1/*1* [75]. Several studies have reported the association of plasma levels of risperidone and 9-hydroxyrisperidone with adverse events, including akathisia and tremor and hyperprolactinemia [76, 77]. The DPWG guidelines have recommended selecting an alternative antipsychotic drug or being vigilant to ADRs and adjusting the risperidone dose for clinical efficacy among the patients who are CYP2D6 poor metabolizers, intermediate metabolizers, or ultrarapid metabolizers for risperidone [27].

13.4 Therapeutic Dose Recommendations for Drugs Based on DMEs Genotypes

A number of pharmacogenomic tests are available for the drugs based on the genetic variability among the metabolic enzymes to minimize toxicity and maximize efficacy of drug therapy. For the drugs mentioned above in the review, the therapeutic dose recommendations by FDA, CPIC, and DPWG are included in Table 13.3 comprising the pharmacogenomic guidelines which can assist the clinicians and pharmacists in individualized drug prescription and dispensing.

Table 13.3 Pharmacogenomic dosing recommendations for selected drugs

Gene	Drug	Genotype or phenotype	Therapeutic dosing recommendation	References
CYP2B6	Efavirenz	c.516GG c.516GT c.516TT	Patients with TT genotypes may have increased plasma concentrations. Reduction of the initial dosages of efavirenz to either 400 mg or 200 mg to prevent severity of efavirenz-associated adverse events	[78, 79]
CYP2C9, *VKORC1*	Warfarin	**1/*1*, **1/*2*, **1/*3*, **2/*2* **2/*3*, **3/*3*, −1639GG, −1639GA, −1639AA	Use the dosing algorithm available on http://www.warfarindosing.org/ or the pharmacogenetic dosing algorithm published by IWPC to achieve an INR of 2–3	[37]
CYP2C19	Clopidogrel	UM, EM	Standard dosage as mentioned on clopidogrel label	[32]
		IM	Consider alternative antiplatelet drug, e.g., prasugrel or ticagrelor	
		PM	Consider alternative antiplatelet drug, e.g., prasugrel or ticagrelor	
	Voriconazole	UM	None	[27]
		IM	Monitor serum concentration	
		PM	Monitor serum concentration	
CYP2D6	Risperidone	UM	Alternative drug (quetiapine, olanzapine, and clozapine) is recommended or to be extra careful to observe adverse events and adjust dose to clinical response	[27]
		IM	Alternative drug (quetiapine, olanzapine, or clozapine) is recommended or be extra careful to observe adverse events and adjust dose to clinical response	
		PM	Alternative drug (quetiapine, olanzapine, or clozapine) is recommended or be extra careful to observe adverse events and adjust dose to clinical response	
	Tamoxifen	UM	None	[27]
		IM	Consider aromatase inhibitor for postmenopausal women due to increased risk of relapse of breast cancer and avoid concomitant use of CYP2D6 inhibitors	
		PM	Consider aromatase inhibitor for postmenopausal women due to increased risk of relapse of breast cancer	

Table 13.3 (continued)

Gene	Drug	Genotype or phenotype	Therapeutic dosing recommendation	References
CYP3A5	Tacrolimus	EM (**1/*1*)	Increase starting dose 1.5–2 times standard recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/da. Use TDM for dose adjustments	[57]
		IM (**1/*3, *1/*6, *1/*7*)	Increase starting dose 1.5–2 times standard recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/da. Use TDM for dose adjustments	
		PM (**3/*3, *6/*6, *7/*7, *3/*6, *3/*7, *6/*7*)	Standard starting dose. Use TDM for dose adjustments	
UGT1A1	Irinotecan	**1/*28*	None	[27]
		**28/*28*	Dose >250 mg/m^2: Reduce starting dose 30%, and increase in response to neutrophil count Dose ≤250 mg/m^2: No dose adjustment	
TPMT	Azathioprine /6-MP	Normal, high activity	Standard starting dose	[70]
		Intermediate activity	Reduce the starting dose 30–70% of target dose, and adjust doses of mercaptopurine based on the degree of myelosuppression and disease-specific guidelines	
		Low or deficient activity	Malignant conditions: Reduce daily dose by tenfold, and reduce frequency to thrice weekly instead of daily Nonmalignant conditions: Alternative nonthiopurine immunosuppressive agent	

IWPC International Warfarin Pharmacogenetics Consortium, *UM* Ultra rapid Metabolizer, *EM* Extensive Metabolizer; *IM* Intermediate Metabolizer, *PM* Poor Metabolizer

13.5 Pharmacogenomic Clinic and Patient Counseling

The pharmacogenomic markers to predict the plasma concentrations of particular drugs have been successfully implemented clinically leading to improved efficacy and reduced ADRs in personalized medicine. Several medical centers and health organizations have begun implementing the pharmacogenomic tests to identify individuals with a high risk of ADRs. The challenges, including pharmacogenomics science, translational incentives, healthcare professional education, and patient

acceptance, are outlined in the earlier review by Weinshilboum et al. [80] and have been addressed in recent years with the development of genotyping technology, analytical techniques, and improvements in study design [81]. The US FDA has incorporated pharmacogenomic drug labels for the product label of some drugs to improve the drug therapy for several metabolizing enzymes, including CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A5, UGT1A1, and TPMT (http://www.fda.gov/). CPIC and DPWG have provided guidelines for the clinicians by interpreting the genetic data for prescribing medications. At present, genetic variants of metabolizing enzymes such as *CYP2C9*, *CYP2C19*, and *CYP2D6* are readily assayed in the clinical setting which assists in making prescribing decisions (Table 13.4). The recommendation for genetic biomarker testing for the anticancer drugs makes up the majority of FDA-approved drug labels as compared to other therapeutic areas [82]. Bringing pharmacogenomic testing to the clinic, however, requires analytical validity, clinical validity, and clinical utility [83]. The adoption of pharmacogenomic-guided prescribing patterns in the clinical setting has been sluggish due to the lack of training among clinicians and health practitioners in genetics and molecular biology and cost-effectiveness analysis of pharmacogenomic tests [84, 85].

Even though the pharmacogenomic contributions have made pharmacotherapy more efficacious with fewer ADRs, the delivery model of interpretation and utilization of the tests to the general public remains uncertain. Healthcare professionals, including genetic counselors, physicians, and pharmacists, play an important role in delivering the pharmacogenomic information confidently to the patients. The healthcare professionals should complement each other and combine the set of skills they possess in deciphering the comprehensive pharmacogenomic knowledge to the patients and facilitate the patients' understanding of the genetic components of testing and results [86].

The clinical utilization of pharmacogenomics at Ramathibodi Hospital, Bangkok, Thailand, has been an inspiring successful story with both pre- and post-pharmacogenomic test counseling for the patients [87]. The Division of Pharmacogenomics and Personalized Medicine (PPM) consists of two main core laboratories, namely, the genotyping core laboratory and the phenotyping core laboratory, as well as a pharmacogenomic clinic. After receiving pharmacogenomic testing, as ordered by clinicians, the genotyping core laboratory extracts DNA and genotypes blood samples of the patients to find the biomarkers involved in altering drug responses and adverse events. In addition, this laboratory not only provides routine clinical services but also provides a service to the local scientific community and pharmaceutical and diagnostic companies. The phenotyping core laboratory deals with therapeutic drug monitoring (TDM) and drug-metabolizing enzyme (DME) activity, which relates to several classes of therapeutic drugs, including antiretroviral drugs nevirapine, efavirenz, zidovudine, stavudine, lamivudine, didanosine, tenofovir, and abacavir; antipsychiatric drugs risperidone and paliperidone; anticancer agents tamoxifen, irinotecan, 6-MP, and gefitinib; and antifungals voriconazole, fluconazole, and itraconazole. Finally, the combined genotype and

Table 13.4 Recommendation for drug adjustment for CYP2C9, CYP2C19, and CYP2D6 genetic variations

			Cytochrome P450									
			2C9			2C19			2D6			
No.	Generic name	Trade name	EM	IM	PM	EM	IM	PM	UM	EM	IM	PM
1	Amiodarone	Cordarone®	Std	Std*	↓							
2	Amitriptyline	Polytanal®							↑	Std	Std*	↓
3	Aripiprazole	Abilify®							↑	Std	Std*	↓
4	Atomoxetine	Strattera®							↑	Std	Std*	↓
5	Bosentan	Tracleer®	Std	Std*	↓							
6	Captopril	Capoten®							↑	Std	Std*	↓
7	Carisoprodol	Soma®				Std	Std*	↑				
8	Carvedilol	Coreg®	Std	Std*	↓				↑	Std	Std*	↓
9	Celecoxib	Celebrex®	Std	Std*	↓							
10	Chloroquine	Aralen®							↑	Std	Std*	↓
11	Chlorpromazine	Largactil®							↑	Std	Std*	↓
12	Citalopram	Cipram®				Std	Std*	↓				
13	Clobazam	Frisium®				Std	Std*	↓				
14	Clomipramine	Anafranil®				Std	Std*	↓	↑	Std	Std*	↓
15	Clonidine	Catapres®							↑	Std	Std*	↓
16	Clopidogrel	Plavix®				Std	Std*	↑				
17	Codeine	Ropect®							↓	Std	Std*	↑
18	Cyclophosphamide	Endoxan®				Std	Std*	↑				
19	Dapsone	Dopsan®	Std	Std*	↓							
20	Desipramine	Norpramin®							↑	Std	Std*	↓
21	Desogestrel	Marvelon®, Mercilon®				Std	Std*					
22	Dextromethorphan	Romilar®						↓	↑	Std	Std*	↓
23	Diazepam	Valium®				Std	Std*	↓				
24	Doxepin	Sinequan®							↑	Std	Std*	↓

(continued)

Table 13.4 (continued)

No.	Generic name	Trade name	Cytochrome P450									
			2C9			2C19			2D6			
			EM	IM	PM	EM	IM	PM	UM	EM	IM	PM
25	Doxorubicin	Adriblastina®							↑	Std	Std*	↓
26	Duloxetine	Cymbalta®							↑	Std	Std*	↓
27	Escitalopram	Lexapro®				Std	Std*	↓				
28	Esomeprazole	Nexiam®				Std	Std*	↓				
29	Flecainide	Tambocor®							↑	Std	Std*	↓
30	Fluoxetine	Prozac®	Std	Std*	↓				↑	Std	Std*	↓
31	Fluphenazine	Pharnazine®							↑	Std	Std*	↓
32	Fluvastatin	Lescol®	Std	Std*	↓							
33	Fluvoxamine	Faverin®							↑	Std	Std*	↓
34	Glibenclamide	Daonil®, Euglucon®	Std	Std*	↓							
35	Glimepiride	Amaryl®	Std	Std*	↓							
36	Glipizide	Gipzide®, Minidiab®	Std	Std*								
37	Haloperidol	Haldol®			↓				↑	Std	Std*	↓
38	Ibuprofen	Brufen®, Nurofen®	Std	Std*								
39	Imipramine	Celamine®, Topramine®			↑	Std	Std*	↓		Std	Std*	
40	Indomethacin	Indocid®				Std	Std*	↓	↑			↓
41	Irbesartan	Aprovel®	Std	Std*	↓							
42	Ketamine	Calypsol®	Std	Std*	↓							
43	Lansoprazole	Prevacid®				Std	Std*	↓				
44	Losartan	Cozaar®, Fortzaar®	Std	Std*	↓							
45	Maprotiline	Ludiomil®							↑	Std	Std*	↓
46	Meloxicam	Melox®	Std	Std*	↓							
47	Mestranol	Necon®	Std	Std*	↑							
48	Methsuximide	Celontin®				Std	Std*	↓				

			Cytochrome P450									
			2C9			2C19			2D6			
No.	Generic name	Trade name	EM	IM	PM	EM	IM	PM	UM	EM	IM	PM
49	Methylphenidate	Concerta®, Ritalin®							↑	Std	Std*	↓
50	Metoprolol	Lopressor®							↑	Std	Std*	↓
51	Mirtazapine	Remeron®							↑	Std	Std*	↓
52	Moclobimide	Auroxic®				Std	Std*	↓	↑	Std	Std*	↓
53	Montelukast	Singulair®	Std	Std*	↓							
54	Naproxen	Synflex®	Std	Std*	↓							
55	Nateglinide	Starlix®	Std	Std*	↓							
56	Nelfinavir	Viracept®				Std	Std*	↓				
57	Nilutamide	Nilandron®				Std	Std*	↓				
58	Nortriptyline	Nortrilen®, Ortrip®								Std	Std*	
59	Omeprazole	Losec®, Miracid®				Std	Std*	↓	↑			↓
60	Pantoprazole	Controloc®				Std	Std*	↓				
61	Paroxetine	Paxil®							↑	Std	Std*	↓
62	Perphenazine	Trilafon, Pernazine®								Std	Std*	
63	Phenobarbitone	Phenobarb®				Std	Std*	↓	↑			
64	Phenytoin	Dilantin®	Std	Std*	↓	Std	Std*	↓				
65	Pioglitazone	Actos®	Std	Std*	↓							
66	Piroxicam	Piroxcin®	Std	Std*	↓							
67	Progesterone	Progestogel®				Std	Std*	↓				
68	Promethazine	Nortuss®							↑	Std	Std*	↓
69	Propafenone	Rytmonorm®							↑	Std	Std*	↓
70	Propofol	Diprivan®	Std	Std*	↓							
71	Propranolol	Inderal®, Betalol®				Std	Std*	↓		Std	Std*	
72	Rabeprazole	Pariet®				Std	Std*	↑	↑			↑
73	Repaglinide	Novonorm®	Std	Std*	↓							

(continued)

Table 13.4 (continued)

No.	Generic name	Trade name	Cytochrome P450									
			2C9			2C19			2D6			
			EM	IM	PM	EM	IM	PM	UM	EM	IM	PM
74	Rifampicin	Rifadin®	Std	Std*	↓							
75	Risperidone	Risperdol®							↑	Std	Std*	↓
76	Selegiline	Jumex®	Std	Std*	↓							
77	Sertraline	Zoloft®							↑	Std	Std*	↓
78	Sulfadiazine	Sulfatril®	Std	Std*	↓							
79	Sulfamethoxazole	Bactrim®	Std	Std*	↓							
80	Sulfinpyrazone	Apo-Sulfinpyrazone®	Std	Std*								
81	Sulfisoxazole	Sulfizole®	Std	Std*	↓							
82	Tamoxifen	Nolvadex®, Novofen®				Std	Std*		↓	Std	Std*	↑
83	Tamsulosin	Harnal®						↑	↑	Std	Std*	↓
84	Thioridazine	Ridazine®							↑	Std	Std*	↓
85	Timolol	Betimol®							↑	Std	Std*	
86	Tolbutamide	Tol-Tab®	Std	Std*	↓							
87	Tolterodine	Detrusitol®							↑	Std	Std*	↓
88	Torsemide	Unat®	Std	Std*	↓							
89	Tramadol	Tramal®							↑	Std	Std*	↓
90	Trimethoprim	Bactrim®	Std	Std*	↓							
91	Trimipramine	Surmontil®				Std	Std*	↓	↑	Std	Std*	↓
92	Venlafaxine	Efexor®							↑	Std	Std*	↓
93	Voriconazole	Vfend®	Std	Std*	↓	Std	Std*	↓				
94	Warfarin	Coumadin®, Orfarin®	Std	Std*								
95	Zafirlukast	Accolate®	Std	Std*	↓							
96	Zopiclone	Zimovane®	Std	Std*	↓							
97	Zuclopenthixol	Clopixol®							↑	Std	Std*	↓

EM extensive metabolizer, *IM* intermediate metabolizer, *PM* poor metabolizer, *UM* ultrarapid metabolizer, *Std* Recommend to use the standard dose, The symbolic of arrow down; Recommend to decrease dose and The symbolic of arrow up; Recommend to increase dose

phenotype test results from the pharmacogenomic clinic provide interpretation and counseling for both clinicians and patients. The pharmacogenomic clinic disseminates results of patient screening for possible therapeutic failure or adverse events, and the patients are provided with a card containing their pharmacogenomic traits and recommendations related to drug use. The pharmacogenomic clinic provides pre-, post-, and bedside counseling to patients seeking services of the PPM. This proves that pharmacogenomics has been integrated successfully into clinical practice. In an exploratory project to develop and pilot a patient education tool for warfarin pharmacogenomics, focus groups of patients taking warfarin praised the idea of pharmacogenomic concepts [88].

13.6 Future Perspectives of Pharmacogenomic Research

In this era of personalized medicine, pharmacogenomic testing has identified biomarkers for a number of drugs which have been applied for selecting various treatment modalities based on the genotype/phenotype correlations and have been validated as a cost-effective treatment [89, 90]. Due to the lack of adoption and incorporation of pharmacogenomics into the routine clinical settings, however, the progress has been underwhelming. To implement pharmacogenomic results into routine health system, more firm scientific grounds and inclusion of multiple variants, well-characterized samples, gene-drug-environment interactions, and gene expression must be incorporated with the pharmacogenomic analyses [81]. It is imperative to study multiple genes to evaluate the response of a particular drug, and this will need the assembly of research experts, high-throughput genotyping technologies, bioinformatics, and sophisticated molecular models to predict drug responses [91].

In a positive note, there have been considerable advancements in genomic technologies: the cost of sequencing has become cheaper; attention to the genome-wide study of epigenetics has been given; and genome-wide association studies have provided more biological mechanisms and identification of traits leading to improved individual therapy. The next decade will see the eventual application of personalized medicine in the clinic as well as in the biopharmaceutical sector, and the countries with advanced healthcare systems and greater investment in genomic research will adopt personalized medicine extensively in routine clinical care [92]. The guidelines by CPIC and the FDA regulations on drug label changes will pave the way for successful implementation of pharmacogenomic discoveries into clinical practice.

Acknowledgments The authors would like to thank (1) Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; (2) Khoon Poom Foundation, The Project of Her Royal Highness Princess Ubonratana Rajakanya Siriwatana Bhanawadee, and (3) Pharmacogenomics for Autistic Children, Office of National Research Council of Thailand.

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14 Epigenetic Patterns/Therapies Associated with Genetic Disorders

Elizabeth Mazzio and Karam F. A. Soliman

Abstract

Within the past three centuries, all-cause disease burden in developed countries has shifted from infectious to non-communicable (NCD)/genetic based diseases including cardiovascular conditions, cancer, neuropsychiatric conditions, and diabetes. Factors accounting for this drift include discoveries in vaccination (e.g., tetanus, cholera, typhoid, plague, anthrax, and tuberculosis), antibiotics, advances in medical diagnostics, lasers, surgical techniques, and routine medicines to treat almost every type of systemic imbalance. Moreover, advances in public health, sanitation, food safety, and geriatric sciences are creating extended life expectancy, where age-related illnesses (osteoarthritis, back pain, neurodegenerative conditions) in addition to NCDs are plaguing an ever-growing elderly population. The age-related risk for these diseases is now worsened by aggregation of global industrial pollutants, where the World Health Organization (WHO) now uses the term "environmental burden of disease" to describe adverse effects of a man-made climate, ecosystem degradation, cumulative rise in pollutants, noise, and electromagnetic fields, etc. While epigenetic environmental triggers can alter disease risk, the epigenome contains a plethora of drug targets which can alter the expression of pathological gene traits.

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_14

14.1 Global Disease Burden

Within the past 300 years, disease burden, especially in developed countries, has shifted from infectious to non-communicable chronic disease. Modern technological advances in vaccines, antibiotics, public health, sanitation, and food safety have reduced plagues (e.g., tetanus, cholera, typhoid, plague, anthrax, and tuberculosis) shifting most of the diseases toward chronic illness much of which is age-related with an underlying genetic component. At risk carriers of a gene trait may not necessarily manifest a disease, where positive or negative environmental epigenetic triggers can modify the risk of capitulating a maladaptive phenotype. This environmental impact on health is referred by the World Health Organization (WHO) as the "environmental burden of disease" which attributes numerous environmental factors including poor diet, toxins, cumulative pollutants, electromagnetic fields, xenobiotics, and other factors on human health.

14.2 Discovery of the Genome

How does the environment influence our phenotype, when the DNA sequence code is consistent in all the cells of our body? To answer this question, a few modern technological advances in this century have enabled researchers to study, not only the DNA, but also how the environment can alter its coding into a specific phenotype. The first major discovery was the elucidation of the basic structure, composition, and nature of DNA in 1953, followed by completion of the human genome project 50 years later. This achievement enabled whole genomic analysis to be carried out defining disease-related genome-wide associations, DNA sequence variants [deletions, insertions, copy number variants, aberrant splicing, and single-nucleotide polymorphisms (SNPs)] [1]. Enormous databanks have been developed such as that of the Wellcome Trust Case Control Consortium being routinely mined by researchers who continue to unveil genomic profiles associated with major diseases such as ischemic stroke, heart disease [2], diabetes [3], rheumatoid arthritis, Crohn's disease, [4] bipolar disorder, and other illnesses. While the genome can account for static disease risk hereditability, it is blatantly obvious that environment plays a large role in preventing or augmenting disease phenotype. Influences like diet, exercise, use of synthetic medicines, nutraceuticals, alcohol, smoking, pollution, poverty, or even stress can affect disease risk (in both positive and negative directions). Further, environmental influence appears most efficacious when introduced during early development from pre-implantation/fertilization, in utero development, and early postnatal periods [5]. The totality of environmental factors that influence the outcome of a transcribed genome to establish a patterned phenotype is referred to as the "epigenome."

14.3 The Epigenome

The epigenome is a function of environmental controls which ultimately mold the tertiary structure of DNA so to enable its decoding through either a patterned expression/transcription, (which genes are turned on) or repression/silencing (which genes

are turned off). Epigenetics, simply put, is a series of switches that activate or silence different genes to enable organisms large and small to adapt to their environment. While the on–off switch conceptualization is easy to understand, there are vast complexities to epigenetic biochemistry which involve millions of combined mechanical/chemical modifications to histones, which comprise larger order nucleosomes morphing into euchromatin (transcription = on) or heterochromatin (silenced = off).

14.4 The Off Position/Silencing

Very briefly, "silencing" is the largest component of epigenetic regulation where only 2% of the genome is transcribed into protein-coding mRNA. Proper silencing must be carried out precisely to enable stem cell commitment from pluripotent stem cells and genomic stability throughout the life cycle. Silencing forces are rigorous and predominate over the entire genome including non-functional, non-coding repetitive elements (pseudogenes) or ancestral genomic code. Major silencing systems involve: proper methylation of DNA at transcription start sites (5′-position of cytosine residues within CpG dinucleotides) by DNA methyltransferases (DNMTs) where DNMTs are themselves subject to the docking attachment by methyl binding domain proteins (MBD proteins), which then adjoin histone repression complexes that house constrictive histone modifying enzymes. Hundreds of histone modification proteins then work collaboratively to stabilize the histone structure (tails, core, and linker) all enhancing the electrostatic affinity of histones for DNA. Meanwhile, histone is "marked" by a modification which is then recognized by chromatin remodeling complexes which can bind to those marks (e.g., chromodomains). Remodeling complexes provide the energy and mechanical force to form compact tightly wound heterochromatin. *See Review* [6]. Silencing is further ensured by non-protein coding functional interfering RNAs (siRNAs) or miRNAs that target and destroy specific transcribed mRNA, by cleavage, shortening of its poly(A) tail, or preventing ribosomal translation.

14.5 The On Position/Gene Expression

Actively transcribed portions of the genome involve demethylation of DNA [ten-eleven translocation (TET) enzymes], enzymatic modifications to the histones that weaken electrostatic affinity for DNA, collapse of histone cores and linker elements, which are marked by modification for recognition by chromatin remodeling complexes (bromodomains) which attach and eject the nucleosome away from transcription start sites. These processes if not properly carried out precisely, and for specific genes can lead to diverse human pathologies. *See Review* [6].

14.6 Epigenetics and Human Pathologies

Epigenetic coding errors can be environmentally induced and can occur in the predominant non-coding dormant section of the genome. Insufficient silencing of pseudogenes can trigger global genomic instability through the activation of jumping

genes which are spliced by reverse transcriptases or DNA transposases and pathologically relocate to a protein-coding region inducing a code error. The release of jumping genes (mobile elements) can occur concomitantly to genome-wide hypomethylation as reportedly associated with diverse human ailments [7–9].

The process of silencing pseudogenes requires amongst many things dietary nutrients. Simple deficiencies in several B complex vitamins (folic acid, vitamins B6 and B12) otherwise required for methylation can lead to genomic instability [10, 11]. A clear example of this can be seen in the offspring of the Agouti pregnant mouse model [12]. In this strain of mouse, the non-coding Agouti viable yellow (Avy) pseudogene (retro-transposon) is not properly silenced (methylated) due to a lack of several vitamins, which becomes subject to splicing, where it can jump on to the transcription start site of a protein- coding gene manifested as a disease phenotype. This type of genomic instability can be resolved by the administration of folate (positive epigenetic trigger) and at the same time introduced by negative environmental toxins such as an endocrine-disrupting compound (EDC) bisphenol A [13, 14]. Numerous studies have corroborated the importance of adequate folic acid intake for positive influences on the developing brain [15], learning/memory capacities, behavioral response to stress [16], and reduced incidence of late-onset neuropsychiatric diseases in adult offspring [17]. It is consistently reported that maternal intake of other epigenetic nutrients (choline, betaine, B12, B6, folinic acid, methionine, folic acid, w-3 fatty acids, iron, and zinc) can lead to a positive outcome in offspring with lower disease risk and healthy birth outcome [18–24]. During the human life cycle, folic acid deficiencies correspond to several disease phenotypes including hyperhomocysteinemia, megaloblastic anemia, hemolytic uremic syndrome, diabetic retinopathy [25, 26], cardiovascular disease, hypertension, increased cholesterol, LDL/HDL ratios [27], thrombosis [28, 29] atherosclerosis, and vascular inflammation [30, 31]. Although this is just one example, use of folic acid, this fundamentally demonstrates that we can introduce both positive and negative influences on forces that alter genomic stability and in essence alter disease risk.

Faulty epigenetic silencing can also occur in the coding section of the genome which transcribes functional siRNAs and mRNAs, often reported as being in association with DNA hypermethylation of a gene promoter. These defects involve a pathological pattern of one or more elements such as DNA methylation, the function of epi-enzymes (DNMTs, histone modifying enzymes), an overexpression of methyl CpG binding proteins or heterochromatin proteins. These forces collectively magnify the stability of histone tails, histone cores, and nucleosomal position to wind closely around DNA. In an equal and opposite manner, overexpression of a pathological gene trait can arise from a promoter hypomethylation or changes to the expression of epi-enzymes (TETs, histone modifying enzymes) or any other process involved with the formation of euchromatin. Both errors can initiate pathological up or downregulation of a corresponding coding mRNA or miRNA that targets a specific transcript.

We now know that thousands of DNA mutations have been identified as being associated with disease risk. Likewise, thousands of aberrant epigenetic patterns

are now being associated with similar disease risk. Epigenomic pathological phenotypes have been documented for inflammatory/neurological diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), cardiovascular diseases, diabetes mellitus type 2, cancer [32], inflammatory bowel diseases [33, 34], and obesity [35]. Just as examples, in the case of obesity, pathological markers such as leptin and adiponectin show aberrant gene methylation profiles in adipose tissues/blood of obese patients, [36] diabetes is associated with methylation in specific CpG sites of the TCF7L2 promoter [37] and for human cancers: hypomethylation of a BRCA1 promoter (breast) [38]/hypermethylation of Dlg5 (bladder) or tumor-suppressor gene TSLC1 gene promoter (cervical cancer) exists [39]. This type of terminology is ubiquitous throughout the literature—as it describes disease-associated "epigenetic codes." Many of these describe a hypomethylation/overexpression of a pathological transcript/oncogene alone or in conjunction with hypermethylation/silencing of a protective transcript/tumor-suppressor gene and altered patterns of miRNA controlling a protein-coding disease element. There are technically billions of combinations of epigenetic elements that control disease-specific areas of the genome, and the complexity has necessitated the development of bio-informatics epigenetic databases such as epifactors [40] (Fig. 14.1).

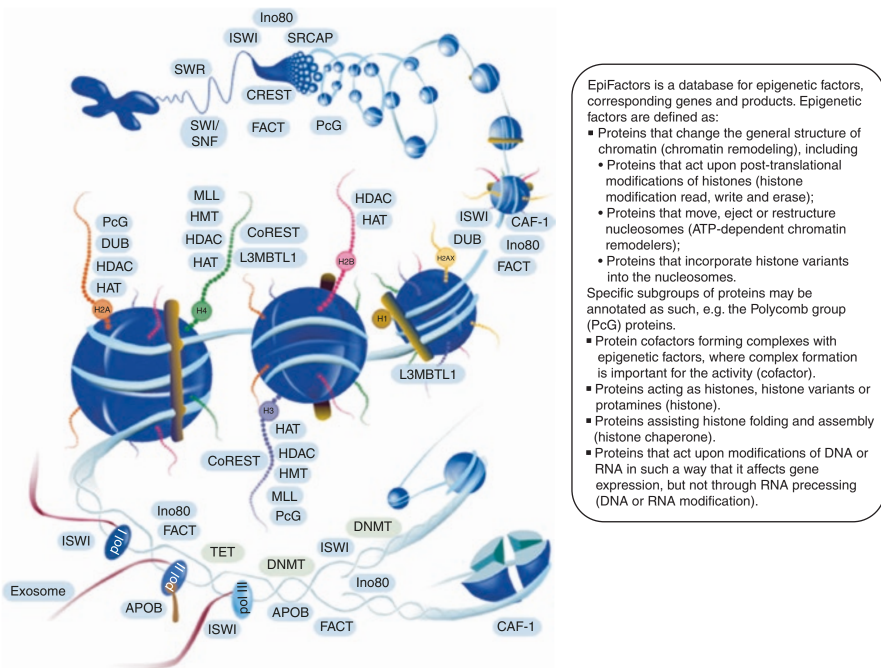

Fig. 14.1 Epifactors: a comprehensive database of human epigenetic factors and complexes [40]

14.7 Genomic Mutations in Epigenetic-Related Proteins and Human Pathologies

There is a second group of epigenetic disorders that needs a separate classification. These include deficiencies in proteins that carry out epigenetic biochemistry, such as the case of gene mutations in mammalian SWI/SNF chromatin remodeling proteins (SMARCB1, SMARCE1, SMARCA4, ARID1A, ARID1B) [41, 42] or nuclear lamins which tether heterochromatin in place, all leading to severe developmental disorders such as Prader-Willi, Angelman, autism, Coffin-Siris and Rett syndromes, or laminopathies [43–46]. Mutations can also occur in proteins circumscribing epigenetic biochemical pathways such as methylenetetrahydrofolate reductase (MTHFR) otherwise required for methylation of DNA and histones [47–49]. An MTHFR mutation or even autoimmune diseases where autoantibodies (AuAbs) target the folate receptor α (FRα) can mimic a folate deficiency, evoking pathological expression of developmental genes [50, 51] associated with low birth weight, preterm birth [52], neural tube defects, encephalopathy, neurological/muscular insufficiency, cognitive impairment [19], and impaired hippocampal plasticity which in later life could elevate risk for neurodegenerative disease [53].

14.8 Epigenetics and the Life Cycle

14.8.1 In Utero

There is little question that the most critical period for establishing a patterned epigenome is in utero. Intrauterine exposure not only influences the epigenome of the fetus but also that of the germ cells in the female infant's ovaries, as these cells develop during this period. Given the influence of the environment during pregnancy, it is expected that future practices and policies will be employed during this critical window to attenuate adult-onset disease risk [54]. The importance of environmental stimuli during early pre and postnatal development on chronic disease risk has been dubbed the Developmental Origins of Health and Disease (DOHaD) paradigm [55, 56]. Some of the challenges to the DOHaD paradigm include the vast and rapid rise in accumulation of pollutants and environmental toxins (e.g., pesticides, heavy metals [57], endocrine disruptors [58]), poverty, societal stress which can leave negative lasting epigenetic patterns, and augment the risk of adverse birth outcome [59]. Of particular concern is the endocrine disruptors, from plastics epoxide resins, printed receipts, or product leaching containers as ubiquitous as bottled water or baby bottles. EDCs can bind/activate the human estrogen receptor (ERalpha) and initiate G-coupled estrogen receptor signaling altering sex steroid hormone-controlled organs and tissues leading to effects on growth and developmental processes, creating risk for later stage onset disease [60, 61]. Specifically, bisphenol A exposure during pregnancy/perinatal initiates deregulated immune homeostasis in offspring [62] corresponding to allergies [63], asthma [64], anxiety-related disorders [65], and precancerous lesions in mammary prostate and uterus tissue [66].

14.8.1.1 Fertilization and Conception

A second major consequence of environmental pollutants is a rise in infertility and low semen quality [67, 68]. To overcome this, new technologies have been developed for in vitro fertilization (IVF), fertility-preserving cryopreservation, or assisted reproductive technology (ART). These techniques are now deployed in about 2–5% of births [69]. Unfortunately, the process of IVF itself is also believed to alter the epigenome of offspring, and aberrant DNA methylation profiles have been found in cord blood [70]. Some research suggests there could be a greater risk of diseases such as cardiovascular disease resulting from IVT-associated altered DNA methylation of eNOS affecting vasodilation function [71, 72]. Other concerns of ART could be poor birth outcome, malformations, and potential genomic imprinting disorders [73] such as Beckwith-Wiedemann syndrome [74]. Even a slight variation in ambient conditions which vary during IVF, such as temperature, culture , media, light quality, hormone applications, and ovarian stimulation protocols may alter DNA methylation patterns. These slight variations may increase risk of epigenetic disease phenotype in offspring [75]. In the future, there must be strategies to ensure avoidance of pollutants and toxins in pregnant women and children, and seek our innovations to live in a cleaner greener world.

14.8.2 Nutrition/Food Supply

Some factors within the environment can be controlled such as diet. Maternal diet plays a hefty role in directing epigenetic programming in offspring. We have learned this by the DNA hypermethylation and abnormal silencing of growth-related genes as demonstrated by the Dutch Famine (1944–1945) Birth Cohort Study [76, 77]. Maternal deficiency in caloric and nutrient dense diet can introduce lifetime risk of metabolic, cardiovascular, asthmatic [78], cancer, depressive, psychological [79] as well as accelerated aging in offspring [77, 80–82]. In contrast, maternal over-nutrition or obesity (gestational diabetes) can equally lead to greater risk of adult-onset metabolic disease, obesity, diabetes, and cardiovascular disease in part due to maternal insulin resistance, hyperglycemia, and development of fetal hyperinsulinism [83]. Maternal intake of epi-micronutrients (e.g., folate, vitamin B6, B12) is critically involved with fetal epigenetic biochemistry. Sadly, epi-nutrient deficiencies still exist today on a global scale, where millions of infants are at risk for impaired cognitive function and behavioral disorders [84, 85]. Severe epi-nutrient deficiencies can lead to congenital defects [86, 87], neural tube defects, and orofacial clefts [88, 89].

14.8.3 Early Postnatal Human Development

After birth, in the early postnatal development phase, a lack of breastfeeding can foster greater risk in offspring for developing obesity, cardiovascular disease, diabetes, gastrointestinal allergies/inflammatory bowel disease, autoimmune disorders [90], and asthma [91, 92]. Positive modifiers in breast milk which epigenetically

nurture the child include secretory IgA, w3 long-chain polyunsaturated fatty acids, growth factors, indigestible oligosaccharides [93] (cytokines- interleukins) [90], and microRNAs [94, 95]. Not only are the components in breast milk epigenetic modifiers, but the nurturing/bonding aspect of breastfeeding has a beneficial effect on neurological development and stress response in offspring [96].

Epigenetic programming in early developmental periods can become permanent, and possibly perpetuated trans-generationally for several generations with potential to span over a century to the future. Historical examples of trans-generational phenotype transmission of the first generation offspring (F1) include the effects of the drug thalidomide, which when used during pregnancy rendered harm to the fetus in utero causing short truncated limbs and other deformities depending on the gestational exposure age [97]. An example of second generation (F2) impact is that of diethylstilbestrol (DES) used during pregnancy [98] where F1 and F2 offspring had an overall greater risk for vaginal adenocarcinoma, infertility, and perpetuation of future adverse outcome pregnancies [99]. Transgenerational epigenetic inheritance to the F3 generation has been demonstrated by fetal impacting agents such as vinclozolin, bisphenol A and methoxychlor [99, 100]. While the impact of a single environmental trigger is most acute in early development, every form of life will uncontrovertibly face an omnipresent environment, subject to variation through its entire life cycle which drives epigenetic change. While there is a certain element of control over how we choose to live, many epigenetic triggers are uncontrollable, such as in those directed by a planetary rotation that drives circadian rhythms [101], the process of aging, hormonal changes, estrous cycles, end-of-life senescence/telomere replication and death [102].

14.8.4 The Human Lifecycle

Although the critical window to establish a healthy epigenome is greatest in utero, it is possible to manipulate the adult epigenome to treat disease in later life. Given that almost all human diseases involve aberrant genome-wide mRNA expression profiles [103, 104], we are now evaluating epigenetic drugs or nutrients to treat these diseases. While the specificity of epigenetic drugs for individual gene promoters is a field in its infancy, we do know that use of DNMT inhibitors (5-azacytidine and 5-aza-20-deoxycytidine) or HDAC inhibitors has proved somewhat successful in experimental models of cancer, heart disease, and neurodegenerative conditions [105–108]. HDACs regulate silencing, and therefore, HDAC inhibitors would serve to initiate re-expression of "potentially anti-disease proteins." There are about 18 mammalian HDACs, 4 classes [zinc-dependent (class I (nucleus), II (cytosol/nucleus), and IV (nucleus)], class III HDACs [also known as the sirtuins, SIRT 1, 6, 7 (nucleus) and SIRT 2 (cytosol) SIRT 3 4 5 (mitochondria)] composed of a family of NAD+-dependent protein-modifying enzymes. Several classes of HDAC drugs currently include, hydroxamic acids (TSA/FDA approved vorinostat (SAHA), short chain fatty acids (sodium butyrate, valproic acid) cyclic peptides, and benzamides.

While the synthetic design of epi-drugs is on the horizon, disease risk can also be attenuated by factors in our diet. Nutri-epigenetics encompasses the area of diet and its positive influence on the epigenetic landscape throughout life [109, 110]. Epi-nutrient daily supplementation may protect against diseases associated with a poor diet, excessive alcohol abuse [111], or other factors linked with genomic instabilities [112–115]. Specific nutrients required for epigenetic processes appear to be most critical and include B vitamins (folate, B6, B12) as well as vitamin A, D, zinc, lysine, methionine, riboflavin, choline, betaine, methionine, and inositol [116, 117]. Some of these, for example, folic acid, could play a role in preventing neurological disease pathologies such as accrual of Aβ oligomers, presenilin 1 (PS1), amyloid precursor protein (APP) [118], and phosphorylated tau [119] in Alzheimer's disease (AD) [120–122] and mitochondrial instability, [123] inherent in Parkinson's disease [124]. Folic acid supplementation can also provide relief in schizophrenia patients who have inherited autoantibodies against FRα at the choroid plexus which block N (5)- MTHF transfer to the brain [125, 126]. Optimal B-vitamin status is believed to result in biologic gains of neurological function, cognitive function [127, 128], academic performance [129], and reduced risk for vascular/neurodegenerative co-morbidities [130, 131].

The macronutrient composition of the diet can also influence epigenetic control and disease risk. Dietary composition of fats and various indigestible fibers can influence the epigenome. Just, for example, a high-fat western style diet will render reduction in global DNA methylation which corresponds to a greater risk of colon cancer [132]. Alternatively, adequate intake of fiber can trigger microbial fermentation and release of butyrate (HDAC inhibitor) into the gut, reducing the risk of colon cancer [133, 134]. Biologic gains can also be realized from intake of omega-3 PUFAs which can synergize with fermentable fiber [135] cruciferous vegetables (kale, cabbage, brussels sprouts, and broccoli), green tea, curry, and black pepper which can change the gut epigenome, and reduce the incidence of colon cancers [136]. While these are just a few examples, many studies are now showing that food chemical components, such as sulforaphane, 3,3′- diindolylmethane, indole-3-carbinol, EGCG, curcumin are direct regulators of miRNAs, HDACs/histone acetyltransferases (HATS) and DNMTs [137, 138] which are likely to reduce risk of disease by maintaining the epigenetic landscaping [139–141].

In conclusion, epigenetic control over the genome is likely to be a means to control the element of risk associated with adult-onset non-communicable diseases. Much of the data suggest intervention strategies are most effective during conception and early life development, likely to involve minimizing exposure to environmental pollutants and optimizing diet and emotional nurturing. As we learn more about dietary epigenetic influences and disease risk in adults, there should be changes in public policy governing strategic initiatives to tailor food ingredients, and food availability to the masses, which is of the largest controllable component to disease risk.

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Neurodegenerative Disease Conditions and Genomic Treatment for Better Health

15

Ravindra V. Badhe, Dharmesh R. Chejara, Pradeep Kumar, Yahya E. Choonara, and Viness Pillay

Abstract

Neurodegenerative diseases are genetic and/or sporadic disease conditions characterized by progressive nervous system dysfunction involving the atrophy of central or peripheral nervous. The neurodegenerative diseases (NDs) like Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) are responsible for more than 1% deaths and more than 2% disabilities of total world population. These NDs also impart huge socioeconomical burden on families of patients. NDs involve complex etiology with different genetic and environmental factors. The understanding of the etiology may help therapists to develop new effective symptomatic and preventive (genetic) treatments for NDs. The development in Human Genome Project helping to detect the genetic mutations causing HDs and advancement in gene and genome therapy are being implemented to correct these mutations. In this chapter, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) are discussed in detail for their pathophysiology, etiology, and latest symptomatic and preventive treatment. In preventive treatment, the latest achievements of the gene and genomic therapies are discussed.

Keywords

Alzheimer's disease · Parkinson's disease · Huntington's disease · Amyotrophic lateral sclerosis · Genomic treatment

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Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_15

15.1 Introduction

The human brain is the master organ of the body, controlling and regulating all functions of the body. The brain is composed of neurons and glial cells and extends out from the skull as the spinal cord and nerves of the central nervous system (Fig. 15.1a). The axon terminals of the central nervous system connect to the dendrites of neuron cells that service different organs and those of the motor neurons. The nerve tracts of the central nervous system that transmit signals to the muscles and glands are called efferent nerves.

The neurons, nerves, and ganglions starting from different organs and connecting to the central nervous system are called peripheral nervous system. The nerves of peripheral nervous system (Fig. 15.1a) are called as afferent nerves that transmit signals from sensory neurons to the central nervous system. The nervous system is functionally differentiated as "autonomic or involuntary" nervous system which works without conscious effort and "somatic or voluntary" nervous system. The autonomic nervous system regulates blood pressure, heart rate, and the breathing rate, while the somatic system operates through the central nervous system consisting of sensory and motor neurons. Thus the neurons and nerve are the basic units controlling all the voluntary and involuntary things happening with our body.

As shown in Fig. 15.1b, neurons are made up of various parts which include the soma (consist of nucleus and extensions called the dendrite tree) and axons (consist of myelin sheath with nucleus and nodes of Ranvier), and axon terminals are fine structures which vary from hundreds to thousands. The axon terminals and dendrites of soma are connected to synapses which are specialized structures where neurotransmitters are released to communicate with target neurons. These neurotransmitters trigger action potential through voltage-dependent sodium channels to start the next wave of electrical impulse. In the brain, glutamate acts as excitatory neurotransmitter, and GABA act as inhibitory neurotransmitter. In motor and sensory neurons, acetylcholine acts as excitatory neurotransmitter, and glycine acts as inhibitory neurotransmitter.

If a nerve is damaged, infected, diseased, autoimmune disrupted, or degenerated, it can lead to various diseases. The causes of damage, infection, immune activation, and degeneration are probably a series of events which include environmental factors, genetic factor, or mixture of damaging genetic and environmental factors. As the age progresses, a person increasingly loses the ability to control this damage, triggering irreversible neurodegeneration or neurodegenerative diseases (NDs).

15.1.1 Neurodegenerative Diseases (NDs)

According to the European Commission Public Health, NDs are defined as genetic and/or sporadic disease conditions characterized by progressive nervous system dysfunction. The atrophy of central or peripheral nervous system is often associated with it. Till date, more than 600 disorders were reported to affect the nervous system and can cause diseases like brain cancer, degenerative nerve diseases, Alzheimer's

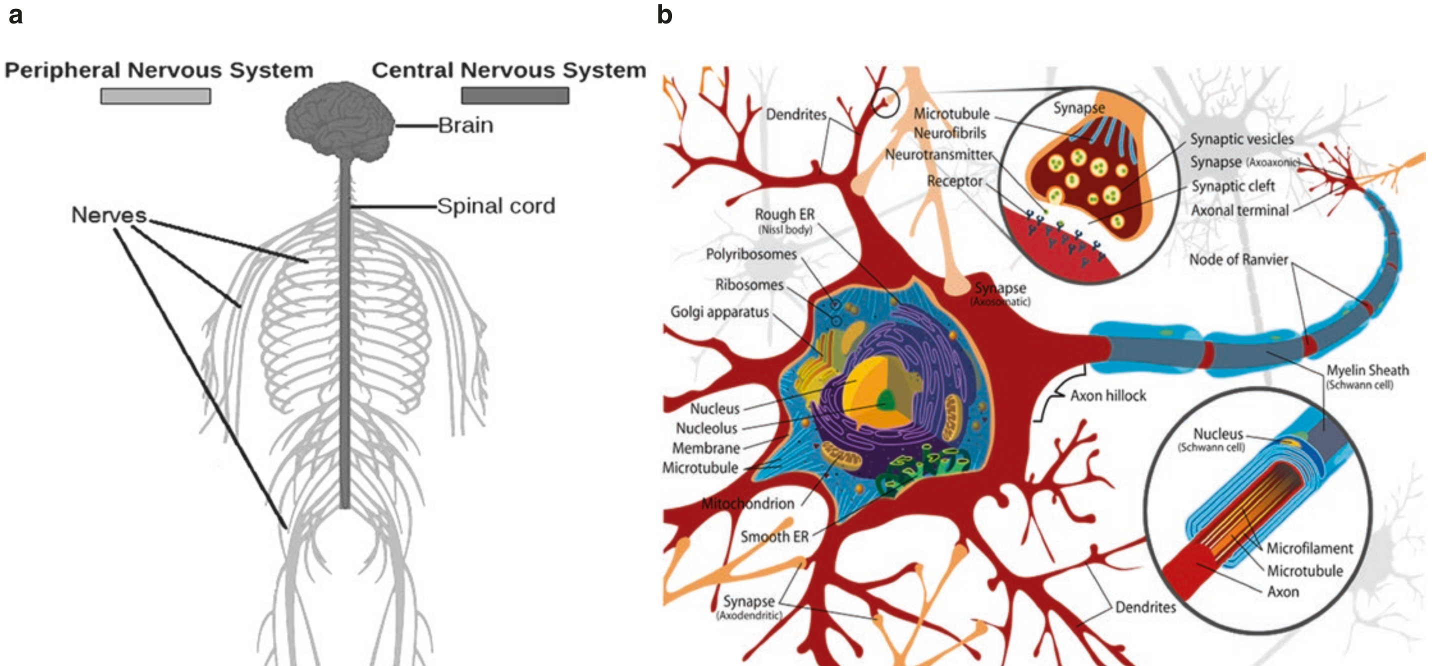

Fig. 15.1 (**a**) Central and peripheral nervous system. (**b**) Neuron (Figure adapted from https://en.wikipedia.org/wiki/Neuron#/media/File:Complete_neuron_cell_diagram_en.svg)

disease and other dementias, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), encephalitis, epilepsy, genetic brain disorders, hydrocephalus, multiple sclerosis, stroke, prion diseases, and others [1, 2].

15.1.2 Mechanisms for Neuron-Related Diseases

15.1.2.1 Misfolded Protein Aggregates

These aggregates are abnormal clumps of protein which are generated inside motor neurons. They are found in nearly all cases of motor neuron disease and may disrupt the normal working of the motor neurons or put the cell under great strain and can be used as marker for the disease. The most common aggregates found are TDP-43, alpha-synuclein, tau, and beta-amyloid mainly found in Alzheimer's disease. The diseases like Alzheimer's disease, Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy are the examples of misfolded protein aggregates [2, 3].

15.1.2.2 Polyglutamine

This disease is caused by genetic mutations leading to formation of multiple CAG nucleotide sequences called polyglutamine (polyQ) tract. CAG is responsible for glutamine amino acid, and its expression results in polyglutamine diseases. The excess glutamine, in the neuron cell, exhibits abnormal properties, such as protein degradation, irregular protein folding, anomalous interactions with other cellular proteins, and altered subcellular localization. Proteasome are the ubiquitin with enzymes which are responsible for degradation of many misfolded proteins like alpha-synuclein and polyQ expansions. If proteasome do not correctly cleave these irregular proteins, it leads to increase in cell toxic protein levels. The diseases like Huntington's disease and spinocerebellar ataxias are the examples of polyglutamine diseases [4, 5].

15.1.2.3 Cell Transport Disruption

Individual cell has its transport system, which transports nutrients and other chemicals into the cells and throws out the waste toxic products in the form of vesicles or destroys them with the help of antioxidants or enzymatic degradation. Due to changing lifestyle and other environmental factors, antioxidants decrease in the body which leads to the cell transport disruption and buildup of toxic waste in the neuron cell. The diseases like Parkinson's disease and Huntington's disease are the examples of cell transport disruption diseases.

15.1.2.4 Glial Cells and Myelin Sheath

The axon of the motor neuron are surrounded and supported by glia cells which provide nutrients to the neuron cells and on outer surface the cells release myelin forming myelin sheath, which is a dielectric fatty white substance. The purpose of a myelin sheath is to increase the speed of impulses propagation through myelinated fiber. Thus, if glial cells or myelin sheath is affected, it leads to hampered nerve

signal transfer leading to abnormality. The diseases like multiple sclerosis, chronic inflammatory demyelinating polyneuropathy, acute disseminated encephalomyelitis, Guillain-Barré syndrome, neuromyelitis optica, transverse myelitis, leukodystrophy, and Charcot-Marie-Tooth disease are the examples of glial cell and myelin sheath disorders [6, 7].

15.1.2.5 Mitochondrial Dysfunction

Mitochondria are known as the powerhouse of the cell; if it is damaged, it initiates the intrinsic mitochondrial apoptotic pathway through activation of caspase-9 (cysteine-aspartic acid protease cascade) which releases the cytochrome C from the mitochondrial intermembrane space (IMS). This cytochrome C binds apoptotic protease-activating factor-1 (Apaf-1) and initiates the apoptotic cycle of the cell. The reactive oxygen species (ROS) helps the cytochrome C to bind with Apaf-1 by activating pore-stabilizing proteins (Bcl-2 and Bcl-xL). In large concentration, ROS can cause both apoptosis and necrosis [8–11]. The diseases like Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis (ALS) are the examples of mitochondrial dysfunction.

15.1.2.6 Programmed Cell Death (PCD)

Programmed cell death (PCD) is an intracellular program-mediated cell death. It is a natural phenomenon used by the cells to commit suicide if cells are infected, diseased, or injured. It involves series of biochemical events which lead to cell death or disruption. Autophagy is a most favored route of self-destruction acquired by any cell if there is presence of aggregate prone proteins in the cell. It is categorized as macro autophagy and chaperone-mediated autophagy (CMA). Both the pathways involve use of lysosome for the destruction of misfolded proteins. The macroautophagy mechanism is involved in macromolecule recycling during starvation, and chaperone-mediated autophagy (CMA) mechanism is involved in macromolecule and other toxic by-product degradations. If any of these cycles are blocked, it leads to accumulation of misfolded proteins and toxic substances leading to cell death. Other PCD like apoptosis involving intrinsic and extrinsic pathways initiated by internal or external cell factors to trigger biochemical events leading to cell death or destruction is also responsible for many nerve diseases [1, 12–14]. The diseases like Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, and Huntington's disease are the examples of programmed cell death mechanism.

15.1.3 Classification of Neurodegenerative Diseases

The neurodegenerative diseases are classified into two main categories:

1. Diseases caused by non-motor neuron degeneration in the cerebral cortex leading to cognitive disturbance like dementia (e.g., Alzheimer disease, Pick disease)

2. Diseases caused by motor neuron degeneration in various parts of the brain and spinal cord, leading to movement disorders which are subcategorized on the basis of the part of the CNS affected as:
   (a) Motor neuron weakness and degeneration (e.g., amyotrophic lateral sclerosis (ALS), spinal muscular atrophy)
   (b) Motor neuron degeneration at the spinal cord and cerebellum junction (e.g., Friedreich ataxia, ataxia-telangiectasia)
   (c) Motor neuron degeneration at substantia nigra and basal ganglia (e.g., Parkinson's disease, progressive supranuclear palsy)
   (d) Motor neuron degeneration at basal ganglia (e.g., Huntington's disease)
   (e) Motor neuron degeneration at multiple areas of CNS leading to complex ND conditions

15.1.4 Severity and Economical Burden of Neurodegenerative Disorders

According to WHO, the diseases like epilepsy, Alzheimer's and other dementias, Parkinson's disease, multiple sclerosis, and migraine are categorized under neuropsychiatric neurological disorders [15].

The economic burden due to nerve diseases and brain disorders are very large. These costs include the cost of treatment and the cost due to lost productivity of patients and their family members. In addition to economic burden, the emotional, practical, and financial burden on family members exacerbates the problem. As per the study conducted by the WHO, the World Bank, and the Harvard School of Public Health since 1993, dementias are responsible for the greatest burden with Alzheimer's disease taking 60–70% share of all dementias and 12% of all neurodegenerative disease cases throughout the world and affecting 0.75% of total world population. Parkinson's disease (PD) is the second most common neurodegenerative disorder followed by AD, taking 1.8% share of all neurodegenerative disease cases throughout the world and affecting 0.11% of total world population. Similarly multiple sclerosis, migraine, and epilepsy contribute 1.6, 8.3, and 7.9%, respectively, of all neurodegenerative disease cases throughout the world and affect 0.10, 0.52, and 0.50% of total world population, respectively, adding huge economic burden to world economy [16–23].

15.1.5 Current Treatments for Neurodegenerative Diseases

The drug therapy for neurodegenerative disorders involve use of anti-inflammatory drugs, antioxidants, steroids (estrogen), neurotransmitters specifically levo-3,4-dihydroxyphenylalanine (L-dopa) in Parkinson's disease, GABAergic for Huntington's disease, and acetylcholine and cholinesterase inhibitors for Alzheimer's disease.

The functional neurosurgery approaches like lesions, stimulation, and transplants toward the treatment of neurodegenerative disorders are also in use. The lesions and stimulation offer only symptomatic alleviation of the disease, but the surgical knowledge is nowadays being used for transplantation. The transplant being tried includes fetal tissue transplants and cellular transplants which can release neuron protecting growth factors and enzymes. Researchers are also trying to transplant embryonic stem cells and pluripotent stem cells in attempt to create therapies for neurodegenerative diseases with limited success.

Gene therapy approaches to treat neurodegenerative diseases (NDs) which might be inherited or acquired offer many advantages over conventional therapies. It is particularly striking for ND due to BBB's restricted bioavailability of conventional drugs. The most effective gene delivery can be achieved through lentiviral vectors which offer postmitotic long-term expression of the genes with high titer levels without immunological complications. This therapy showed exciting results in Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and spinal muscular atrophy [24–26].

15.1.5.1 Limitations for Neurodegenerative Diseases Treatments

All the major neurodegenerative diseases (NDs) have their origin in CNS, and the majority of treatments need to concentrate toward the brain and spinal cord. However CNS is strongly protected by several barriers which inhibit the entry of any foreign material. Thus the entry of the drug molecules to the site of action in the CNS is restricted, and it modifies the disease symptoms to a short period of time and does not show any inhibitory effect on the progression of the ND. The main barrier for the drug delivery in the treatment of ND is blood-brain barrier (BBB), which affects the drug release kinetics and many times leads to peripheral side effects. Furthermore, the death of the neurons in any type of NDs is generally caused by multiple factors as mentioned above, thus adding difficulties in ND treatment and management.

15.1.5.2 Way Out for Neurodegenerative Disease Treatments

The major hurdle for ND treatment is BBB, as it limits the drug molecules to reach the target sites in the brain. A vast attention was acquired by nanoparticles as they have a capacity to pass through BBB and carry the drug molecules with it to deliver at target site. More efforts are being made to improve the efficiency of the NP to improve their capacity to carry different drug molecules to treat the symptoms of ND effectively. The other hurdle is to stop the recurrence of the symptoms, which need to be tackled at genetic level. For this the gene therapy approach was tried, as the mechanisms of ND were better understood; it was confirmed that it has more complex biochemical mechanisms involving more than one gene. Thus gene therapy was not very successful in treating the NDs. So the way-out researchers are looking at its genomic treatment along with NP-based symptomatic treatment as cotherapy.

15.1.5.3 Genomic Treatment

Genomic therapy is the branch of medicine which utilizes the maps of human DNA, called the genome, to understand and correlate the biochemistry and pathogenesis of diseases to patients' genes. It is different from gene therapy, as gene therapy involves modification of just one pair of gene on chromosome resulting in modification of disease condition. This is not much useful in NDs as they involve multiple genetic mutations, while genomic treatment uses many pieces of genetic information to modify disease condition. Genomic treatment is based on three main mechanisms—diagnosing disease, preventing disease, and treating disease. Thus genomic treatment refines diagnoses, prevents adverse drug effects, manages epidemics, develops new therapies, and can individualize the treatments [27–29].

The mutations in genes cause diseases by modulating the cell function. Recent successful genomic advances helped to predict few changed phenotype from mutated genotype in single cells [30], but we are still far from being able to relate each mutation to its synthesized molecule and, ultimately, phenotypic outcome in humans.

Detecting and understanding the genetic mutation(s) is necessary for subsequent therapeutic and preventive actions. Thus the major challenge in genome-based medicine is segregating the disease-related mutated and non-mutated genes from a large number of genes and correlating them with the disease. Once the mutations and their phenotypic expressions are identified, the planning and process of designing targeted treatment start. Though this process is slow, little progress has been made to modify the actionable mutations to modify the disease conditions [31]. Genomic treatment is being extensively explored for infectious disease, cardiovascular diseases, cancer, and NDs. In this chapter, the recent trends in genomic treatment of the major four neurodegenerative diseases, namely, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), are discussed.

15.2 Alzheimer's Disease

Alzheimer's disease (AD) is the most common non-motor neurodegenerative disease. It is also the most common type of dementia, involving decline in cognition, specifically in memory, language, and thinking, due to gradual death of brain cells [32]. In the progressed AD, the brain tissue shrinks compared to normal brain, hampering all the cognitive functions, and specifically, the hippocampus and cortex shrinks, damaging the areas involving thinking, planning, remembering, and forming of new memories (Fig. 15.2). It was also observed that the ventricles (fluid-filled spaces within the brain) grow larger. It affects mainly the people aged 65 and older.

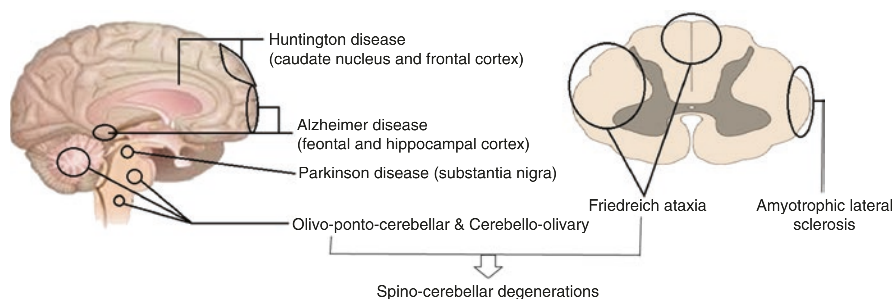

Fig. 15.2 Areas in the brain and spinal cord marked for pathogenesis of different neurodegenerative disorders

Table 15.1 Number of DALYs[a] for neurological disorders and percentage of global DALYs and deaths attributable to neurological disorders as percentage of total deaths projected for 2015 and 2030 (WHO report on neurological disorders: public health challenges)

	2015			2030		
Cause category	No. of DALYs (000)	Percentage of total DALYs	Deaths (%)	No. of DALYs (000)	Percentage of total DALYs	Deaths (%)
Epilepsy	7419	0.5	0.21	7442	0.49	0.19
Alzheimer's and other dementias	13,540	0.91	0.81	18,394	1.20	0.92
Parkinson's disease	1762	0.12	0.20	2015	0.13	0.23
Multiple sclerosis	1586	0.11	0.03	1648	0.11	0.02
Migraine	7736	0.52	0.00	7596	0.50	0.00

[a]Disability-adjusted life years or DALYs—years of healthy life that are lost to disability as well as death

15.2.1 Global Burden of AD

AD is taking 12% share of all neurodegenerative disease cases throughout the world and affecting 0.75% of total world population. In the USA only, the collective direct and indirect cost of PD, including treatment and lost job income due to work inability, was estimated to be nearly $214 billion per year. According to WHO, the projected deaths attributable to AD as percentage of total deaths for 2015 and 2030 are 0.81 and 0.92%, respectively (Table 15.1).

15.2.2 Pathophysiology

The autopsy samples of AD patients show the presence of plaques and tangles in the brain tissue. The plaques are depositions of the protein called beta-amyloid

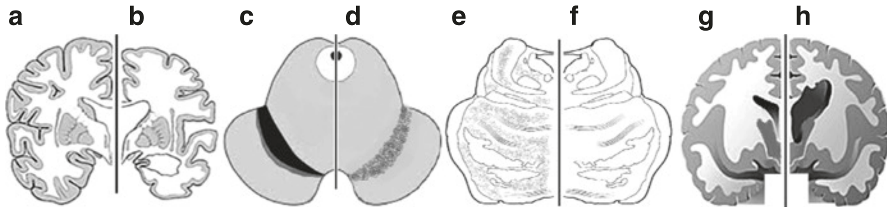

Fig. 15.3 (**a**) Normal and (**b**) Alzheimer's brain TS observed in Alzheimer's disease; (**c**) normal and (**d**) Parkinson's pallor (depigmentation) of the substantia nigra observed in Parkinson's disease; (**e**) normal and (**f**) Parkinson's pallor (depigmentation) of the locus coeruleus observed in Parkinson's disease; (**g**) normal and (**h**) Huntington brain TS observed in Huntington's disease

between the dying cells in the brain and also termed as "amyloid plaques (AP)." The tangles occur between the neurons in the brain and happen due to disintegrated tau protein [33]. In early stages of AD, AP accumulation was observed followed by tau protein tangles and degradation of neurons leading to progressive loss of cognition as the disease progresses [34]. There were different theories proposed based on the AP and tau protein mechanisms of degradation of the nerve cells, and tau protein tangle theory was proved more appropriate for dementia observed in AD (Fig. 15.3a and b) [35].

15.2.3 Etiology

The causes of AD are still not clear, but there are several hypotheses put forth by different investigators ranging from environmental factors to genetic risk factors triggering the pathophysiologic cascade which, over the decades, leads to AD:

1. *Genetic causes*—Genetic mutations in amyloid precursor protein (APP) gene (chromosome 21), presenilin-1 (PS1) gene (chromosome 14), and presenilin-2 (PS2) gene (chromosome 1) cause early-onset AD (Table 15.2). These faulty genes start producing beta-plated amyloid protein with more sticky 42 amino acid residues instead of normal nonsticky 40 amino acid residues initiating the AP deposition on the nerves [36].
2. *Insulin resistance*—Researcher observed that there is relation between decreased cerebral glucose metabolic rate due to insulin resistance and onset of AD. Thus, researchers are trying to develop a correlation between extent of insulin resistance and onset of AD as a marker for early detection of AD [37].
3. *Infection*—It was known that amyloid protein has antimicrobial property. Hence when there is an infection specifically with *spirochetes* sp., *Treponema*, and *Borrelia burgdorferi* resulting in chronic inflammation and neuronal destruction, it was related to the release of amyloid protein. This observation suggests the direct relation between infection and amyloid protein release which might lead to AD if infection becomes chronic [38].

Table 15.2 Major genes responsible for pathogenesis in different neurodegenerative disorders

Gene	Location	Protein	Relevance to pathogenesis	Reference
Alzheimer's disease (AD)				
APP (*AAA*; *AD1*; *PN2*; *ABPP*; *APPI*; *CVAP*; *ABETA*; *PN-II*; *CTFgamma*)	21q21.3	Aβ precursor protein	Altered Aβ production (Aβ42/Aβ40 ratio increased) and aggregation	Goate et al. (1991)
PSEN1 (*AD3*; *FAD*; *PS1*; *PS-1*; *S182*)	14q24.3	Presenilin 1	Altered Aβ production (Aβ42/Aβ40 ratio increased)	Sherrington et al. (1995)
PSEN2 (*AD4*; *PS2*; *AD3L*; *STM2*; *CMD1V*)	1q42.13	Presenilin 2	Altered Aβ production (Aβ42/Aβ40 ratio increased)	Rogaev et al. (1995) and Levy-Lahad et al. (1995)
APOE (*AD2*; *LPG*; *APO-E*; *LDLCQ5*)	19q13.2	Apolipoprotein E	Unknown (Aβ aggregation and lipid metabolism)	Strittmatter et al. (1993) and Schmechel et al. (1993)
TREM2 (*Term2a*; *Term2b*; *Term2c*)	6p21.1	Triggering receptor expressed on myeloid cells 2	TYROBP-TREM2 complex activates these cells, triggering an inflammatory response to injury or disease	Forabosco et al. (2013)
Parkinson's disease (PD)				
SNCA (*PD1*; *NACP*; *PARK1*; *PARK4*)	4q21	α-Synuclein	Neurotoxicity by aggregation of α-synuclein	Polymeropoulos et al. (1997)
PARK2 (*PDJ*; *PRKN*; *AR-JP*; *LPRS2*	6q25.2-q27	Parkin	Impaired protein degradation via proteasome	Kitada et al. (1998)
PARK7 (*DJ1*; *DJ-1*; *HEL-S-67p*)	1p36.23	Peptidase C56	Impaired oxidative stress response	Bonifati et al. (2003)
PINK1 (*BRPK*; *PARK6*)	1p36	Serine/threonine protein kinase	Mitochondrial dysfunction	Valente et al. (2004)
Fbx07	22q12.3	F-box proteins	Mitophagy	Burchell et al. (2013)
LRRK2 (*PARK8*; *RIPK7*; *ROCO2*; *AURA17*; *DARDARIN*)	12q12	Leucine-rich repeat kinase 2; dardarin	Impaired oxidative stress response	Zimprich et al. (2004) and Paisan-Ruiz et al. (2004)
PKPS (*PARK15*)	22q12.3	Ubiquitin protein ligase	Hamper ubiquitination and mitophagy	
Amyotrophic lateral sclerosis (ALS)				
SOD1 (*ALS*; *SOD*; *ALS1*; *IPOA*; *hSod1*; *HEL-S-44*; *homodimer*)	21q22.11	Superoxide dismutase 1	Protein misfolding/aggregation and/or impaired oxidative stress response	Brun (1987)

(continued)

Table 15.2 (continued)

ALS2 (*ALSJ*; *PLSJ*; *IAHSP*; *ALS2CR6*)	2q33.1	Alsin; ATS1/RCC1-like domain, a RhoGEF domain	Impaired neuroprotection	Ingelsson and Hyman (2002) and Morris et al. (2002)
C9orf72, *ALSFTD*, *FTDALS*	9p21.2	TDP-43 and FUS	Protein aggregates in sporadic ALS	Frag et al. (2014)
Huntington's disease (HD)				
HD (IT15)	4p16.3	Huntingtin-associated protein-1	Modulate neurodegeneration by expanded polyglutamine repeats	Kato et al. (2003)
PRNP (*CJD*; *GSS*; *PrP*; *ASCR*; *KURU*; *PRIP*; *PrPc*; *CD230*; *AltPrP*; *p27-30*; *PrP27-30*; *PrP33-35C*)	20p13	Prion protein (PrP)	Defective cell protection, communication and Cu transport, Huntington disease-like 1 (HDL1)	Cardone et al. (2014)
JPH3 (JP3; HDL2; JP-3; TNRC22; CAGL237)	16q24.3	Junctional complexes protein	Miss communication between plasma membrane and ER, Huntington's disease (HD)-like 2 (HDL2)	Schneider et al. (2012)
TBP (HDL4; GTF2D; SCA17; TFIID; GTF2D1)	6q27	TATA-binding protein (TBP)	Transcription factor IID (TFIID) dysfunction leading to faulty translation of mRNA	Savinkova et al. (2013)
ATXN1 (ATX1; SCA1; D6S504E)	6p23	Elongated polyglutamine tract in the corresponding protein	Spinocerebellar ataxia type 1 (SCA1)	Bergeron et al. (2013)
ATXN2 (ATX2; SCA2; ASL13; TNRC13)	12q24.1	Golgi apparatus protein	Affects golgi apparatus—ER signals. spinocerebellar ataxia-2 (SCA2)	Neuenschwander et al. (2014)
ATXN3 (*AT3*; *JOS*; *MJD*; *ATX3*; *MJD1*; *SCA3*	14q21	(CAG)n repeat proteins	Spinocerebellar ataxia-3 (SCA3)	Chou et al. (2014)
ATN1 (*B37*; *HRS*; *NOD*; *DRPLA*; *D12S755E*)	12p13.31	CAG/CAA mediated serine repeat proteins	Dentatorubral pallidoluysian atrophy (DRPLA)	Yapijakis et al. (2014)

APP amyloid beta (A4) precursor protein, *PSEN1* presenilin 1, *PSEN2* presenilin 2, *APOE* apolipoprotein E, *TREM2* triggering receptor expressed on myeloid cells 2, *SNCA* synuclein, alpha (non-A4 component of amyloid precursor), *PARK2* parkin RBR E3 ubiquitin protein ligase, *PARK7* Parkinson protein 7, *PINK1* PTEN (phosphatase and tensin homolog) induced putative kinase 1, *Fbx07* F-box protein 7, *LRRK2* leucine-rich repeat kinase 2, *SOD1* superoxide dismutase 1, soluble, *ALS2* amyotrophic lateral sclerosis 2 (juvenile), *C9orf72* chromosome 9 open reading frame 72, *HD* (*IT15*) huntingtin gene/interesting transcript 15, *PRNP* major prion protein, *JPH3* junctophilin 3, *TBP* TATA box binding protein, *ATXN1* ataxin 1, *ATXN2* ataxin 2, *ATXN3* ataxin 3, *ATN1* atrophin 1

4. *Head trauma*—Researchers reported that a traumatic brain injury triggers the release of amyloid precursor protein (APP) from axons in extracellular space, and this APP starts depositing amyloid protein on the neurons in the form of amyloid plaques [39].

15.2.4 Symptoms of Alzheimer's Disease

AD is characterized by shrinking of hippocampus and cortex area of brain tissue. Hippocampus is associated with developing new memories, and thus loss of neurons at this region leads to loss of recent memories (short-term memory loss) which is the earliest and most prominent symptom of AD. This situation worsens over the period of time affecting remote or old memories. As the disease progresses, parietal and temporal lobe starts losing function leading to language dysfunction mainly affecting word search. Thus communication ability of the patient becomes compromised. Posterior cerebral dysfunction causes difficulty in performing simple practiced functions like brushing teeth and using remote control [40]. Patients experience behavioral problems like depression and sleep disturbance. In later stages, verbal and physical aggression, psychomotor agitation, inappropriate sexual behavior, and psychotic symptoms are observed in patients. In advanced stages, patients develop motor signs such as gait disturbance, tremor, and urinary incontinence [41, 42].

15.2.5 Treatment

15.2.5.1 Symptomatic Therapy

There is no cure for Alzheimer's disease, but some FDA-approved medications can dramatically improve the symptomatic conditions. The first class of symptomatic drug therapy reported to reduce AD symptoms is anticholinesterase inhibitors (AchEI) like donepezil. Other classes are memory booster drugs like galantamine. The parasympathomimetic or cholinergic agent, like rivastigmine, is also used in the treatment of AD. Recently NMDA (N-methyl-D-aspartate) glutamate receptor antagonist drugs, like memantine, are also reported to be used for symptomatic treatment of AD [43].

15.2.5.2 Preventive Therapy

Currently, there are many research-based and clinical trials going on to reduce, delay, or prevent symptoms of AD before they start appearing. One clinical trial ongoing (by Dominantly Inherited Alzheimer Network) on the high-risk genetic AD patients to avoid or delay the AD symptoms includes the use of specific antibodies against beta-amyloid protein which are supposed to neutralize the mutated beta-amyloid and reduce or delay the appearing AD symptoms. Similarly, a clinical trial, known as A4 trial, on the patients aged between 65 and 85 without AD symptoms is being carried out with antibodies against beta-amyloid to see the effect toward avoiding or delaying the appearance of AD symptoms due to age [44].

Tuszynski and coworkers at the University of California performed a clinical trial in 2001 in which they isolated the long-living skin cells from patients and infected them with viral vector containing nerve growth factor (NGF) gene. These cells then started secreting nerve growth factor and acted as mini-bio-pumps of NGF. These NFG-secreting cells were then transferred into patient's basal forebrain, and patients were studied for 10 years. The studies revealed that patient's brain started supporting new nerve fibers and the neuron cell size also enhanced. The same group in 2009 reported a gene therapy using brain-derived neurotrophic factor (BDNF)-expressing gene transferred via viral vector to cortical neurons of hippocampus in rat models [45]. They observed that the expression of BDNF gene reverses synapse loss, improves cell signaling, and restores learning and memory (Table 15.2).

In another study, researchers at St. Jude Children's Hospital developed a gene therapy involving the enzyme neuraminidase 1 (NEU1) gene and tested it in mice. NEU1 is responsible for carrying recycling of unneeded proteins in cells. Researchers observed that the AP buildup on neurons declined dramatically after a few weeks of gene therapy designed to boost NEU1 activity [46]. The effect of low levels of progranulin protein in the brain which increased the formation of amyloid-beta plaques on the neurons leads to memory impairment and triggers the immune response. These researchers then developed the gene therapy in mouse using lentivirus-mediated progranulin (PGRN) gene (GRN) transferred in brain cells of frontotemporal lobe of mouse. The overexpression of this gene lowered amyloid plaque load in AD mice brain [47]. Additionally, CREB-regulated transcription coactivator-1 protein expressed by Crtc 1 gene (released by brain cells mainly at hippocampus region and involved in long-term memory development) is blocked from expression in patients with Alzheimer's. These researchers then developed an adeno-associated viral-mediated gene therapy for the delivery of Crtc 1 gene in brain cells at hippocampus region of mouse brain. They observed that the gene expression in the hippocampus efficiently reverses Alzheimer-induced spatial learning and memory deficits [48].

15.3 Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease followed by Alzheimer's disease, affecting more than ten million people worldwide. It is a chronic, progressive neurological disorder affecting mainly dopaminergic neurons in substantia nigra area of the brain and development of Lewy bodies (a pathologic hallmark) in dopaminergic neurons (Fig. 15.2). These neurons in substantia nigra are responsible for producing dopamine which is a major neurotransmitter that controls movement and coordination. With the progression of PD, the amount of dopamine produced in substantia nigra goes on decreasing, leading to decreased control of body movements. Primary motor signs of PD are tremor in the hands, arms, legs, jaw, and face, rigidity of limbs and trunk, slowness of movement, and postural instability due to impaired balance and coordination. PD may be

diagnosed in some patients in their early 40s and is most common in patients over 60s. It affects men and women equally, and at present there is no known cure for Parkinson's disease [49–55].

15.3.1 Global Burden of PD

PD is taking 1.8% share of all neurodegenerative disease cases throughout the world and affecting 0.11% of total world population. In the USA only, the collective direct and indirect cost of PD, including treatment and lost job income due to work inability, was estimated to be nearly $25 billion per year. According to WHO, the projected deaths attributable to PD as percentage of total deaths for 2015 and 2030 are 0.20 and 0.23%, respectively (Table 15.1).

15.3.2 Pathophysiology

As shown in Fig. 15.3, the substantia nigra pars compacta and the pontine locus coeruleus of the brain are affected by typical abnormalities including depigmentation, neuronal loss, and gliosis (Fig. 15.3d and 15.3f). PD is also characterized by Lewy body in a pigmented neuron in substantia nigra. The PD symptoms are prominently observed when 60–70% of the neurons in the substantia nigra pars compacta are degenerated, thus making the treatment more difficult [56, 57].

15.3.3 Etiology

Genetic Mutations The first and most understood cause of PD is the neuronal deaths at substantia nigra region of the brain which is mainly due to the presence of Lewy bodies in those nerves. These Lewy bodies are composed of defectively aggregated, insoluble alpha-synuclein also known as the non-Abeta component of amyloid plaques [58]. Although the normal function of synuclein remains poorly understood, it was studied for its presence at the nerve terminal and in membrane remodeling. When it is related to PD in Lewy bodies, in contrast to its helical conformation on membranes, synuclein adopts a β-sheet structure in aggregates [59]. Thus, it is a clear mechanism of misfolded protein aggregates as explained in the introduction, and it is clearly the outcome of some genetic mutations that code this protein.

Cell Transport Disruption Ubiquitin-proteasome system is the system present in the nerve cell which is designed to break down and throw out the abnormal proteins like insoluble alpha-synuclein aggregates that become impaired in PD.

Mitochondrial Dysfunction The neuronal degeneration can be caused by abnormal oxidative stress through reactive oxygen species, which generally occurs when there is a mitochondrial dysfunction [60].

Viral Infection Braak's hypothesis claims that pathophysiological progression of PD starts with an unknown, possibly viral, pathogen entering the brain through the olfactory route or the swallowing of nasal secretions introduces the pathogen to the gut from where it enters the vagus nerve and the CNS. It was reported that non-motor symptoms, like sleep disorders, loss of sense of smell, hyposmia, and constipation, may indicate the onset of PD several years earlier to actual motor symptoms. The hypothesis was substantiated by pathologic proofs of presence of Lewy bodies in the intestinal structures, vagus nerve, and brain structures [61].

Environmental Factors After Braak's hypothesis, scientists explored possible environmental factors, and their research work suggested possible role of environmental stress and aging in the promotion of neurodegeneration. The researchers found that the exposure to environmental toxins (e.g., pesticides) [62] and abusive drugs or the aging stress leads to a chronic low-level inflammation in the brain. This chronic inflammatory condition might initiate the neuron deterioration in the brain leading to PD [49, 63].

15.3.4 Symptoms of Parkinson's Disease

Parkinson's disease is a chronic, progressive neurological disorder affecting mainly dopaminergic neurons in substantia nigra area of the brain which is mainly affecting motor and non-motor functions of the body. The signs and symptoms differ from person to person. Early signs are often mild and remain unnoticed. The motor symptoms often begin from one side of body and progressively worsen as both sides get affected. As per Braak's hypothesis, we can find out the onset of PD several years earlier to actual motor neuron dysfunction by observing the simple symptoms like sleep disorders, loss of sense of smell, hyposmia, and constipation.

In the early stages of motor neuron dysfunction, patient is unable to show facial expression, the speech becomes soft and slurred, and patient does not swing his/her arms while walking. As condition progresses with time, the following symptoms become worse:

Tremors A prominent motor symptom characterized with shaking of body; it generally begins with terminal limb organs like fingers or hand. An unintentional back and forth rubbing movement between the thumb and forefinger called pill-rolling tremor or tremor of the hand when at rest is the characteristic of Parkinson's disease.

Slowed Movement (Bradykinesia) and Muscle Stiffness As disease progresses, reduced and slower movements and muscle stiffness characterize the disease, as it makes simple tasks difficult and time-consuming. The walking steps may become shorter, patient finds it difficult to get out of a chair, and many times patient drags his/her feet as he/she tries to walk.

Impaired Posture and Balance and Loss of Automatic Movements In the extreme conditions, maintaining body balance and performing unconscious movements like blinking, smiling, or swinging of arms do not take place naturally.

Other neurodegenerative disorders that can mimic the conditions of PD include dementia with Lewy bodies (DLB), multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD).

15.3.5 Treatment

15.3.5.1 Symptomatic Therapy

Parkinson's disease cannot be cured, but some medications can dramatically improve the symptomatic conditions, sometimes surgery, to regulate certain regions of the brain. The main medication which is currently available and most effective against motor symptoms is levodopa, but the other medications available are monoamine oxidase type B inhibitors [MAOBIs], amantadine, anticholinergics, β-blockers, or dopamine agonists which are mainly used to avoid levodopa-related motor complications. The non-motor symptoms like hallucinations can be treated by clozapine, dementia can be treated with cholinesterase inhibitors, and depression can be treated with antidepressants and pramipexole. These medications are generally used along with the specific PD symptomatic medications. The specific PD medications available in the market for single or combination therapies are Sinemet (carbidopa/levodopa), Sinemet+Comtan (entacapone), Parlodel (bromocriptine), Permax (pergolide), Requip (ropinirole), Mirapex (pramipexole), and Casbar (cabergoline) [64, 65].

15.3.5.2 Preventive Therapy

Recently, scientists from Nanyang Technological University, Singapore, reported that the existing antimalarial drugs could be a potential treatment for PD. Researchers at NTU reported that activating Nurr1 gene releases protein which protects brain's ability to generate dopamine. These scientists further reported that potent antimalarial drug molecules like chloroquine and amodiaquine can directly bind to Nurr1 gene and activate it to revert or to stabilize the PD conditions (stop progression) [66]. In addition to pharmacological treatment, gene and genomic therapies are also tried and reported to be successful preclinically and clinically in reverting PD condition.

The scientists from the University of Pittsburgh developed a new gene therapy for PD using the viral vector AAV2 which is in clinical trial phase. They proposed that mitochondria and α-synuclein (encoded by *SCNA* gene—Table 15.2) can interact and damage the neuron, and if we target the α-synuclein synthesis, it might modulate the PD condition. To prove their hypothesis, they used a harmless virus called adeno-associated virus type 2 (AAV2) which was engineered to transport a *SCNA* gene silencer code [short hairpin RNA (shRNA)] into the neuron and block the production of α-synuclein. The blocked α-synuclein production ultimately

results in avoiding aggregation of faulty Lewy bodies in neurons. It also avoids the interaction of α-synuclein with mitochondria and thus avoids the PD progression [67]. Professor Nicholas Mazarakis and his team from Imperial College London developed a new genomic therapy with a strategy to deliver three different genes (not disclosed) which are coded for enzymes that produce dopamine. He used lentivirus vector which is closely related to HIV to incorporate the set of genetic material into the genome of the neuron cells it infects, ensuring a long-lasting effect. The team reported that the treatment corrected the movement defects of the monkeys for more than 3 years, without any adverse effects. The clinical trials with 15 patients also showed promising results in recovering from motor symptoms and stopping the progression of PD [68].

15.4 Huntington's Disease

Huntington's disease (HD) is caused by a defective gene which results in the programmed degeneration of neurons (brain cells) (Fig. 15.2), particularly in basal ganglia and the cerebral cortex. It is an incurable, hereditary brain disorder. HD is named after George Huntington, the physician who described it as hereditary chorea in 1872 [69, 70].

15.4.1 Global Burden of HD

HD is taking 0.3% share of all neurodegenerative disease cases throughout the world and affecting 0.018% of total world population. HD and other hyperactivity disorders (ADHD) also called as Huntington's disease-like syndrome are showing 5.29% prevalence in the global population. In the USA only, the collective direct and indirect cost of HD, including treatment, and lost job income due to work inability, is estimated to be nearly $1.3 billion per year [71, 72].

15.4.2 Pathophysiology

Pathophysiological evidences can be seen microscopically in the infected brain tissue only at grade 2 HD where neuropathy with striatal atrophy and convex caudate nucleus at neostriatum was observed. Thus the extent of gross striatal atrophy, neuronal loss, and gliosis (Fig. 15.3g and h) is generally used for grading the severity of HD pathology (grades 0–4) [73–76].

The neuropathy with striatal atrophy in HD is caused due to the expansion and expression of a Nterminus cysteineadenosineguanine (CAG)n repeat sequences in Huntington protein synthesizing gene (e.g., ATXN3, SCA-1) (Table 15.2). This Huntington protein is located in the cytoplasm and is associated with the range of organelles, like transport vesicles, mitochondria, microtubules, synaptic

vesicles, etc. The expression of Nterminus cysteineadenosineguanine (CAG) repeat sequences in the Huntington protein might destroy the actual function of the protein. In addition to this, there are some evidences suggesting the mutant Huntington accumulation and formation of inclusions in the brain cell nucleus called as "neuronal intranuclear inclusions (NIIs)" which are toxic/pathogenic and cause HD [77–80].

15.4.3 Etiology

The disease affects movement, behavior, and cognition in later part, but as discussed, there are microscopic and biochemical clues available for the prediction of the HD. There are different mechanisms like excitotoxicity, oxidative stress, impaired energy metabolism, and apoptosis, which cause these microscopic and biochemical changes in neurons [81]:

1. *Excitotoxicity*—It is the excessive activation of postsynaptic receptors by excitatory amino acids leading to the neurotoxic effects of HD. This mechanism was proved by injecting kainic acid, an agonist of glutamate receptor, and quinolinic acid, an agonist of NmethylDaspartate (NMDA) receptor in rat, and observing the mimicking neuropathology similar to HD. In addition to excitotoxicity, reduced uptake of glutamate by glial cells was also proposed to play a role in the pathogenesis of HD.
2. *Oxidative stress*—It is caused by the presence of free radicals like highly reactive oxygen derivatives (ROS) in large amounts. The mechanism was proved by the observation that quinolinic acid-induced striatal damage can be reduced by administration of antioxidants. Oxidative stress can occur as a consequence of mitochondrial breakdown or excitotoxicity and can trigger apoptosis. The probable mechanism of the oxidative stress proposes reduction in the activity of the respiratory chain complexes II and III of mitochondria of neurons which show the increased lactate levels in the basal ganglia and occipital cortex of patients with HD. This mechanism was proved by Revilla and coworkers by injecting 3nitroproprionic acid (3NP) which is an inhibitor of succinate dehydrogenase or complex II of the respiratory chain. This experiment caused dose-dependent ATP depletion, increased lactate concentration in neurons, and neuronal loss in the striatum in rats [81].
3. *Apoptosis*—It is called as programmed cell death and is the natural phenomenon which the body experiences from embryogenesis where this phenomenon is used to remove supernumerary neurons as part of natural development. Naturally the neurons are protected from apoptosis by CREB-binding protein (CBP), which is a major mediator of survival signals in mature neurons. One theory suggests that expanded polyglutamine repeats interact with other proteins containing short polyglutamine tracts and interference with transcription and expression of CREB-binding protein (CBP) leading to HD [80].

15.4.4 Symptoms of Huntington's Disease

Huntington's disease is an autosomal genetic disorder, it requires only one copy of the defective gene to develop the disorder, and there are 50–50% chances of developing HD in children of HD parents. The disease causes the programmed breakdown (degeneration) of neurons in the brain. The HD signs and symptoms generally appear in patients at the age 30s or 40s, but the onset of disease may be earlier (in some cases it was observed in 20s).

This degeneration of neurons in basal ganglia and the cerebral cortex causes uncontrolled movements, loss of thinking (cognitive) ability, and psychiatric disorders. Broadly, the patient gradually loses his/her abilities to walk, talk, think, and reason, and they become totally dependent on other people for their care. Thus, HD has a major negative physical, emotional, and socioeconomic impact on patients and their families' lives.

15.4.5 Treatment

15.4.5.1 Symptomatic Therapy

HD is not curable but some symptoms of the disease can be treated with medications. Symptoms like depression, obsessive-compulsive behaviors (OCBs), agitation, and irritability can be treated by selective serotonin reuptake inhibitor type of antidepressants. Irritability and impulsive behavior can be treated with anticonvulsants like valproic acid or carbamazepine. Anxiety can be treated with anxiolytic drugs, and delusions and behavioral outbursts can be treated by antipsychotic drugs. Chorea can be treated with dopamine-blocking agents like tetrabenazine. Cognitive disorders are now being treated with new drugs, namely, memantine [82], rivastigmine [83], and donepezil [82–84].

15.4.5.2 Preventive Therapy

The molecular and biochemical understanding of the disease suggested the following causes for HD: changes in protein homeostasis, mitochondrial dysfunction, excessive or abnormal neurotransmitter input, and hampered axonal trafficking in brain neuron cells.

Based on the same understanding, new compounds and strategies were designed as HD preventive treatment which include histone deacetylase inhibitors (e.g., phenylbutyrate); neuroprotective compounds (e.g., lithium); antioxidants, mitochondrial enhancers, and energy substrates (e.g., coenzyme Q and creatine); anti-apoptotic compounds (e.g., minocycline); transglutaminase inhibitors; chemicals that inhibit protein aggregation or support protein folding; molecules that enhance clearance of mutant protein; molecules that inhibit the kynurenine 3monooxygenase pathway; cell or gene replacement therapy; and RNA silencing agents that "knock down" disease gene expression [85].

Different genetic mutations lead to faulty protein expression (Table 15.2) and ultimately lead to the disease. HD is mainly caused by faulty production of

Huntington protein in brain neurons. These faulty proteins contain (CAG)n repeat sequences because of mutant gene transcribing faulty mRNA. Prof. Stephanie Liou developed an antisense gene therapy which is a gene silencing technique, where he inserted short single-stranded pieces of chemically modified nucleotides, known as oligonucleotides (oligos), into the cells. These oligos contain complimentary sequences to that of faulty mRNA and either physically block translation, or they stimulate RNAse H enzyme to degrade the mRNA complex and thus stop synthesis of faulty Huntington protein.

Similar to gene silencing technique, RNA interference (RNAi) technique is also being developed which utilizes large strand of complimentary RNA molecule which binds to faulty mRNA of Huntington protein and activates "dicer" enzyme which cuts this complex into smaller fragments and thus avoids translation of the faulty Huntington protein. Researchers found that inserting the large complimentary RNA fragment is difficult, so they came up with the new idea of small interfering RNA (siRNA), which is a double-stranded RNA fragment with complimentary sequence to the mRNA for Huntington protein. These siRNA are easy to insert in cells, and after entering the cells, they separate, and the complimentary piece attaches to mRNA strand initiating the mRNA degradation by "dicer" enzyme, thus avoiding translation of Huntington protein.

Researchers also found that the caspase enzyme is essential for the specific cleavages of Huntington protein. This enzyme cleaves the protein at 3rd and 6th position from N-terminal. If this enzyme is allowed to cleave the mutated Huntington protein selectively at caspase3 site, but not caspase6 site, it resulted in protection of neuron from neuronal dysfunction and neurodegeneration. These observations suggest that preventing caspase6 cleavage of Huntington protein may be studied for therapeutic interest [86].

15.5 Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, Charcot disease, or motor neuron disease (MND), is a rapidly progressive motor neuron disease which attacks and kills the neurons controlling voluntary muscles such as the diaphragm, face, arms, and legs. The motor neurons in the brain, brain stem, and spinal cord maintain and control the vital communication between voluntary muscles of the body. In ALS the motor neurons transmitting signals from the brain (upper motor neurons) to the spinal cord (lower motor neurons) degenerate or die ultimately failing to send the messages to muscles and ultimately showing no voluntary muscular movement. As there are no movements, muscles gradually weaken and undergo atrophy which is characterized by fine twitches (fasciculations). After 3–5 years from the onset of symptoms, the brain completely loses the ability to control voluntary movement of the body. When voluntary muscles in the diaphragm and chest wall fail, patients lose the ability to breathe without external support and eventually die.

15.5.1 Global Burden of ALS

ALS is taking 0.072% share of all neurodegenerative disease cases throughout the world and affecting 0.002% of total world population. In the USA only, the collective direct and indirect cost of ALS, including treatment and lost job income from work inability, is estimated to be nearly $1.03 billion per year [87].

15.5.2 Pathophysiology

The brain stem and upper spinal cord neurons undergo axonal loss with secondary myelin pallor and gliosis which extend throughout the spinal cord. It was also observed that deeper layers of the gray matter which is underlying subcortical white matter undergo astrocytic gliosis. The presence of CD68 (lysosomal marker) suggest that the glial response at the cortical and spinal tracts are due to microglia activation and presence of active macrophages. It was also observed that ventral roots become thin due to the huge loss of myelinated fibers from motor nerves leading to denervation atrophy (Fig. 15.3g) [88].

15.5.3 Etiology

The exact mechanism of the neuron degradation in ALS is not clear, but researchers believe that there are multiple factors individually or in combination affecting the motor neurons or the cells that support them.

1. *Aggregates*—As reported earlier, if the mRNA translation is faulty, it leads to generation of faulty protein. TDP-43 is a protein which regulates the expression of the mRNA in neuron. In ALS this TDP-43 protein itself expressed faulty forming aggregates and thus started the cascade of faulty protein expressions which ultimately disrupt the normal working of the motor neurons and destroy it.
2. *Cell transport disruption*—The normal cell transportation gets rid of metabolic and other toxic substances by packaging them in microvesicles and throwing them out of cells. Antioxidants help the cells in this reaction. This normal function is disrupted in the ALS, and toxic waste buildup in cells will destroy the normal functioning and ultimately complete neuron cell.
3. *Glial cells*—Glial cells are the nurse cells of the neuron fibers. They supply nutrients and support and excrete lipid layer around it, which help neuron cells to function normally and transfer electrical impulses efficiently. If these glial cells are hampered, neurons will not receive nutrition and support, and lipid layer will be destroyed, resulting in complete loss of nerve conduction and normal function of neuron.

4. *Glutamate*—If the neuron cells become sensitive (immunosensitive) to the neurotransmitter (glutamate), immune system will destroy the neurons and a complete loss of motor neuron activity will be observed.
5. *Mitochondria*—They are the energy house of the cells and contain SOD1 protein in intermembrane space, matrix, and outer membrane. If there is a presence of abnormal misfolded SOD1 protein, it can cause mitochondrial dysfunction with damaged mitochondrial membrane, and it leads to motor neuron diseases.

15.5.4 Symptoms of Amyotrophic Lateral Sclerosis Disease

The early symptoms like cramps, stiff muscles, muscle weakness affecting arms and legs, slurred speech, and difficulty in chewing and swallowing were very difficult to distinguish from non-ALS progressing patients. As the disease progresses, patients find it difficult to perform simple tasks such as buttoning a shirt, writing, or turning a key in a lock. Many patients experience tripping or stumbling while walking or running. There is muscle weakness, and atrophy spreads through other parts of the body which is indicative of lower motor neuron degeneration. A characteristic abnormal reflex action, Babinski's sign (the large toe extends upward as the sole of the foot is stimulated in a certain way), is an indication of upper motor neuron damage. Thus, to confirm the diagnosis of ALS, patients must show signs and symptoms of both upper and lower motor neuron damages which cannot be confused with other disease symptoms. In the later part of disease, severe conditions were observed where patients find it difficult to stand, walk, use their hands and arms, and get in or out of bed on their own. There is a risk of choking due to the difficulty in chewing and swallowing. As ALS is mainly a motor neuron disease, the cognitive abilities of the patients remain relatively intact and as patients are aware of their progressive loss of function, they become anxious and depressed. In end stages of the disease, patients feel difficulty in breathing as the muscles of the respiratory system weaken and ultimately lose the ability to breathe on their own [89].

15.5.5 Treatment

15.5.5.1 Symptomatic Therapy

There is no cure for ALS to date, but a symptomatic treatment for glutamate-related motor neuron degeneration is available in the market. FDA approved riluzole (Rilutek) in 1995 which decreases the release of glutamate and thus reduces damage to motor neurons. This drug does not reverse the damage already done to motor neurons but slows down the damage further and helps the patients with swallowing difficulty. It also helps the patients to delay the ventilation support as the disease progresses. Scientists are trying to develop the combination therapy to further slow

down the progression of ALS. Other symptoms like fatigue, muscle cramps, excess saliva and phlegm, pain, depression, sleep disturbances, and constipation can be treated with the help of available medications. Physical therapy, speech therapy, nutrition support, ventilators and Diaphragm Pacing System, respirators, home care, and hospice nurses can help patients to some extent, but it does not help in ALS progression.

15.5.5.2 Preventive Therapy

The understanding of molecular mechanisms of the ALS helps the scientist to develop the preventive therapy for ALS. Scientists are focusing on the molecular mechanisms of RNA molecules and recycling of proteins, impaired energy metabolism, hyperactivation of motor neurons, and degradation of glial cells. Scientists are working on developing gene/genome therapy to correct single or multiple mutations in the gene responsible for ALS. One of the important and most understood mutations responsible for ALS is antioxidant enzyme Cu/Zn superoxide dismutase 1 (SOD1). Recently researchers found TDP-43 and FUS protein aggregates in the spinal cord which are responsible for ALS, and the respective mutations were observed in chromosome 9 (C9orf72). Scientists used adeno-associated virus serotype 6 (AAV6) to deliver small hairpin RNAs (shRNAs) to knockdown mutant superoxide dismutase 1 (mSOD1) in mouse models, and the treatment showed neuroprotection and halted muscle atrophy in mouse [90]. Scientists also tested neuron protection action of expressed PRDX3 or NRF2 genes by transferring these genes to NSC34 cells lines (ALS tissue culture model expressing the human SOD1^{G93A} mutation) using lentiviral vectors. The cell lines showed the overexpression of PRDX3 or NRF2 with 40 and 50% decrease in endogenous oxidation stress levels [91]. Some researchers used antisense oligonucleotides (ASOs) to bind to the antisense (GGCCCC) direction C9orf72 RNA foci which specifically reduced the expression C9orf72 and showed significant neuron protection action. In the same study, researchers also observed that siRNAs inserted in cells with viral vectors fail to reduce nuclear RNA foci but showed marked reduction in C9orf72 RNAs which is well tolerated by mice brain with no symptoms of ALS [92].

Conclusion

This chapter summarizes the pathophysiology, etiology, and latest symptomatic (drug) and preventive (gene and genomic) therapies for Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. The etiology of the HDs suggested that there are multiple genes responsible for these conditions. The recent studies suggested that genomic treatment (multiple gene treatment) can reverse or at least stop further neuron degeneration and effectively provide the cure for different HDs. These treatments are site-specific with no or little side effects, suggesting genes can be ultimately be used as medicine. Thus it can be concluded that gene and genome therapy can provide effective treatment for HDs.

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16 Recent Trends in -Omics-Based Methods and Techniques for Lung Disease Prevention

Raisah Salhab and Yashwant Pathak

Abstract

As the use of engineered nanoparticles (ENMs) in the manufacturing environment and consumer products increases, the concern over human exposure to ENMs is also increased. Due to varying physical and chemical characteristics of ENMs, the level of toxicity varies based on the shape, size, solubility, surface area, and surface charges of the ENM that is synthesized. However, with the lack of reference materials and inconsistent protocols, the validation of novel methods in order to determine toxicity has been deemed challenging; thus, there is an inability for an accurate assessment based on the human health risk assessment (HHRA) of environmental chemicals when exposure has occurred [1]. Also, current methods for chemical risk assessments are not without additional limitations as their high costs and the reliance on observing the effects of toxicity in animals lead to very few assessments done on chemicals that are in use in manufacturing [2]. With the use of toxicogenomics, there is the ability to determine the level of toxicity that is associated with certain properties of ENMs as well as assist in the identification of potential health hazards [2, 3]. DNA microarray, large-scale real-time quantitative polymerase chain reaction, and RNA sequencing are among the most commonly used technology within toxicogenomics [2].

16.1 Transcriptomics

As the use of engineered nanoparticles (ENMs) in the manufacturing environment and consumer products increases, the concern over human exposure to ENMs is also increased. Due to varying physical and chemical characteristics of ENMs, the

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_16

level of toxicity varies based on the shape, size, solubility, surface area, and surface charges of the ENM that is synthesized. However, with the lack of reference materials and inconsistent protocols, the validation of novel methods in order to determine toxicity has been deemed challenging; thus, there is an inability for an accurate assessment based on the human health risk assessment (HHRA) of environmental chemicals when exposure has occurred [1]. Also, current methods for chemical risk assessments are not without additional limitations as their high costs and the reliance on observing the effects of toxicity in animals lead to very few assessments done on chemicals that are in use in manufacturing [2]. With the use of toxicogenomics, there is the ability to determine the level of toxicity that is associated with certain properties of ENMs as well as assist in the identification of potential health hazards [2, 3]. DNA microarray, large-scale real-time quantitative polymerase chain reaction, and RNA sequencing are among the most commonly used technology within toxicogenomics [2].

Measurement of gene expression using DNA microarray is done through the binding of complimentary DNA to probes attached to array in order to detect mRNA. This then allows the comparison of the levels of gene expression. Although this method is well validated and has the ability to measure gene expression of large numbers of genes, there is still the disadvantage that the number of genes measured is fixed [2].

Gene expression profiling has been utilized in studies in order to explore either lung injury or lung disease caused by exposure to ENMs specifically if nanoTiO_2, carbon black, or multi-walled carbon nanotubes (MWCNTs) present an association with the development of certain lung diseases. As a result of its wide use in commercial and biomedical applications, carbon nanotubes (CNTs) and their effects on pulmonary pathology have been studied. After exposure to CNTs, more particularly multi-walled carbon nanotubes (MWCNTs), rodents have experienced effects on their lungs such as pulmonary inflammation, granulomas, and lung fibrosis. Certain features of MWCNTs, such as persistence in lung tissue after exposure, being composed of fiber-like structures, and chemical groups found on their surface, may lead to the development of pulmonary fibrosis. However, the underlying mechanism of how MWCNTs cause pulmonary fibrosis has yet to be understood and proven to be challenging [1]. Data from other studies have shown that MWCNTs have the potential to induce fibrosis through Th2-mediated signaling. Analyses of microarray studies have shown that the initial response to MWCNTs is similar to the response of exposure to substances such as bleomycin or bacterial challenge. Bleomycin exposure models exhibit the initial response is characterized by the disruption of innate immune mechanisms which progresses to the activation of CD4+ T cells triggering Th2 cytokines. Th2 cytokines, when upregulated, engage in the development of fibrosis [1]. There is also a difference in a later response as compared to the initial response where the later response of MCWNT exposure is similar to Th2 response after exposure to allergens as altered Th2 gene expression of chemokines, cytokines, and growth factors play a role in lung fibrosis [3]. Unlike MWCNTs, which were classified by Nikota et al. [3] as potentially disease-causing ENMs, nano-TiO_2 and carbon black were not classified as such although exposure by both ENMs can

induce lung inflammation as well as change the expression of inflammatory genes. However, the instance of change in the expression of genes through exposure of nano-TiO_2 and carbon black was lower as well as reversible. Compared to MWCNTs that had multicell-type involvement in inflammation, nano-TiO_2 and carbon black exposure exhibited less inflammation with primarily neutrophil cell involvement.

The concept of RNA sequencing is that there is the ability to count how many times a transcript has been sequenced which then provides the level of expression of each exon in a gene. RNA sequencing can also detect both known and unknown transcripts that have low signal/noise ratio as well as high degree of sensitivity, unlike microarray technology [4]. Due to its reliance on counting transcripts, this method becomes more quantitative and can theoretically measure any potential transcripts. However, it is not as validated as microarray due to the continuing evolution of RNA sequencing technology [2].

RNA sequencing has been used to exhibit the differences between the transcriptomic profiles of in vivo whole human endobronchial biopsies between patients with asthma and healthy nonatopic controls. Between asthma patients and controls, 46 genes were differentially expressed, and a number of those genes have shown to have differing effects on biological processes such as mRNA degradation and translation which go on to affect cellular functions, such as STAU2 and WARS, within the airways. When the phrase "differentially expressed" is used, it means that the number of copies of the gene's transcript is increased (upregulated) or decreased (downregulated). The number of genes that are differentially expressed presents information on the magnitude of a transcriptional response [2]. Pendrin, BCL2, and periostin were among the 46 genes that were differentially expressed. From the genes that were differentially expressed, a large number have yet to be linked to asthma; thus, there is the potential for novel disease-related genes [5]. Due to the complexity of asthma, as well as the difference in the biological processes between patients that have asthma and subjects who are healthy as exhibited by this study, these findings may be pertinent to the pathogenesis of asthma.

Gene expression profiling with the use of RNA sequencing of lung tissues was also done to understand the molecular mechanisms that take part in the pathogenesis of COPD. Lung tissue samples were acquired from patients who required resection for lung cancer, and total RNA was isolated from lung tissue that was away from the area with lung cancer. The subjects were made up of 98 patients with COPD and 91 control subjects. Genes that had expression levels that were found to be related to COPD status by RNA-seq was validated using TaqMan real-time PCR. A total of 2312 genes were identified as differentially expressed between the lung tissues of COPD patients and control subjects. MICAL2 and NOTCH2 were upregulated in the resected lung tissue of patients with COPD while S100A6 and genes encoding ribosomal proteins had lower expression in the COPD group compared to the control subjects [6]. The expression of these genes was also reduced in the small airway epithelium of smokers as previously shown by RNA-seq. Protein catabolism pathway and ubiquitination-proteasome pathway were impaired in the lung tissues of patients with COPD. There was also downregulation of genes that are related to the 20S proteasome such as PSMA2, PSMB1, PSMC5, PSMD4, and PSMD13.

The genes for chromatin modification, an important mechanism in epigenetics, were found to be upregulated in lung tissue of COPD patients. The finding of this study may help in the understanding of the mechanistic implications of COPD as oxidative phosphorylation, protein degradation, and chromatin modification were the most altered pathways in COPD lung tissues [6].

Quantitative reverse transcription PCR, or RT-qPCR, is a method that is as well validated as microarray but is more sensitive. Due to the fact that analyses are done separately, the drawback lies in that a finite number of genes can be examined at a time. RT-qPCR was used in order to validate microarray results when assessing if miRNA expression was altered in the sputum of patients who had active pulmonary tuberculosis. Other studies have shown that miRNA is present in sputum and there is alteration of unique miRNA signatures in lung diseases such as COPD. To understand the role of miRNA in active pulmonary TB, the study showed that there were 95 differentially expressed miRNAs: 43 miRNAs were overexpressed and 52 miRNAs were underexpressed in the tuberculosis group as compared to the control. In order to validate microarray results, miR-19b-2*, miR-3179, and miR-147 were chosen due to the results showing that miR-19b-2* was the most underexpressed, miR-3179 was the most overexpressed, and miR-147 was both overexpressed and is a negative regulator of inflammatory response. With use of RT-qPCR for validation of microarray results, the results were consistent with microarray showing that miR-147 was overexpressed in the tuberculosis group as compared to the control group [7].

With the concern that the microbial microenvironment related to the development of pulmonary diseases, RT-qPCR has also been used to study the potential relationship of TLR4 and endothelial PAS domain-containing protein 1, a key regulator of COPD. Bronchoalveolar fluid was collected from 55 patients who had COPD and 25 healthy subjects. The detection of the expression levels of TLR4 and TLR5 was done with the use of RT-qPCR. In the lower respiratory track of COPD patients, the expression of TLR4 was significantly increased as well as the levels of neutrophils, lymphocytes, and macrophages. Associated with the increase in TLR4 expression, EPAS1 mRNA was decreased while EPAS1 promoter methylation was increased in COPD. Thus the study suggests that overexpression of TLR4 leads to decreased expression in EPAS1 expression leading to the progression of COPD [8].

Innate immunity of COPD patients who were stable was investigated using RT-qPCR. Patients were divided into four groups based on risk and symptoms exhibited. Patients were also classified based on sputum cellularity. If sputum neutrophil count was ≥76%, then they were classified as neutrophilic phenotype. Subjects who were healthy did not display any respiratory symptoms or airway responsiveness. The expression of IL-29 was positive in 16 of the 51 COPD patients as well as in 9 out of 35 healthy subjects. IFN-β was found in 6 of the 51 COPD patients while 2 out of the 35 healthy patients had IFN-β detected in sputum samples. Interferon-stimulated genes were expressed in both patients with COPD and healthy subjects. In terms of the severity of airway obstruction, there was no difference detected in the expression of IL-29 or IFN-β between the two groups, but patients who had severe COPD did exhibit lower expression of OAS. OAS is an enzyme that activates the latent form of RNaseL which leads to viral and hot RNA

degradation. Compared to patients with mild to moderate COPD, patients with severe COPD had low expression of interferon-stimulated genes. Due to the results showing that there is a correlation with MxA and OAS expression with post-bronchodilator FEV_1/FVC ration, it demonstrates that the expression of interferon-stimulated genes is lowered as airway obstruction progresses [9].

16.2 Proteomics

Proteomics allows the characterization of proteins and the role they play in biological processes as alterations in proteins hint an involvement in the development of disease [10]. Unlike transcriptomics where gene expression is studied by measuring transcriptional regulation of genes through messenger levels, proteomics focuses on proteins that take part in the establishment of the function of genes through enzymatic catalysis, molecular signaling, and physical interactions [11]. Mass spectrometry is a tool that is widely used in large-scale proteomics. A tool that is comprehensive, mass spectrometry has the capability to quantify proteins as this provides a depiction of concentrations of proteins. Due to its dependency on concentration, molecules with highest concentration in samples are detected over lower abundant proteins [10].

The CF transmembrane conductance regulator (CFTR) gene codes for a protein transmembrane conductance regulator that functions as a chloride channel and is regulated by cyclic adenosine monophosphate (cAMP) in epithelial tissue. Mutations in this gene lead to the development of cystic fibrosis as cAMP-regulated chloride transport function in the lung epithelial cells becomes defective leading to chronic bacterial infections that incite inflammation, the most common mutation of this gene being ΔF508CFTR. Whether a mutation in CFTR results in the alteration of protein levels in CF airway epithelial secretome in the absence of infection and inflammatory cells has been tested through comparison of CF and non-CF lung epithelial secretions. Proteins that were secreted were identified by Secretome P and Signal P databases. From 666 proteins that were identified as well as quantified, 70 were significant indicating that CF epithelium displays a unique apical secretome without the presence of immune and inflammatory cells with the structural/molecular function of these proteins being primarily innate immunity (24%), protease/anti-protease activity (17%), cytoskeleton structure (14%), extracellular matrix (ECM) organization (10%), energy metabolism (12%), and ion-dependent activity (10%). Western blot or ELISA was used to validate specific proteins. ELISA analysis done on the levels of matrix metalloprotease (MMP)-9 in apical secretions from life-extended HBE ALI and primary HBE ALI cultures showed a trend of increased MMP-9 in CF secretion in both which were not significant [12].

Due to the inability to predict which individuals are more likely to develop irreversible airflow obstruction, novel markers for COPD as well as a more thorough understanding of the underlying biological mechanism are required. Proteomic screening of induced sputum from smokers and patients who have been diagnosed with COPD has shown increased levels of bactericidal/permeability-increasing

protein fold-containing protein B1 (BPIFB1) as compared to non-smokers. In order to determine how smoking effects BPIFB1, current smokers and ex-smokers were compared. BPIFB1 levels were found to be higher in COPD patients who were current smokers as compared to current smokers without COPD, while there was no difference in the levels of BPIFB1 between ex-smokers with and without COPD [13]. As well as having higher sputum levels of BPIFB1, there was an association between increased sputum levels and changes in lung function found most apparently with COPD patients who were current smokers. Such information can be used to show that BPIFB1 may take part in the pathogenesis of smoking-related lung diseases. However, further studies are required in order to validate the role of BPIFB1 in the pathophysiology of COPD.

Cigarette smoking is the main risk factor for the development of COPD; however, only 20–30% of smokers end up developing COPD; thus, this puts into consideration that there could be a specific genetic background that takes part in the pathogenesis of COPD. The family members of patients who developed severe early-onset COPD are considered "susceptible individuals" as they have an increased risk of developing COPD. An assessment was done to determine if there was a difference between "susceptible individuals" and age-matched "non-susceptible individuals." Epithelial lining fluid was collected from young susceptible and young non-susceptible individuals and old COPD patients who took part in acute smoking experiments. Epithelial lining fluid was also collected from healthy smoking and non-smoking individuals, but they did not participate in the smoking experiments and were labeled as controls for COPD patients at baseline. Bronchoscopies were performed at 24 h after smoking as well as 6 weeks later and epithelial lining fluid was using microsampling probes. Peroxiredoxin I, uteroglobin serpinB3, S100A8, S100A9, and aldehyde dehydrogenase 3A1 were chosen for further analysis because they were significantly up- or downregulated in iTRAQ experiments, had a biological function that may take part in the pathogenesis of COPD, or had quantification with two or more statistically significantly different peptides. When comparing the groups at baseline, there was no difference in peroxiredoxin I, uteroglobin, ALDH3A1, between young susceptible individuals and young non-susceptible individuals and I. The levels of serpinB3, S100A9, and S100A8 were higher in patients who were in the young susceptible group. ALDH3A1 and peroxiredoxin I levels were found to be higher in old healthy smokers when compared to old healthy non-smokers. When comparing before and after acute smoking, there was a decrease in peroxiredoxin I, S100A9, S100A8, and ALDH3A1 levels in young susceptible patients after acute smoke exposure. In young non-susceptible individuals, all of the proteins were downregulated. Peroxiredoxin I, serpinB3, and ALDH3A1 were upregulated in old COPD patients, while uteroglobin was downregulated after acute smoke exposure [14]. Due to serpinB3 and uteroglobin showing decreased levels strictly in young non-susceptible individuals, these two proteins may play an important role in the beginning development of COPD. The study also supports the use of younger individuals in order to understand what contributes and how it contributes to the beginning development of COPD. Although the study was based on family history, which could have been a weakness, this is not the first time that family

Table 16.1 Proteins that were identified in the study by Franciosi et al. [14]

Protein	Description	Accession number
S100A8	Plays a role in antimicrobial activity as well as pro-inflammatory mediators in acute and chronic inflammation	P05109
S100A9	Also plays a role in antimicrobial activity as well as pro-inflammatory mediators in acute and chronic inflammation	P06702
Uteroglobin	Has immunosuppressive and antitumor qualities but may take part in reducing airway inflammation and providing protection from oxidative stress	P11684
Peroxiredoxins	Controls the response oxidants and has an anti-inflammatory role as well	Q06830
Aldehyde dehydrogenase 3A1	Involved in the detoxification of carcinogenic aldehydes that are associated with smoking	P30838
SerpinB3	Inhibits several types of proteases. Also plays a role in modulating inflammation, fibrosis, and apoptosis	P29508

history has been used and was able to provide clues for the genetic component of the disease [14] (Table 16.1).

The use of sputum can provide information about the presence of inflammatory cells and mediators within the airways as it is considered to represent bronchial lining fluid. This information can be used for phenotypic characterization of patients who are diagnosed with chronic respiratory diseases. There is difficulty with use of sputum as there is much needed effort in obtaining healthy control samples as well as the presence of highly charged mucins within the sputum makes it difficult to separate sputum proteins using techniques such as two-dimensional gel electrophoresis [15]. Saliva and nasal lavage fluid (NLF) samples can also be obtained noninvasively but are more closely related to the upper airways; thus, it is mainly used for investigation of upper respiratory diseases such as allergic rhinitis. The least commonly used samples are lung and bronchial tissue samples as they are obtained during surgical procedures making this method invasive.

Hypersensitivity pneumonitis is an inflammation of the lung alveoli caused by exposure to airborne substances such as bacteria and fungi. However, very few people who are exposed to these airborne substances are actually diagnosed with hypersensitivity pneumonitis which goes on to suggest that genetic factors and exposure patterns are required in order for exposure to noninfectious microbial particles to cause hypersensitivity pneumonitis. Damp building-related illnesses, although less established as an illness compared to hypersensitivity pneumonitis, can still be a risk factor in the development of adverse health effects. Yet, there is difficulty in diagnosing damp building-related illnesses and assessing effects due to exposure from noninfectious microbial particles that do not meet the criteria for hypersensitivity pneumonitis. To discover diagnostic markers for pathologic conditions due to exposure to noninfectious microbial particles as well as to examine if there is a relationship between hypersensitivity pneumonitis, agricultural NIMP exposures,

and damp building-related illnesses in terms of proteomics, bronchoalveolar lavage fluid was collected. Unlike the noninvasive collection of sputum, bronchoalveolar lavage fluid is collected through a more invasive procedure [15]. 2D gel analysis and the immunoblot validation studies have shown that there is a difference in protein expression between damp building-related illnesses and hypersensitivity pneumonitis/agricultural NIMP exposure even though both are due to exposure of noninfectious microbial particles, but protein expression showed that there is a close association between hypersensitivity pneumonitis and agricultural NIMP exposures. Semenogelin and histone 4 were found to be possible diagnostic markers for differential diagnosis between damp building-related illnesses and hypersensitivity pneumonitis-like conditions [16]. Although semenogelins are proteins that take part in the formation of sperm coagulum, it has also been found in the lungs and small cell lung carcinoma. DeCyder analysis showed that it was upregulated in all of the studied disease patient groups, and immunoblot validation confirmed that semenoglobin levels were also increased in agricultural NIMP exposure, hypersensitivity pneumonitis, and sarcoidosis patients when compared to the healthy controls. There was no difference in semenoglobin levels between the damp building-related illnesses group and the healthy controls. Histone variants H2B and H4 were found to be upregulated in all experimental groups but were slightly less in the damp building-related illnesses group. Histone component H4 had increased expression in bronchoalveolar lavage fluid associated with AME, HP, and SARC patient samples. H2B, another histone variant, also had increased expression in plasma samples of HP and SARC patients while H4 could not be detected from plasma. Two-dimensional gel electrophoresis is not without its limitations. Like mass spectrometry, high-abundant proteins are detected over less abundant proteins which leads to a less comprehensive proteomic profile. The difficulty in reproducing experiments and protein identification limits its use in large-scale proteomics analysis [17].

16.3 Metabolomics

Metabolomics, the field of study that analyzes molecules or metabolites present within in organism, allows a snapshot reading of gene function, enzyme activity, and physiological landscape. As previous -omics studies, metabolomics is not limited to just one method in order to assess endogenous metabolites within a biologic system. Metabolic finger- or footprinting, target isotope-based analysis or targeted metabolomics, and metabolic targeted profiling are all different methods utilized in metabolomics. Lipids, carbohydrates, peptides, and proteins that are of different molecular size and charge, whether they are of exogenous or endogenous origin, are metabolites that can be detected [18] Metabolites have possibility to be good biomarkers due to being easily detectable with analytical methods [19]. The metabolome interacts with transcriptome, genome, or proteome, and any changes that occur within those "-omes" are believed to be reflected on the metabolome as this then leads to changes in metabolite concentrations in biological fluids [20]. Its close relationship with other "-omes"

demonstrates that metabolomics is important for connecting systems biology [21]. Use of metabolomics, also known as metabonomics, allows detection of changes that result from biological or environmental events over short periods which proves to be useful in monitoring disease progression or drug response and be predictive of disease severity as most acute illnesses are due to disruption in biochemical homeostasis [20]. These changes that are linked to biological events can provide information to the pathogenesis of disease with use of bioinformatics models. Thus any information collected through metabolomics must be linked to biochemical causes and physiological consequences [21].

With use of metabolomics evaluation of cystic fibrosis airway secretions, biomarkers could be found with the identification of metabolites and metabolic pathways that take part in neutrophilic inflammation, a hallmark of cystic fibrosis. Cellular metabolism can be altered by neutrophilic inflammation, and studies suggest that cystic fibrosis is associated with changes patterns and concentrations of metabolites in airway secretions [22]. Mass spectrometry-based metabolomics was done bronchoalveolar lavage fluid samples and targeted mass spectrometry methods were used in order to identify as well as quantify metabolite related to neutrophilic inflammation. Mass spectrometry determines the composition of a particle based on the mass-to-charge ratio in charged particles. Although its analysis requires more work, it has a higher sensitivity for metabolite detection as compared to NMR spectroscopy as well as specificity in metabolite identification at low concentrations. This is especially useful when bronchoalveolar lavage fluid is used because it contains low levels of metabolites. With the use of positive-mode MS metabolomics discovery profiling, 338 of the 7791 individual peaks that were detected were associated with neutrophilic inflammation and identified as potential biomarkers. From the metabolites detected, the majority were related to pathways that take part in the metabolism of purines, polyamines, proteins, and nicotinamide. Metabolite identification was done using online resources such as Human Metabolite Database and comparing with published literature [22].

Metabolomics has been applied to urine samples in order to characterize asthma phenotypes and identify metabolites. Urine samples obtained from 41 atopic asthmatic children and 12 healthy controls were profiled using liquid chromatography–mass spectrometry (LC–MS) [23]. LC–mass spectrometry is widely used due to its ability to detect a broad range of different classes of metabolites because it is sensitive to nanomolar concentrations and has good coverage of mass. Its disadvantages, however, are that there is no standardized metabolite library and that it has high variability [20]. With the use of urine, unlike other biological samples, it does not require the removal of macromolecules. Prior to the use of LC–mass spectrometry, samples must be ionized in order for metabolites to be detected with electrospray ionization being the most common technique [20]. Coupled with LC–mass spectrometry, a quadrupole-time-of-flight (Q-TOF) analyzer was used (Figs. 16.1 and 16.2).

Untargeted metabolic profiles were studied by multivariate analysis. In multivariate analysis, all metabolites in the data are analyzed simultaneously in one analysis and metabolic variations are detected through dimension reductions [18].

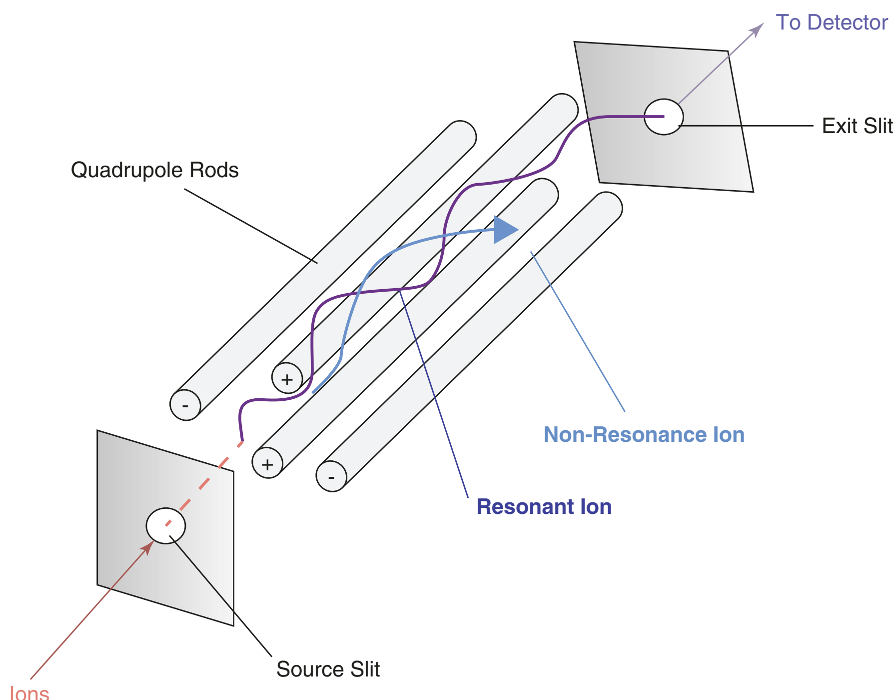

Fig. 16.1 Quadrupole mass analyzer

A reduction was found in the excretion of urocanic acid and methyl-imidazoleacetic acid contents. Methyl-imidazoleacetic acid is specific marker of histamine metabolism and urocanic acid is an intermediate produced by histidase in the catabolism of histidine. The reduction of urocanic acid in urine may postulate that it can affect the resolution of the inflammation process of asthma. Through this study, metabolic profiles from urine may be able to characterize asthma and urine may be used as a target for metabolic studies for pulmonary disorders in contrast to it being used only for systemic disorders.

Serum has also been used to identify the underlying mechanism of asthma as well as potential biomarkers. Serum samples were collected from 39 asthma patients and 17 healthy controls. NMR analysis, using H-NMR spectroscopy more specifically, was then done on these samples. NMR spectroscopy allows the identification of different nuclei based on their resonant frequency when molecules are placed in a magnetic field. Advantages in the use of H-NMR are that it has unbiased metabolite detection and is quantitative and the experiment and results can be reproduced. “Binning” was done in order to reduce the effect of shifting peaks. In “binning,” NMR spectra are divided into “bins” that are of equal chemical shift intervals [20]. The use of “binning” in this study did provide to be a bit difficult when assigning metabolites using targeted profiling because the resolution of the spectra from the integration process was reduced. The metabolites that were detected in the sera of

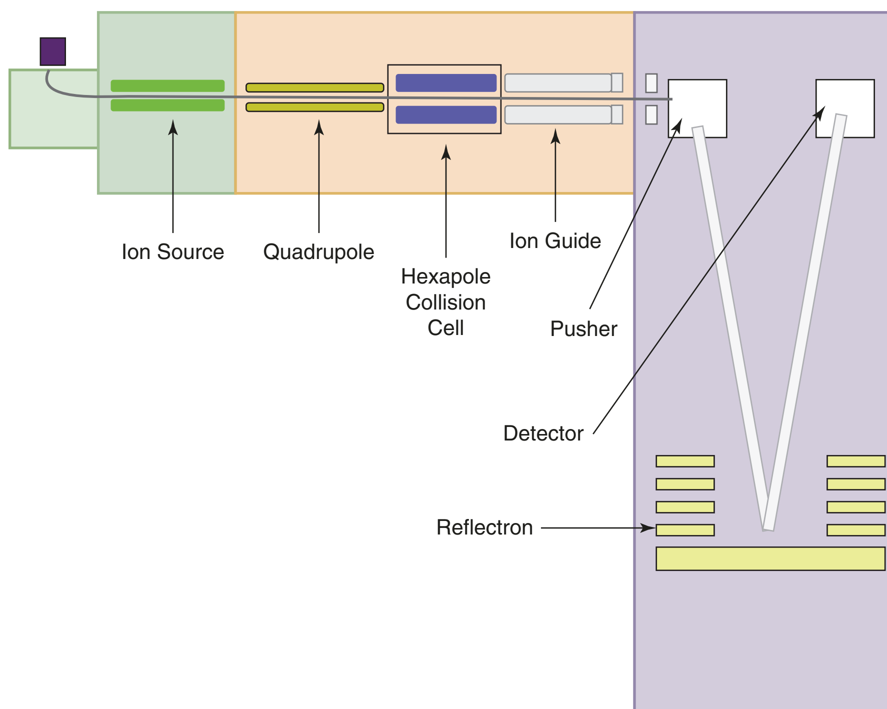

Fig. 16.2 Q-TOF mass analyzer

asthmatic patients were involved in the hypermethylation, immune reaction, as well as response to hypoxia. From the ten metabolites that were identified, five were related to the enhancement of the methyl-transfer pathway [24]. These five metabolites include formate, choline, methionine, *O*-phosphocholine, and methanol. An increase in arginine methylation has been previously described as a key process in asthma; therefore, it is possible that increased methylation takes part in the pathogenesis of asthma. Sera from patients with asthma who had lower FEV_1% predicted values also had higher levels of VLDL/LDL products. Also shown with the correlation with FEV_1% predicted values was that the breakdown of these lipids under insufficient glucose conditions can lead to an increase in levels of acetone in patients who have severe asthma [24]. Therefore, H-NMR-based metabolite profiling of serum may be useful in understanding the pathogenesis of asthma.

Platelet-activating factor, as well as other inflammatory lipids, has been identified as possible biomarkers for COPD. Sputum samples were collected from 11 patients in order to assess the effect of exacerbations on the sputum metabolome. In order to do that, liquid chromatography–tandem mass spectrometry (LC–MS/MS) was used and data was analyzed with MS/MS molecular networking and multivariate statistics. From the two sputum sample sets that were collected, 4639 unique MS/MS spectra were detected. From the 4639 unique MS/MS spectra detected, 556 unique metabolites were found in exacerbations samples, 132 metabolites in Tr

samples, 781 metabolites in Pt samples, and 100 metabolites in St samples. Across all clinical states, 1222 metabolites were common. Experiments that use iTRAQ typically use MS/MS spectra in order to infer peptide levels [11]. Bray–Curtis distance matrix was done on the metabolite abundance matrix for each set separately as LC–MS/MS batch effects did not allow the similarity of each metabolome to be compared across two datasets. Comparison could only have been done within each dataset. From the 556 unique metabolites that were found in exacerbation samples, platelet-activating factor and a related molecule PC were significantly elevated across patient cohort. This implies that lipid remodeling may occur during exacerbation. Platelet-activating factor has previously been reported to be at increased levels in exacerbations of inflammatory lung diseases such as asthma. Due to platelet-activating factor having the ability to activate neutrophils, changes in levels of platelet-activating factor can be used to monitor increased neutrophilic inflammation as well as possible onsets of exacerbations [19].

16.4 Breathomics

Breathomics is the noninvasive metabolomics study of exhaled air where the main aim of breathomics is to find patterns of volatile organic compounds that are related to metabolic processes that occur within the human body [25]. Exhaled breath has shown to contain hundreds of volatile organic compounds that can be analyzed by high-throughput assessment [26, 27]. However, exhaled breath volatile organic compounds do not just strictly represent metabolism in the lungs. The collection of exhaled breath is noninvasive as the subject has a noseclip applied and breaths quietly through a mouthpiece for 10 min. The mouthpiece has a salivary trap and single-way valve attached to it; this diverts the airflow through a Teflon or polypropylene tube that is contained inside a cooling container. The exhaled air is converted from droplets to exhaled breath condensate [28]. Albeit confusing, the terms "exogenous" and "endogenous" have been sometimes used in order to describe the origins of volatile organic compounds. Exogenous volatile organic compounds come from the environment mainly through inhalation, ingestion of food or drink, and drugs. Endogenous volatile organic compounds reflect metabolites as they arise from processes that occur within the body and will vary based on the airway compartment that is under study [29]. There are considerations that should be taken into account when dealing with both breath sampling and analysis. For example, in analytical methodology, there are methods based on mass spectrometry and electronic noses. For pathophysiological research, methods based on mass spectrometry are done to detect as well as identify volatile organic compounds especially when the volatile organic compounds of interest have yet to be known. Electronic noses, or e-nose, are usually less specific and based on cross-reactive, non-specific sensors. Sensor properties are changed to respond to patterns of volatile organic compound mixtures which then produces a quantitative signal change based on pattern recognition algorithms [29] (Table 16.2).

Table 16.2 Description of EBC collection devices [28]

EBC collection device	Description
EcoScreen 2	Allows fractionated collection of EBC from varying parts of the bronchial tree. EBC is collected in two disposable polyethylene bags which allows the disposal of dead space condensate which have no biomarkers with clinical relevance. Device is not portable
TurboDECCS	Consists of a turbo unit that is portable and a DECCS collection system that is disposable. The DECCS is equipped with a mouthpiece, a one-way valve, a tube, and a collection cell that is put in an electrical cooling system
RTube	A portable device that can be used by subjects at home without supervision. The large tee section made from polypropylene separates saliva from the exhaled breath. The one-way valve is made from silicon and a PP collection tube which has a cooling sleeve placed around it
ANACON	A device that can be attached to the expiratory branch of a ventilator circuit via two adaptors and two elastomeric connectors. The exhaled air goes through the condensation tubes that pass through the body of the condenser. Condensation temperature can be monitored through the thermometer

Metabolomic profiling of exhaled breath condensate was shown to have the ability to distinguish different biochemical-metabolic profiles of children with asthma as well as differentiate children who have severe asthma from those who have nonsevere asthma and from healthy controls. The study subjects that were recruited were 42 atopic asthmatic children, 31 of the children had nonsevere asthma, 11 children had severe asthma, and 15 children were healthy with no history of respiratory diseases. LC–mass spectrometry was used in the analysis of metabolites in the exhaled breath condensate samples, and Bidirectional–Orthogonal Projections to Latent Structures–Discriminant Analysis (O2PLS-DA) was used in order to differentiate between the groups of children. O2PLS-DA is a classification technique that creates classification models in which the information that is produced is summarized in a few predictive scores that are a result of a combination of measured variables [30]. Variable 225, a compound that is chemically related to retinoic acid, and variable 127, a compound that is chemically related to deoxyadenosine, were shown to be variable characteristic of severe asthma. Variable 412 was found to be a variable that characterizes the healthy controls and nonsevere asthmatic patients but was lacking in the severe asthma group. This variable was found to be ercalcitriol which is the active metabolite of vitamin D2. Data in this study showed that unlike lung function and FE_{NO}, metabolomic analysis was able to differentiate between nonsevere and healthy children as well as between severe and nonsevere asthmatic patients [30].

Assessment of inflammatory subtype in mild and moderate COPD by exhaled breath metabolomics has been studied. Exhaled breath was collected from 32 patients who have mild to moderately severe COPD by having the patients breathe normally for 5 min while having the nose clipped through a mouthpiece that was connected to a three-way non-rebreathing valve, an inspiratory VOC filter, and an expiratory silica reservoir [26, 27]. Breath analysis was done using gas

chromatography–mass spectrometry (GC-mass spectrometry). GC-mass spectrometry is the most commonly used analytical method in order to trace gases within complex mixtures [25]. With GC-mass spectrometry, there is the advantage that it is highly sensitive as well specific with being used to separate and detect volatile metabolites. However, this technique is done for thermally stable volatile compounds that have low polarity as well as those where derivatization can be done. This allows compounds to become less polar and more stable [20]. Electronic nose was also used to examine the relationship of exhaled molecular profile as it has previously been able to differentiate well-defined COPD from asthma. Measurements were performed twice due deviant sensor deflections, and the first analysis done for every measurement was excluded in the analysis. From the exhaled breath samples from the study, 26 volatile organic compounds were found to be correlated to markers of airway inflammation. Also found to be associated with markers of inflammatory cell activation were exhaled compounds. These compounds were eosinophil cationic protein (ECP) for eosinophils and myeloperoxidase (MPO) for neutrophils. From the compounds found, 18 were significantly correlated with ECP and 4 with MPO for patients with GOLD stage I. GOLD stage II had nine different compounds correlated with ECP while one identical compound was found compared with GOLD stage I. GOLD stage II also had one different compound that correlated with MPO and one identical compound was found compared with GOLD stage I. This study suggests that exhaled breath profiling using the quantitative GC-mass spectrometry method has ability to identify the type and activation of inflammation whether it is eosinophilic or neutrophilic. However, multi-compound breath profiling using e-nose has more appropriate use for detecting the activation of inflammatory cells.

16.5 Lipidomics

Recently, it has been shown that changes in lipid contents within the airway epithelium may play an important role in chronic airway diseases such as asthma, cystic fibrosis, and COPD. The purpose of lipidomics is to characterize the full lipid complement that is produced by cells, organisms, or tissues. The surfactant that covers the epithelium toward the air spaces is a mixture of approximately 80–90% lipids and surfactant-specific proteins. The roles of pulmonary surfactant are to lower surface tension as well as stop the occurrence of end-expiratory collapse of the alveoli. The importance of pulmonary surfactant is stressed as the continued synthesis and secretion of surfactant is important for the maintenance of lung function [31]. If surface tension is not lowered by the surfactant, high surface tension leads to pulmonary edema, where intra-alveolar fluid continues to accumulate and cause impairment of gas exchange and lung mechanical disturbances [32]. However, the composition and function can be affected by the occurrence of respiratory diseases. Primary surfactant deficiency in the lungs of preterm infants is known to be the major cause of neonatal respiratory syndrome; secondary surfactant deficiency takes part in the pathology of respiratory disease of the mature lung such as acute

Table 16.3 The components of pulmonary surfactants as well as the amount composition of each found in pulmonary surfactant [32]

Pulmonary surfactant component	% Composition
Dipalmitoylphosphatidylcholine (DPPC)	50%
Unsaturated phosphatidylcholine	21%
Proteins (SP-A, SP-B, SP-C, SP-D)	10%
Phosphatidylglycerol (PC) and phosphatidylinositol (PI)	9%
Phosphatidylserine (PS), phosphatidylethanolamine (PE), and sphingomyelin (SM)	6%
Other lipids	4%

lung injury (ALI)/acute respiratory distress syndrome (ARDS), asthma, COPD, and cystic fibrosis [31]. To help recognize the individual molecules that vary in molecular lipid structure, lipidomics constantly uses mass spectrometry [33]. LC–mass spectrometry-based methods are used due to the fact that lipid extracts contain high amounts of impurities and thus cannot be analyzed by shotgun lipidomics because of ion suppression effects [34] (Table 16.3).

16.6 Phosphatidylcholine

Phosphatidylcholine (PC) is the major surfactant phospholipid, comprising 80% of surfactant lipids, where PC16:0/16:0 is the principle phosphatidylcholine among several species. This phosphatidylcholine is thought to take part in surface reduction at the air–liquid interface. In patients who have respiratory failure that is secondary to acute respiratory distress syndrome (ARDS), the pulmonary surfactant complex shows a change in composition as well as lack of adequate surface activity. These changes may then cause severe hypoxemia, poor lung compliance, and lung atelectasis, all of which are characteristic of ARDS. In a study to characterize surfactant phosphatidylcholine kinetics in patients with ARDS, patients and controls had an intravenous infusion of *methyl*-D9 choline chloride as *methyl*-D9 choline chloride has been used to quantify surfactant PC flux via the CDP-choline pathway. Bronchoalveolar lavage fluid was then obtained with the use of a fiber-optic bronchoscope. There was a significant reduction in the total bronchoalveolar lavage fluid phosphatidylcholine isolated in patients with ARDS as compared to healthy controls. Bronchoalveolar lavage fluid fractional PC16:0/16:0 absolute concentrations were also found to be lower in patients. Low concentrations of total PC and fractional PC16:0/16:0 may be attributed to reduced synthesis, increased breakdown, or dilution by pulmonary edema [31].

As part of a study to determine sensitive biomarkers in peripheral blood for the identification of interstitial lung abnormalities, phosphatidylcholine was found to be both a sensitive and reliable biomarker. Metabolomics-based liquid chromatography quadruple time-of-flight mass spectrometry (LC–Q–TOF–MS) technique was used to show serum metabolic characteristics. Metabolic changes were seen in subjects who were initially healthy and were identified with interstitial lung

abnormalities a year later, and confirmation of the metabolites took part in the progression from healthy to development of interstitial lung abnormalities. When compared to the initial stage when the subjects were healthy, nine metabolites were identified in the outcome stage when they developed interstitial lung abnormalities. Phosphatidylcholine, phosphatidic acid (PA), phosphatidylethanolamine (PE), and betaine aldehyde (BA) were all found to be upregulated. The upregulation of phosphatidylcholine may be associated with epithelial injury in interstitial lung abnormalities [35].

16.7 Phosphatidylglycerol

Phosphatidylglycerol is the second most abundant surfactant phospholipid and is involved in absorbing and spreading of surfactant over the epithelial surface after surfactant films are compressed. The concentration of phosphatidylglycerol is much higher in the lungs when compared to other mammalian tissue [32]. Bronchoalveolar lavage samples were collected from ARDS patients in order to assess if elevation of secretory phospholipases A_2, which hydrolyzes phospholipids in cell membranes and extracellular structures such as pulmonary surfactant, in human lungs during ARDS and if the levels of secretory phospholipases A_2 are associated with surfactant injury. In ARDS, the alveolar inflammation leads to changes in the biophysical and biochemical properties of pulmonary surfactant which takes part in how severe the disease becomes. Secretory phospholipases A_2 enzymatic activities were increased when compared to healthy controls. Although phosphatidylcholine was found to be the most abundant in terms of the composition of surfactant phospholipids when comparing ARDS patient to healthy controls, there was a decrease of phosphatidylglycerol in the ARDS samples. The decrease of phosphatidylglycerol may take part in the mechanism for surfactant injury in ALI/ARDS [36].

Lipidomic profiling has been done to see if it could identify certain forms of interstitial lung disease in children with surfactant alterations. Many chronic childhood interstitial lung diseases directly affect different components of pulmonary surfactant which includes genetically caused deficiency in ABCA3, a lipid transporter. Phosphatidylglycerol was found to be low in the interstitial lung disease group and characteristic changes were seen in species 35:1 and 36:1 in interstitial lung disease related to growth abnormalities as well. Patients who had ABCA3 deficiency from two interstitial lung disease-causing mutations had decreases in phosphatidylglycerols PG 32:1, PG 36:1, PG 36:4, PG 38:4, and PG 38:5 [37].

16.8 Sphingolipids

Sphingolipids play an important role in signaling molecules and components of other membranes and extracellular fluid whether it is in normal functioning or pathological settings that involved inflammation. Sphingolipids and altered sphingolipid metabolism have demonstrated to be potential key contributors to the pathogenesis

of asthma. The most studied sphingolipids, ceramides and sphingosine-1-phosphate, also play an important role as signaling molecules. Ceramide is a substrate for the production of complex sphingolipids while sphingosine-1-phosphate acts as both an intracellular second messenger and extracellular ligand for S1P1–S1P5, specific G-protein-coupled receptors. Sphingosine-1-phosphate levels are increased in bronchoalveolar lavage from patients with asthma as compared to control subjects. In exhaled breath collections, ceramide levels were increased in very ill patients with asthma. In patients with emphysema, lung ceramide levels were also found to be increased when compared to control subjects without emphysema. Alterations in sphingolipid homeostasis may possibly link ORMDL3 to asthma [38]. This suggests that smoking and COPD cause impairment in the regulation of sphingolipid metabolism. In a study by [39], 26 sphingolipids were identified to be significantly associated with emphysema and 11 sphingolipids were associated with severe COPD exacerbations. Trihexosylceramides had the strongest association with COPD exacerbations; however, it is a lipid class with unknown effects on the lung. Mass spectrometry showed that emphysema was inversely associated with 10 sphingomyelins and that low baseline plasma of sphingomyelin was associated with worse COPD, defined as lower (lower FEV_1). Ceramides, gangliosides, and monohexosylceramides, specifically, showed inverse correlation with emphysema. This may be due to an increase in activity of sphingomyelinase and recycling (from gangliosides) pathways, which is then followed by ceramide consumption/degradation. This study was the largest as 250 subjects took part and is the study that combined targeted and nontargeted metabolomics study of plasma sphingolipid [39].

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Impact of Genomics on Personalization of Breast Cancer Care

17

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Abstract

Over the past 50 years, progress on multiple fronts has dramatically altered the nature of the disease known as breast cancer. The initiation of randomized prospective clinical trials in 1959, a novel concept at the time, by the National Surgical Adjuvant Breast and Bowel Project (NSABP) under the guidance of Bernard Fisher established a scientific philosophy as the guiding force in breast cancer treatment. Since 1975, multiple innovations have increased the therapeutic options and improved the outcomes available to women with breast cancer. Increased awareness of breast cancer, improvements in breast imaging, and the development of screening programs have made early diagnosis commonplace. The de-radicalization of surgical techniques used to obtain local control and the application of plastic surgical techniques for breast reconstruction have dramatically reduced the morbidity associated with mastectomy and axillary dissection. The development of pharmacologic hormonal therapy, more effective cytotoxic chemotherapy, and targeted HER2 therapy has improved survival for women with the most common types of breast cancer as well as less common but highly aggressive cancers. The development of predictive assays for response to chemotherapy has spared many patients from unnecessary toxicity and improved their quality of life. Critical to these advances has been the recognition that all breast cancers are not the same and the belief that treatment should be tailored so that every patient receives the best chance of survival with the least morbidity. New

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© Springer Nature Singapore Pte Ltd. 2018
Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_17

insights into the genomic heterogeneity of breast cancer offer the prospect for improved outcomes for patients with breast cancer by further personalization of breast cancer care.

17.1 Introduction

It is impossible to overstate the impact the Human Genome Project will have on future generations of scientists, physicians, and philosophers. The reference human genome sequence is the first chapter in the Atlas of Human Molecular Anatomy, and like the Atlas of Human Anatomy produced by Vesalius 500 years ago, the human genome sequence will be the foundation of biological and medical science for centuries to come. The first draft of the reference human genome sequence [1] took over one decade, countless man hours, and a billion dollars to complete. Since then, massively parallel sequencing (MPS; also called next generation sequencing) has reduced the cost of determining the 6000 MB of DNA sequence in a human genome more than 10^6-fold [2] and spawned efforts to sequence thousands of human genomes [3]. The ability to sequence archived cancer samples from patients enrolled in prospective randomized trials now offers the real possibility of correlating genomic alterations at single base-pair resolution with outcomes in the context of specific cancer treatment regimens. The availability of genomic analysis at relatively low cost also provides the opportunity to offer false hope to the public and to individual patients. Different subspecialties of medicine will likely use genetic information about their patients in different ways. To anticipate the potential impact genomic analysis will have on breast cancer patients and breast cancer physicians, it is essential to understand the current state of the art of breast cancer care and how other technological advances have improved breast cancer outcomes over the past 100 years.

Progress in the war on breast cancer has advanced on multiple fronts: early detection, the development of targeted therapies for estrogen receptor (ER)-positive and HER2-positive breast cancer, the development of more effective cytotoxic drugs, the discovery of genetic risk factors for breast cancer, and effective strategies for chemoprevention of breast cancer are some of the most important [4]. But perhaps the most important improvement has been the reduction in unnecessary treatment related morbidity. Carefully designed randomized prospective clinical trials have defined, primarily in terms of pathologic criteria, patient subgroups that do not benefit from specific interventions thereby sparing many patients from unnecessary disfiguring surgery and cytotoxic drug exposure. Optimal treatment planning for each individual patient now requires consideration of numerous variables. Clinical factors (age, menopausal status, premorbid conditions, clinical stage at presentation, future plans to bear children, and desires regarding physical appearance), radiologic findings (size and location of mammographic, ultrasound, and MRI abnormalities in the breast and axilla), and pathologic criteria (tumor size, histologic grade, lymph node involvement, and molecular subtype, i.e., expression levels of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)) are integrated into a treatment plan for each patient. Thus,

the diversity of breast cancer patients is apparent before we consider genomic heterogeneity. The relevance of genomic analysis to patients with breast cancer or at risk for the development of breast cancer will likely be context dependent in ways that cannot be foreseen.

The availability at relatively low cost of targeted exome, whole exome, or even whole-genome tumor DNA sequencing has led to calls for "personalized medicine" or "precision medicine." The concept of integrating sequence information from a particular patient's tumor DNA into their treatment plan is grand, but may not be readily adapted to the clinic. One of the overarching insights learned from the thousands of breast cancer genomes analyzed to date is that breast cancer genomes are incredibly heterogeneous. In fact, every breast cancer is genetically unique, and many are composed of multiple genetically unique subclones. The activation and deactivation of multiple (often hundreds) of genes in unique combinations promote the progression of breast cancer in a patient-specific way [5]. Some of the genomic alterations implicated as drivers of cancer progression are "actionable" meaning they are intuitively predictive of sensitivity or resistance to established or experimental therapies [6]. But until there is evidence from appropriately designed studies that demonstrates the efficacy of targeted therapies in specific subsets of patients, identifying and targeting "actionable mutations" are only appropriate in the setting of a clinical trial. Advertisements by healthcare systems that imply better outcomes through genomic analysis and targeted therapy can be misleading. Acting on "actionable" genomic alterations outside the realm of a clinical trial runs the risk of backsliding into anecdotal and unscientific methods. This is one risk in the Paradox of Accomplishment described by Bernard Fisher [7]. The extraordinary genomic diversity of breast cancer also has profound implications for the design of future clinical trials [8, 9].

The breast cancer classifications used clinically in 2016 to direct patient care are pregenomic—the additional insights into the molecular diversity and biologic basis of breast cancer discerned from genomic profiling have not yet been translated into clinical practice. Understanding the potential utility of new knowledge generated by genomic analysis of breast cancer requires a detailed understanding of breast cancer pathophysiology and the current approach to patients with breast cancer. Toward that end, we present a perspective on the anatomic, histologic, and molecular classifications of breast cancer that currently guide therapeutic decision-making. With that background, we review some of the recently published results of genomic breast cancer research and discuss suggestions that have been offered as to routes to improve breast cancer outcomes in the future.

17.2 Clinical and Molecular Classifications of Breast Cancer

17.2.1 Anatomic/Clinical Classification

From clinical, histologic, and molecular perspectives, breast cancer is a heterogeneous disease, but all breast cancers share some basic features in their origin and natural history. Breast cancers originate as cellular proliferations within the

terminal duct lobular unit (TDLU) and evolve over time by the accumulation of various genetic and epigenetic changes [10]. By mechanisms that are still unclear, a subset of cells within these intraductal neoplastic proliferations traverse the basement membrane of the TDLU establishing an invasive cancer. Following a variable period of local growth, by mechanisms that are poorly understood, modulated hypothetically by the accumulation of other genetic and epigenetic changes and interactions with the host microenvironment, neoplastic cells enter the bloodstream usually by way of the lymphatic system. In some patients, subsets of these metastatic cells develop into clinical metastases. The extent of disease, in particular lymph node or distant metastatic disease, at the time of diagnosis is strongly predictive of survival. Statistically, patients with large tumors fare worse than patients with small tumors, and patients with positive lymph nodes fare worse than those with negative nodes. Patients with distant metastatic disease generally fare worst of all. These findings led to the development of the tumor node metastasis (TNM) staging system by the American Joint Committee on Cancer (AJCC). Patients are classified by histologic evaluation of tissue removed at surgery to determine tumor size (T) and number of positive lymph nodes (N). The AJCC TNM stage classification provides a concise language to communicate the anatomic extent of disease and provides an index of average risk for patients with a defined stage of disease. But the TNM system by itself masks enormous heterogeneity in the biology of breast cancers. This heterogeneity is reflected to some extent in the histologic grade of a cancer as discussed below. The impact of genomic data on our understanding of breast cancer heterogeneity is discussed in later sections of this chapter.

Involvement of lymph nodes has long been recognized as the most significant prognostic factor in “operable” breast cancer [11]. The number of positive nodes is also highly prognostic [12]. Furthermore, the size of the largest lymph node metastasis is prognostic when only one or two nodes are involved [13, 14]. The survival rate of patients with micrometastases (<2 mm) in one lymph node is statistically the same as patients with histologically negative lymph nodes. Physical examination and radiologic evaluation of the axilla have a high positive predictive value, but about 20% of patients with a clinically negative axilla have positive nodes by histologic evaluation [15].

17.2.2 Histologic Classification of Breast Cancer

Histologic analysis of tissue samples is used to make or confirm a diagnosis of breast cancer and to determine the histologic grade and pathologic stage of disease. Prior to the advent of screening mammography, breast cancer was detected by physical examination, and diagnosis of breast cancer was confirmed histologically by frozen section of an open biopsy with the patient on the operating table. Today, the diagnosis of breast cancer is made by a pathologist based on histologic evaluation of a formalin-fixed paraffin-embedded tissue biopsy, usually an image-guided core biopsy. If the biopsy demonstrates an epithelial proliferation, the pathologist must determine whether the proliferation is neoplastic and whether neoplastic cells are

confined within breast ducts (intraductal carcinoma) or if they have invaded through the basement membrane into the breast stroma (invasive carcinoma). The distinction between intraductal carcinoma (also called carcinoma in situ) and invasive carcinoma is critical, because tumor cells confined within the ducts and lobules have no access to lymphatic channels and cannot metastasize. Carcinoma in situ has many histologic forms and like invasive breast cancer may exhibit high degree of intratumoral diversity as a result of clonal evolution [16]. A full discussion of the heterogeneity of ductal and lobular carcinoma in situ is outside the scope of this chapter. Suffice it to say that the great morphological diversity observed in invasive carcinoma, to be described in detail shortly, mirrors the diversity of morphologic patterns seen in carcinoma in situ. The molecular basis of invasion, like metastasis, is still unknown and, again like metastasis, thought to most likely be epigenetic rather than genetic. Consistent with this hypothesis, the mutational profiles of high-grade ductal carcinoma in situ are quite similar to the profiles of invasive carcinoma [17].

Once a diagnosis of invasive carcinoma is made, the pathologist performs two tasks that are critical for the development of an optimal treatment plan. The first task is to determine the histologic grade, and the second task is to determine the expression of three proteins in the neoplastic cells: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). These three proteins are predictive of benefit from the molecularly targeted therapies that are available today. It is possible, perhaps even likely, that additional biologic markers will be added to the armamentarium of predictive tests as additional targeted therapies are proven useful and the utility of current therapies is better defined.

The method used to determine histologic grade of an invasive breast carcinoma and estimate its malignant potential was conceptualized in the 1920s and refined in the 1950s by Bloom and Richardson [18]. Their genius was to quantize three more or less subjective histologic features of breast cancer. They validated the prognostic power of their method in a retrospective study of 1408 patients treated with radical mastectomy and/or radiation therapy. Figure 17.1 is a reproduction of Table I from the 1957 publication of Bloom and Richardson. It shows that in experienced hands, histologic grading can be remarkably predictive of outcome in surgically treated patients. Eighty-five percent of patients with the lowest histologic score were alive at 5 years, whereas only 33% of patients with the highest score survived for 5 years. The prognostic power of histologic grade indicates that morphologic variations in breast cancer reflect meaningful biologic diversity. Bloom and Richardson stated

	Total points						
	3	4	5	6	7	8	9
Grade . . .	I (Low)			II (Intermediate)		III (High)	
Number of cases . .	39	121	202	307	333	335	72
5-year survivors . .	33	93	146	141	157	106	24
" survivors % .	85	77	72	46	47	32	33

Fig. 17.1 Survival rates according to histologic grading

that the three classes of tumor (grades 1, 2, and 3) are not disparate pathological entities and that the lines of cleavage between the grades are merely arbitrary divisions in a continuous scale of malignancy. This perspective is of interest in light of recent high-throughput studies suggesting the existence of multiple intrinsic subtypes of breast cancer based on mRNA expression profiles and genomic architecture [19–21]. In addition to its prognostic power, histologic grade is also highly predictive of response to endocrine therapy, but this is seldom emphasized. Charles Huggins, whose work is described in some detail later, noted that "adenocarcinoma," a term used historically for cancers with a high degree of tubule formation, was highly sensitive to hormonal therapy [22]. Histologic grade never gained acceptance as a predictive test for endocrine therapy, perhaps because a more specific biochemical test was soon developed.

The Bloom-Richardson criteria for histologic grading were later refined giving rise to what is now known at the Nottingham histologic score (NHS) or Elston-Ellis modification of the Scarff-Bloom-Richardson (SBR) score [23]. Three aspects of the epithelial elements of a tumor are assessed to determine the SBR score of a breast cancer. The histologic aspects of the neoplastic epithelium that go into the NHS are tubule formation, nuclear pleomorphism, and mitotic activity. Each of these elements is assigned a score of 1–3, and the individual scores are summed to give an overall histologic score from 3 to 9. Tumors with an overall score of 3–5 are considered low grade (grade 1), tumors with an overall score of 6 or 7 are considered intermediate (grade 2), and tumors with a combined score of 8 or 9 are considered high grade (grade 3). The stromal/microenvironmental elements are not considered. It is worth noting that the mRNA expression profiles discussed in later sections of this chapter are derived from whole tumor extracts and therefore represent stromal and inflammatory (the microenvironment) as well as the epithelial cells. Such profiles might therefore be expected to demonstrate greater diversity than profiles of the epithelial cells alone.

The three aspects of tumor epithelial cells considered in the SBR score represent very distinct biologic properties of the neoplastic cells. Tubule formation is a measure of the degree to which the epithelial cells retain the tendency of normal breast epithelium to form polarized single layer epithelial sheets. Tumors with a high degree of tubule formation maintain a near normal cell-matrix orientation. Normal epithelia have a cellular program called anoikis that triggers cell death when cell-matrix contacts are disrupted [24]. Breast cancers with a high degree of tubule formation may therefore retain anoikis signaling pathways. Conversely, tumors that infiltrate as single cells or solid nests appear to have developed resistance to anoikis. This hypothesis is supported by the observation that cell lines with the simple genomic pattern characteristic of well-differentiated breast cancer are conspicuously absent among available breast cancer cell lines [25]. As noted previously, most tumors with a high degree of tubule formation are highly sensitive to endocrine therapy suggesting that anoikis signaling may be coupled to estrogen signaling.

Nuclear pleomorphism is a subjective assessment of the variation in size, shape, and staining of tumor cell nuclei. It is the factor with greatest interobserver variation. A high degree of nuclear pleomorphism is associated with aneuploidy as

assessed by flow cytometry [26] and may reflect the high somatic mutational load and chromosomal instability characteristic of some breast cancers. Tumors with grade 1 nuclei are almost always ER-positive and Her2-negative, whereas Her2+ and triple-negative breast cancers almost always have grade 2–3 nuclei. At the genomic level, ER-negative and Her2+ tumors have the highest degree of mutations revealed by mutational profiling in high-grade tumors [27]. Interestingly, high mutational load was associated with increased breast cancer death in ER+ tumors but not ER− tumors [27], so this broad measure of genomic heterogeneity is particularly relevant to one subset of breast cancers.

Lastly, the mitotic score component of the SBR score provides a measure of the proliferative activity of a particular tumor. This aspect of tumor histology has the highest correlation with outcome. Interestingly, molecular markers of cellular proliferation are the most critical elements in the bevy of targeted mRNA expression profile tests that have been developed as prognostic tools since 2004 [28, 29]. They are discussed further in Sect. 17.3.

17.2.3 Molecular Classifications of Breast Cancer

Molecular classifications of breast cancer have arisen in two ways. Clinical molecular classifications are driven by clinical demand for predictive assays to direct breast cancer therapy. The second type of molecular classification of breast cancer, exemplified first by comparative genomic hybridization (CGH) and mRNA profiling and more recently by genome sequencing and integrated molecular profiling, is driven by the research goal of fully describing the molecular heterogeneity of breast cancer. One hopes that defining breast cancer at the genomic level and integrated "multiomic" level will translate into advances in the clinic, but that remains to be seen. We expect that hypotheses generated by unbiased molecular profiles of breast cancer will lead to the development of new targeted therapies and predictive tests and that some of these efforts will improve outcome for specific subsets of breast cancer patients. In order to put those efforts in perspective, it is useful to review the development of the molecular markers currently in use.

Estrogen Receptor Recognition that some breast cancers are dependent on estrogen for growth and survival goes back to the nineteenth century as described in detail in Sect. 17.3. Prior to the development of pharmacologic endocrine therapy, patients with advanced primary breast cancer, locally recurrent breast cancer, or metastatic breast cancer were treated empirically with surgical endocrine ablation procedures including oophorectomy, adrenalectomy, and hypophysectomy. One-third of premenopausal women experienced tumor regression following oophorectomy, and some patients achieved long-lasting remissions [30]. Elwood Jensen's discovery of high-affinity estrogen-binding proteins in hormonally sensitive tissues of rats led him to develop the estrogen receptor (ER) theory of estrogen action [31]. This then led to the development of assays sensitive enough to measure ER in tumor tissue. In a seminal study reported by Jensen and colleagues in 1975 [32], an analy-

sis of ER levels in tumor samples of women who underwent endocrine ablative therapy revealed that two-thirds of patients with ER+ tumors experienced objective remissions. No patients with ER− tumors responded to endocrine ablative procedures. This study indicated three molecular subtypes of breast cancer: (1) ER-negative, (2) ER-positive/estrogen dependent, and (3) ER-positive/estrogen independent. Surgical ablation of the ovaries, adrenal glands, and pituitary gland gave way to pharmacologic endocrine therapies (tamoxifen and aromatase inhibitors), and complex ligand-binding assays for ER gave way to immunohistochemical (IHC) determinations of ER, but the three molecular subtypes of breast cancer identified by Jensen and Huggins still represent the critical subgroups of breast cancer patients. Furthermore, the challenges defined by Jensen and Huggins are still at the center of breast cancer research today. First, how can we distinguish ER-positive tumors that are dependent on estrogen and sensitive to hormonal therapy from estrogen-independent (hormone-resistant) ER-positive cancer? And, secondly, how are ER-negative breast cancer and hormone-resistant ER-positive cancer optimally treated?

Progesterone Receptor The next advance in the clinical molecular classification of breast cancer came from studies of progesterone receptor (PR). The observation that expression of PR is induced by estrogen in breast cancer cells in culture gave rise to the notion of an estrogen-signaling pathway and to the hypothesis that estrogen signaling is defective in patients with ER+ tumors who do not respond to endocrine therapy [33]. Early PR assays performed on fresh frozen tumor samples partially confirmed this hypothesis: 77% of patients with ER+/PR+ tumors responded to endocrine therapy, a significant improvement over the predictive value of ER alone, but, surprisingly, 27% of patients with ER+/PR− tumors also responded [34]. In any event, measuring PR improved our ability to predict who would respond to endocrine therapy and became a part of the clinical classification of breast cancer, albeit somewhat a stepchild to ER. Of note, early PR assays, like ER assays, were based on biochemical analysis of whole tumor extracts and were subject to false-positive results due to contamination of the sample with normal breast tissue. This accounts for the identification of a small but significant number of tumors as ER-negative/PR-positive. In the era of IHC assays for ER and PR, ER-negative/PR-positive tumors are extremely rare, and the existence of a real ER-PR+ subtype has been questioned [35]. Expression of PR has been validated in multiple studies as a marker of improved prognosis, but all ER+ patients are currently considered candidates for endocrine therapy regardless of PR status [36].

Human Epidermal Growth Factor 2 (HER2) William Huggins received the Nobel Prize for his work demonstrating the hormone dependency of human cancers in 1966. Interestingly, he shared the Nobel that year with Francis Peyton Rous for his work in tumor viruses. Huggins' work had clear implications for the treatment of breast cancer, but the direct relevance of Rous' work to breast cancer would not be fully apparent for many years. Following the discovery that normal cells contain a protein homologous to the viral oncogene in Rous sarcoma virus and the publica-

tion of the proto-oncogene hypothesis [37], several laboratories began looking for homologues of viral oncogenes in human tumor samples. In 1985, King et al. reported that a gene related to the avian erythroblastosis viral oncogene v-erbB was amplified in a human mammary carcinoma [38]. The v-erbB protein had previously been shown to be homologous to human epidermal growth factor [39], a receptor tyrosine kinase structurally related to v-Src. The same v-erbB homologue had been identified in neuroblastoma cells the year before and dubbed the "neu" oncogene [40]. Dennis Slamon's laboratory at UCLA went on to confirm that the Her2/neu oncogene was amplified in a large subset (30%) of breast cancers and that Her2/neu (HER2) gene amplification was associated with poor prognosis independent of other risk factors [41]. Within several years, a monoclonal antibody that inhibited the growth of HER2 overexpressing cells in culture was developed by Genentech [42]. This led to the development of Herceptin, a humanized monoclonal antibody targeted to the extracellular domain of HER2, which proved to be effective in the treatment metastatic HER2+ breast cancer in combination with cytotoxic chemotherapy [43]. Once Herceptin was approved for clinical use outside of clinical trials, HER2 expression became an essential component of the clinical molecular classification of breast cancer. Herceptin has since been shown effective for the adjuvant treatment of patients with early-stage HER2-positive breast cancer [44]. Of note, HER2-positive breast cancers that are ER-positive (about two-thirds of all Her2+ cancers) are relatively resistant to hormonal therapy and probably account for a significant percentage of the tumors Jensen and Huggins identified as ER-positive/estrogen independent.

Ki67 Mitotic count is the component of histologic grade that most correlates strongly with prognosis [45]. Proliferation rate is also predictive of response to chemotherapy [46]. In general, tumors with a low proliferation index do not benefit from cytotoxic chemotherapy, and patients with highly proliferative ER-positive tumors do benefit from adjuvant cytotoxic chemotherapy. Ki67 is a nuclear antigen expressed during all stages of the cell cycle except G0 [47] and therefore should be an excellent marker of proliferation as well as a prognostic marker and predictive marker for chemotherapy response. The percentage of tumor cells in a breast cancer that express Ki67 (known as the Ki67 proliferation index) is highly variable and ranges from less than 5% in some low-grade breast cancers to greater than 90% in some high-grade breast cancers. Not surprisingly, the Ki67 PI (PI) correlates strongly with mitotic index [48]. Unfortunately, the clinical use of Ki67 is plagued by several problems. First the distribution of Ki67 proliferation index of ER-positive tumors is unimodal with the median between 10 and 20%—precisely where the cut point for distinguishing luminal A from luminal B tumors lies [49, 50]. Secondly, there is frequently a high degree of intratumoral heterogeneity in the number of Ki67-positive cells in breast cancers. In particular, the percentage of Ki67-positive cells is often much higher at the tumor-stromal interface than in the center of the tumor. Other tumors have seemingly random areas of increased Ki67 expression ("hotspots"). Lastly, accurate Ki67 indexes obtained by manual counting are tedious and time-consuming. These factors have limited the clinical utility of IHC staining

for Ki67, and manual reads of Ki67 have not been wholeheartedly accepted as a valid biomarker for chemotherapy benefit. At the very least though, Ki67 serves as a check on the accuracy of the mitotic index of a breast cancer, as it also is subject to interobserver and intraobserver variation.

Classifications Based on mRNA Expression Profiles Two types of breast cancer mRNA expression profiles are important in 2016. One has revolutionized breast cancer research, and the second has revolutionized the clinical practice of breast cancer medicine. In 2000 and 2001, breast cancer research teams in Norway and the United States published a breast cancer classification based on mRNA expression profiles [21, 51]. The 456 cDNA clones that formed the basis for the classification were selected from the set of 8102 genes on the Affymetrix microarrays used to analyze the samples. The clones were selected "to include those with significantly greater variation in expression between different tumors than between paired samples from the same tumor." The investigators reasoned that this subset of genes should represent inherent properties of the tumors themselves rather than just differences between different samplings. The expression of these 456 mRNAs was then analyzed by hierarchical clustering [52]. What emerged was the observation that the gene expression profiles (GEP) of breast cancer cluster naturally into two main groups each with three subgroups. The two main groups were distinguished by expression of estrogen receptor and related genes, confirming the division of breast cancer into ER-positive and ER-negative subtypes by Jensen 25 years earlier. The principle subgroups of ER-negative tumors are distinguished by expression of HER2 and related genes. The ER-negative/Her2-negative subgroup was designated the basal subtype because it uniquely expresses keratin isoforms generally found in basal cells of skin (as well myoepithelial cells in the breast) and not found in luminal breast cells. The ER-negative/Her2-positive subgroup was designated Her2-enriched. The ER-positive tumors were named luminal subtypes A, B, and C based on their expression of keratin isoforms found in luminal cells. Luminal A type tumors were distinguished from luminal B and C tumors primarily by high levels of ER in luminal A and lower levels of ER in luminal B and C subtypes. In addition, luminal C was distinguished by the expression of a novel set of genes that was also highly expressed in ER-negative tumors including at least one marker associated with cellular proliferation, *MYBL2*. Luminal B and C subtypes were combined as luminal B in subsequent refinements of the intrinsic subtype classification of breast cancer. Critically, the luminal A subgroup had a much better prognosis than any of the other subgroups [21]. The intrinsic subtype classification focused attention on the heterogeneity of breast cancer and in particular the existence of clinically and molecularly distinct ER-positive subtypes and a unique highly aggressive basal subtype.

The heterogeneity of breast cancers identified clinically as triple negative (ER-negative/PR-negative/HER2-negative) has been dissected using gene expression profiling into six subtypes [53]. Cell line models of these subtypes showed differential response to drugs, setting the stage for clinical trials. It is important to

recognize the molecular heterogeneity in the triple-negative breast cancers because, as a group, they are highly aggressive and tend to occur in younger patients, but there are subgroups of TNBC with unique clinical and pathologic features [54]. The most common type of triple-negative breast carcinoma is the basal subtype identified by Perou et al. Basal subtype triple-negative breast cancer is a distinct group of cancers based on clinical, morphologic, and gene expression analysis. Not surprisingly, they also turn out to be distinctive at the genomic level [25].

The molecular classification proposed by Sorlie et al. based on mRNA expression profiles (basal, Her2-enriched, luminal A/B) does not correspond exactly to the groups defined clinically as ER-negative/Her2-negative, ER-negative/Her-positive, ER-positive/Her2-negative, and ER-positive/Her2-positive and has not been found to be clinically useful. In contrast, mRNA profiles designed to identify ER+ tumors that do not benefit from adjuvant chemotherapy have become an important part of clinical breast cancer classification [55, 56]. The 21 gene expression panel developed by Genomic Health (Oncotype Dx Recurrence Score) and the 70 gene panel developed by Agendia (MammaPrint) are both used extensively by oncologists as a guide in the treatment of low-stage ER+ breast cancer patients. Clinical trials are underway to better define their predictive value. Recent results of these trials have confirmed that breast cancers with low-risk scores in either test do not benefit from chemotherapy [57, 58]. Thus these tests have followed in the path of improving breast care by defining groups of patients who do not benefit from therapy and can therefore avoid the unnecessary morbidity of ineffective therapies.

Genomic Classifications of Breast Cancer Classical karyotyping provided a catalogue of the genomic changes in breast cancer and identified double-minute (DM) chromosomes and homogeneously staining regions (HSRs) in the chromosomes of many carcinoma cell lines, but their significance was unknown. Following the discovery that the human genome contains genes homologous to viral oncogenes, several viral oncogene homologues were found to be amplified in breast cancer cell lines including *MYC*, *EGFR*, *NRAS*, and *HER2* [59]. The significance of DMs and HSRs in cancer cells was revealed when Bishops laboratory showed that the HSRs in a colon cancer cell line contained numerous copies of the *MYC* gene [60]. Subsequently, the development of comparative genomic hybridization (CGH) techniques provided the first genome-wide method to examine tumor DNA for amplified genes. In the original method, differentially labeled normal and tumor DNA was hybridized simultaneously to normal chromosome spreads [61]. Using this method, Kallioniemi et al. identified 26 chromosomal subregions with increased DNA copy number in a survey of 5 breast cancer cell lines and 33 primary tumors [62]. The regions of gene amplification included the loci of genes known to be amplified in breast cancer including HER2 (17q12), CCND1 (11q13), and MYC (8q24) as well as numerous foci whose genetic content was unknown. Some loci such as 17q22-q24 and 20q13 were amplified in multiple tumors, but no tumors showed the exact same pattern of amplified DNA sequences, a harbinger of the enormous heterogeneity of breast cancer genomes [62].

In a second generation of CGH, differentially labeled normal and tumor DNA was hybridized on DNA microarrays (array CGH; aCGH) instead of normal chromosome spreads [63, 64]. The introduction of aCGH improved the resolution of the emerging map of recurrent amplifications and deletions in the breast cancer genome. Using high-resolution aCGH to study of 99 "diploid" tumors, Hicks et al. [65] identified three characteristic patterns of genomic rearrangements: simple, highly complex, and "firestorm," the latter characterized by tightly packed high-level amplicons on single chromosome arms. The firestorm pattern was highly correlated with poor survival. Also in 2006, Pollack's group at Stanford used aCGH to characterize 89 breast tumors and linked patterns of DNA copy number alteration with the intrinsic breast cancer subtypes [66]. Basal-like breast cancer was reported to have gains and losses in numerous genes, whereas luminal B carcinomas tended to have high-level gene amplification, particularly in chromosomes 8 (8q11-q24) and 20 (20q13). Thus three patterns of copy number aberrations (CNAs) are reproducible across different platforms and are associated with distinct gene expression subtypes. In a recent study, Curtis et al. [19] integrated aCGH analysis of genome-wide copy number alteration with transcriptional profiles in 997 primary tumors with long-term clinical follow-up and identified ten molecular subgroups (integrated clusters or "IntClusts") with distinct clinical outcomes. Of greatest interest, they identified an ER-positive subgroup characterized by CNAs at 11q13/14 that is at particularly high risk for cancer-related mortality. Several known putative driver genes reside in this region, namely, *CCND1* (11q13.3), *EMSY* (11q13.5), *PAK1* (11q14.1), and *RSF1* (11q14.1). In hint of the complexity yet to be revealed, a recent study characterized *EMSY* as an oncogene in a pathway involving epigenetic repression of the anti-metastatic microRNA miR-31 [67].

Genomic aCGH analysis can produce a map of DNA CNAs but misses genetic changes that do not result in a copy number alteration. This includes oncogenic changes such as activating single base substitution in oncogenes (e.g., *KRAS*, multiple cancer types), inactivating single base substitutions in tumor suppressor genes (p53, multiple cancer types), balanced translocations common in non-epithelial malignancies, and inversions (e.g., Inv(2)(p21;p23), 2–5% of lung cancers). With completion of the human genome sequence in 2003, it became theoretically possible but enormously expensive and labor intensive to sequence the DNA of cancer samples using the same methods used to complete the reference human genome sequence. The first attempt to comprehensively analyze the genome of cancer samples for mutated genes at single base resolution was reported in 2006 [68]. This effort, led by Bert Vogelstein and Ken Kinzler at Johns Hopkins, sequenced 13,023 well-characterized genes one at a time by PCR amplification and Sanger sequencing methodology and described results for 11 breast and 11 colorectal carcinomas. They reported that individual tumors contain an average of ~90 mutated genes (excluding silent mutations, i.e., those not expected to result in an amino acid substitution) and identified 189 genes that were mutated at a frequency higher than expected by chance and therefore predicted to provide a survival advantage to the neoplastic cell. Each cancer sample carried its own distinct set of putative oncogenic mutations as well as a much larger set of unique passenger mutations. Most of the 189 genes in the Sjoblom set of candidate oncogenes (CAN genes) had not previously been

thought of as cancer-causing genes but were associated with cellular functions altered in cancer cells. Cellular adhesion and motility, signal transduction, and transcriptional regulation were the most frequently "mutated" pathways in both breast and colon cancers. The high level of mutations reported by Sjoblom was met with some skepticism, because of the small sample size. However, large-scale cancer genome sequencing studies using massively parallel sequencing (MPS) have confirmed the essential conclusions of the Sjoblom study: the genome of cancer cells is highly mutated and highly diverse.

The genetic landscapes of breast cancer depicted by aCGH and targeted exome Sanger sequencing are incomplete on their own. A more complete picture of the genomic alterations in cancer requires a method capable of detecting both copy number alterations and smaller changes such as single base substitutions and small insertions and deletions. Massively parallel sequencing provides that capability. In one of the first reported MPS studies of breast cancer, Stephens et al. [69] analyzed the exome of 100 tumors (79 ER+ and 21 ER−) and identified 7241 somatic point mutations or ~72 point mutations per genome (slightly less than the Sjoblom study). Driver alterations were identified in 40 cancer genes: 25 genes were altered by point mutation, 8 were altered by CNA, and 7 were altered by both. Only *TP53*, *PIK3CA*, and *GATA3* were activated by point mutation in more than 10% of tumors; the most frequent copy number alterations were in chromosome 17 (*HER2*, 21%) and chromosome 8 (*MYC*, 15% and *FGFR1/ZNF703* 15%). Point mutations in *TP53* were identified in 90% of ER− cancers; *PIK3CA* mutations were identified in 34% of ER+ cancers. Nine novel candidate driver genes (*AKT2*, *ARIDIB*, *CASP8*, *CDKN1B*, *MAP 3K1*, *MAP 3K13*, *NCOR1*, *SMARCD1*, and *TBX3*) were identified; *MAP 3K1* was mutated in six of the cancers. Most cancers had more than one driver mutation identified. In a "multiomics" approach, The Cancer Genome Atlas Network studied hundreds of tumors by exome sequencing and genomic DNA copy number arrays as well as other methods and identified 30,626 somatic mutations in 510 tumors (average 60 mutations per genome) [70]. In addition to identifying nearly all genes previously implicated in breast cancer (*PIK3CA*, *PTEN*, *AKT1*, *TP53*, *GATA3*, *CDH1*, *RB1*, *MLL3*, *MAP 3K1*, and *CDKN1B*), a number of novel genes were implicated in the TCGA study including *TBX3*, *RUNX1*, *CBFB*, *AFF2*, *PIK3R1*, *PTPN22*, *PTPRD*, *NF1*, *SF3B1*, and *CCND3*. Only *TP53*, *PIK3CA*, and *GATA3* were mutated in more than 10% of cancers. Most of the cancer gene mutations identified occurred in only 1–3% of the tumors. Both of these groundbreaking studies highlight the high mutational load, genetic diversity, and oncogenic driver redundancy in breast cancer.

17.3 Current Methods of Breast Cancer Treatment and Prevention

17.3.1 Local Control: Surgery and Radiation

Prior to 1975, most patients with breast cancer, even those with preinvasive disease, underwent a radical Halsted mastectomy including both pectoralis muscles and all axillary nodal tissue based on the mistaken belief that breast cancer spread entirely

by local invasion. At that time, breast cancers were detected by physical exam, and many patients had skin and lymph node involvement at the time of surgical intervention. Aggressive surgery for some of these women was certainly logical. But a one size fits all mentality was pervasive, and many women were overtreated. The National Surgical Adjuvant Breast and Bowel Project—Protocol 4 (NSABP-04), initiated in 1971, proved that for women with clinically negative axillary lymph nodes, radical mastectomy did not provide better local control or survival than three less aggressive alternatives: simple mastectomy and axillary dissection, total mastectomy without axillary dissection but with postoperative irradiation, or total mastectomy and delayed axillary dissection only if the nodes became positive. And with that Halsted's radical mastectomy, a debilitating and disfiguring procedure, was placed in the ashbin of medical history. NSABP-04 was an important step toward personalized medicine for breast cancer patients. NSABP-04 also provided the first clue that complete axillary dissection did not provide a significant therapeutic benefit. Amazingly, the final results of NSABP-04 with 25 years of follow-up were published less than 15 years ago in 2002 [71].

NSABP-06, a trial to define the efficacy of breast-conserving surgery (lumpectomy) in patients with tumors less than 4 cm in diameter, was initiated in 1976 on the heels of NSABP-04. Half of the patients who had lumpectomy also received whole breast radiation. All patients had axillary dissections, and patients with positive lymph nodes received chemotherapy (melphalan and fluorouracil). With 20 years of follow-up in 2002, the overall survival (OS), disease-free survival (DFS), and distant DFS among women who underwent lumpectomy were not significantly different from patients treated with mastectomy [72]. This study and similar studies carried out by the Milan group in Italy initiated the era of breast-conserving surgery for breast cancer. Critically, NSABP-06 did not simply replace one procedure with another as NSABP-04 did. Instead it created a bifurcation in the breast surgery decision-making process that required surgeons to make the following judgment call. Is it possible to completely excise this tumor with clear margins and obtain an acceptable cosmetic result?

Another key finding in NSABP-06 is that radiation after lumpectomy prevented local recurrence. Among patients with negative margins, 39.2% of patients treated with lumpectomy alone developed an ipsilateral recurrence, usually within 5 years, compared with a 14% local recurrence rate following lumpectomy and radiation. Essentially all patients who developed a recurrence went on to mastectomy. In 2016, patients who elect breast-conserving therapy for invasive carcinoma are generally advised to accept total breast irradiation to lower the risk of recurrence and subsequent mastectomy. It is worth noting however that 60% of patients in NSABP-06 achieved adequate local control without radiation. This observation suggests that some patients may be able to avoid whole breast radiation.

In 1994, a clinical trial comparing the efficacy of tamoxifen alone (Tam) with tamoxifen plus radiation therapy (RT) (TamRT) in older women with ER-positive, clinical stage I breast cancer was initiated by the Cancer and Leukemia Group B (CALGB 9343). With median follow-up of 12.6 years, radiation provided a small improvement in locoregional recurrence that has not yet translated into an

advantage in overall survival [73]. Thus, older patients with small low-grade invasive carcinomas excised with wide margins can reasonably opt to decline whole breast radiation—another option in the medical decision-making process that personalizes breast cancer care today. Patients with noninvasive breast cancer (ductal carcinoma in situ, DCIS) may also avoid radiation altogether. Clinical-pathologic criteria that are predictive of local recurrence following lumpectomy without radiation have been developed for DCIS [74] and incorporated into the Van Nuys prognostic index (VNPI). Risk factors for recurrence following excision of DCIS without radiation include age < 50, tumor size >2 cm, high histologic grade, and close margins. Application of the VNPI to determine the need for whole breast radiation after breast-conserving surgery is a paradigm for breast cancer decision-making: how much risk is acceptable to avoid a certain therapeutic option? When the additional risk does not affect overall survival or is small and the morbidity/cost associated with the therapeutic option is subjective or difficult to predict, the decision can become highly personal.

Another approach to avoid whole breast radiation in patients with early breast cancer that is being evaluated is immediate intraoperative partial breast radiotherapy (IORT). Two recent trials to evaluate the efficacy of IORT have been reported [75, 76]. Additional trials are underway. Thus the local control treatment options for patients with early breast cancer are expanding again. It is worth noting that the criteria used to select patients who can avoid radiotherapy or opt for IORT are highly dependent on pathologic data of tumor size, histologic grade, and margin width. All of these data points are to some degree subjective and subject to error. Improvements in breast cancer classification emerging from genomic studies may help guide these therapeutic decisions in the future.

Involvement of the axillary lymph nodes has long been recognized as the most significant biomarker for risk of death from breast cancer, and until the end of the twentieth century, it was commonly believed that removing the axillary lymph nodes provided a therapeutic benefit as well as prognostic information. Consequently, a complete axillary lymph node dissection (ALND) was a standard component of breast cancer surgery. But ALND is associated with significant morbidity, and it was increasingly clear that axillary dissection did not provide a therapeutic benefit as some had claimed. Furthermore, increased breast cancer screening and technical improvements in breast imaging have shifted the stage at which breast cancer is discovered during its natural history so that the large majority of patients were node negative at the time of axillary dissection. In 1994, Giuliano et al. reported their initial experience with a lymph node biopsy technique that they had developed for patients with melanoma that targeted removal of the first lymph node in a particular area of lymphatic drainage (the "sentinel node") prior to completing ALND. Sentinel lymph node biopsy accurately predicted the results of ALND in more than 95% of patients. The SLN technique was rapidly adopted and is now the standard of care for patients with clinically negative nodes. Despite the 5–7% false-negative rate of the SLN method, early studies indicated that the rate of axillary recurrence following SLN alone is comparable to the recurrence rate following ALND if the SLN is negative. This conclusion was confirmed by the NSABP-32 randomized prospective

trial: after 10 years of follow-up, patients with a negative SLN did not benefit from ALND. This left open the question of what to do when the sentinel node is positive. The benefit of ALND to patients with positive SLN was addressed in the Z0011 randomized prospective trial organized by the American College of Surgeons Oncology Group (ACOSOG). In Z0011, patients with tumors less than 5 cm and clinically negative axilla underwent breast-conserving therapy and whole breast radiation. Patients with 1 or 2 positive SLN were randomized to ALND or no further axillary treatment. The use of SLND alone compared with ALND did not result in inferior survival [77].

Surgical prevention: Patients at very high risk of developing breast cancer on the basis of defined genetic risk, e.g., BRCA1/2 mutation, or undefined increased risk on the basis of a strong family may opt to undergo prophylactic mastectomy as a means to prevent breast cancer. While this intervention undoubtedly prevents the development of breast cancer in many patients, its effect on overall survival has not been adequately studied. Likewise, patients with breast cancer may opt to undergo prophylactic mastectomy of the unaffected breast.

17.3.2 Systemic Control: Medical Adjuvant Therapies

Hormonal therapy (also called endocrine therapy), chemotherapy, and molecular targeted therapy are important components for breast cancer management. The broad use of systemic therapies, for both primary and adjuvant therapies, have effectively contributed to reduced mortality of breast cancer. In patients with early stages of breast cancer that is operable, preoperative and postoperative systemic therapies have shown evidence of reducing the risk of recurrence and improving patient survival. For patients with inoperable tumors, systemic therapy plays a crucial role. The choice of agents should be based not only on the staging of disease, tumor grades but also on the prediction using the presence of biomarkers discussed above, such as ER and HER2 [78]. The genomic analysis for individual patients brings a hope to make available more predictive biomarkers as a guidance for selection of optimal systemic therapies.

Hormonal Agents Breast cancer was the first type of cancer to show response to hormonal therapy. The status of ER strongly predicts responsiveness to hormonal therapies [79]. As a well-accepted biomarker, ER expression has been shown to have a high negative predictive value, i.e., patients who have ER-negative tumors do not respond to hormonal therapies [80]. However, not all ER-positive tumors benefit from hormonal therapies. There are several approaches to treat hormone-responsive breast cancer, including blocking ovarian function (i.e., ovarian ablation), blocking estrogen production, or blocking estrogen's effects [81]. Among these approaches, the latter two are currently more commonly used in clinical practice.

Tamoxifen and Other SERMs The first FDA-approved selective estrogen receptor modulator (SERMs) is tamoxifen (Nolvadex, approved by US FDA in 1977), which

was approved for use in both treatment and prevention of breast cancer. Up to now, tamoxifen remains the most widely used anticancer drug. It is used for the treatment of metastatic ER-positive breast cancer or as adjuvant to surgery. Tamoxifen binds to the ER in the same manner as estrogen but triggers reduced gene transcription and cell proliferation compared to estrogen [79]. However, resistance to tamoxifen is a major obstacle in hormonal therapy [82]. The other limitation of tamoxifen is the side effects associated with long-term use. Due to its effect as agonist in the uterus, long-term use of tamoxifen may stimulate the endometrial growth and increase the risk of endometrial cancer [83]. In order to overcome these obstacles, other SERMs have been developed such as raloxifene (Evista). Acting as an antagonist in uterus, raloxifene does not increase the incidence of endometrial cancer but showed similar effect as tamoxifen in the prevention of breast cancer in women at high risk [84]. Another SERM, fulvestrant (Faslodex), which acts as a pure ER antagonist, has different mechanisms as other SERMs because upon binding to the ER, it causes receptor destruction [81].

Aromatase Inhibitors Aromatase is an enzyme involved in estrogen biosynthesis by converting testosterone to estrogens. In postmenopausal women, because ovarian function is reduced, the main source of estrogen is derived from androgens produced by the adrenal glands which is an enzymatic reaction dependent on aromatase. Thus, targeting estrogen production by inhibiting aromatase provides an alternative approach of treating ER-positive breast cancer. Currently, there are three aromatase inhibitors in clinical use, anastrozole (Arimidex), letrozole (Femara), and exemestane (Aromasin). They are approved by US FDA for postmenopausal women with ER-positive breast cancer in both the adjuvant and metastatic setting [85]. These agents are not effective for premenopausal women, because they are not sufficiently potent to inhibit aromatase in the ovaries of these women [81]. In general, aromatase inhibitors are well tolerated with some preventable side effects such as vasomotor symptoms, musculoskeletal symptoms, bone loss, and osteoporosis after long-term use [86].

Cytotoxic Chemotherapies A broad range of chemotherapy agents have demonstrated activity in breast cancers, including anthracyclines, taxanes, and others. These agents can be used alone or more commonly in combination in various chemotherapy regimens or combined with hormonal or targeted therapies.

Early Clinical Trials and First-Generation Chemotherapy Regimen CMF The earliest clinical trials of adjuvant chemotherapy in breast cancer were initiated in 1958. These trials evaluated the single alkylating agents thiotepa or L-phenylalanine mustard given after surgery. Both of the trials reported that the adjuvant alkylating agents significantly decreased rate of recurrence [11, 87]. Later on, the first prospective clinical trial using 12 cycles of the combinational chemotherapy regimen named "CMF" which include the alkylating agent cyclophosphamide and the antimetabolites methotrexate and 5-fluorouracil showed significant benefit to patients [88]. The success of this trial represents the beginning of combinational chemotherapy which is now commonly used in clinical practice [89]. Later studies confirmed that six

cycles were as effective as 12 cycles of CMF and the CMF was added to tamoxifen for ER-positive patients and also showed improved outcome [89]. The adjuvant chemotherapy regimens can be classified as first, second, and third generation. The results of clinical trials and clinical benefits for the three generations of chemotherapeutic agents can be found in a nice review of these regiments [89]. Because these regimens commonly included anthracyclines and taxanes, the most active class of chemotherapeutic agents for both early-stage and advanced breast cancer, these two classes are reviewed briefly below.

Anthracyclines Anthracyclines belong to a class of chemotherapy agents including the natural compounds isolated from soil bacteria doxorubicin and its synthetic analog epirubicin. Anthracycline-based chemotherapy regimens have shown improved outcomes in comparison with other regimens such as CMF [90]. Doxorubicin therefore became the essential component of the first-generation chemotherapy regimens known as AC (doxorubicin and cyclophosphamide), the second-generation regimen such as CAF (cyclophosphamide, doxorubicin, and 5-fluorouracil), and the third-generation regimen DAC (docetaxel, doxorubicin, and cyclophosphamide). Although doxorubicin is the most effective chemotherapeutic agent for breast cancer and other types of solid tumors, dose-dependent cardiotoxicity limits its long-term use. Epirubicin has showed less cardiotoxicity compared with doxorubicin and thus replaces doxorubicin in some of the regiments [91]. For example, epirubicin is the component of the first-generation regimen FEC50 (5-fluorouracil, epirubicin 50 mg/m^2, and cyclophosphamide) and second-generation regimen FEC100 (5-fluorouracil, epirubicin 100 mg/m^2, and cyclophosphamide).

Taxanes Another class of cytotoxic drugs are taxanes, in which the natural compound derived from Pacific yew tree, paclitaxel, and its semisynthetic analog docetaxel are the major agents. Taxanes work by binding to microtubules and inhibiting depolymerization, thereby leading to mitotic arrest. Both of these agents have been extensively examined in various clinical trials in breast cancer in monotherapy or in combination [89]. For example, docetaxel is used in combinations known as TAC (docetaxel, doxorubicin, and cyclophosphamide). One limitation for paclitaxel and docetaxel is poor solubility which requires the addition of solvents such as Cremophor EL. Premedication is required to reduce the risk of acute hypersensitivity reactions associated with the solvents. To overcome this issue, albumin-bound paclitaxel (nab-paclitaxel, Abraxane) was developed as a solvent-free formulation of paclitaxel. Nab-paclitaxel, approved for the treatment of metastatic breast cancer in 2005, enables the delivery of higher doses of paclitaxel with reduced hypersensitivity reactions [92]. The current ongoing trials are trying to solve the issues such as the optimal dose of taxanes and treatment schedule [93].

Targeted Agents The molecular targeted therapy designed to inhibit HER2 and its signaling represents a prototypical example of precision medicine for breast cancer. The success of HER2-targeted therapies has stimulated a beginning to identify more valid therapeutic targets for various types of breast cancer. Here we focus on HER2-targeted therapies, while other novel targeted agents will be discussed in Sect. 17.6.

Trastuzumab (Herceptin) The discovery of the role of human epidermal growth factor receptor 2 (HER2) in the development of breast cancer and the subsequent development of HER2-targeted therapies have dramatically improved clinical outcomes for women with HER2-positive breast cancer [94]. Approximately 25% of metastatic breast cancers are known to be HER2-positive, due to amplification and/or overexpression of the gene which encodes a transmembrane receptor with tyrosine kinase activity [94]. Trastuzumab was the first HER2-targeted agent approved for HER2-positive metastatic breast cancer in 1998 and for early-stage disease in 2006 [95]. Trastuzumab is a humanized monoclonal antibody that selectively binds to the extracellular domain of HER2 protein on the tumor cells. Trastuzumab showed benefit as monotherapy or when added to chemotherapy for HER2-positive breast cancer resulting in a significantly reduced risk of recurrence and mortality compared to chemotherapy alone [96]. The limitations for trastuzumab include primary and secondary resistance and cardiotoxicity [97]. Therefore, other agents have been developed to target HER2.

Other Agents Targeting HER2 Lapatinib is an oral, small molecule inhibitor of the intracellular tyrosine kinase domain of HER2 (also targeting HER1, e.g., EGFR) [95]. A third agent, pertuzumab, is a fully humanized monoclonal antibody, working by inhibiting HER2 dimerization with other HER family members [98]. In 2013, the FDA approved ado-trastuzumab emtansine (T-DM1, Kadcyla) for use as a single agent for the treatment of patients with HER2-positive metastatic breast cancer who previously received treatment with trastuzumab and a taxane. T-DM1 is a novel antibody drug conjugate composed of trastuzumab linked to emtansine (DM1, a derivative of maytansine), a highly potent antimicrotubule cytotoxic agent, through a nonreducible thioether linkage [95]. Such design offers the potential to improve tumor targeting while at the same time minimizing exposure of normal tissue to the cytotoxic agent. In clinical trials, T-DM1 showed evidence of improving efficacy associated with lower overall toxicity [95]. The FDA approval for these agents as standard care for breast cancer is based on the significant improvements of outcome when applied in HER2-positive breast cancer patients [79]. However, although preclinical studies demonstrating synergy and lack of cross-resistance between lapatinib and trastuzumab, the addition of lapatinib did not improve outcomes when added to trastuzumab [99]. To date, only the combining of trastuzumab and pertuzumab has indication in the first-line setting [94].

17.4 Genetic Risk Factors for Breast Cancer and Incorporation of a Genetic Program into Clinical Practice

Breast cancer is overall a multifactorial disease. However, in a subset of the breast cancer population in the United States the cause is largely genetic. Hereditary breast and ovarian cancer syndrome (HBOC) has been thoroughly discussed in the medical literature and in mainstream popular media. The awareness of physicians and patients has increased over the last decade with several prominent celebrities

developing breast or ovarian cancer and/or undergoing prophylactic surgery for HBOC [100]. The risk of breast cancer over a lifetime varies depending upon the genes involved. Because of the very high penetrance of breast cancer in the population of patients with deleterious mutations of *BRCA1* and *BRCA2*, these are the genes that have been the most studied and discussed. There are also many other genes that have the potential to increase the risk of breast cancer that should also be considered. A typical breast cancer panel would include the following genes: *ATM*, *BRCA1*, *BRCA2*, *BARD1*, *BRIP1*, *CDH1*, *CHEK2*, *NBN*, *NF1*, *PALB2*, *PTEN*, *RAD50*, *STK11*, and *TP53*. With the advent of panel testing, we are now able to offer patients a relatively affordable and quick test for a variety of genetic mutations that are clinically actionable. This can be done in many settings including the primary care office, the imaging center, and in the surgeon's or medical oncologist's office after the patient has been diagnosed.

The front line of breast care and screening remains the primary care physician who is burdened by a busy clinic and may not be aware of the frequent guideline changes that may suggest that a patient warrants testing for deleterious mutations. The advent of so-called high-risk clinics has helped to alleviate this problem. Some breast imaging centers have now taken on this issue by having all patients including those who are there for routine screening undergo a risk evaluation with a computer model such as Tyrer-Cuzick (IBIS) or BRCApro, Hughes Risk App, or others that allow the Breast Center staff to inform patients and their primary care physicians of their risk status and to recommend genetic testing to those who meet National Comprehensive Cancer Network (NCCN) guidelines (NCCN Guidelines, http://www.nccn.org). This requires participation and cooperation between radiologists and other clinicians such as surgeons and primary care physicians.

The established genes that are associated with a significantly increased risk of breast cancer and have medical management guidelines are *BRCA1*, *BRCA2*, *CDH1*, *PALB2*, *PTEN*, *STK11*, *TP53*, and *CHEK2*. For genetic syndromes involving breast cancer, the penetrance may vary according to the presence of other risk factors such as family history. Particularly in deleterious mutations of the *CHEK2* gene, the presence or absence of a family history can affect penetrance and should be considered in any risk calculations and in patient counseling. The *CHEK2* gene deserves attention because its incidence in the population is estimated to be equal to the incidence of HBOC although the penetrance is less. The data on *CHEK2* suggest that the incidence of a second cancer is approximately 30% and that these patients may benefit from prolonged endocrine therapy for the primary breast cancer [101].

The major risk factors that are red flags for hereditary breast and ovarian cancer syndrome (HBOC or deleterious mutations of the *BRCA1* or *BRCA2* genes) are listed here (www.invitae.com):

1. Breast cancer diagnosed before age 50
2. Ovarian cancer at any age
3. Bilateral breast cancer or two primary breast cancers
4. Male breast cancer at any age
5. Triple-negative breast cancer

6. Ashkenazi Jewish ancestry with an HBOC-associated cancer (HBOC-associated cancers include breast invasive or DCIS, ovarian, and pancreatic, and aggressive prostate cancer with a Gleason score of >7)
7. Three or more HBOC-associated cancers at any age
8. A previously identified HBOC syndrome mutation in the family
9. A clustering of cancers that may suggest an inherited cancer syndrome, such as breast cancer, thyroid cancer, pancreatic cancer, prostate cancer, bone of soft tissue cancer, sarcoma, adrenocortical carcinoma, or leukemia/lymphoma—all on the same side of your family

Recently, clinicians have become interested in an array of other genetic syndromes that are related to breast cancer such as Cowden's syndrome, Li-Fraumeni syndrome, and others that may be clinically actionable in the breast cancer setting or in the high-risk clinic. Other genes that are relevant are listed in Ref (26 genes) which generated larger panels used to screen for other cancer genes, but these should be used judiciously as there may not be adequate clinical guidelines for screening or other interventions. Single site testing should be utilized in families with a known mutation. With the advent of the Supreme Court decision of Association of Molecular Pathology vs. Myriad Genetic in 2013, there are now several other companies that offer genetic testing using blood and/or saliva samples. The scientific methods and quality of these companies can vary widely and should be vetted carefully by clinicians prior to deciding which ones to utilize.

As more breast centers adopt formal high-risk programs and routinely screen for indications for genetic testing, prophylactic risk-reducing surgery is increasing and is a part of the armamentarium of most breast surgical oncologists and GYN oncologists (https://www.cancer.gov/types/breast/risk-reducing-surgery-fact-sheet). Other risk-reducing strategies including the use of tamoxifen and Evista as prophylaxis are increasingly utilized in the high-risk population in addition to their uses in preventing breast cancer recurrences. These medications can reduce the risk of breast cancer or recurrent breast cancer by up to 50% in some studies but do have side effects. They are used with genetic syndromes and other high-risk scenarios such as the presence of atypical ductal or lobular hyperplasia or strong family histories with negative genetic testing.

Genetics and genomics have left the laboratory and are insinuating themselves into clinical practice in many fields of medicine. Nowhere is this more apparent than in a breast surgical oncology practice. The type of operation and the timing of an operation are dependent on having genetic and genomic information available. These factors can also determine the chronological order of treatment modalities. At times surgery may be the first-line treatment, and at times it may be judicious to use preoperative chemotherapy. The specific genomics of the cancer may dictate this decision. Reconstructive surgeries may need to be delayed or modified depending on this information. It is incumbent upon the entire breast team including radiologists, oncologic and plastic surgeons, pathologists, and radiation oncologists to have this information available and to react accordingly. GYN oncologists operating in tandem with breast surgical oncologists is increasingly common and can expedite a

patient return to a high functioning, normal life. Of additional importance is the impact this information may have on entire families. Preventing disease in future generations is now possible. It has been our experience that patients are increasingly open to and accepting of this kind of information as part of their care.

17.5 Commonly Observed Somatic Alterations in Breast Cancer and Strategies for Targeted Therapies

Recent next-generation sequencing projects have analyzed thousands of breast cancers and found that only a few somatic mutation and copy number alterations (CNAs) occur with any degree of frequency. The most common genomic alterations in breast cancer are *TP53* point mutations, detected in ~90% of triple-negative breast cancer, and *PIK3CA* mutations, found in 30–40% of ER-positive breast cancer. Although different studies have detected different sets of significantly mutated genes in breast cancer, *TP53*, *PIK3CA*, *GATA3*, *MAP 3K1*, *AKT1*, and *CBFB* have been identified in independent studies and may represent the most significant drivers of breast cancer [80]. Among these genes, only *TP53*, *PIK3CA*, and *GATA3* were found to be consistently mutated in >10% of breast cancer [80]. The involvement of multiple genes in various combinations with each combination found only in a small subset of cancers indicates that breast cancer is a highly heterogeneous disease. However, most of the genes significantly affected by mutations in breast cancer belong to a relatively small number of signaling pathways, which simplifies matters somewhat. Significant pathways that are frequently "mutated" in breast cancer include the p53 (*TP53*) pathway, the PI3K pathways (*PIK3CA*, *PTEN*, *AKT1*), MAPK/JNK signaling (*MAP 3K1*, *MAP 2K4*, *NF1*), and transcriptional factors and regulators (*GATA3*, *RUNX1*, *CBFB*).

17.5.1 Treatment Strategies for Loss of the Tumor Suppressor p53

The tumor suppressor p53 is encoded by the *TP53* gene. It is a cell's and the genome's principal guardian against cancer [102]. The mutations detected in *TP53* vary with tumor subtypes (Table 17.1). It is the most commonly mutated gene in triple-negative breast cancer with a frequency of 79–82%. In HER2+ tumors, *TP53* is mutated in 42–75%. And in ER+ tumors, *TP53* is reported to be mutated in only 20%. The Cancer and Genome Atlas Project (TCGA) reported an overall frequency of *TP53* mutation of 37% [70]. *TP53* mutation was statistically associated with shorter relapse-free survival and overall survival. In addition, *TP53* mutation has been identified as a significant predictor of outcomes in all breast cancer patients [106]. It is currently unclear if the presence of a *TP53* mutation directly causes a worse prognosis or if factors associated with p53 mutation play a role. In a focused analysis of the prognostic significance of *TP53* mutations with respect to breast cancer subtypes in the METABRIC cohort, *TP53* mutations were associated with

Table 17.1 Somatic mutations discovered by genomic sequencing of breast cancer with frequencies

Gene	Gene function	Pathway	Subtype	Mutation frequency	Refs
TP53	Tumor suppressor	Genomic instability	HER2+	75%	[80]
			TNBC	82%	[80]
			HER2+/ER-	67.5%	[93]
			HER2+/ER+	42.6%	[93]
			HER2	73.7%	[70]
			ER+/HER2-	20.6%	[70]
			HER2+	54.7%	[70]
			TNBC	79.1%	[70]
			All	37%	[70]
PIK3CA	Makes p110 alpha protein, which is a subunit of phosphatidylinositol 3-kinase (PI3K)	Cell proliferation, survival, angiogenesis, transformation	ER+ and PR+	32.8%	[103]
			HER2+	22.7%	[103]
			TNBC	8.3%	[103]
			HER2+	38.6%	[70]
			TNBC	10.5%	[104]
			HER2+	30.7%	[104]
			ER+/HER2-	43.9%	[104]
PTEN	Tumor suppressor	Cell division	HER2	1.75%	[70]
			ER+/HER2-	4.8%	[104]
			HER2+	0%	[104]
			TNBC	1.2%	[104]
AKT1	Serine-threonine protein kinase		HER2	1.75%	[70]
			ER+/HER2-	3.3%	[104]
			HER2+	1.3%	[104]
			TNBC	0%	[104]

(continued)

Table 17.1 (continued)

Gene	Gene function	Pathway	Subtype	Mutation frequency	Refs
GATA3	Transcription factor	Transcription factor for normal development of mammary gland	HER2+/ER+	8.2%	[105]
			HER2+/ER-	0.5%	[105]
			HER2	1.75%	[70]
			ER + HER2-	13.6%	[104]
			HER2+	10.7%	[104]
			TNBC	0%	[104]
MAP3K1	Encodes a serine/threonine kinase that is part of signal transduction cascade, including ERK, JNK, and NFkB	Regulates cell migration, apoptosis	HER2	3.51%	[70]
			ER+/HER2-	10.9%	[104]
			HER2+	2.7%	[104]
			TNBC	0%	[104]
CBFB	Beta subunit of a heterodimeric core-binding transcription factor belonging to the PEBP2/CBF transcription factor		HER2	1.75%	[70]
			ER+/HER2-	2.1%	[104]
			HER2+	0%	[104]
			TNBC	1.2%	[104]

Subtypes of breast cancer: ER+, HER2+, triple negative (TNBC)

increased mortality in patients with ER-positive disease classified as luminal B by PAM50 expression profiling, but not in patients with luminal A and basal-like tumors [107]. Because p53 is a multifunctional protein, mutations in different domains may result in different functional changes. Interestingly, there was a higher incidence of nonsense/frameshift *TP53* mutations in the triple-negative breast cancer subtype compared to other subtypes [106, 108]. The mechanism by which *TP53* mutations increase mortality of breast cancer patients is unclear, but *TP53* mutations were recently associated with resistance to endocrine therapies [109].

The p53 pathway can be inactivated in tumors without *TP53* mutation. For example, p53 can be inactivated by the binding to MDM2 or MDM4 [110]. Both *MDM2* and *MDM4* have been found overexpressed in breast cancers [111]. The high frequency of TP53 mutation in TNBC and its association with poor outcome in specific subtypes of ER-positive breast cancer makes the p53 pathway an attractive target for the development of novel breast cancer therapies. Preclinical experiments have shown that established cancers require p53 being persistently inactivated and are potentially vulnerable to the recovered p53 function [102]. p53 was regarded as "undruggable" before. Recently, however, several approaches have been developed to reactivate or restore normal function of p53 inactivated by mutation [110]. These studies have been reviewed in detail elsewhere [110]. In one approach, PRIMA-1 (APR-017) and PRIMA-1^{MET} (APR-246), compounds were reported to reactivate mutant p53 and exert anticancer effects in breast cancer cell lines carrying *TP53* mutations [112] and xenograft animal models [113]. However, no clinical studies have been reported for the use of these agents in breast cancer. Another strategy is to block the interaction of the wild-type p53 with MDM2 and/or MDM4 [110]. Several such antagonists have been characterized in cell line and animal models, but the results have not yet translated into clinical trials for breast cancer.

17.5.2 Treatment Strategies for Aberrant PI3K/AKT/mTOR Pathway Activation

The phosphatidylinositol 3-kinase (PI3K) pathway consists of multiple intracellular signal transducing enzymes with three key regulatory components—PI3K, AKT, and mammalian target of rapamycin (mTOR) [114]. Somatic mutations have been identified in *PIK3CA* (8.3%–43.9%), *PTEN* (0–4.8%), and *AKT1* (0–3.3%) in breast cancer (Table 17.1). *PIK3CA* is the most frequently mutated gene in breast cancer, in particular in ER-positive subtypes [115]. The TCGA breast cancer analysis found *PIK3CA* mutation rates of 45% in luminal A, 29% in luminal B, 39% in HER2+, and 9% in the basal-like subtypes [70]. All of these mutations activate AKT which regulate cellular activities such as cell proliferation, differentiation, and metabolism [116]. This pathway is not only implicated in tumorigenesis of breast cancer but also may confer resistance to endocrine therapy, chemotherapy, and HER2-targeted therapies [116]. The PI3K/AKT/mTOR pathway is activated physiologically by insulin and growth factor receptors and is regulated by feedback inhibition mediated in part by downregulation insulin receptor substrate 1 (IRS-1)

[117]. In neoplastic cells, the mTOR pathway is activated not only by mutations in its components but also by activating mutations or amplification of genes encoding receptor tyrosine kinases such as *HER2*, *EGFR*, or *KRAS* [118]. Activation of this pathway has been estimated to occur in 70% of breast cancer overall [119], and several inhibitors of the PI3K/AKT/mTOR pathways are currently in clinical development (Table 17.2).

PI3K inhibitors include the pan-PI3K inhibitor BKM120 (buparlisib) that targets all four isoforms of PI3K and the isoform-selective inhibitors such as GDC-0941 (pictilisib), GDC-0032 (taselisib), and BYL719 (alpelisib) (Table 17.2). Clinical response to PI3K inhibitors in the metastatic setting has been observed in patients with and without activating *PIK3CA* mutations, and therefore a number of strategies are being evaluated to optimize the clinical benefits of these agents [118]. Because clinical efficacy of PI3K inhibitors as single agents has been modest [120], PI3K inhibitors are now being tested in combination regimens (Table 17.2) in trials that are ongoing. Initial data from these clinical trials are summarized in review paper [118].

An alternative approach to target the PI3K/AKT/mTOR pathway is the use of AKT antagonists. Clinically relevant AKT inhibitors are MK-2206 and GSK2141795. MK-2206 is an allosteric inhibitor of AKT. Preclinical data has suggested that inhibition of PI3K with either MK-2206 or GSK2141795 enhances the antitumor effect of trastuzumab in cell lines carrying amplification of HER2 amplified and activating mutations in PI3K [121, 122], and trials are ongoing to test this combination in patients who have developed Herceptin resistance (Table 17.2). AZD5363, a pan-AKT kinase catalytic inhibitor, has also demonstrated antitumor effect in several xenograft models [123] and has been tested in phase I clinical trials where antitumor activity was observed and AKT1 mutation was found as predictor for clinical response to AZD5365 [124].

Another important class of agents being tested are inhibitors of mTOR kinase. Rapamycin (sirolimus) was the first clinically available mTOR inhibitor. It was originally developed as an immunosuppressant for transplant recipients. Everolimus (Afinitor) is an oral mTOR inhibitor that was approved by US FDA in 2012 to be used in combination with aromatase inhibitors for postmenopausal women with ER-positive tumor who are resistant to single-agent hormonal therapies [125]. The efficacy of everolimus in this setting was independent of the status of PIK3CA, FGFR1, and CCND1 or related pathways [125]. Despite promising results in preclinical models, clinical responses to PI3K inhibitors have been modest in part due to release of feedback inhibition of signaling through the insulin receptor and insulin-like growth factor receptor [126]. Currently, everolimus in combination with other drugs has been tested against other subtypes of breast cancer [127] (Table 17.2).

Table 17.2 Targeted agents for breast cancer in ongoing clinical trails

Target	Representative agents	Subtype	Trial ID	Latest phase	Combined treatment	Genomic biomarkers
Kinase inhibitors						
MEK	Trametinib (GSK1120212)	Triple-negative breast cancer **(TNBC)**	NCT01964924	II	AKT inhibitor	
	Selumetinib (AZD6244)	Metastatic breast cancer **(mBC)**	NCT00780676	II		
AKT	**Uprosertib** (GSK2141795)	TNBC	NCT01964924	II	MEK inhibitor	
	MK2206	Advanced breast cancer **(ABC)**	NCT01277757	II		PI3K, PIK3CA, AKT, or PTEN mutations
		Stage I/II/III HER2 (−)	NCT01776008	II	Nonsteroidal aromatase inhibitor	PIK3CA mutant estrogen receptor positive
		mBC HER2 (+)	NCT01281163	I	Lapatinib ditosylate (tyrosine kinase inhibitor)	
		ABC	NCT01263145	I	Paclitaxel (disruption of microtubule breakdown)	
	AZD5363	Recurrent endometrial and TNBC	NCT02208375	I/II	mTORC1/2 inhibitor	
	IPATASERTIB (GDC-0068)	TNBC	NCT02301988/ NCT02162719	II	Paclitaxel	

(continued)

Table 17.2 (continued)

Target	Representative agents	Subtype	Trial ID	Latest phase	Combined treatment	Genomic biomarkers
PI3K	GDC-0941 (Pictilisib)	mBC	NCT00960960	I	Bevacizumab (angiogenesis inhibitor) or trastuzumab (HER2/neu receptor inhibitor)	
	BYL719 (alpelisib)	ER(+), PR (+), HER2(−) mBC	NCT01791478	I	Letrozole (nonsteroidal aromatase inhibitor)	
		HER2(−) mBC	NCT02379247	I/II	Nab-paclitaxel	
		TNBC	NCT01623349	I	Olaparib (PARP inhibitor)	
	BKM120 (buparlisib)	Metastatic TNBC	NCT01629615	II		
		ER (+), PR (+) mBC	NCT01248494	I	BEZ235 (PI3K/mTOR inhibitor)	
		Advanced MBC HR+, HER2-	NCT01633060	III	Fulvestrant (ER antagonist)	
	GDC-0032 (Taselisib)	Metastatic TNBC	NCT02457910	I/II	Enzalutamide (nonsteroidal antiandrogen, NSAA)	Androgen receptor positive
mTOR	Everolimus	HER2(−) mBC/ABC	NCT01520103	II	Vinorelbine (anti-tubulin)	
		HER2(+)	NCT00876395	III		
		mBC	NCT00574366	I	Erlotinib (EGFR inhibitor)	
	Ridaforolimus	HER2(+) mBC	NCT00736970	II	Trastuzumab (HER/neu receptor inhibitor)	
		ER(+) HER2(−)	NCT01605396	II	Exemestane (aromatase inhibitor) Dalotuzumab (monoclonal antibody)	

Agents targeting genomic instability						
PARP	Rucaparib	TNBC	NCT01074970	II	Cisplatin (alkylating agent)	BRCA1/2 mutations (gBRCAm)
	Veliparib (ABT-888)	HER2(−)	NCT02163694	III	Carboplatin (alkylating agent) and paclitaxel	Unresectable BRCA-associated
		HER2(−)/neu(−)	NCT01351909	I	Cyclophosphamide (alkylating agent)	
			NCT01506609	II	Temozolomide Carboplatin Paclitaxel	BRCA1/2 mutations (gBRCAm)
	KU-0059436 (AZD2281)		NCT00494234	II		BRCA1/2 mutations (gBRCAm)
	Talazoparib (BMN 673)		NCT01945775	III		BRCA1/2 mutations (gBRCAm)
	Olaparib (AZD2281)	TNBC	NCT01116648	I/II	Cediranib Maleate (inhibitor of VEGF receptor tyrosine kinase)	
			NCT02000622	III		BRCA1/2 mutations (gBRCAm)
Epigenetics						
HDAC	Entinostat (SNDX-275/ MS-275)	ER+, HER/neu (−) locally advanced or metastatic	NCT02115282	III	Exemestane (aromatase inhibitor)	
		HER/NEU (−) ABC	NCT01349959	II	Azacitidine	
	Romidepsin (FK228)	TNBC	NCT02393794	I/II	Cisplatin	BRCA1/2 mutations (gBRCAm)
	Panobinostat(LBH589)	MBC	NCT01105312	I/II	Letrozole (nonsteroidal aromatase inhibitor)	
		HER2(−) MBC	NCT00777049	II		
	Vorinostat	MBC	NCT01084057	I	Ixabepilone (microtubule stabilizer)	

(continued)

Table 17.2 (continued)

Target	Representative agents	Subtype	Trial ID	Latest phase	Combined treatment	Genomic biomarkers
Immunity						
CTLA4	Ipilimumab (monoclonal antibody)	HER2/neu (−) locally advanced or metastatic	NCT02453620	I	Entinostat (HDAC inhibitor) Nivolumab (human IgG4 monoclonal antibody)	
	Tremelimumab (monoclonal antibody)	ER (−)/ER(+), HER2/neu (−) MBC	NCT02536794	II	MEDI4736 (monoclonal antibody)	
		Locally recurrent breast cancer	NCT02643303	I	Durvalumab (monoclonal antibody) Poly ICLC	
PD-1	Pembrolizumab (MK-3475)	TNBC	NCT02452424	I/II	PLX3397 (CSF1R inhibitor)	
		TNBC/HR+ HER2- mBC	NCT02648477	II	Anthracycline (aromatase inhibitor)	
		TNBC	NCT02819518	III	Nab-paclitaxel Paclitaxel Gemcitabine Carboplatin Normal saline solution	
	Nivolumab (BMS-936558)	HER2(−) mBC	NCT02309177	I	Nab-paclitaxel Gemcitabine Carboplatin	
	Pembrolizumab (human antibody)	TNBC	NCT02657889	I/II	Niraparib (PARP inhibitor)	
	PDR001	TNBC	NCT02404441	I/II		
PD-L1	Durvalumab (MEDI4736)	TNBC	NCT02826434	I	PVX-410 (peptide vaccine)	
	Atezolizumab (MPDL3280A)	TNBC	NCT02655822	I	CPI-444 (adenosine-A2A receptor antagonist)	
	Pembrolizumab (MK-3475)	TNBC	NCT02452424	I/II	PLX3397(CSF1 inhibitor)	

Angiogenesis						
VEGF	Bevacizumab	TNBC	NCT00733408	II		
		HER(+) mBC	NCT00095706	I/II	Herceptin (HER/neu inhibitor)	
		HER2(−)	NCT00567554	III	Lapatinib Everolimus(mTOR inhibitor)	
		Hormone receptor-positive ABC	NCT00601900	III	Letrozole (aromatase inhibitor) Tamoxifen citrate (ER inhibitor)	
	Motesanib (AMG 706)	Locally recurrent or mBC	NCT01349088	I/II	Ixabepilone (stabilizes microtubules) Capecitabine (TYMS inhibitor)	
		HER2 (−) mBC	NCT00356681	II	Paclitaxel	
	Pazopanib (Votrient)	Malignant neoplasm	NCT01639911	I	Alisertib (Aurora A kinase inhibitor)	
VEGFR	Cediranib (ZD-217)	TNBC	NCT02484404	I/II	Olaparib (PARP inhibitor) MEDI4736 (anti-CTLA-4)	BRCA1 and BRCA2 mutation
	Lucitanib	mBC	NCT02202746	II		FGF aberrant
	Vandetanib (ZD6474)	ER(+), HER2 (−) mBC	NCT02530411	II	Fulvestrant (anti estrogen)	
	Cabozantinib (XL184)	TNBC	NCT01738438	II		
Checkpoint						
CHK1/2	LY2606368	TNBC	NCT02203513	II		BRCA1/2 mutations (gBRCAm)
Others						
XXX						

Subtypes of breast cancer: ER+, HER2+, triple negative (TNBC)

17.5.3 Treatment Strategies for BRCA1/2 Inactivation (PARP Inhibitors)

Major progress has been made in the treatment of ER-positive and HER2-positive breast cancer, but treatment options are still limited for triple-negative breast cancer [128]. Promising avenue for targeted therapy in TNBC has come from the study of patients with hereditary *BRCA1* mutation. Breast cancers that develop in patients with hereditary breast cancer due to *BRCA1* mutations and (to a lesser extent) *BRCA2* mutations are frequently triple negative [129]. BRCA1/2 genes play a key role in double-strand DNA repair through homologous recombination (HR). Potentially, tumor cells with an HR repair deficiency might be particularly dependent on alternate mechanisms of DNA repair such as the base excision repair (BER) pathway and particularly susceptible to inhibition of a second DNA repair pathway. This concept for targeting the loss of BRCA function led to the development of poly (ADP-ribose) polymerase (PARP) inhibitors. PARPs are a large family of enzymes active in the BER pathway of single-strand DNA breaks. DNA damage caused by alkylating agents is repaired predominantly by the BER pathway, and thus PARP inhibitors might sensitize the tumors to these agents. Normal cells would be protected, theoretically, by an intact HR DNA repair mechanism. Currently, several PARP inhibitors such as olaparib, veliparib, rucaparib, and talazoparib (BMN673) are undergoing clinical development in combination with chemotherapy or targeted therapy (Table 17.2). Sporadic basal-like TNBC and TNBC that occur in the setting of hereditary BRCA1 mutation have similar genetic profiles of genomic instability and truncating p53 mutations [108]. This suggests that sporadic basal-like TNBC may be due in part to inactivation of HR DNA repair and sensitive PARP inhibitors as well. PARP inhibitors represent a milestone in the treatment of TNBC, and promising results have been achieved with these agents in cancers with BRCA mutations [130]. Further progress may depend on the development of predictive biomarkers for PARP inhibitor sensitivity [131].

17.5.4 Other Targeted Treatment Strategies Suggested by Genomic Analysis of Breast Cancer

Many driver mutations have been detected in breast cancer at much lower incidence than PI3K, TP53, and BRCA1/2. Some of these encode targetable proteins (such as kinases) and are amenable to evaluation in clinical trials because effective inhibitors have already been developed (Table 17.2). For example, aberrations in the mitogen-activated protein kinase (MAPK) pathway frequently coexist with aberrations predicted to activate the PI3K/AKT/mTOR pathway. Several inhibitors of MAPK are currently under clinical evaluation alone or in combination with PI3K/AKT/mTOR inhibitors (Table 17.2).

Many low-frequency driver mutations do not already have effective inhibitors, and many represent inactivated tumor suppressor genes that might be considered "undruggable" in the usual sense. One approach to targeting tumor suppressor

mutations is to target pathways activated by loss of tumor suppressor functions [5]. This concept represents an opportunity for innovative "small laboratory" studies of the cell biology and biochemistry of tumor suppressor pathways.

Characterization of breast cancer genomes by aCGH has revealed unexpected heterogeneity and identified numerous recurrent foci of gene amplification. Many foci of recurrent amplification (amplicons) in breast cancer contain multiple genes whose expression is driven by amplification (Supplemental Table 1 in Ref. [80]). Sorting out the drivers from the passengers in amplicons is a critical task for the future. Once driver genes are identified, efforts to develop targeted therapies will follow. The observation that the most aggressive breast cancers are driven by multiple genetic changes is daunting and indicates the need for combination targeted therapies. The observations that the most aggressive tumors are polyclonal and that drivers in a single pathway are often mutually excluded from activation suggest that targeting multiple pathways simultaneously might prevent the emergence of resistant disease from small preexisting subclones.

The observation that every breast cancer contains a high level of unique mutations suggests that personalized active specific immunotherapy based on an individual's cancer genome DNA sequence is a real possibility [5]. The concept of immunotherapy is particularly appealing for triple-negative breast cancer (TNBC) patients for several reasons. First, no targeted therapies exist currently for TNBC. Second, TNBC patients who relapse have a dismal prognosis. Third, TNBC carry a high mutational burden and are therefore an immunotherapy "target-rich environment." Fourth, a subset of TNBC is associated with an immune response that portends a better prognosis. The use of immune checkpoint inhibitors to nonspecifically enhance immune response to breast cancer is in early stages of development and has already shown some promise in advanced-stage TNBC [132]. The biologic basis for immune response to some breast cancer and the lack of such response to other tumors are an active area of research and still poorly understood. Hypothetically, coupling immune checkpoint inhibitors with personalized vaccines based on the mutational profile in an individual exome sequence might activate the immune system and convert an aggressive basal-type triple-negative carcinoma into the prognostically better immune modulatory subtype.

Conclusion

Over the past 40 years, breast cancer surgery, radiotherapy, endocrine therapy, and chemotherapy have all been tailored to the heterogeneity of the disease. The paradigm for improvement in all treatment modalities has been similar. As new methods were employed, each was applied with maximal force until clinical trials identified subgroups that achieved equivalent survival with less therapy and thus less treatment-related morbidity. Radical Halsted mastectomy gave way to modified radical mastectomy and then to breast-conserving therapy (lumpectomy) with radiation. Initially, all patients treated with lumpectomy and radiation received whole breast radiation. Recent trials of intraoperative partial breast radiotherapy have identified subsets of patients who can avoid whole breast radiation. Other trials have suggested that selected patients can avoid radiation

altogether and be treated with excision alone. In the era of surgical endocrine ablation, all patients with advanced breast cancer were candidates for therapy until it was discovered that patients with ER-negative tumors did not benefit. In the late twentieth century, after clinical trials demonstrated improved survival of patients treated with adjuvant chemotherapy, all patients with tumors greater than 10 mm in diameter received multi-agent chemotherapy, regardless of tumor type. Now, several multigene assays have been validated as clinical tools to identify patients with ER-positive node-negative cancer who can be adequately treated with tamoxifen alone and avoid the toxicity of chemotherapy [57, 58]. Breast cancer physicians have already adapted their treatment approaches to the heterogeneity of breast cancer. In order to understand how breast cancer genomic profiling might lead to further improvements, we must focus on specific clinical situations where an opportunity for significant improvement exists.

Early diagnosis, preferably before invasion has occurred, and outright prevention, may be the most effective paths to better outcome. A comprehensive library of cancer-specific mutations and improvements in the capture of circulating tumor DNA may lead to screening blood tests for breast cancer that do not exist today. However, it is unlikely that such tests would be able to detect preinvasive disease. Toward that end, coupling sensitive tests for cancer-specific mutations or mutational profiles with better methods for sampling intraductal breast fluid might enable detection of mammographically occult intraductal neoplasia.

For patients diagnosed with early-stage breast cancer, genomic analysis may eventually be an adjuvant guide to treatment planning. For example, patients with ER-positive breast cancer are currently stratified by various assays that essentially measure proliferation rate to predict which patients will benefit from chemotherapy. How the proliferation rate interacts with genomic stability in ER-positive tumors is not entirely clear but should be elucidated by analysis of genomic data in the context of classical clinicopathologic classifications. The relevance of a genomics-informed breast cancer pathology approach was demonstrated by an in silico analysis of the TCGA data set [133]. This analysis demonstrated a strong correlation between Nottingham histologic grade (NHG) and mutational load in breast cancer. All three parameters of the NHG were correlated with mutational load; nuclear grade correlated most strongly. This is reassuring and expected. Just as the intrinsic subgroups of breast cancer identified by Sorlie, Perou, and colleagues by mRNA expression profiles define at the molecular level the clinical subgroups identified by Jensen and Huggins in 1975, the number of mutated genes (mutation index) identified by exome sequencing validates the histologic grading system defined by Bloom and Richardson 60 years ago.

In their analysis of the TCGA data, Budczies et al. also note that mutational load is strongly correlated with immunohistochemical subtype of breast cancer and that specific mutations are associated with specific immunohistochemically defined breast cancer subtypes. In particular, ER-positive cancers with a low mutation index are particularly affected by mutations in the PI3K pathway and transcription factors of the ER pathway. Cancers with a high mutation index are characterized by overexpression of genes controlling cellular proliferation (*Ki67*,

MYBL2, *BIRC5*, and *AURKA*) and mutations in genes controlling genomic integrity including *TP53*, *BRCA1/2*, and *RB1*. The results reported by Budczies et al. suggest that genomic analysis may be uninformative, when it corroborates the histologic grade and immunohistochemical subtype. The interesting subgroups identified by comparative analysis of genomic and classical clinicopathologic features are the subsets in which conventional subtyping conflicts with genomic results. For example, *TP53* mutations occurred in 8.8% of luminal A tumors in the TCGA cohort. Do these tumors represent an early time point on an evolutionary path to genomic instability, loss of hormone receptors, and triple negativity? And if so, do a *TP53* mutation or high mutational load in an early-stage, grade 1 ER-positive breast cancer portend a worse prognosis and predict benefit from chemotherapy? A recent report showing genomic instability is a stronger prognostic marker than proliferation in early ER-positive breast cancer [134] suggests that they may. Future generations of genomic analysis that provide an expression profile and mutation profile/index could be used to confirm the histologic findings and immunohistochemical subtype reported by pathologists. Outliers would trigger a review of the pathologic findings. Appropriate treatment of true outliers is undefined at present and would require additional study.

For advanced-stage breast cancer patients, the hope provided by genomic analysis of breast cancer is that numerous new targets for therapy have been identified and new strategies are being developed to test combinations of targeted therapies to prevent the emergence of resistant clones. The possibility of targeting a patient's own immune system against the unique antigenic profile of their tumor is the holy grail of personalized medicine for the patient with advanced breast cancer. Thus, new insights into breast cancer derived from MPS and other genomic analyses have the potential to improve breast cancer care in myriad ways from improving early detection to better prognostic and predictive markers for early-stage breast cancer to the development of novel therapies for advanced disease. At some point in the future, it might be possible to design a treatment plan based entirely on sequence analysis of circulating tumor DNA. In the interim however, it is likely that genomic analysis will be integrated into breast cancer care in a variety of ways depending on the clinicopathologic context of the patient.

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Pharmacogenomics: A New Approach for Preventing Severe Cutaneous Adverse Drug Reactions

18

Chonlaphat Sukasem and Therdpong Tempark

Abstract

Pharmacogenomics can be used to identify genetic predisposing factors for serious cutaneous adverse reactions and personalize drug therapy accordingly. Pharmacogenetic screening for severe cutaneous adverse reactions (SCARs) is a key opportunity and potential paradigm for reducing morbidity and mortality and finally eliminating some of the most devastating of adverse drug reactions. This chapter focuses on the current state of surveillance know-how, pathogenesis, and treatment of SCARs. The role of genomics and pharmacogenomics in the etiology, treatment, and eradication of preventable causes of drug-induced SCARs is discussed. Drugs associated with hypersensitivity reactions with strong genetic predisposing factors include abacavir, nevirapine, carbamazepine, allopurinol, etc. The gaps, unmet needs, and priorities for future research are identified in order to eliminate genetically mediated SCARs globally.

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Y. Pathak (ed.), *Genomics-Driven Healthcare*,
https://doi.org/10.1007/978-981-10-7506-3_18

18.1 Introduction

Delayed drug hypersensitivity reactions, also referred to as type IV hypersensitivity reactions, usually occur after 2–3 days of exposure to a xenobiotic [1]. Clinical manifestations of drug hypersensitivity include milder forms of cutaneous adverse drug reactions (cADRs), such as urticaria, exanthema, angioedema, and maculopapular exanthema (MPE), and severe cutaneous adverse reactions (SCARs), such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug reactions with eosinophilia and systemic symptoms (DRESS), drug-induced hypersensitivity syndrome (DIHS) or hypersensitivity syndrome (HSS), and acute generalized exanthematous pustulosis (AGEP) [2, 3].

Drug hypersensitivity reactions are mediated by T cells but the mechanisms are not well understood [1, 4]. On initial exposure to the drugs, T cells are primed and, upon continuous exposure, the memory pool is restimulated. Human leukocyte antigen (HLA) molecules have a crucial role in the development of drug hypersensitivity because they are the key elements in T-cell-mediated immune responses [4–6]. There are several mechanistic hypotheses to describe the interactions between particular drugs, HLA, and T-cell receptors (TCRs) in the activation of multiple immunological and cytotoxic signals [7–10]. Genotyping of HLA alleles has been beneficial in screening for populations at risk of drug-induced SCARs and avoiding prescribing certain drugs to them [3, 11, 12]. In addition, drug metabolism and the underlying conditions of patients have been found to play a role in the pathogenesis of SCARs, such as the variation of *CYP2C9 *3* in phenytoin-induced SCARs [13–16] and the impairment of renal function in allopurinol-induced SCARs [17, 18].

Pharmacogenomic studies of drug-induced SCARs have made important steps for prevention of SCARs by identifying predisposing HLAs and genetic variants for genes encoding drug-metabolizing enzymes [2, 3, 5, 13–16]. The association between specific HLAs and sensitivities to particular drugs has been identified, such as *HLA-B* 15:02* with carbamazepine and oxcarbazepine [19–23], *HLA-B* 57:01* with abacavir [24, 25], and *HLA-B* 58:01* with allopurinol [26–29]. In this chapter, current cutting-edge findings concerning the discovery of pharmacogenetic markers for SCARs are highlighted. The relationship between HLA alleles and drug-induced SCARs has been discussed in terms of their clinical potential for prediction and prevention.

This chapter focuses on clinical manifestations and differential diagnoses of the SCARs including SJS/TEN, DRESS, and AGEP to provide an informative summary for pharmacogenomics researchers.

18.2 Severe Cutaneous Adverse Reactions (SCARs)

The clinical manifestations of drug hypersensitivity reactions can range from minor exanthematous skin rashes to life-threatening reactions including SJS/TEN, DRESS, and AGEP, as shown in Fig. 18.1.

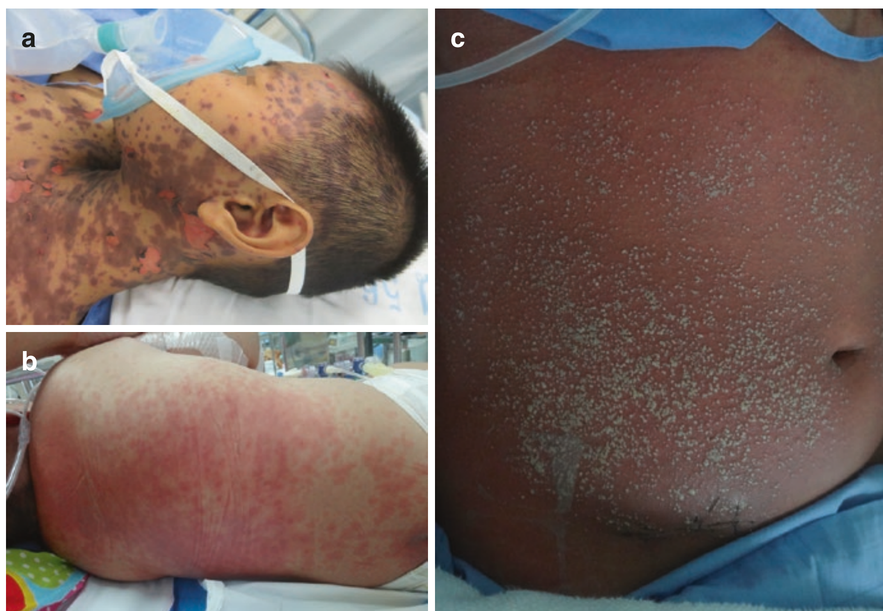

Fig. 18.1 Clinical features of (**a**) Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN). The skin is characterized by erythroderma, blister and extensive skin erosion. The lesions usually start on the face, often affects the mucosa such as eyelid, mouth and genital area. The mucosal membrane of the lip typically develops erosion. The extensive skin usually detaches and denudes to black sheet. Nikolsky sign is positive; (**b**) Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS). The skin reveals generalized erythematous macules, papules on the chest wall, abdomen and lower extremities and some are coalesce to large plaques on the back and extremities; (**c**) Acute Generalized Exanthematous Pustulosis (AGEP). The skin typically presents with generalized erythroderma with numerous small, sterile, non-follicular pustules on the abdomen and intertriginous area

18.2.1 Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN)

SJS and TEN are life-threatening mucocutaneous reactions which are part of a delayed type of hypersensitivity. The majority of cases are drug-induced. These conditions result from an association of the structure of the drug, patients' genetic factors (human leukocyte antigen (HLA) alleles), drug metabolism, and interaction of T-cell clonotypes [30, 31].

The overall incidence of SJS/TEN is estimated to be 2–7 per million per year [32, 33]. SJS/TEN occurs in all age groups, including infants and children, although the incidence increases with age owing to higher drug use and comorbidity from drug metabolism. TEN and SJS/TEN overlap occurs mainly in older patients, approximately 75% of patients with TEN or SJS/TEN overlap were over age 40, whereas 40% of patients with SJS were over age 40 as reported by Mockenhaupt [34]. An important factor in cADRs is the underlying preexisting disease, particularly in

immunocompromised patients due to increased drug prescription use and comorbidities from drug effects. The incidence of TEN in HIV-infected patients is approximately 1000 times that in the HIV-negative population [35].

18.2.1.1 Etiology and Pathogenesis of SJS/TEN

There are multiple factors associated with the etiology of SJS/TEN including genetic susceptibilities: HLA profiles, individual drug use, drug metabolism, ethnicity-specific association, and underlying diseases [2–5]. Drug administration is the primary etiologic factor. Only 5% of cases cannot be identified as drug-related [36]. In children, the most common drugs that contribute to this condition are sulfonamides, aromatic anticonvulsants (carbamazepine, phenytoin, and phenobarbital), penicillin, and NSAIDs. The major causative agents in adults are allopurinol, oxicam, NSAIDs, and nevirapine [33, 37]. *Mycoplasma pneumoniae* infection has been reported to cause SJS [38]. Potential pathogenic triggers associated with an infection and/or comorbidity with drug administration in SJS/TEN of children include coxsackie virus, influenza, herpes simplex virus, cytomegalovirus, parvovirus, varicella zoster virus, Epstein-Barr virus, measles virus, human herpesvirus types 6 and 7 (HHV-6, HHV-7), streptococcus group A, mycobacterium, and rickettsia [33, 39].

SJS/TEN is considered to be associated with medication when the patient has ingested the suspected agent within 8 weeks prior to the onset of the rash [33]. Conversely, the presumptive cause of SJS/TEN from an infection is noted to occur 1 week prior to the onset of the rash. It is crucial to determine cause of SJS/TEN as being attributed to a drug versus an infection due to the fact that the patients who get an infection might immediately start the medication (antipyretic or anti-infective drugs) preceding the skin rash. The immunologic investigation, such as the immunologic titer, cold agglutinin, and IgM of the infective agents, is also helpful to diagnose these infections [40]. Differentiation of the time interval between the start of drug use and onset of rash is extremely important in diagnosing drug hypersensitivity. Not only do the effects of specific drugs and causes of infections differ between children and adults, but children also have a lower incidence of SJS/TEN and better outcomes than adults [33].

Recent studies have shown that some of the genetic predisposing factors of SJS/TEN are associated with specific human leukocyte antigens (HLAs). Non-HLA risk alleles have also been identified to cause drug-induced SJS/TEN. The exact immunehistopathology of SJS/TEN remains unclear. Specific T-cell receptors recognize a metabolized drug presented by specific HLA alleles, followed by the activation of cytotoxic T lymphocytes (CTLs) and the release of multiple cytokines, chemokines, signals, and mediators, causing widespread apoptosis of keratinocytes leading to epidermal necrosis in SJS/TEN [31].

18.2.1.2 Clinical Manifestation of SJS/TEN

SJS and TEN are life-threatening conditions that typically present as severe mucocutaneous adverse drug reactions (ADRs). They are characterized by erythroderma, extensive skin lesions, aggressive detachment of the epidermis, and erosion of

mucous membranes. They are differentiated by the percentage of skin detachment on the body surface area: SJS is defined as epidermal detachment in which less than 10% of the body surface areas affected, whereas TEN is defined as epidermal detachment in which more than 30% is affected. If the affected area is between 10 and 30%, the condition is defined as SJS/TEN overlap.

SJS/TEN is initially preceded by non-specific prodromal symptoms for 1–7 days in approximately one third of cases. These symptoms include painful mucous membranes, stinging eyes, malaise, fever, headache, anorexia, sore throat, and pseudomembrane formation of the eyes and genitalia. Erythroderma and inflammation of the mouth and genitalia commonly occur within the first few days [32, 34].

Cutaneous manifestations are initially present at the presternal region of the trunk, the face, and proximal parts of the limbs, palms, and soles [41]. Dermatological examination reveals morbilliform exanthema, which is defined as discrete erythematous macules, papules that occasionally coalesce to become generalized erythematous to dusky dark red plaques, and rash with or without targeted lesions. The rash can potentially progress to form inflammatory vesicles and bullae that rapidly coalesce primarily to painful erosion, necrotic tissue, and then large, denuded black sheets of the total epidermal layer over a period ranging from 1 day to 2 weeks [32]. The Nikolsky sign is characterized by an epidermal detachment when applying lateral pressure on non-lesional erythrodermic skin causing dermal-epidermal cleavage. This sign and cutaneous tenderness have been used as a guide for diagnosing this condition. The extension of a blister to adjacent skin whenever pressure is applied on top of the bullae is termed the Asboe-Hansen sign.

The involvement of at least two mucous membrane sites occurs in approximately 95% of SJS/TEN cases. Lesions usually start with painful, burning sensation of the conjunctivae, lips, and genitalia followed by edematous, erythematous, and flaccid bullae lesions. These bullae modify to a painful hemorrhagic crust which involve the mucosa of many body parts, such as the eye, mouth, pharynx, nose, respiratory tracheal and bronchial airways, and anogenital area, and can lead to life-threatening complications [32, 34]. Ocular involvement often includes conjunctivitis, photophobia, lacrimation, and chemosis. The severe presentations include corneal ulceration, anterior uveitis, purulent conjunctivitis, and blindness [41].

Systemic symptoms related to internal organ involvement are respiratory, gastrointestinal and renal systems. Pulmonary involvement usually includes bronchial hypersecretion, respiratory distress syndrome, bronchiolitis obliterans, and breathing difficulties. Gastrointestinal involvement includes transaminitis, diarrhea, abdominal distension, and colonic intestinal epithelium excretion followed by bowel perforation. Renal involvement has been reported as proximal tubular necrosis, microalbuminemia, hematuria, interstitial nephritis, and acute renal failure. Other clinical manifestations may include anemia, leukopenia, encephalopathy and myocarditis. [32].

In the acute period of skin detachment, aggressive transepidermal water loss may lead to hypovolemia and electrolyte imbalance. Skin detachment and open wounds often result in serum oozing, which can lead to hypoalbuminemia and become a source of secondary bacterial skin infection. Septicemia is the most frequent cause of death in SJS/TEN patients, while hypovolemia, hypoalbuminemia, transaminitis,

and septicemia increase the risk of multi-organ failure [42]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most frequent pathogens involved in septicemia in SJS/TEN. Morbidity from multiple organ sequelae and pneumonitis has been frequently observed [42].

The prognosis for SJS/TEN patients can be assessed using SCORTEN, a severity score that involves seven independent factors: age, skin detachment from the body surface, underlying malignant disease, tachycardia, blood urea nitrogen, serum glucose, and serum bicarbonate [32, 34]. To maximize its predictive value, SCORTEN should be assessed on days 1 and 3 after admission [42].

18.2.1.3 Differential Diagnosis of SJS/TEN

Differential diagnosis for SJS/TEN includes DRESS, drug-induced maculopapular eruption (MPE), staphylococcal scalded skin syndrome, erythema multiforme and drug-induced linear IgA dermatosis, and generalized bullous fixed drug eruptions [42, 43]. The diagnosis is based on the history of the drug used, the time of rash onset, and the time of dermatological examination.

DRESS causes various cutaneous eruptions, from maculopapular rash to blisters with multisystemic involvement. Patients usually have peripheral eosinophilia (eosinophil count of>1500 cells/mm^3) or atypical lymphocytes as well as systemic conditions such as lymphadenopathy, transaminitis (hepatitis), interstitial nephropathy, interstitial lung diseases, and myocarditis [33, 42]. The generalized bullous fixed drug eruption presents with well-defined round or oval plaques with a dusky violaceous color and/or bullae in the central area of the plaques. A previous history of recurrent fixed drug eruption is common in patients with this condition [39].

18.2.1.4 Histopathology of SJS/TEN

Histopathologic features are key factors for diagnosis of SJS/TEN and to distinguish it from alternative conditions that mimic SJS/TEN. The characterized histological features include extensive keratinocyte destruction with separation of the epidermis from the dermis at the dermoepidermal junction [32].

18.2.1.5 Diagnosis of SJS/TEN

The diagnosis is manifested by three clinical elements: cutaneous and mucous membrane manifestations, systemic involvement, and histological findings. A comprehensive history of drug use and physical examination are essential for diagnosis of SJS/TEN. The initially classic manifestation of SJS/TEN consists of prodromal symptoms such as malaise, fever, anorexia, and pharyngitis followed by cutaneous and mucous membrane inflammation with pain at the ocular, oral, and genital areas and other systemic involvements.

18.2.1.6 Laboratory Investigation of SJS/TEN

The use of lymphocyte transformation tests to find the causative drugs is controversial because of false positive and negative results of these tests. Lymphocyte transformation tests have been used in vitro to assess regulatory T-cell activation. These

tests have been used frequently in cases of DRESS or drug-induced hypersensitivity syndrome (DIHS), and they were mostly positive at 5–7 weeks to 1 year after onset of the rash [44, 45] Even when the lymphocyte transformation tests for SJS/TEN were performed within the first week after onset of rash [33, 45], their sensitivity was still too low for diagnosis [45].

Patch testing is optional for investigation but is not diagnostic, owing to its low sensitivity [46]. It has been performed in DRESS/DIHS patients between 6 weeks and 6 months after the skin lesions have healed [47]. The benefit of this test is still controversial for diagnosing SJS/TEN [33, 45].

18.2.1.7 Treatment of SJS/TEN

The treatment of SJS/TEN includes a multidisciplinary approach. Early identification and withdrawal of the culprit drug and all nonessential medications are critically important. The patients should be transferred to intensive care and/or the burn unit. The physicians usually provide supportive care involving thermoregulation, airway protection, fluid replacement, and assessment of fluid balance. Nutritional support and pain management are mandatory. Secondary bacterial skin infection should be monitored to reduce the risk of death.

Medical treatment includes systemic immunomodulatory treatment such as systemic corticosteroid, intravenous immunoglobulin (IVIG), cyclosporine, tumor necrosis factor (TNF) antagonists, and cyclophosphamide. There is, however, still controversy over the efficacy of systemic corticosteroids and IVIG and also no consensus on standard dosages for treatment of SJS/TEN.

18.2.1.8 Prognosis of SJS/TEN

SJS/TEN is a potentially life-threatening drug reaction. There are approximately 1–5%, 5–25%, and 25–30% mortalities from multiple complications of patients with SJS, SJS/TEN overlapping, and TEN, respectively [42]. Long-term dermatologic sequelae include atrophic or hypertrophic scars, hypo- or hyperpigmentation, eruptive melanocytic nevi, hyperhidrosis, and xerosis. Hair thinning frequently occurs. Nail involvement can include anonychia, onycholysis, partial to total nail loss, and onychodystrophy [34, 42]. Mucous membrane involvement can include vaginal adhesion, mucosal dryness, pruritus, reduced salivary flow, gingival inflammation, and synechiae. Adhesion, introital stenosis, and urethral and anal strictures can sometimes occur as anogenital sequelae. Buccal and dental sequelae have been reported with gingival synechiae, gingival recession, dental alteration, xerostomia, and increased saliva acidity. Ocular sequelae are usually the most severe, derived from the functional change of conjunctival epithelium, i.e., chronic inflammation, sicca syndrome, entropion, subconjunctival fibrosis, trichiasis, symblepharon, corneal ulceration, corneal xerosis, photophobia, visual loss or impairment, and blindness [41, 42, 48].

Mortality rates in children are widely variable and increase by age of affected patient and related with drug comorbidities.

18.2.2 Drug Reactions with Eosinophilia and Systemic Symptoms/Drug-Induced Hypersensitivity Syndrome/Hypersensitivity Syndrome (DRESS/DIHS/HSS)

Drug reactions with eosinophilia and systemic symptoms (DRESS) are rare life-threatening adverse drug reactions characterized by manifestations of fever, rash, lymphadenopathy, eosinophilia and/or other leukocyte abnormalities, and internal organ involvement.

Many medical terms have been proposed to identify this condition in the previous literature, such as DHS, drug-induced pseudolymphoma, and drug-induced delayed multi-organ hypersensitivity syndrome [49, 50].

It is categorized as one of the severe cutaneous adverse reactions (SCARs) along with Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and acute generalized exanthematous pustulosis (AGEP).

The exact incidence of this condition is still unknown; the estimated overall population risk is about 1:1000 and 1:10,000 drug exposures [49, 51].

18.2.2.1 Etiology and Pathogenesis of DRESS/DIHS/HSS

The etiology of DRESS syndrome is commonly revealed to be severe drug hypersensitivity. The causative agents include aromatic/nonaromatic anticonvulsants (carbamazepine, phenobarbital, phenytoin, valproic acid), antimicrobial agents (ampicillin, cefotaxime, dapsone, ethambutol, isoniazid, trimethoprim-sulfamethoxazole, minocycline, metronidazole), antiviral agents (abacavir, nevirapine), allopurinol, antihypertensive (amlodipine, captopril), antidepressant (fluoxetine), and NSAIDs (ibuprofen) [52]. Aromatic anticonvulsants such as phenytoin, carbamazepine, and phenobarbital are the most common causative agents [49].

DRESS syndrome usually occurs within 2 months, particularly in 2–8 weeks after administration of the medication [49, 53]. Drug-specific immune responses and reactivation of human herpesvirus (HHV)-6 are proposed to be causative. A previous study reported the association of HHV-6, 7 and Epstein-Barr virus (EBV) with DRESS syndrome [54].

The pathogenesis of DRESS syndrome is not exactly known. The hypotheses that had been suggested included pharmacokinetic drug metabolism, reactive metabolites, sequential reaction of the herpesvirus family, and pharmacogenetic and associated HLA alleles [44, 49, 52, 54]. Mutation of genes related to drug detoxification enzymes causing drug reactive metabolites which alter cellular protein and autoimmune response to skin and liver cells is also postulated [54].

Aromatic anticonvulsants are metabolized by the hepatic cytochrome P450 (CYP) enzymes and aromatic hydroxylase and form toxic arene oxide metabolites [52]. These reactive substances are usually modified to nontoxic metabolites by epoxide hydroxylase or glutathione transferase. Deficiency of these enzymes leads to accumulation of these toxic metabolites, which can affect the immune response, causing cell necrosis and/or apoptosis [55].

Reactivation of herpesvirus probably explains the clinical relapse despite cessation of the culprit drug [56]. The complexity of drug dosage, genetic polymorphisms, and potential for environmental factors to change the detoxification process have been proposed to be possible causes of this condition [52].

18.2.2.2 Clinical Manifestation of DRESS/DIHS/HSS

Prodromal symptoms such as fever, malaise, and pruritus are commonly found in DRESS syndrome presentation for several days. Dermatologic manifestations are variable including diffuse pruritic macular, erythroderma, and exfoliative dermatitis. Morbilliform rash is the most common presentation [52]. The development of indurated skin and edema primarily occur first on the face and upper trunk followed by lower extremities. Edema of the face and periorbital areas has been found in approximately 25% of the cases [57, 58]. Vesicles, bullae, typical targetoid, plaques, and purpura have also been presented.

Rash probably involves the mucosa as cheilitis, erosion, pharynx, or enlarged tonsils. The rash usually modifies from morbilliform to violaceous color and diffuse scaling. Even upon discontinuation of the offending drug, the rash is commonly recurrent and persists for several weeks to months. Patients typically present with milder symptoms than during the primitive presentation.

Internal organ involvement is usually affected in the DRESS syndrome and occurs as lymphatic, hematologic, or hepatic involvements (the most common systemic manifestations) followed by renal, pulmonary, and cardiac involvements [59]. Lymphadenopathy is commonly found in approximately 75% of DRESS patients, particularly in the cervical, axillary, and inguinal areas [60].

Hematologic involvement is usually indicated by marked leukocytosis or atypical lymphocytes. Eosinophilia has been found in approximately 30% of the cases 1–2 weeks later. Eosinophilic granules are profuse from hypereosinophilia which possibly causes the visceral manifestations [49].

Hepatic involvement is the most frequently affected visceral organ in the DRESS syndrome, with presentations including hepatitis, hepatosplenomegaly, elevated alanine transaminase (AST), and alkaline phosphatase [52]. Elevated serum alanine aminotransferase (ALT) has been found in approximately 70% of the patients and persists for several days after withdrawal of the suspected drug [58]. The most serious sequela is hepatic necrosis leading to hepatic failure, coagulopathy, sepsis, and potentially death [49].

Kidney involvement is also common and has been found in 11% of cases [61]. Allopurinol is the drug most commonly associated, followed by carbamazepine and dapsone [62]. A majority of the cases are asymptomatic; some can present with mild hematuria and proteinuria [52]. Elevated blood urea nitrogen (BUN) and creatinine levels and diminished creatinine clearance a re commonly present in renal involvement. Nevertheless, most cases have shown only mild renal impairment. Interstitial nephritis progressing to kidney failure has, however, been observed [63].

Pulmonary involvement is occasionally found in DRESS syndrome, presenting as impaired pulmonary function, pneumonitis, lymphocytic interstitial pneumonia,

Table 18.1 Internal organ involvement with abnormalities and specific drug associations in DRESS syndrome

Organ involvement	Frequency	Abnormalities	Drug-associated
Hematology	Common	– Leukocytosis – Atypical lymphocytes – Eosinophilia – Aplastic anemia/agranulocytosis	Multiple
Hepatology	Common	– Hepatosplenomegaly – Hepatitis – Liver function test abnormalities – Hepatic failure	Phenytoin Minocycline Dapsone
Renal	Common	– Acute kidney injury – Mild hematuria/proteinuria – Renal function test abnormalities – Kidney failure (rare)	Allopurinol Carbamazepine Dapsone
Pulmonary	Uncommon	– Impaired pulmonary function – Interstitial pneumonia – Lymphocytic interstitial pneumonitis – Pleuritis	Minocycline
Cardiology	Uncommon	– Myocarditis – Cardiomegaly – Cardiac enzymes abnormalities	Ampicillin Minocycline
Neurology	Uncommon	– Meningitis – Encephalitis – Headache, seizure, coma – Cranial nerve palsy – Muscle weakness	–
Others	Uncommon	– Pancreatitis – Myositis – Thyroiditis	–

Adapted from table of Husain et al. [52]

pleuritis, and acute respiratory distress syndrome [62]. Minocycline has been reported as the culprit drug associated with these reactions [63].

Cardiovascular involvement of DRESS syndrome can present as myocarditis, chest pain, tachycardia, dyspnea, and hypotension. Cardiac enzymes such as creatinine kinase and troponin I may be elevated. Ampicillin and minocycline are most commonly found to be the associated drugs in the literature [64].

Neurological involvement is infrequent but can include meningitis or encephalitis. Other symptoms observed are headache, muscle weakness, seizure, coma, and cranial palsy [63].

Gastrointestinal involvement uncommonly occurs in DRESS syndrome. Gastroenteritis with dehydration is the most common presentation [63].

Endocrinological involvement occurs occasionally but presents as long-term sequelae. The thyroid gland is the most commonly affected [63]. Thyroid function tests, such as those for thyroid-stimulating hormone (TSH) and free thyroxine (FT4), are recommended to screen and monitor this involvement [58]. Systemic

abnormalities include sick euthyroid syndrome, thyroiditis, an isolated increase of free T4, and isolated low thyrotropin. Drug-induced hypothyroidism can possibly develop up to 2 months after the reaction onset [65]. The list of drug-associated internal organ involvements of DRESS syndrome is revealed in Table 18.1

18.2.2.3 Histopathology of DRESS/DIHS/HSS

Histopathology may help to confirm the diagnosis of DRESS syndrome. The most common findings are a dense, perivascular lymphocytic infiltration in the papillary dermis with extravasated erythrocytes, eosinophils, and dermal edema. Eosinophilia, atypical lymphocytes, and spongiosis occur occasionally [52].

18.2.2.4 Diagnostic of DRESS/DIHS/HSS

There is no standard for the diagnosis of DRESS syndrome. The diagnosis has been conducted by excluding other serious conditions, such as infection, neoplastic process, autoimmune diseases, and connective tissue diseases. At least three categories have been proposed to be the diagnostic and are shown in Table 18.2 [49, 62, 75]. The differential diagnosis of DRESS and other conditions of SCARs has been revealed in Table 18.3.

18.2.2.5 Laboratory Investigation of DRESS/DIHS/HSS

The skin patch test and lymphocyte transformation test (LTT) are two helpful methods that can be used to identify the drug responsible for DRESS syndrome [66].

Skin Patch Tests

Patch testing reveals the localized inflammatory response by activation of drug-specific T cells that lead to the recruitment of inflammatory cells causing clinically observed red spots on the skin [67]. Multiple factors can interfere the interpretation of patch test reading, including type and concentration of drug, vehicle used, and time after exposure. The patch test should be conducted at 2–6 months after clinical recovery [67] and at least 1 month after cessation of corticosteroid [47].

A positive patch test highly supports an inflammatory cutaneous hypersensitivity reaction, whereas a negative test cannot exclude the offending drugs [66]. The most notable culprit drugs associated with DRESS syndrome and patch testing were anti-epileptic drug such as carbamazepine and phenytoin [47, 67].

Lymphocyte Transformation Tests (LTT)

The lymphocyte transformation in vitro test reveals the activated drug-specific T cells that react to the culprit drug in solution. This test measures ^{3}H-thymidine uptake by selecting T cells which are proliferative after encountering the antigen [45]. The average sensitivity ranges from 60 to 70%; the specificity reveals at least 85% [68]. The appropriate time to conduct this test is 5–8 weeks after onset of DRESS syndrome [45, 66, 68]. A positive LTT can help to identify the suspected drug for diagnosis, but a negative LTT cannot exclude the culprit hypersensitivity drug [66].

Table 18.2 Diagnostic criteria for drug reactions with eosinophilia and systemic symptoms (DRESS)

Bocquet et al. [49]	RegiSCAR [75]	J-SCAR [62]
Criteria: All three (one of each clinical sign)	*Criteria*: Diagnosis when all 3 items +3/4 of all of the following clinical signs	*Criteria*: Typical seven clinical signs Atypical five first clinical signs
1. Cutaneous drug eruption	1. Acute rash 2. Reaction suspected to drug-related 3. Hospitalization	1. Maculopapular rash develops >3 weeks after starting offending drug 2. Prolonged clinical symptoms after discontinuation of the causative drug 3. Fever >38 °C 4. ALT >100 U/L or other organ involvement 5. Lymphocyte abnormalities (≥1) – Leukocytosis (>11 × 10^9/L) – Atypical lymphocytes (>5%) – Eosinophilia (>1.5 × 10^9/L)
2. Hematologic abnormalities – Eosinophil >1.5 × 10^9/L – Atypical lymphocytes	1. Fever >38 °C 2. Enlarged lymph nodes ≥2 sites 3. Involvement ≥1 internal organ 4. Blood count abnormalities – Lymphocytes above or below normal limit – Eosinophils above normal limit – Platelets under normal limit	6. Lymphadenopathy 7. HHV-6 reactivation
3. Systemic involvement – Lymphadenopathy ≥2 cm – Hepatitis: transaminase ≥2X – Interstitial nephritis – Interstitial pneumonitis – Carditis		

Adapted from table of Husain et al. [52]

18.2.2.6 Treatment of DRESS/DIHS/HSS

The most important issues are early recognition and immediate withdrawal of the suspected drug. Physicians and medical personnel should provide information about supportive care and prescribe anti-inflammatory drugs during the onset of DRESS syndrome.

Table 18.3 Differential diagnosis of severe cutaneous adverse reactions (SCARs)

	SJS/TEN	DRESS	AGEP
Etiology	– Drug – *Mycoplasma pneumoniae* – Others	– Drug – HHV-6 reactivation	– Drug – Infection (viral, bacteria) – Others: spider bite, agents
Onset of eruption	1–3 weeks	2–6 weeks	48 hours
Duration of eruption	1–3 weeks	Several weeks	< 1 week
Clinical presentation			
• Fever	+++	+++	+++
• Characteristic features	Bullae, atypical target	Facial edema	Facial edema
• Cutaneous features	Bullae, atypical target, mucocutaneous erosion	Morbilliform eruptions, pustules, exfoliative dermatitis	Multiple pustules, bullae, possible mucosa involvement
• Lymph node enlargement	–	+++	+
Other organ involvement	Hepatitis, tubular nephritis, tracheobronchial necrosis	Hepatitis, interstitial nephritis, pneumonitis, myocarditis	Hepatitis
Laboratory			
• Neutrophils	Decrease	Increase +	Increase +++
• Eosinophils	–	Increase +++	Increase +
• Atypical lymphocytes	–	Increase +	–
Histopathology	Epidermal necrosis	Perivascular lymphocytic infiltration	Subcorneal pustules
Prognosis/ mortality	5–35%	10%	5%

Adapted from table of Husain et al. [66]

Supportive care includes fluid replacement, antipyretic drugs for fever, correction of electrolyte imbalance, control of environmental factors, skin care with appropriate dressings, and prevention of secondary bacterial infections [66].

Systemic corticosteroids are accepted as a valuable treatment. Early administration of a corticosteroid is basically recommended for all cases of DRESS syndrome, usually beginning with prednisolone (1 mg/kg/day) or equivalent [51]. Oral prednisolone should be gradually tapered off, in a period ranging from 6–8 weeks [44] to 3–6 months [66] due to the possible recurrence and prolonged course of DRESS syndrome.

If life-threatening signs are present, such as hemophagocytic syndrome, encephalitis, severe hepatitis, and renal failure, oral prednisolone and/or intravenous immunoglobulin (IVIG) 2 g/kg over 5 days might be considered [44, 69].

Laboratory tests should be monitored including urine analyses, complete blood counts, alanine aminotransferase, aspartate aminotransferase, creatinine, and lactate dehydrogenase to prevent organ-specific complications. Thyroid function tests should be obtained and repeated after 2–3 months [66]. The patients should be educated to strictly avoid the culprit drug, and structurally cross-reactive drugs, in the future.

18.2.2.7 Prognosis of DRESS/DIHS/HSS

The clinical course is variable. The dermatologic presentation usually regresses, and the average time to recovery in the literature is 6–9 weeks [53, 59]. DRESS syndrome is a potentially life-threatening drug reaction; there is an approximate 10% mortality from patients with the hepatic complication [51, 70]. Some reports revealed the development of septicemia and fungemia during hospitalization [71]. Poor prognostic factors that had been proposed include high absolute eosinophil count >600/UL, thrombocytopenia, pancytopenia, a history of chronic renal insufficiency, multiple organ involvement, and underlying diseases [71].

A majority of the cases have complete recovery several weeks after discontinuation of the culprit drug. Cutaneous sequelae have been found in DRESS syndrome that are characterized as chronic exfoliative dermatitis and turn to dyspigmentation and scarring [72].

18.2.3 Acute Generalized Exanthematous Pustulosis (AGEP)

AGEP is a SCAR commonly attributed to drugs. This SCAR is typically characterized by the accepted SCAR clinical presentation, histologic findings, and clinical course.

The incidence rate of this condition had been estimated one to five per million per year [73]. AGEP has been found at any age; the mean age varies by the data from previous serial case studies from 40.8 to 56 years (±21 years) [74, 75]. There appears to be no sex difference in terms of the prevalence in this study [73]. The comprehensive review of the literature represented female predominance as well as the predominance in all drug eruptions for general practice [61].

18.2.3.1 Etiology and Pathogenesis of AGEP

AGEP is mainly attributed to drugs. Antibiotics are the most common cause of AGEP in over 90% of the cases [76, 77]. Others include broad spectrum of medication, systemic and topical medication, corticosteroids, and herbal remedies [76, 78].

The large multinational EuroSCAR case-control study revealed a range of causative agents including ampicillin/amoxicillin, quinolones, hydroxychloroquines, sulfonamides, terbinafine, and diltiazem. Less associated were corticosteroids, macrolides, oxicam, NSAIDs, and antiepileptic drugs [74, 77].

The time interval from drug exposure to presentation is approximately 2–3 days with a range 1–5 days [76]. Nevertheless, the latent period for AGEP varies wildly for each different drug.

AGEP has also been described as resulting from contact sensitivity with mercury, bufexamac, and potent topical NSAIDs [76, 77]. AGEP had been proposed to be associated with spider bite, viral infection (coxsackie B4, cytomegalovirus, parvovirus B19), and bacterial infection (*Chlamydia pneumoniae*, *Escherichia coli*) [76–78].

These associations with a preceding infection, however, possibly reflect antibiotic usage that finally causes AGEP. Atopy and pregnancy without preceding medication or illness have also been proposed to be the cause of AGEP in the literature [79, 80].

The pathogenesis of AGEP may involve immune mechanism changes after exposure with the culprit agent. Antigen-presenting cells present foreign antigens at MHC molecules leading to activation of specific CD4 and CD8 T cells that react as drug-specific T cells. Drug-specific CD8 T cells recruit perforin/granzyme B and Fas ligand to induce apoptosis of keratinocytes within the dermis causing epidermal vesicle formation [81].

Drug-specific CD4 T cells predominantly release Th1 cytokines, such as interferon gamma (IFN-gamma) and granulocytes/macrophage colony-stimulating factor. These agents induce release of IL-8/CXCL 8 by keratinocytes, a potent neutrophilic cytokine, causing transformation of vesicles into sterile pustules [76, 82]. CD4 T cells, however, occasionally stimulate the Th2 cytokine pattern to produce Il-4 and Il-5, potent stimulators of eosinophilic differentiation, causing eosinophilia, and this has been found in approximately 30% of AGEP patients [77].

18.2.3.2 Clinical Manifestation of AGEP

Typical presentation is an acute erythroderma, followed by numerous small, sterile, non-follicular pustules, particularly in the intertriginous regions of the neck fold, groin, and axillary area with widespread distribution. Mucosal involvement has been found in about 20% of the cases [76], usually confined to a single site, most commonly the lip or buccal mucosa [77].

Atypical presentation may occur including edema of the face, purpura, and blisters as TEN-like AGEP (TEN-AGEP overlap) or AGEP-DIHS overlap. Localized AGEP had been reported as localized AGEP over the mid-sternal scar and on the cheek, at the distal limb from diltiazem, and AGEP-like contact dermatitis from methylisothiazolinone (MI) sensitivity [83].

Associated findings include fever >38 °C and an elevated neutrophil count. Internal organ involvement, such as hepatic, renal, and pulmonary dysfunction, is uncommon having been observed in about 17% of the cases. Abnormal liver function tests may reveal high aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and/or a cholestatic pattern [76].

18.2.3.3 Histopathology of AGEP

The characterized histopathologic features are intracorneal, subcorneal, and/or intraepidermal pustules with dermal papillary edema involving neutrophilic and eosinophilic infiltration. Spongioses have also been found in subcorneal and intracorneal pustules. The spongiotic change of the epidermis includes exocytosis of neutrophils and necrotic keratinocytes.

18.2.3.4 Diagnosis of AGEP

Diagnosis of AGEP is conducted based upon clinical presentation. Histopathology is also helpful for differentiating this from other conditions even though there are no significant differences in histopathology between pustular psoriasis and AGEP.

18.2.3.5 Laboratory Investigation of AGEP

In vitro tests, such as the lymphocyte transformation test (LTT), have been used to identify the culprit drugs in AGEP. Both positive and negative findings have been reported in cases of AGEP [76]. Further studies beyond LTT are required to confirm the causative drugs. In vivo tests, such as a patch test, can help to identify the culprit drugs with a strongly positive patch test reaction [84, 85]. Also, patch testing is a well-tolerated procedure and of little risk to the patients.

18.2.3.6 Treatment and Prognosis of AGEP

AGEP is a self-limiting disease with a good prognosis. The necessary treatment is removal of the suspected drug and supportive treatment. The clinical course is usually resolved over a period of up to 15 days [73, 86]. A majority of the cases require only supportive care. A moist dressing is an appropriate treatment that should be performed during the pustular phase and also is appropriate for prevention of secondary bacterial skin infections. Topical corticosteroids have been used for inflammatory or pruritic lesions and can reduce duration of hospitalization.

18.3 Interaction of Human Leukocyte Antigen (HLA), Drugs, and the T-Cell Receptor (TCR)

A small-sized chemical-like drug may stimulate the immune system to induce SJS/TEN, based on the following three mechanistic models: hapten/prohapten, pharmacological interaction (p-i) with immune receptors, and altered repertoire [3, 87, 88]. The hapten/prohapten hypothesis proposed that the drug/drug metabolite could organize covalent binding with a peptide carrier to form a complex, which is presented by the HLA to the TCRs, resulting in activation of the classical peptide antigen pathway. The other hypothesis nominated is that the non-covalent drug metabolite may directly bound to TCRs or peptide-loaded HLAs. Wei CY et al. performed a series of studies to prove this hypothesis and found that endogenous peptide-loaded *HLA-B*15:02* could bind to carbamazepine or its metabolites directly and also present to CTLs [89].

The role of TCRs that stimulate T cells is another important pathology associated with drug-induced SJS/TEN [6]. The mechanism of T-cell stimulation necessitates APCs, and commonly follicular B cells in lymphoid follicles, to present the drug molecule in MHC class II molecules. Combined reactivity of B cells and T helper (Th) cells, with matching TCRs, results in immune reactions [90, 91]. Ko et al. demonstrated the key role of specific TCRs in the pathogenic mechanism of SJS/TEN [92]. In recent years, the immune mechanisms of T-cell-mediated drug hypersensitivity have been elucidated. The presumptive hypotheses on the interaction of *HLA* alleles, drug antigens, and TCRs in drug hypersensitivity are [1] the pharmacological interaction with immune receptors concept, [2] the hapten/prohapten theory, and [3] the altered peptide repertoire model (Fig. 18.2).

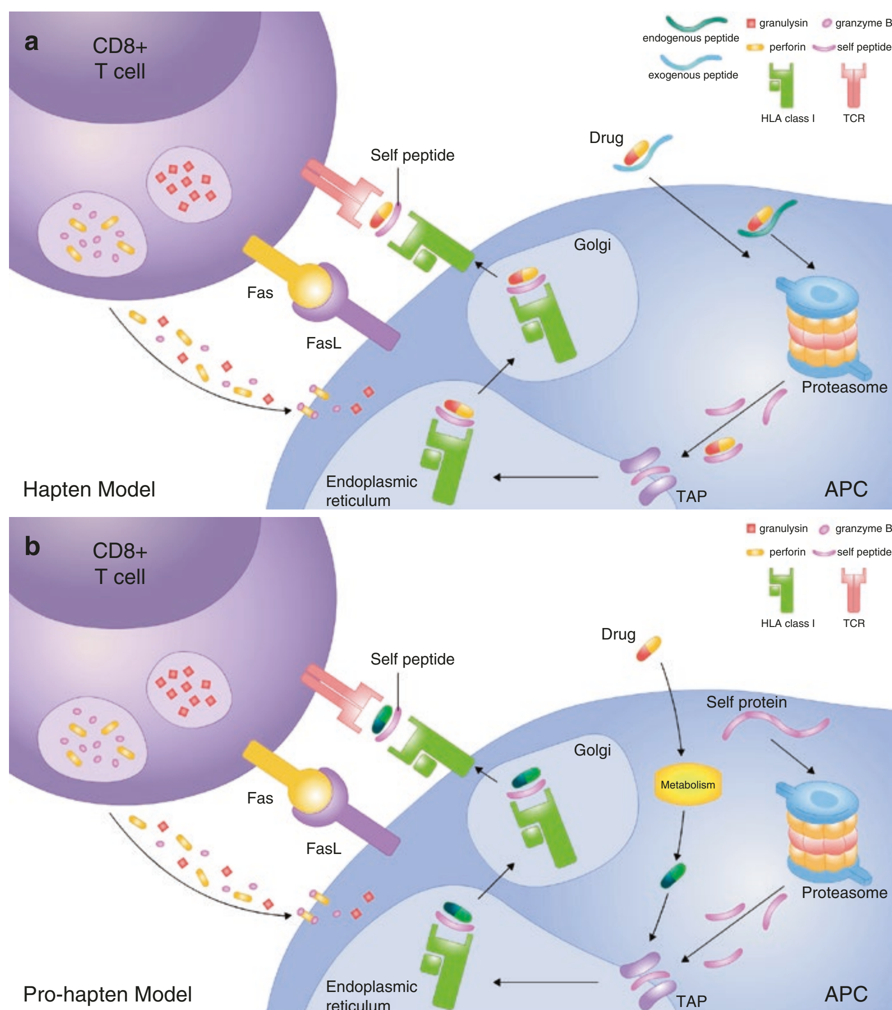

Fig. 18.2 Three major mechanistic models have been suggested to explain how a small-sized chemical-like drug can stimulate the immune system to induce SJS/TEN: hapten/prohapten model, pharmacological interaction (p-i) with immune receptors model, and altered repertoire model. The hapten/prohapten hypothesis proposes that a drug or drug metabolite could organize covalent binding with a peptide carrier to form a complex that HLA then presents to the T-cell receptors, resulting in the classical peptide antigen pathway (**a** and **b**). The p-i hypothesis proposes that the non-covalent drug metabolite directly binds to T-cell receptors or peptide-loaded HLAs (**c**). The altered repertoire model proposes that a drug or drug metabolite binds non-covalently within the pocket of the peptide-binding groove of a specific HLA and alters the repertoire of endogenous self-peptides, resulting in novel self-peptides being displayed on antigen-presenting cells, which leads to an immunological response (**d**)

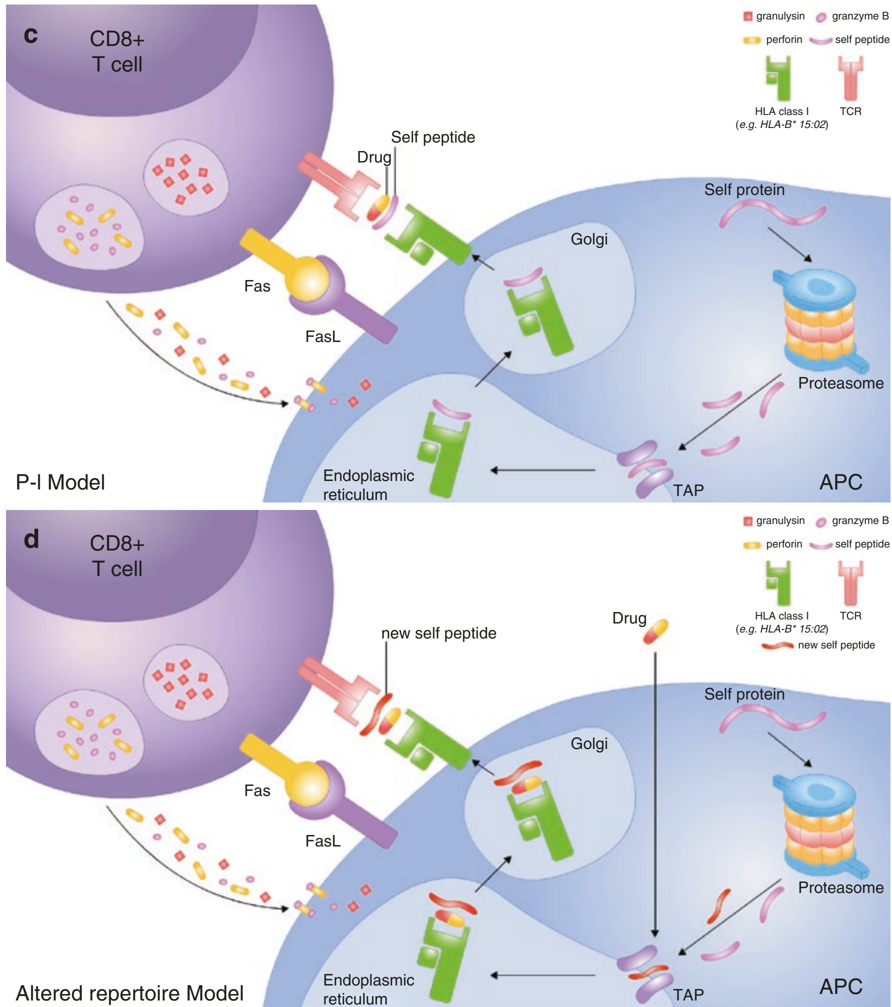

Fig. 18.2 (continued)

18.3.1 The Pharmacological Interaction (p-i) with Immune Receptors Concept

The pharmacological interaction (p-i) with the immune receptors concept explains the ability of a drug that can bind directly, specifically, and reversibly to immune receptors, either the major histocompatibility complex (MHC) or the T-cell receptors (TCRs), in order to trigger the cells just like pharmacological activation of other receptors (Fig. 18.2a). The p-i concept is based on the non-covalent binding of the drug itself to HLA (drug binding to HLA; p-i HLA model) or TCR (drug binding to

TCR; p-i TCR model). The binding sites on HLA and TCR are variable and this leads to different mechanisms [1, 93]. In the case of carbamazepine-induced SCARs in a *HLA-B*15:02* carrier, it can be explained by both p-i HLA and p-i TCR models [8], while for abacavir in *HLA-B*57:01* and allopurinol in HLA-B*58:01 carriers, only the p-i HLA model with the drugs binding to the peptide groove has been described. Alteration of the self-peptide repertoire additionally takes place in abacavir and *HLA-B*57:01*, whereas no evidence for alteration of self-peptide occurs in allopurinol and *HLA-B*58:01* [30].

18.3.2 Hapten/Prohapten Theory

The hapten/prohapten theory is described for small molecule drugs with a molecular weight of less than 1000 Daltons, in which antigenicity is gained by covalent binding with exogenous or endogenous cellular proteins to be a protein-drug complexes. The hapten is a chemically reactive small compound such as penicillin, with spontaneous binding to a carrier protein (Fig. 18.2b), while prohaptens, such as sulfonamides and acetaminophen, are chemically inert drugs that undergo metabolic bioactivation to become a protein-reactive molecule (Fig. 18.2c). The antigenic hapten-carrier complex (large modified protein) undergoes the proteasome-dependent antigen-processing pathway in the antigen-presenting cells to generate a major histocompatibility complex (MHC) ligand. The binding of a particular ligand with a T-cell receptor (TCR) can stimulate an inflammatory immune response and T-lymphocytes proliferation [7, 94, 95].

18.3.3 The Altered Peptide Repertoire Model

The altered peptide repertoire model postulates that a drug binds non-covalently within the antigen-binding cleft of a specific HLA and leads to presentation of altered endogenous self-peptides, which is subsequently stimulated in polyclonal T-cell proliferation [95]. Illing et al. studied the mechanisms involving abacavir-induced drug hypersensitivity and carbamazepine-induced SJS [96]. They demonstrated the non-covalent binding of abacavir into the peptide-binding groove, the F pocket, of *HLA-B*57:01* and a resulting conformation change of the peptide-binding cleft, thus altering the potential peptide repertoire loaded onto *HLA-B*57:01*. Consequently, a new altered peptide is exhibited on APCs with immunogenic neo-epitopes that initiate polyclonal CD8 T-cell activation and immunological reactions. Correspondingly, it was proposed that carbamazepine binds specifically to *HLA-B*15:02*, resulting in alteration of self-peptides. Interestingly, not all patients carrying the risk allele *HLA-B*15:02* exhibit immunological reactions, suggesting the involvement of additional factors that contribute to the mechanism of carbamazepine-induced SJS/TEN.

18.4 Influence of Drug Metabolism and Transporter Genes in Severe Cutaneous Adverse Drug Reactions

Dose and duration of drug use are important determinants of SJS/TEN, and recent studies have supported a dose-dependent T-cell response in abacavir-, carbamazepine-, and oxypurinol-induced SCARs. Of note, Halevy et al.'s EuroSCAR multinational study reported the incidence of allopurinol-associated SJS or TEN among the patients who were treated with daily doses equal to or greater than 200 mg of allopurinol, suggesting the risk for allopurinol-associated SJS or TEN could be dose dependent [37]. Lamotrigine was reported to be the likely cause of SJS in a case report of one woman [97]. Burkhart et al. performed a FDA Adverse Event Reporting System (FAERS) analysis and presented a hypothesis showing the association of drug-related targets, enzymes, and transporters with SJS. The analysis found *cyclooxygenases 1* and *2* (*COX-1* and *COX-2*), *carbonic anhydrase 2*, *sodium channel 2 alpha*, *CYP3A4*, *CYP2C9*, *MRP-1*, *OAT1*, and *PEPT2* as potentially contributing to the development of SJS [98].

Variability among the genes encoding drug-metabolizing enzymes has been suggested as being responsible for drug-induced SJS/TEN. *CYP2C9*3* allele was associated with phenytoin-induced SCARs in a genome-wide association study among Taiwanese, Japanese, and Malaysian populations [16]. Further, *CYP2C9*3* showed an increased risk (odds ratio = 11) for phenytoin-induced SCARs in a meta-analysis for these three populations. This association was also observed in Thai epileptic children who were treated with phenytoin, thus suggesting *CYP2C9*3* as a genetic marker for phenytoin-induced SCARs [13]. Associations between nevirapine-induced SJS/TEN susceptibility and *CYP2B6 G516T* and *T983C* polymorphisms were reported among HIV patients from Mozambique treated with nevirapine [99]. A strong correlation of *CYP2B6 T983C* polymorphism with nevirapine-induced SJS/TEN was also replicated in Malawian and Ugandan HIV populations [100]. Tanno et al. [101] described the normal CYP3A5 activity as a protective factor to aromatic antiepileptics-induced hypersensitivity reactions in Brazilian subjects. Evidence of the roles of metabolizing enzymes in drug-induced SCARs supports genetic testing to prevent adverse drug reactions before drug prescription.

18.5 Pharmacogenomics of SCARs

Several genetic studies have been performed to discover the genetic predisposition to drug hypersensitivity (Table 18.4) and gain insight into phenotypic diversity. There is considerable interest in the potential implications of genetic variations in association studies for ADRs. The genotype-phenotype correlation is still lacking due to low incidence, difficulty of patient enrollment, and small sample size. With genetic research findings, ADRs which are currently unpredictable could become both predictable and preventable and facilitate development of a better definition of drug response phenotypes.

Table 18.4 The genetic predisposition to severe cutaneous adverse reactions (SCARs)

Drugs	Major HLA-markers	Other factors
Carbamazepine	HLA-B*15:02, HLA-B*15:08, HLA-B*15:11, HLA-B*15:21 HLA-A*31:01	
Lamotrigine	HLA-A*02:07, HLA-A*33:03, HLA-B*15:02, HLA-B*44:03	Co-medication (Depakine)
Phenytoin	HLA-B*15:02, HLA-B*15:13	*CYP2C9*, *CYP2C19*, co-medication (Omeprazole)
Ox-carbazepine	HLA-B*15:02	
Phenobarbital	HLA-A*01:01, HLA-B*13:01	
Allopurinol	HLA-B*58:01	High dose, female, renal impairment, elderly
Abacavir	HLA-B*57:01	
Co-trimoxazole	HLA-B*15:02, HLA-C*06:02, HLA-C*08:01 HLA-B*13:01	
Dapsone	HLA-B*13:01	

18.5.1 Pharmacogenomics of Carbamazepine-Induced SCARs

Carbamazepine-induced SCARs are frequently reported and have shown strong associations with HLA alleles in several populations. Chung et al. [20] originally reported the strongest association between the carbamazepine-induced SJS and *HLA-B*15:02* allele in the Han Chinese population. They found that all the carbamazepine-induced SJS patients carried *HLA-B*15:02* (44/44), but only 3% of the carbamazepine-tolerant patients carried *HLA-B*15:02*. In the Thai population, 88.10% (37/42) of carbamazepine-induced SJS/TEN patients carried *HLA-B*15:02*, and carbamazepine-tolerant patients showed a frequency of 11.90% in a case-control study conducted to examine the association between *HLA-B*15:02* and carbamazepine-induced SJS/TEN [23]. Notably, *HLA-B*15:02* allele has shown a strong association with carbamazepine-induced SJS/TEN among Asian populations, including Indians, Malay, central Chinese, and Vietnamese [102–105]. The frequency of *HLA-B*15:02* is ethnicity-specific and has a low frequency in the Caucasian populations; therefore, it is not considered a strong risk factor for carbamazepine-induced SJS/TEN in these populations [106]. Similarly, the association of *HLA-B*15:02* with carbamazepine-induced SJS/TEN is lacking in Korean and Japanese populations [107, 108].

In addition to the strong association between *HLA-B*15:02* and carbamazepine hypersensitivity, there is evidence of other HLA alleles being involved. *HLA-B*15:21* allele was found to be significantly associated with carbamazepine-induced SJS in a pooled data and in silico analysis [109]. In a North American population-based cohort study among children, the *HLA-A*31:01* allele was associated with carbamazepine-induced HSS (odds ratio (OR), 26.4, $P = 0.0025$) and MPE (OR, 8.6, $P = 0.0037$), but not with carbamazepine-induced SJS [110]. *HLA-B*15:02* allele, which has a lower prevalence outside Asian populations, was linked with

carbamazepine-induced SJS (OR, 38.6, $P = 0.002$), but not HSS and MPE in this study. An association between the *HLA-A*31:01* allele and carbamazepine-induced SCARs has been reported in Japanese, Korean, and Caucasian populations. The strongest association of *HLA-A*31:01* allele and carbamazepine-induced SCARs among Japanese population has come from the genome-wide association study conducted by Ozeki et al. [111]. In this study, *HLA-A*31:01* allele was present in 60.7% (37/61) of the patients with carbamazepine-induced SCARs, but in only 12.5% (47/376) of the carbamazepine-tolerant controls. An association study between the HLA class I genotype and carbamazepine-induced SCARs in Koreans and *HLA-B*15:11* and *A*31:01* showed significant associations with carbamazepine-induced SJS and HSS/SCAR [112]. A genome-wide study conducted in Europeans showed a significant association of the *HLA-A*31:01* allele with carbamazepine-induced HSS ($P = 3.5 \times 10–8$) [113]. Follow-up genotyping of the *HLA-A*31:01* allele found *HLA-A*31:01* allele as a risk factor for carbamazepine-induced HSS (OR, 12.41), MPE (OR, 8.33), and SJS/TEN (OR, 25.93). An international study and meta-analysis of carbamazepine-induced SCARs revealed *HLA-A*31:01* as a specific biomarker for carbamazepine-induced DRESS in Europeans (OR, 57.6, $P < 0.001$) and Chinese (OR, 23.0, $P < 0.001$), but not for carbamazepine-induced SJS/TEN in Europeans or Chinese [114]. The frequency of *HLA-A*31:01* allele is >10% in Japanese and Korean population, and this risk allele frequency ranges from 3 to 10% among Caucasian, Thai, Han Chinese, Vietnamese, Indian, and Hispanic populations [115].

Aromatic anticonvulsant agents have a high degree of cross-reactivity explained by the hapten or p-i concept leading to hypersensitivity reactions [116]. A recent investigation of association of *HLA* alleles and oxcarbazepine-induced SCARs in Chinese and Thai populations showed significant associations of *HLA-B*15:02* with oxcarbazepine-induced SJS in these populations [19]. Previously, northern Han Chinese patients carrying *HLA-B*38:02* allele were reported to be at risk for oxcarbazepine-induced MPE [117]. *HLA-B*40:02* and *DRB1*04:03* were shown to be associated with oxcarbazepine-induced MPE in Koreans [118].

18.5.2 Pharmacogenomics of Lamotrigine-Induced SCARs

Lamotrigine-induced SJS/TEN has been reported in several pharmacogenomic studies regarding the association of HLA alleles. *HLA-B*15:02* is not considered to be a significant risk allele for lamotrigine-induced SJS/TEN in a Han Chinese population [119, 120]. A recent meta-analysis, however, showed a significant association between *HLA-B*15:02* and lamotrigine-induced SJS/TEN (OR = 5) in the Han Chinese population [121]. In an unpublished study conducted on Thai patients, two *HLA* alleles are found to be associated with lamotrigine-induced SCAR which includes *HLA-B*15:02* and *HLA-B*44:0*3. *HLA-B*15:02* showed a significant OR of 3.6 (SCAR cases vs. lamotrigine-tolerant controls), while *HLA-B*44:0*3 was evident among cases as compared to tolerant controls with an OR = 8.9 and gave a significant OR of 4.3 when comparison was made between SCAR cases and the general Thai population [122].

The occurrence of SCARs, such as DRESS, MPE, and AGEP after lamotrigine therapy, has been reported in several clinical and pharmacogenomic studies [123]. In a case-control association study among the Mexican Mestizos, *HLA-A*02:01:01/HLA-B*35:01:01/HLA-C*04:01:01* haplotype was found to predispose patients to lamotrigine-induced MPE [124]. In a Korean population, *HLA-A*2402*, *HLA-Cw*0102*, and *HLA-Cw*0702* alleles were found to be significantly associated with lamotrigine-induced MPE, and the haplotype *HLA-A*2402/Cw*0102* with an OR of 7.8 was considered a strongest predictive marker for lamotrigine-induced MPE ($P = 0.007$) [125]. Among the Han Chinese population, *HLA-A*3001* and *HLA-B*1302* allele-carrying patients showed a significant association with lamotrigine-induced MPE, while *HLA-A*3303* was found to be a protective marker for lamotrigine-induced MPE [126]. A recently published study showed significant association of *HLA-A*24:02* with lamotrigine-induced DRESS in the Spanish population [127]. These findings suggest the need for pharmacogenomic testing before initiating lamotrigine therapy to prevent SCARs.

18.5.3 Pharmacogenomics of Phenytoin-Induced SCARs

CPIC guidelines have issued recommendations regarding *CYP2C9* and/or *HLA-B* genotyping before initiation of phenytoin in certain at-risk populations [128]. Genetic testing for the presence of *HLA-B*15:02* alleles to predict the risk of phenytoin-induced SJS/TEN is recommended in Asian populations. Based on *CYP2C9* genotyping, CYP2C9 intermediate metabolizers are recommended for at least a 25% reduction of the standard starting maintenance dose of phenytoin and subsequent maintenance doses to be adjusted based on therapeutic drug monitoring and drug response. On the other hand, for CYP2C9 poor metabolizers, a 50% reduction of starting maintenance dose of phenytoin is recommended and subsequent maintenance doses adjusted based on therapeutic drug monitoring or drug response.

Evidence of association between phenytoin-induced SCARs and *HLA* alleles is found across populations including Han Chinese, Taiwanese, Malays, Thai, Mexicans, and Spanish [13–16, 129]. *HLA-B*15:02* showed a significant association with phenytoin-induced SJS/TEN in a case-control and meta-analysis study among the Han Chinese population [130]. Presence of HLA-B*40:01 and *HLA-B*58:01* alleles were found to be protective against SCARs induced by antiepileptic drugs. SJS/TEN and DRESS due to phenytoin treatment were associated with the *HLA-B*15:13* allele; however, *HLA-B*15:02* was only associated with phenytoin-induced SJS/TEN in Malays [131]. In a case-control analysis examining phenytoin-induced SCARs in Thai patients, *HLA-A*33:03*, *HLA-B*38:02*, *HLA-B*51:01*, *HLA-B*56:02*, *HLA-B*58:01*, and *HLA-C*14:02* alleles were associated with phenytoin-induced SJS/TEN, and *HLA-B*51:01* allele was significantly associated with phenytoin-induced DRESS [14, 15]. The author did not find significant association of *HLA-B*15:02* with phenytoin-induced SCARs which is in contrast to the significant findings of the association of *HLA-B*15:02* allele with phenytoin-induced SJS by Locharernkul et al. [124]. A study among Mexican Mestizo patients implicated the *HLA-C*08:01* allele

for phenytoin-induced MPE [131]. *HLA-A*02:01/Cw*15:02* haplotype and *HLA-B*38:01* allele were associated with phenytoin-induced SJS/TEN, and *HLA-A*24:02* allele was associated with phenytoin-induced DRESS in a Spanish population [132].

CYP2C9 is the major enzyme responsible for metabolism of phenytoin, and genetic polymorphisms in *CYP2C9* are associated with altered plasma concentrations of phenytoin and the safety profile. A study by Suvichapanich et al. found that in Thai epileptic children, the risk for phenytoin-induced SCARs was highest for patients carrying *CYP2C9*3* allele (OR = 14.52) [13]. *CYP2C9*3* is an allele resulting in a loss of function of CYP2C9 activity, and this allele is also associated with phenytoin-induced SCARs in the Taiwanese population [16].

18.5.4 Pharmacogenomics of Allopurinol-Induced SCARs

Allopurinol is therapeutically used as a urate-lowering drug in the treatment of gout and has been observed to cause SJS/TEN, DRESS, and MPE. *HLA-B*58:01* allele has been established as a risk factor associated with allopurinol hypersensitivity [26, 133]. A meta-analysis of the pharmacogenomic evidence confirming the strong association of *HLA-B*58:01* allele with SCARs induced by allopurinol across different ethnicities showed *HLA-B*58:01* allele as a risk factor for allopurinol-induced SCARs in matched studies and population-based studies with ORs of 82.77 and 100.87 [134]. A stronger association was found among Asians (allele frequency of 10–15%) as compared to Caucasians (allele frequency of 1–3%). Studies among Thai, Koreans, Japanese, Han Chinese, and Caucasians have shown a strong association of *HLA-B*58:01* allele with allopurinol-induced SCARs after the first published report by Hung et al. describing the association of *HLA-B*58:01* with allopurinol-induced SCARs in the Taiwanese Han Chinese population [26]. A multicenter retrospective case-control clinical study among Han Chinese patients found *HLA-B*58:01* allele responsible for allopurinol-induced SCARs with sensitivity of 94.6% and specificity of 88.0% for the prediction of allopurinol-induced SCARs [135]. In the Japanese population, a whole-genome association study showed several polymorphisms across genes located in 6p21, including rs2734583, rs309401, GA005234, and rs9263726, which showed a strong linkage disequilibrium with *HLA-B*58:01* [136]. A case-control association study in a Thai population examined the association of *HLA-B*58:01* allele with allopurinol-induced SCARs (SJS, TEN, DRESS, and MPE) and found that *HLA-B*58:01* was present in 100% of the patients with allopurinol-induced SJS/TEN (OR = 579.0) and DRESS (OR = 430.3) and 85.7% of the patients with allopurinol-induced MPE (OR = 144.0) [28]. *HLA-B*58:01* was present in 66.7% of the Portuguese patients with allopurinol-induced SJS/TEN (OR = 99.59) and 63.2% of patients with allopurinol-induced DRESS (OR = 85.36) but only 1.96% of normal controls who tested positive for *HLA-B*58:01* [43]. Ng et al. found that poor renal function and presence of homozygous *HLA-B*58:01* in Han Chinese patients increased the risk of allopurinol-induced cADRs (OR = 1269.45, specificity = 100%) as compared to the normal renal function and heterozygous *HLA-B*58:01* carrying patients (OR = 15.25, specificity = 82%) [27]. Osabe et al. performed in silico analysis to investigate the binding mode and affinities between

allopurinol-related compounds and *HLA-B*58:01* and found that the binding of oxypurinol, the active metabolite of allopurinol, to *HLA-B*58:01* was stronger than allopurinol, suggesting the events of SCARs mainly due to oxypurinol [137].

18.5.5 Pharmacogenomics of Abacavir-Induced SCARs

Approximately 2–9% of patients receiving abacavir may experience hypersensitivity reactions that usually occur within the first 6 weeks of therapy [138]. HLA-*B*57:01* allele is considered a risk variant strongly associated with abacavir hypersensitivity reactions in several populations [139]. Illing et al. investigated the mechanism for the association of *HLA-B*57:01* with abacavir hypersensitivity reactions [96]. The hypersensitivity reaction occurs due to the binding of abacavir to *HLA-B*57:01* subsequently modulating the repertoire of endogenous peptides that can bind *HLA-B*57:01* and inducing altered T-cell immunity. CPIC recommends *HLA-B*57:01* screening before initiating abacavir therapy, and abacavir is not recommended to those individuals who are *HLA-B*57:01*-positive [140]. Mallal et al. reported the first evidence of risk of abacavir hypersensitivity reactions in 2002 among Western Australian HIV patients who carried the haplotypes *HLA-B*5701, HLA-DR7,* and *HLA-DQ3* [141]. In subsequent studies, the association of *HLA-B*57:01* with abacavir hypersensitivity reactions was confirmed in white males and females, and Hispanics, but lacked significant associations in the black population [142, 143]. A NORA sub-study of the DART trial in Ugandan patients reported no evidence to suggest the association of *HLA-B*57:01* allele with abacavir hypersensitivity reactions [144]. Saag et al. previously reported 100% sensitivity of *HLA-B*57:01* as a predictive marker for immunologically confirmed abacavir hypersensitivity reactions in both US white and black patients [145]. A PREDICT-1 study, a prospective, randomized, multicenter, double-blind study examined the effectiveness of prospective *HLA-B*57:01* screening to prevent abacavir hypersensitivity reactions; screening produced a negative predictive value of 100% and positive predictive value of 47.9% for abacavir hypersensitivity reactions [25]. *HLA-B*57:01* screening, combined with skin patch testing, can eliminate abacavir hypersensitivity reactions with cost-effect assurance and has been successfully implemented globally in HIV clinical practices [146].

18.5.6 Pharmacogenomics of Sulfonamide Antibiotic-Induced SCARs

The incidence of SJS was documented in patients undergoing co-trimoxazole treatment. Co-trimoxazole (a combination of the drugs, trimethoprim and sulfamethoxazole) is an antibiotic prescribed for the treatment of several microbial infections. A multicenter case-control study, carried out in a Thai population involving 43 patients with co-trimoxazole-induced SJS/TEN and 91 being co-trimoxazole tolerant, found significant associations of *HLA-B*15:02*, *HLA-C*06:02*, and *HLA-C*08:01* with co-trimoxazole-induced SJS/TEN, as compared to tolerant controls with an OR ranging from 4 to 12 [147]. The HLA-*B*15:02* allele was present in 32.56% of the

43 patients with co-trimoxazole-induced SJS/TEN, but it was evident in only 11% of the 91 tolerant patients. Haplotype analysis found that *HLA-B*15:02-C*08:01* and *HLA-B*15:02-C*08:01-DRB1*12:02* haplotypes were associated with co-trimoxazole-induced SJS/TEN, with a significant OR of 5 [147].

The pathophysiology of SJS and TEN remains unclear during sulfamethoxazole treatment. Previous studies have suggested an immune mechanism involving the T lymphocytes via the human leukocyte antigen (HLA) pathway. *HLA-B* genotyping was performed on 25 European patients, who were diagnosed with sulfamethoxazole-induced SJS or TEN by RegiSCAR criteria. They found an association between *HLA-B*38:01* and sulfamethoxazole-induced SJS/TEN with an OR 4.3 (95% CI 1.4–12.7) and a *p*-value 0.022. Moreover, *HLA-B*38:02* allele is highly associated with sulfamethoxazole-induced SJS/TEN by an OR 76 (95% CI 4.6–1250) and a *p*-value 0.027 [148].

18.5.7 Pharmacogenomics of Dapsone-Induced SCARs

Dapsone (diaminodiphenylsulfone) is widely used for the treatment of inflammatory disease and infections, such as leprosy, *Pneumocystis jiroveci* pneumonia in patients with HIV infection, dermatitis herpetiformis, and autoimmune bullous disease. Dapsone treatment of patients at 0.5–3.6% developed dapsone hypersensitivity syndrome (DHS) with a mortality rate of 9.9%. A study found that *HLA-B*13:01* was associated with dapsone-induced hypersensitivity reactions among leprosy patients in China with an OR 122.1, *p*-value 6.038×10^{-12}, and OR 20.53, *p*-value 6.84×10^{-25}. Furthermore, the sensitivity and specificity of *HLA-B*13:01* were 85.5% and 85.7%, which are predictors of DHS in Chinese patients with leprosy [149].

In addition, the association between *HLA-B*13:01* and dapsone-induced SCARs in 15 Thai patients has been reported. The *HLA-B*13:01* allele was significantly associated with dapsone-induced SCARs compared with dapsone-tolerant controls (OR value of 54.00, 95% CI 7.96–366.16, *p*-value = 0.0001) and the general population (OR value of 26.11, 95% CI 7.27–93.75, *p*-value = 0.0001). This study demonstrated an association between *HLA-B*13:01* and dapsone-induced SCARs including SJS-TEN and DRESS in non-leprosy patients [150].

18.5.8 Pharmacogenomics of Non-antibiotic Sulfonamide-Induced SCARs

Acetazolamide and methazolamide, a sulfonamide derivate and a carbonic anhydrase inhibitor, are used therapeutically to reduce intraocular pressure in glaucomatous patients [151, 152]. Studies have reported the association of methazolamide treatment with SJS [153, 154]. The genetic basis of methazolamide-induced SJS/TEN has been described in case reports, and a strong association was observed between *HLA-B*59:01* allele and methazolamide-induced SJS/TEN (OR = 249.8) in patients of Korean and Japanese ancestry [155]. A strong correlation between *HLA-B*59:01* allele and methazolamide-induced TEN in a Han Chinese woman

was first reported by Xu et al. in 2015 [156]. Similarly, a case study of a Chinese-Korean patient with methazolamide-induced TEN found the presence of *HLA-B*59:01* allele in the patient [152]. Recently, *HLA-B*59:01* allele was shown to be highly associated with methazolamide-induced SJS/TEN in Han Chinese patients with an OR of 305.0 [157]. Jee et al. described the immunologic mechanism of methazolamide-induced SJS/TEN through the hapten theory [158]. An allelic predisposition for individuals with *HLA-B*59:01* to develop SJS after acetazolamide intake was reported in a case study of a female Korean patient [151].

18.5.9 Pharmacogenomics of Phenobarbital-Induced SCARs

Phenobarbital is a broad-spectrum antiepileptic drug used for the treatment of epileptic seizures [159]. The use of phenobarbital has been associated with cADRs which is a concern before initiating the phenobarbital therapy [160]. The EuroSCAR study, a case-control study conducted in Europe, evaluated the risk of medications to induce SCARs and found that phenobarbital was associated with high risks of SJS/TEN [161]. A case study in seven Iranian children confirmed phenobarbital as a causative drug for erythema multiforme, SJS, and TEN [162]. A pharmacogenomic study was conducted to explore the association of *CYP2C19*1*, *CYP2C19*2*, and *HLA-B*15:02* with phenobarbital-induced SCARs in Thai children, and the *CYP2C19*2* variant was significantly associated with the onset of SCARs following phenobarbital treatment (OR = 4.97, $P = 0.025$) [163]. Another study in Thai children showed the significant association of *HLA-A*01:01* (OR = 11.66, $P = 0.01$) and *HLA-B*13:01* (OR = 4.60, $P = 0.009$) with phenobarbital-induced SCARs [164]. *HLA-B*15:02* was associated with phenobarbital-induced SJS in a Han Chinese patient suggesting the cross-reactivity between aromatic antiepileptic drugs [165].

18.6 Implementation of Pharmacogenetics in Clinical Practice

At the present time, *HLA-B* genotyping is considered the standard of care in clinical practices before starting therapy with the previously discussed drugs. *HLA-B* genotyping is available in clinical practice, providing appropriate clinical monitoring and patient counseling about phenotypic findings and recommendations about therapy. Currently, “pharmacogenetic tests” and “pharmacogenomic cards” have been successfully implemented in clinical practice in Thailand at the Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center, Ramathibodi Hospital. The results of the pharmacogenetic tests are provided along with the interpretation associated with *HLA-B* alleles and SCARs for a particular drug. All pertinent information required for the clinician and the patient is provided. The patients are also screened for the alleles present which are associated with the ADRs related to the use of concerned drugs. The patients and clinicians are informed about the presence of such alleles on the pharmacogenomic card which will aid in preventing the drug-induced ADRs in case the patient uses the drug in the future.

The interpretations of clinical *HLA-B* genotyping tests provide useful information with regard to abacavir, allopurinol, and carbamazepine treatment. The *HLA-B* variants do not affect pharmacokinetics and pharmacodynamics of the aforementioned drugs. The specific drug/pharmacogenetic marker (specific *HLA-B* marker) results are presented as either "positive" or "negative" for the particular *HLA-B* allele, with no intermediate phenotype (Fig. 18.3).

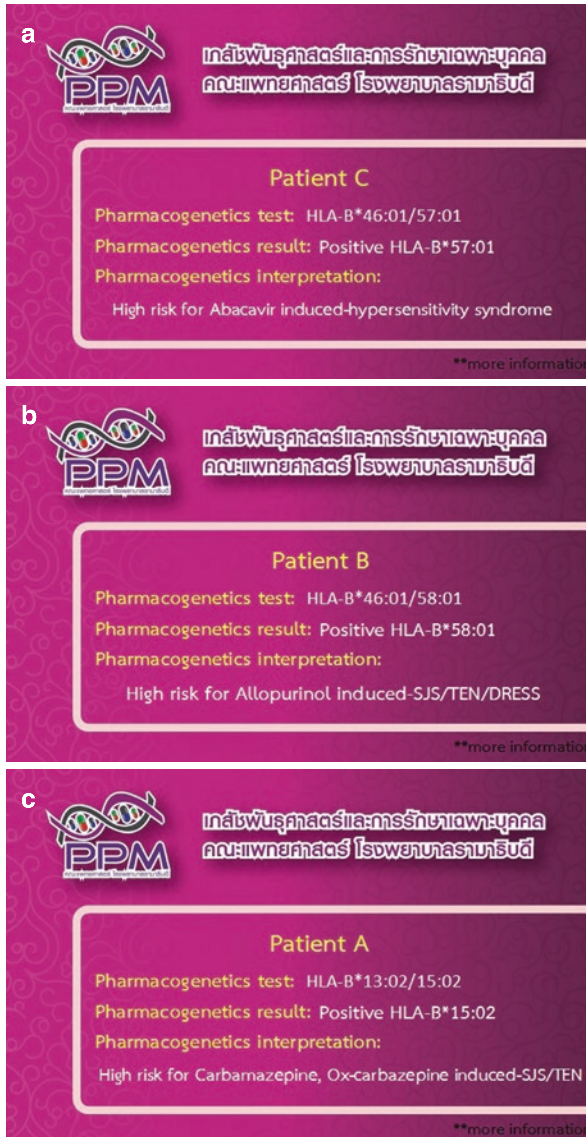

Fig. 18.3 Pharmacogenetic card of a patient with *HLA-B*57:01* (**a**), *HLA-B*58:01* (**b**), and *HLA-B*15:02* (**c**) positive

For *HLA-B**57:01 screening, the absence of *HLA-B*57:01* alleles, reported as "negative" on a specific *HLA-B* genotype test, has a very low risk of abacavir hypersensitivity reactions, whereas for the individuals who are *HLA-B*57:01*-positive with the presence of at least one *HLA-B**57:01 allele, abacavir is not recommended because of the high risk of abacavir-induced hypersensitivity. Both the heterozygote and homozygous variants are reported as "positive" on a specific *HLA-B* genotyping test (Fig. 18.3a).

For *HLA-B*58:01* screening, similar guidelines for the pharmacogenetic test for allopurinol are recommended, with *HLA-B*58:01*-positive individuals contraindicated for allopurinol due to the significantly increased risk of allopurinol-induced SCAR (Fig. 18.3b).

For *HLA-B*15:02* screening, genotyping results are presented as "positive" with the presence of one or two copies of *HLA-B*15:02* and "negative" if no copies of *HLA-B*15:02* are present in the recommendations to prevent carbamazepine-induced SJS/TEN for the carbamazepine therapy (Fig. 18.3c).

To strengthen the use of pharmacogenetic testing in hospital, Sukasem C. at Bangkok's Ramathibodi Hospital has invented and launched a low-tech approach. After patients have taken an *HLA* test, their results are entered into a plastic "pharmacogenomic wallet card," which basically contains the genomic results of those persons related to the risks of SJS/TEN. This card can be carried and shown to appropriate doctors. In order to serve the needs of patients, such pharmacogenetic cards have been expanded to more drug/pharmacogenetic biomarkers, such as *cytochrome P450* genes depending on the medication used, which is simple and cost-effective. Focused screening of HLA-B alleles, such as *HLA-B*15:02, HLA-B*58:01, HLA-B*57:01*, and *HLA-B*13:01*, before high-risk populations begin carbamazepine, allopurinol, abacavir, and dapsone therapy, could significantly avert occurrence of SCARs globally.

18.7 Summary

This chapter has provided the evidence of the genetic associations of drug hypersensitivity reactions with reference to commonly used drugs like abacavir, nevirapine, carbamazepine, and allopurinol for different indications. The highly positive predictive value of *HLA-B*57:01* in abacavir-induced cutaneous adverse reactions demands implementation of pharmacogenetic screening in routine clinical settings. Abacavir should not be used in patients who test positive for *HLA-B*57:01*. Similarly, a screening test to detect the presence of an *HLA-B*58:01* allele could be useful to prevent allopurinol-induced SCARs. The US FDA recommendation for genetic screening of *HLA-B*15:02* before prescribing carbamazepine might be useful and cost-effective only for the patients of Asian ancestry. Ethnicity has an important role in inducing the adverse events by the alleles in question.

Although rare, SCARs have a high morbidity and mortality rate. The discovery of potential implicated genes will help develop preventive strategies and make medications safer earlier. From these impressive findings, it is just a matter of time before these results can be used in clinical practice to prevent specific toxic effects

of a drug. Several issues like equity in health, ethical principles, and legal challenges need to be considered in clinical practice. There are several factors related to the patient and drugs which have effects on the frequency and severity of drug hypersensitivity. It has to be noted, however, that without the exposure of an individual to the drug, there will be no adverse effects even if an individual carries the risk gene. Since most drug hypersensitivity reactions are rare, it is imperative that a multicenter, multinational collaboration is developed to collect enough case and control samples across various ethnic populations to ensure statistical power for the detection of genetic biomarkers, both in exploratory and validation studies. To successfully translate the discovery into clinical practice, the accurate phenotypic characterization of patients is essential. From a drug safety standpoint, the negative predictive values of pharmacogenetic tests should be approximately 100%. The laboratory tests should be cost-effective, widely available, and easy to implement.

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