Advances in Predictive, Preventive and Personalised Medicine
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Godfrey Grech
Iris Grossman *Editors*

Preventive and Predictive Genetics: Towards Personalised Medicine

EPMA European Association for Predictive Preventive & Personalised Medicine

Springer

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Advances in Predictive, Preventive and Personalised Medicine

Volume 9

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Prof. Dr. Olga Golubnitschaja

Book Series Editor

Dr. Golubnitschaja, Department of Radiology, Medical Faculty of the University in Bonn, Germany, has studied journalism, biotechnology and medicine and has been awarded fellowships for biomedical research in Paediatrics and Neuro-sciences (Medical Centres in Austria, Russia, UK, Germany, the Netherlands, and Switzerland). She is well-cited in the research fields of "gene hunting" and "subtractive hybridisation" applied to predictive prenatal and postnatal diagnostics published as

O. Labudova in years 1990–2000. Dr. Golubnitschaja is an expert in molecular diagnostics actively publishing in the fields of perinatal diagnostics, Down syndrome, diabetes mellitus, hyperhomocysteinemia, cardiovascular disease, neurodegenerative pathologies and cancer. She is the *cofounder* of the theory of multi-pathway organ-related blood fingerprintingwith specific molecular patterns at epi/genomic, transcriptional and post/translational levels and author of fundamental works in *integrative medicine.* Dr. Golubnitschaja holds appointments, at the rank of Professor, at several European Universities and in International Programmes for Personalised Medicine and is author of more than 300 international publications in the field. Awards: National and International Fellowship of the Alexander von Humboldt-Foundation; Highest Prize in Medicine and Eiselsberg-Prize in Austria; She is *Secretary-General* of the "European Association for Predictive, Preventive and Personalised Medicine" (EPMA in Brussels, www.epmanet.eu), Editor-in-Chief of *The EPMA-Journal* (BMC in London); Book Editor of *Predictive Diagnostics and Personalized Treatment: Dream or Reality,* Nova Science Publishers, New York 2009; Book Co-editor *Personalisierte Medizin,* Health Academy, Dresden 2010; Book Series Editor *Advances in Predictive, Preventive and Personalised Medicine,* Springer 2012; *European Representative* in the EDR-Network at the NIH/NCI, http://edrn.nci.nih.gov/; and *Advisor and Evaluator* of projects dedicated to personalisedmedicine at the EU-Commission in Brussels, NIH/NCI,Washington, DC, USA, and at Foundations and National Ministries of Health in several countries worldwide.

Preface

Traditionally, medical research comprised of the identification of the pathological causes of a disease, its epidemiology and empirical investigation of treatment response. Intensive genetic research, marked by the completion of the human genome project in 2003, heralded a new era in medical research. While epidemiology and gross pathology are still mainstay useful tools, genetics and genomics have gradually been shown to increase the resolution of drug response research, showing great potential in also informing and identifying the role of genes and their encoded products in the pathophysiology of diseases. This information is already being applied to effective early diagnosis, better risk assessment (prognosis), as well as targeted effective and safe treatment allocation (prediction and monitoring).

Genetic testing and genomics support personalised medicine by translating genome-based knowledge into clinical practice, offering enhanced benefit for patients and health-care systems at large. Current routine practice for diagnosing and treating patients is conducted by correlating parameters such as age, gender and weight with risks and expected treatment outcomes. In the new era of personalised medicine the healthcare provider is equipped with improved ability to prevent, diagnose, treat and predict outcomes on the basis of complex information sources, including genetic and genomic data. The support of regulatory bodies and policy makers internationally has been critical for the rapid translation of personalised medicines into the clinic. Notwithstanding, inequality in the utilisation of targeted therapies in different health care systems across the world exists, and ethical considerations, as well as economic cost-effectiveness analyses are in need to inform decision making. In addition to the benefits of pharmacogenomics in diagnosis and treatment, prevention of illness using genomic information is important to reduce the burden on the healthcare system, a methodology proven effective in many therapeutic areas, but, paradoxically still facing challenges in others. In current settings screening programmes (e.g. BRCA1/2 screening) address this by identifying susceptible families and preventive measures or ensuring appropriate treatment at the earliest stages of disease, hence increasing health management effectiveness.

The integration of pharmacogenomics into the various health care systems have been the responsibility of the respective national health authorities, which in turn follow recommendations by leading regulatory bodies such as the European

Medicines Agency and the US FDA. The dynamics and logistics of this integration therefore vary substantially across the globe. To this end, implementation of pharmacogenomics is an important component of PPPM (Predictive, Preventive and Personalised Medicine), which is the main focus of the European Association for Predictive, Preventive and Personalised Medicine (EPMA). Since 2009, EPMA (http://www.epmanet.eu/) embarked on various initiatives to promote PPPM including the launch of the EPMA Journal to ensure dissemination of current aspects of PPPM, and the organisation of the first World Congress in September 2011 bringing together participants from over 40 countries to discuss education, policy and implementation of PPPM. In addition, EPMA took the initiative to publish a series of books in advances in PPPM, including the present one entitled "**Preventive and Predictive Genetics: Towards Personalised Medicine**". The editors take this opportunity to thank all contributing authors and trust that the content meets the expectation of all readers.

The Editors

Godfrey Grech, PhD
and Iris Grossman, PhD

Acknowledgement of Reviewers

The Editors wish to acknowledge the reviewers for their generous contribution in assessing the Chapters providing constructive critique and recommendations. The reviewers provided the means to enhance the quality of the book and hence we sincerely are grateful.

Beena Koshy, Peter Shaw, Ann Daly, Anderson Wayne H, Dr Alex Gatt, Aruna Bansal, Michael Barnes, Godfrey Grech, Joseph Borg, George Patrinos, Christian Scerri.

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Dr. Godfrey Grech is Senior Lecturer at the University of Malta. Since 2006, Dr. Godfrey Grech is responsible for the coordination of an MSc in Biomedical Sciences and currently runs the Molecular Pathology laboratory at the University of Malta. Following his doctorate studies at Erasmus Medical Centre, Rotterdam, he published in high impact journals and presented in International conferences such as the American Society of Hematology, the EPMA Congress and the European Pathology Congress. In the last few years, Dr. Godfrey Grech was selected to lead the research arm of the National Transfusion Centre and was given the role of a Principle Investigator on the National Breast Cancer Research Project. In addition, he is highly recognised by the clinical sector and runs numerous projects with Mater Dei Hospital and was instrumental to set up infrastructures that allow the use of fresh surgical sections in molecular pathology research. Currently he runs various collaborative projects with International Institutions including Erasmus Medical Centre and the Molecular Medicine Institute in Leeds. The main research topic aims to identify biomarkers to classify breast cancer patients into a specific therapeutic group that shall benefit from activation of phosphatases as a main therapeutic option.

Dr. Godfrey Grech was nominated and is elected on international scientific committees including the International Scientific Council of the European Group for Molecular Pathology (EMP); Global Leader at the Genomic Medicine within the National Human Genome Research Institute (NHGRI) of the U.S. National Institutes of Health (NIH); member of the Pharmacogenomics Working Group of the Global Genomic Medicine Consortium (G2MC); Leader of the Cancer Position Paper at the European Association for Predictive, Preventive & Personalised Medicine (EPMA); and national contact point for the PharmacoGenetics for Every Nation Initiative (PGENI).

Iris Grossman, PhD

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Dr. Iris Grossman is CEO and president of the pharmacogenetics management consultancy IsraGene Ltd., as well as VP, global head of the Personalized Medicine and Pharmacogenomics (PMP) unit for Teva Global R&D. She has dedicated her research career, in both industry and academia, to the advancement of the field of personalised medicine. Dr. Grossman is currently charged with defining and implementing the global PMP strategy for Teva, a top-10 global pharmaceutical company, covering both discovery and development R&D programmes. Israel's leading financial magazine, Globes Magazine, selected Dr. Grossman as one of the country's top 40 professionals under 40 years of age in 2013.

This followed several years of spearheading pipeline pharmacogenetic programmes for industry and academia as director of pharmacogenetics at Cabernet Pharmaceuticals Inc. Dr. Grossman moved into consultancy having been responsible for running large-scale pharmacogenetic programmes at GlaxoSmithKline, with an emphasis on infectious and neurological diseases.

In academia, Dr. Grossman was a key member of Professor David Goldstein's team at the Center for Population Genomics and Pharmacogenetics, Institute for Genome Sciences and Policy, at Duke University. Dr. Grossman received her PhD from the Technion – Israel Institute of Technology, where her research project, conducted in collaboration with the Weizmann Institute for Science, investigated pharmacogenetic markers of multiple sclerosis treatment response.

Introduction

Godfrey Grech and Iris Grossman

Abstract Genetics evolved rapidly in the past decade, characterising genes that directly cause specific traits in monogenic diseases, as well as modifier genes that are associated with specific disorders but are not sufficient for causality, rather work in concert with additional gene and environmental factors to elicit the disease, mostly referred to as susceptibility genes. The science has also evolved from bench and lab discoveries to bedside implementations and further gathered momentum as knowledge acquired about allele and genotype frequencies in specific populations (via epidemiological studies) resulting in effective genetic disease prevention programmes. In parallel, numerous polymorphisms have by now been characterised and often formulated into drug labels, which play a role in the pharmacokinetics and pharmacodynamics of available therapeutics, predicting the efficacy of drugs in patients, and minimising the occurrence of drug adverse reactions.

The main two fronts of genetic contribution to personalised medicine address the preventive and the predictive aspects of medicine. Preventive genetics plays a major role in the characterisation of specific genetic disorders through populations-wide policies. Preventive programmes include population screening for carriers of rare, fully penetrant alleles that cause monogenic diseases, prenatal diagnosis of specific syndromes, and genotyping of susceptibility genes within families with high risk of developing a specific disease, providing the basis of Public Health Genetics. Carrier testing programmes go beyond the science and empirical testing, accompanied by premarital counselling aiming to provide necessary information to prevent the occurrence of disease, as well as support effective therapy and improved quality of life when such diseases are expressed. Preventing clinical manifestations by early

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diagnosis and intervention provides another level of preventive strategies that are not directed towards the prevention of the genetic defect, but avoiding emergence of symptoms by excluding exposure to exacerbating specific allergens/nutrients in disorders such as Glucose-6-Phosphate Dehydrogenase deficiency and phenylketonuria. Other strategies include the prevention of secondary complications of a disease, such as the use of antibiotics to prevent life-threatening bacterial infection in sickle cell disease. Overall, the multi-faceted approaches to newborn screening programmes are essential to prevent clinical manifestations or secondary complication of disease.

In complex diseases, genetic testing provides a means for measuring risk of manifesting disease given demographic and environmental risk factors. A "high risk" individual is then provided with recommendations for risk reduction interventions and/or access to screening and monitoring programmes at an earlier age, to prevent the occurrence or to delay onset of the disease. The efficiency of genetic testing to prevent disease depends on the weight of evidence, the predictive value of the variant(s) screened in the programme and the frequency of such alleles and genotypes in a specific population.

Another area of predictive genetics deals with the efficacy and toxicity of drugs in individuals. Pharmacogenomics is defined as the impact of the individual's genetic and genomic make-up on the body's response to drug therapy. This response depends on various factors additional to genotype and genomic expression, including environment, lifestyle and demographics (e.g. age and gender). Currently, the use of genetic information to treat patients is still in its early stages, with some clear success mostly in the oncology and infectious diseases therapeutic areas. Some successful examples include the targeting of tailored pharmaceuticals developed for the treatment of patients with a particular disease subtype or according to a specific genetic make-up pertaining to the drug's mode of action (e.g. zelboraf). In other examples, genetic information is being used to help determine the effective and safe dose of specific pharmaceuticals (e.g. warfarin). However, implementation of this pharmacogenetic knowledge to the clinic has proven to be challenging and to require collaboration between the various stakeholders throughout the discovery, development and validation stages so as to ensure the utility of actionable genetic testing in a cost-effective manner. Targeted therapy and reliable prediction of expected outcomes offer patients access to better healthcare management, by way of identifying the therapies effective for the relevant patient group, avoiding prescription of unnecessary treatment and reducing the likelihood of developing adverse drug reactions.

In accordance with the main themes that define preventive and predictive genetics and its utility and wide-spread adoption world-wide, the chapters of this book walk the reader through the principles of this discipline and the state of the art across key therapeutic areas. To this end, the second chapter (i.e. the one preceding the introduction) discusses the broad definition of public health and the specific role that genetic testing plays in advancing population-level health outcomes. Indeed, preventive genetics has demonstrated utility as a crucial component in the success of population-wide health policies that promote improved health outcomes. The

identification of subjects at risk at the earliest age possible provides opportunities for tailoring actionable medical solutions when needed. Principles of preventive genetic programmes are outlined in details, and specific examples reviewed, including phenylketonuria, MCADD, homocystinuria, maple syrup urine disease, glutaric aciduria type 1, cystic fibrosis, haemoglobinopathies, hereditary haemochromatosis, familial hypercholesterolaemia, familial adenomatous polyposis and familial cancer predisposing syndromes. In addition, while complex traits are mostly yet to have been fully characterised in terms of the exact proportion explained by genetics on a population basis, characterisation of monogenic subtypes within complex diseases can be easily taken up into genetic testing programmes, as exemplified in detail by BRCA1 and BRCA2 for risk of breast and ovarian cancers, as well as other relevant cases. The chapter also touches on the practical, ethical and clinical aspects associated with biobanking of the required samples that facilitate the research, as well as application of screening genetics over time.

In the third chapter Bishop et al. describe the use of pharmacogenetics to the development of safer, more effective and differentiated therapies. This chapter describes the principles and requirements of an efficient and valuable pharmacogenetic strategy throughout the course of developing innovative medicines. This strategy combines a proven methodology with rigorous genetic science to create a "Pipeline Pharmacogenetic Program". By describing the pharma industry and the market forces shaping its drivers, pharmacogenetic applications are portrayed as aides to reduce attrition and enhance the scientific rigor, and over all benefit/risk profile of novel therapies. The authors review the scientific requirements, as well as sample collection and practical decision making perspectives that must be taken into account during R&D. Specific examples are shared throughout the drug development continuum and across a variety of therapeutic areas, including Alcohol Dependence, Oncology and Rare Diseases. Finally, unique features associated with the contemporenous development of drug and companion diagnostic are reviewed by way of describing an example dealing with Alzheimer's disease management.

The type of data and design of pharmacogenetic studies is a requirement to provide the necessary outcome and define actionable markers. In Chap. 4 Flynn et al. summarise the key statistical consideration required for successful and meaningful pharmacogenetic programmes. As a scientific discipline pharmacogenomics must demonstrate rigor of study design and significance of statistical findings, additional to biological and clinical relevance of variants identified. In addition, consideration of statistical factors unique to pharmacogenetics must be examined over the course of biomarker studies aspiring to implement prospective analyses. Furthermore, the nature of the biomarkers studied, whether predictive or prognostic, dictates a different suit of statistical considerations, as examplified by Flynn et al. This chapter provides the elements of good statistical practices in the pharmacogenomic space, spanning the entire field, from study design, source of variability, dimensionality, confirmation, model building, bioinformatics and ultimate development of diagnostics.

In Chap. 5 Mifsud et al. summarise decades of pharmacogenetic research dedicated to the various pharmacokinetics processes that drugs are subject to *in vivo* i.e.

absorption, distribution, metabolism and elimination. Examples span the earliest reports on succinylcholine from the 1950s through to the latest advances. In addition, authors provide a window into regulatory perspectives world-wide, which contribute to the adoption of existing knowledge, as well as dissemination of ADME genetics into novel drug development.

The critical role of genetics in predicting adverse drug reactions is described in Chap. 6 by Turner et al. Paradigm setting examples of the high predictive value of genetic variations for Immune-Mediated Adverse Drug Reaction are described in detail. However, the authors provide ample evidence that genetics can be a key determinant of adverse drug reactions associated with virtually any disease area and any drug mode of action, including analgesia, coagulation, cancer and cholesterol levels. These seminal examples have affected the medical profession in a profound fashion, ushering an industry of diagnostics that is widely accepted world-wide.

Chapter 7 builds on the insights revealed in the previous chapters and adds the first example of applied pharmacogenomics, describe the state of research and utility of pharmacogenomics in prescription of haemoglobinopathies therapeutics. Here, Gravias et al. describe the available treatment options and the genetic factors that have thus far been linked mostly to the β-globin gene cluster. These are believed to act by modulating HbF levels. The authors' analysis is concluded by the observation that the use of pharmacogenomics for haemoglobinopathies therapeutics are currently very limited, requiring larger studies in ethnically diverse patients groups.

Cacabelos et al. review the state of research and applicability of pharmacogenetics to neurodegenerative diseases in Chap. 8. The five categories of genetic variants associated with this field are defined as: (i) genes associated with disease pathogenesis (pathogenic genes); (ii) genes associated with the mechanism of action of drugs (mechanistic genes); (iii) genes associated with drug metabolism; (iv) genes associated with drug transporters; and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions. The role of each of these categories is then examined within the prototypic neurodegenerative diseases, i.e. Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis and Huntington's disease. The authors conclude by postulating future areas of focus for pharmacogenomic research, as well as overall policies associated with chronic, debilitating, late-onset diseases that affect the nervous system and expected to affect significant proportions of the aging world population.

The genetics and pharmacogenetics of asthma is reviewed in Chap. 9. Current approaches to asthma management call for clinical severity assessments, with regular re-evaluations of treatment, that is subsequently often redosed or switched. Specific pharmacogenetic considerations are provided which address the major drug classes in current use for asthma and/or chronic obstructive pulmonary disease (COPD), including leukotriene modifiers, glucocorticoids and anti-muscarinic agents. The evolving use of pharmacogenetic tools in novel drug development in respiratory indications in further details, providing great promise for patients.

Patel et al. provide an overview on the pharmacogenetics of antineoplastics in Chap. 10. In this context, the authors explain the unique attributes associated with germ-line versus somatic mutations, and the associated prognostic versus predic-

tive value of the reported biomarkers within each category. Specifically, examples are shared which directly address clinical decision making for a variety of solid and liquid tumour types, including the use of 6-mercaptopurine (6-MP), irinotecan, tamoxifen, fluorouracil, crizotinib, imatinib, ATRA, vemurafenib. erlotinib, herceptin, panitumumab and cetuximab. With the reduction in sequencing technology cost it is expected that tumour profiling will be used for patient classification and drug development, as well as for identification of driver mutations that define causality, diagnosis, prognosis and life-saving treatment choices.

Chapter 11 entails the utility of pharmacogenomics to anticoagulant therapy. Here, van Schie et al. describe both the basic science and clinical evidence associated with multi-marker testing for coumarin anti-coagulant therapy. Furthermore, the authors expand the scope of their analysis to coumarin derivatives, clinical trials investigating the effectiveness of pre-treatment genotyping and the cost-effectiveness of pharmacogenetic-guided dosing. These are critical studies required for adoption of pharmacogenetics to common practice and serve as precedents for the entire field.

Having reviewed each therapeutic area separately, Chap. 12 serves to provide a common vision to lessons learned and remaining challenges associated with the practice of genomic medicine. Here, Grech et al. focus on the needs and recommendations to promote patient molecular classification; stratification of well-defined subgroups of predicted responders to specific therapies; the development of technologies and integrative information systems to provide the healthcare system with optimised and sustainable genetic testing protocols; the need of harmonised guidelines for the proper selection of patient groups for clinical trials; and advances in research to generate evidence based knowledge that can be smoothly translated for healthcare use. Key gaps in the uptake of Genomic Medicine in the Health Care System are attributed by the authors to insufficient education of the Healthcare professionals and lack of mechanisms for appropriate dissemination of genomic information within the healthcare system. Lastly, the research community is still lagging behind in providing real-world, validated evidence to the validity of pharmacogenomic findings.

The book is concluded by Ellul, summarising ethical considerations associated with pharmacogenomics. The chapter focuses on ethical issues affecting the individual patient through his or her experience undergoing pharmacogenetic testing for personalised treatment, enrolling in clinical trials, participating in genomic research or donating biological material for biobanking and research. Core concept leading the ethical discussion center around perceived and actual benefits and risks, and the relative relationship between the two. The ubiquitously acceptable tool of informed consent is presented, including its variable applications and its existing and evolving guiding rules. Aspects of discrimination, ethnicity, privacy, confidentiality and the responsibilities of each of the associated stakeholders is detailed as well, providing a rounded account of the complexities and opportunities associated with pharmacogenomic utilisation.

In summary, this book collates a comprehensive account of the state of the pharmacogenomic science and its application to the management of most common dis-

ease areas, as pertaining to available therapies as well as those in development for future, better patient use. The book provides an outstanding didactic content in both preventive and predictive genetics and intended for use in postgraduate courses in Molecular Biology and Genetics, Bioinformatics and other life sciences programmes that focus on applied Genetics for future medicine. The common theme concluded by each of the expert authors converges into the vision that targeted therapies will become mainstay across all disease areas. Further, with the exponential growth of omics Big Data, our ability to translate sequence variation into useful tools for drug development and utilisation will ensure a speedy, safe and efficaceuos drug development process for future generations.

Preventive and Predictive Genetics: A perspective

Godfrey Grech, Christian Scerri, Jeanesse Scerri and Tomris Cesuroglu

Abstract Public Health practices focus on the implementation of programmes for health improvement and disease prevention (Khoury et al., Am J Prev Med 40(4):486–493, 2011). Public health initiatives in diseases were initially targeted to prevent infectious diseases. Partly due to the availability of vaccines and antimicrobial therapy and partly due to better standard of living, the world is free of diseases such as small pox, almost free of polio and the prevalence of infections such as malaria and HIV is steadily on the decline. This has meant that the human race is living longer with the result that non-communicable diseases have become a global public health priority. Preventing non-communicable diseases is a more logical approach than treating them, even more so when modifiable, common lifestyle risk factors share a role in the onset and progression of the disease. Preventive genetics plays a crucial role in the identification of subjects at risk at a very early age, which would thus give public health officials the necessary time to take appropriate action.

Genetic tests can be classified into carrier, diagnostic and predictive testing. In carrier testing, the tests are directed towards the identification of carriers of autosomal recessive or X-linked genetic disorders to prevent disease. Preventive genetics can be defined as using genetics for the prevention of a future disease that has a genetic component either in the individual tested or in future offspring. Diagnostic testing is the process that identifies the current disease status of the subject and includes, among others, prenatal and newborn screening. The implementation of screening programmes allow the detection of genetic disorders at an early stage, so as to prevent these conditions or their serious consequences. Predictive testing

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determines whether a subject with a positive history but no symptoms of the disease, is at risk of developing the disorder at a future date. In this chapter, we will discuss the application of genetic screening tests to monogenic disorders and complex disorders with monogenic subsets, in view of the current practices. The multifactorial aetiology of complex disorders involves multiple gene effects and gene-lifestyle interactions that cannot be singled out to give a strong predictive value. However, a subset of the complex disorders are caused by highly penetrant genetic mutations. Hence, in this chapter we shall also address predisposing syndromes with high predictive value. In addition, the need of biobanks will be discussed.

Keywords Public health genomics · Preventive genetics · Predictive genetics · Screening programmes · Monogenic disorders · Monogenic syndromes · Biobanks

1 Screening Programmes

According to the UK National Health Services, the term *screening* signifies a public health service aimed at identifying individuals that, though apparently healthy, are at risk of or are already affected by a particular disease or its complications. This identification could be through a particular medical or biochemical test or a questionnaire. Once identified, the affected individuals could then be offered further information, complementary tests or treatment in a bid to reduce their risk of developing the disease or its complication. The use of genetic testing to improve healthcare, requires implementation of programmes as part of public health practice [1]. In their 2004 paper on the history of medical screening, Morabia and Zhang [2] identified the US army's 1917 screening programme, aimed to exclude individuals with clear psychological disorders from joining the army, as the first reported instance of a "screening programme." This programme consisted of the administration of psychological tests to officers, drafted and enlisted soldiers. Since this early example of a screening programme, other initiatives mostly directed towards the general public and aimed at the prevention or early treatment of important health conditions, have spread across the world.

There are three main types of organised screening programmes, namely population screening, newborn screening (NBS) and cascade screening. Population-based screening involves testing the majority of the population, which may either be defined as the whole population of a country or a specific population at risk (such as Ashkenazi Jews for Tay-Sachs disease, or women over a specific age for breast cancer screening). A highly targeted population for screening of genetic disorders is that of women at the prenatal or pre-conception stage, due to their high accessibility and ease of retraceability. Whereas pre-conception screening allows a wider choice of reproductive options than prenatal screening (i.e. opting to have no children, using a sperm donor, or pre-implantation genetic diagnosis (PGD) and selective implantation of embryos created through in-vitro fertilisation (IVF) in the former, against elective termination of pregnancy in the latter), antenatal groups are easier to target.

Meanwhile, NBS involves screening of all newborns in order to detect (generally and preferably) early-onset diseases before the occurrence of overt symptoms. Several genetic metabolic disorders, diagnosed through biochemical tests rather than genetic tests, constitute the core group of disorders for which established newborn screening programmes exist worldwide. These include phenylketonuria (PKU), for which the first NBS programme was set up in the early 1960s, maple syrup urine disease (MSUD) and congenital hypothyroidism (CH). Other specific disorders that are tested for within certain regions or ethnic groups include the haemoglobinopathies (by isoelectric focusing [IEF] and high-performance liquid chromatography [HPLC]) and Cystic Fibrosis (CF; by immunoreactive trypsinogen [IRT]). Cascade screening starts with an index case showing symptoms (the proband) and testing family members for mutations predisposing them to the same disease. Unlike the other forms of screening, cascade screening specifically targets individuals considered at high risk of acquiring the disease due to family history and is restricted to genetic (i.e. DNA) tests.

In the late 1960s, technological advances in medicine enabled the spread of screening in various fields of medicine but, at the same time, brought forward topics of controversy as well as ethical implications. Under this scenario, the World Health Organisation commissioned a report on screening from James M. G. Wilson and Gunner Jungner. In their 1968 report, Wilson and Jungner [3] recounted their pre-occupation that, while the "central idea of early disease detection and treatment is essentially simple", achieving its success of "bringing to treatment those with previously undetected disease", as opposed to "avoiding harm to those persons not in need of treatment", is not as easy as it might appear. In an attempt to simplify the process of screening, Wilson and Jungner proposed a set of criteria that has been adopted as the gold standard in the establishment of all screening programmes. According to these criteria, screening programmes should be considered for conditions fitting in within the following:

1. The condition should be an important health problem.
2. There should be a treatment for the condition.
3. Facilities for diagnosis and treatment should be available.
4. There should be a latent stage of the disease.
5. There should be a test or examination for the condition.
6. The test should be acceptable to the population.
7. The natural history of the disease should be adequately understood.
8. There should be an agreed policy on whom to treat.
9. The total cost of finding a case should be economically balanced in relation to medical expenditure as a whole.
10. Case-finding should be a continuous process, not just a "once and for all" project.

However, these criteria were targeted towards screening for diseases of significant burden in general and did not take into account certain aspects pertaining to genetic diseases, such as the serious debilitating nature of certain rare genetic conditions and the inheritable nature of these disorders. The accelerated rate of discovery of

new disease-causing genes has opened up a whole new dimension of diagnosis by using genetic testing to detect diseases before the first clinical signs and/or symptoms appear, even before the disease starts its pathological course. Conversely, these advancements have also caused public health to lag behind in the introduction or expansion of genetic screening programmes, mostly because the decision-making process requires extensive risk-assessments and the implementation of pilot studies, as well as control and standardisation of such programmes [4]. This has resulted in different countries applying different criteria, most of which are based on the original ones by Wilson and Jungner but which take into account the mentioned additional factors, leading to a lack of standardisation or consensus. In the following sections, disorders for which screening programmes are either already in place or may be considered in the near future will be described, with the application of screening criteria to the decision-making process. In addition, the use of next-generation sequencing for newborn screening will be discussed as a future strategy in public health genomics.

2 Monogenic Disorders

Monogenic disorders are mainly rare disorders, caused by single-gene modifications that are present in all the cells of the body and following Mendelian modes of inheritance, i.e. dominant, recessive or X-linked [5]. Though the phenotype in monogenic disorders is almost dependent on a single genetic component, it is also influenced by the individual's genome (modifier genes), as well as environmental and lifestyle factors. Rare, or orphan, disorders are most commonly defined as disorders affecting 5/10,000 persons or less, but collectively they contribute to a significant degree of morbidity and mortality. Furthermore, eighty percent of rare disorders have genetic origins [6], and the advent of next-generation sequencing has brought about the accelerated discovery of new causative mutations [7]. There are also several monogenic disorders which are relatively common, either worldwide or in specific populations. Being easier to identify, either by biochemical or genetic tests, these disorders were the first genetic conditions for which population screening programmes were established.

2.1 Inborn Errors of Metabolism

An important subset of monogenic disorders consists of the inborn errors of metabolism. Neonatal screening for inborn errors of metabolism has been in place since the early 1960s, following the development of a fast and cheap blood test for phenylketonuria (PKU) [8]. After the success of the first newborn screening programme, maple syrup urine disease (MSUD) screening by the Guthrie ("heel prick") method was soon added. Up to the introduction of tandem mass spectrometry (MS/MS) technology in newborn screening, individual diseases where tested on a one test,

one disorder system that is both expensive and relatively inefficient. The use of MS/MS has expanded the number of inborn errors of metabolism disorders that can be tested from a single dried blood spot, in one single analytical run. To date, MS/MS can identify 45 different disorders, including amino acid disorders, fatty acid oxidation disorders, and organic acidemias, from a single blood spot taken in the neonatal period [9]. Almost all of the national newborn screening programmes in the world include PKU, with medium-chain acyl-coA dehydrogenase deficiency (MCADD) found in a majority of programmes. Other disorders that are actively being added to the list are homocystinuria (HCU), maple syrup urine disease (MSUD) and glutaric aciduria type 1 (GA1).

2.1.1 Phenylketonuria (PKU)

PKU is an autosomal recessive disorder with a reported prevalence as high as 1 in 4500 in Ireland [10] to as low as less than 1 in 100,000 in Finland [11]. In its typical form, it is a result of a mutant phenylalanine hydroxylase (PAH) enzyme, which catalyses the conversion of phenylalanine to tyrosine. Reduced activity of PAH results in an accumulation of phenylalanine and its metabolite phenylpyruvate. This accumulation of phenylalanine leads to intellectual disability, seizures and other symptoms. The major problem is an inability of the brain to utilise other large neutral amino acids (LNAA), such as tryptophan and tyrosine, since the large quantities of phenylalanine block the blood-brain barrier's large neutral amino acid transporter (LNAAT). Thus, the brain is starved from amino acids that are essential for proper synthesis of neurotransmitters. Management of PKU consists in low phenylalanine diet and possibly the oral administration of tetrahydrobiopterin that is a cofactor in the oxidation process of phenylalanine. To be successful in achieving normal brain development, the diet needs to be initiated as early as possible and continued throughout life.

2.1.2 Medium-Chain Acyl-coA Dehydrogenase Deficiency (MCADD)

Medium-chain acyl-coA dehydrogenase deficiency (MCADD) is a disorder of fatty acid oxidation, in particular the conversion of medium chain fatty acids (MCFA) into acetyl-CoA. It is most prevalent in individuals of Northern European Caucasian descent, with reported worldwide prevalence of between 1:10000 and 1: 27000 [12]. The inability to convert MCFA into Acetyl-CoA results in a deficiency of fatty acid breakdown during periods of metabolic stress, such as a period of fasting or illness. The presenting symptoms include hypoglycaemic attacks, vomiting and encephalopathy that can lead to coma and sudden death. As studies have shown that over 25% of undiagnosed children die during their first attack, with 16% of the survivors having severe neurological deficiencies [13], early diagnosis and start of treatment is essential. The latter consists of a diet which is high in carbohydrates and low in fatty acids, in particular during periods of risk, and avoidance of fasting [14].

2.1.3 Homocystinuria (HCU)

Homocystinuria (HCU) is another rare, autosomal recessive condition, where the body is unable to convert homocysteine (derived from methionine) into cystathionine due to the reduced activity of the enzyme cystathionine beta-synthase. Thus, in a similar way to phenylketonuria, homocysteine and to an extent methionine, accumulate in the body while there is a deficiency of cystathionine. The reported worldwide prevalence is of 1 in 344,000, with certain countries such as Ireland (1: 65000) reporting comparatively very high rates [15]. The symptoms of HCU include developmental delay of the brain, behavioural changes such as mood and personality disturbances, dislocation of the lens of the eye, disproportionate length of limbs compared to the body, osteoporosis and higher risk of vascular thrombosis. Treatment consists of vitamin B6 (a cofactor of cystathionine beta-synthase) and a low methionine diet. In addition, the administration of oral betaine helps to convert homocysteine back into methionine and thus reduce the levels of homocysteine.

2.1.4 Maple Syrup Urine Disease (MSUD)

Maple syrup urine disease (MSUD) gets its name from the presence of a distinctive sweet smelling urine reminiscent of maple syrup. The condition can be the result of mutations in any one of the four genes that code for the 4 subunits ($E_1\alpha$, $E_1\beta$, E_2, and E_3) of the branched-chain alpha-keto acid dehydrogenase complex (BCKDC). The lack of BCKDC leads to a gradual increase of the branched-chain amino acids isoleucine, leucine and valine, resulting in a build-up of toxic ketoacids in the blood and consequently in the urine. Like most of the other inborn errors of metabolism, the condition is inherited in an autosomal recessive way. As the disorder can be due to the lack or reduced activity of any one of the 4 subunits, its clinical picture and prognosis is variable, but brain damage with mental problems as well as physical problems, such as lethargy, hypotonia, seizures, pancreatitis and ketoacidosis, are common events. The management of MSUD requires a life-long diet where the level of these essential branched chain amino acids is kept under strict control and at the barest minimum. If well managed from early years, those afflicted are usually able to live a normal life with little or no neurological damage. The prevalence of this disorder is reported to be approximately 1 in 200,000 [9], with increased incidence in persons of Amish, Mennonite, and Ashkenazi Jewish descent [16–18].

2.1.5 Glutaric Aciduria Type 1 (GA1)

Glutaric aciduria type 1 (GA1) is another inherited error of metabolism involving the reduced capability of the breakdown of amino acids. Its worldwide prevalence is reported as 1:100,000 [19]. GA1 is caused by mutations within the *GCDH* gene that encodes for the enzyme glutaryl-CoA dehydrogenase. The enzyme is required for the metabolism of the amino acids hydroxylysine, lysine and tryptophan. This reduction of effective enzyme activity results in a build-up of intermediate me-

tabolites 3-hydroxyglutaric acid and glutaconic acid. Both these metabolites are toxic to the basal ganglia, with initial symptoms such as macrocephaly occurring at birth in some affected individuals, though it is not unusual for symptoms to become apparent during adolescence or early adulthood. Without treatment, the condition invariably leads to encephalitis-like crisis that leaves severe *sequelae* such as developmental delay, neurologic deterioration, and cerebral palsy. Encephalitis is usually triggered by an intercurrent illness such as infection, fever or prolonged fasting. Treatment consists of dietary manipulation to ensure a low level of lysine and tryptophan, avoidance of prolonged fasting (less than 4–6 h) as well as treatment with supplements such as riboflavin and L-carnitine.

The collective importance of the health burden constituted by inborn errors of metabolism, together with the cost-effective and acceptable patient testing and management strategies, make them ideal candidates for newborn testing programmes; in fact, several countries already have such programmes set-up, with a significant number offering extended screening through MS/MS (Table 3).

2.2 Cystic Fibrosis

Cystic fibrosis (CF) is the most common lethal autosomal recessive condition among Caucasians, with a prevalence of around 1/2500 live births [20] and a high mutation carrier rate at 1/25 [21]. It is caused by mutations in the *CFTR* gene encoding the cystic fibrosis transmembrane conductance regulator protein. The latter functions mainly as a chloride ion channel and is present in the epithelial cell membranes of several tissues having secretory functions, such as the lungs, sweat glands, gastrointestinal tract and pancreas [22]. Mutations in *CFTR* result in the loss or impaired function of the channel, leading to the pathological hallmarks of the disease. The main manifestations are in the lung and pancreas: 85–90 % of affected children develop pancreatic insufficiency by the first year of life, while pulmonary insufficiency causes more than 80 % of CF-related mortality. In the lungs, increased mucous viscosity inhibits its normal clearance by cilia and coughing; the excess mucous forms plaques with hypoxic spaces which can be colonised by opportunistic bacteria such as *Pseudomonas aeruginosa*. Chronic infections of the airways with inflammation and infiltration by polymorphonuclear cells are followed by bronchiectasis (irreversible dilation of the airways), hypoxaemia (low blood oxygen concentration) and hypercarbia (abnormally high levels of circulating carbon dioxide). Pancreatic insufficiency occurs due to intrapancreatic duct obstruction by viscous secretions, with autolysis and fat replacement; insulin insufficiency or carbohydrate intolerance are in fact commonly present in CF patients [23]. Most affected males are also infertile [24].

Fortunately, life expectancy for CF patients has increased to an average of 37 years through new management strategies, although no definitive cure is yet available, and new models predict newborns with CF today to have a life expectancy of around 50 years [25]. Since it is very important to diagnose the disease and provide treatment at an early stage, CF is one of the most important conditions included in

newborn screening programmes (Table 3). The main testing strategy involves the measurement of immunoreactive trypsinogen (IRT) from heel-prick blood samples, followed by the sweat test and genetic mutation analyses [24]. More than 95% of affected newborns have no recorded family history of CF [21], which provides an argument in favour of prenatal or (preferably) pre-conception screening for mutations to predict the risks of having an affected child. However, carrier screening for *CFTR* mutations has its disadvantages. Firstly, around 1000 mutations in the *CFTR* gene have been reported since its association with the disease; although in most populations, up to 90% of affected individuals should have one of a few mutations included in a pan-ethnic gene panel, there may still be mutations which are not covered by the panel and this residual risk must be clearly stated both while obtaining informed consent and while reporting the results, to avoid instilling a false sense of security in mutation-negative patients [26, 27]. This might be overcome in the near future by high-throughput screening of the whole *CFTR* gene, but the latter in turn will identify sequence variations of undetermined significance and thus more research into each detectable variant will be required [21]. Secondly, not all mutations have been associated with classical CF; other *CFTR*-related conditions such as male infertility may constitute incidental findings and require careful genetic counselling [26]. Only the functional effects of a few mutations have been established to date, and associations between genotype and phenotype are weak, due to variability in the environmental and genetic background as well as phenotype heterogeneity in patients having the same mutations [23].

The recent discovery of mutation-specific orphan drugs for CF, most notably VX-770 for patients carrying the G551D mutation [28] and PTC124 for patients expressing premature stop codons [29], further highlights the importance of genetic testing for CF for early detection and treatment in target patients. Although these mutations constitute only a small fraction of CF patients (ca. 5 and 10%, respectively) and, subsequently, treatment costs are still very high, research for new drugs is ongoing and will undoubtedly make population genetic screening more feasible in the near future.

2.3 The Haemoglobinopathies

Heamoglobinopathies comprise a heterogeneous group of autosomal recessive inherited disorders resulting in the production of abnormal or reduced synthesis of normal globin chains that constitute the building blocks of haemoglobin. Those conditions that completely or partially abolish the production of globin chains are known as thalassaemia, while the conditions that result in abnormal globin chain production constitute chain variants. The haemoglobin molecule is composed of two pairs of globin chains with each globin attached to a haem moiety. In adult haemoglobin, the two pairs of globin chains are known as alpha (α) and beta (β). The alpha chains are coded for by a pair of identical alpha genes (*HBA1 and HBA2*) on chromosome 16, thus an individual has four alleles. In contrast, the beta globin chains are encoded by a single gene (*HBB*) on chromosome 11 with an individual

having two alleles. The worldwide distribution of haemoglobinopathies follows the worldwide distribution of endemic malaria [30]. As a result of human migratory patterns, haemoglobinopathies have become a major health issues in developed countries where the disease was not endemic. Since early identification and treatment of haemoglobinopathies improves morbidity and mortality rates, newborn, antenatal and/or cascade screening for these conditions has been established in various countries in Europe (Table 3) and around the world.

2.3.1 Haemoglobin Variants

In general, haemoglobin variants are due to mutations within the β or α globin genes, though foetal haemoglobin variants as a result of mutations in one of the two γ globin genes (*HBG1 and HBG2*) have also been reported. Individuals that carry only one mutated gene are known as carriers. Though considered as an autosomal recessive condition, it is more accurate to define a trait as autosomal codominant, as the globin chain variants are expressed and are present in the heamoglobin of 'carriers'. In contrast, carriers are usually considered healthy as the trait does not normally result in clinical consequences. Haemoglobin variant homozygotes or compound heterozygotes that result in significant functional alterations of the resultant haemoglobin would usually present with symptoms. The clinically relevant variants are easily identified through simple and relatively cheap electrophoretic techniques supplemented by HPLC methods to verify and quantify the variants. The worldwide clinically important variants include sickle cell haemoglobin, also known as HbS (β6 (A3) glutamic acid→valine), haemoglobin C (β6 (A3) glutamic acid→lysine) and haemoglobin E (β26 (B8) glutamic acid→lysine).

HbS is the most widespread haemoglobin variant, occurring mostly in persons of African origin but also found in persons of Mediterranean ethnicity and in the Indian subcontinent. The single point mutation substitutes a valine residue for glutamic acid in codon 6. This substitution induces the polymerisation of the deoxygenated haemoglobin variant through the formation of hydrophobic bonds between the inserted valine residues of adjacent haemoglobin molecules. This polymerisation results in deformation of the red blood cells that take the sickle cell shape form. Such deformed blood cells obstruct the microcirculation of sickle cell patients. Thus, any physiological stress that reduces oxygenation or increases oxygen requirements, results in the rapid polymerisation of HbS and the precipitation of sickle cell crises, with risks of strokes at a very young age. Treatment involves daily prophylactic antibiotics, transfusions and hydroxyurea treatment to reduce sickle cell crises.

In haemoglobin C, the glutamic acid to lysine change is in the same position to that of HbS but instead of polymerisation, the haemoglobin precipitates within the red cell and damages the membrane. Damaged red blood cells increase the viscosity of the blood, are haemolysed and sequestered both in the bone marrow and spleen. HbC disease is not as severe as HbS and might not be diagnosed before adulthood. Though mild, patients can still experience joint pains as well as gall stone problems. HbC is most common in persons of African ancestry.

Haemoglobin E is the most common beta globin variant in the Far East, where carrier frequency can reach 25%, mainly in Thai and Chinese. The HbE mutation activates a cryptic splice site, leading to a slightly reduced rate of synthesis in addition to some instability. Thus in the homozygous state, HbE is considered a very mild haemoglobinopathy with mild anaemia that does not usually require treatment. In contrast, the condition is very severe in compound β^{E}/β^{Thal} heterozygotes.

2.3.2 α-Thalassaemia

As humans inherit two *HBA* genes from each parent and thus the normal genotype is αα/αα, the genetics of α-thalassaemia is somewhat complicated. Most of the alpha-thalassaemia abnormalities are the result of deletions of either one or two of the α-genes. Mutations that result in the inactivation of a single α-gene exist but are not common. The α-thalassaemias can be classified into two groups, α^{+}-thalassaemia and α^{0}-thalassaemia (Table 1).

The homozygous α^{0}-thalassaemia state is incompatible with life as no α-globin chains are produced from around the 6th week of foetal life, the α^{0}-thalassaemic foetus is severely anaemic, oedematous and has all the features of severe intrauterine hypoxia. The child is usually stillborn late in pregnancy and the pregnancy is usually complicated with toxaemia and difficulty during delivery, in part due to an enlarged placenta. The compound heterozygous state (- -/- α) is a condition known as HbH disease, which is characterised by anaemia and an enlarged spleen and might require lifelong treatment with transfusions and iron chelation. The α^{+}-thalassaemia and the heterozygous α^{0}-thalassaemia do not require treatment and can be considered as healthy carriers. α-thalassaemia can be identified during newborn screening through the identification of an abnormal haemoglobin made up of tetramers of γ globin chains (γ_4) called Hb Bart's and in adults through the identification of HbH (β_4).

2.3.3 β-Thalassaemia

Similar to α-thalassaemia, β-thalassaemia is prevalent in Mediterranean countries (with Cyprus having the highest prevalence), the Middle East, Central Asia, India, Southern China, and the Far East, as well as in countries along the north coast of Africa and in South America [31]. β-thalassaemia is mostly due to point mutations or small deletions, though rarely large deletions can also be the cause. Over 270 *HBB* gene mutations that give rise to a β-thalassaemia phenotype are listed in the database of human haemoglobin variants and thalassaemias (http://globin.bx.psu.edu/hbvar/menu.html). Each ethnic group has a small set of predominant mutations.

Table 1 The classification and genotypes of the α-thalassaemias

	Heterozygous state	Homozygous State
α^{+}-thalassaemia	−α/αα	−α/−α
α^{0}-thalassaemia	− −/αα	− −/− −

β-Thalassaemia can be classified into three groups, β-thalassaemia trait or carriers (β^A/β^{Thal}), β^+-thalassaemia (homozygous state for mild or moderate mutations or compound heterozygous for a moderate and severe mutation) and β^0-thalassaemia (homozygous state for severe mutations). Persons with the trait are considered healthy carriers. β^+- and β^0-thalassaemia patients, require regular transfusions, depending on the severity of the conditions, together with regular iron chelation. Newborn screening for β-thalassaemia is complicated by the fact that the majority of haemoglobin in a normal newborn is HbF and thus is not affected by the presence of the thalassaemia. The early identification of β-thalassaemia compound heterozygotes and homozygotes requires the establishment of antenatal screening programmes so as to identify β-thalassaemia carrier expectant mothers. Antenatal diagnosis offers the possibility of counselling and allows targeting of the baby once it is born to avoid complications of the disease.

2.4 Hereditary Haemochromatosis

Hereditary haemochromatosis (HH) is a rather common monogenic disorder, affecting 1 in 200 to 1 in 500 of Caucasians [32] and characterised by increased absorption of dietary iron, with subsequent progressive deposition in organs including the liver, pancreas and heart [33]. It is most prevalent in Northern European populations but is found worldwide [34]. The associated gene, *HFE*, is related to the human leukocyte antigen *(HLA)-A3* complex; two missense mutations, namely C282Y and H63D account for up to 95% of probands. The HFE protein localises to the duodenal crypt cells, where dietary iron is absorbed, and negatively regulates absorption through association with cell-surface transferrin receptors. *HFE* mutations cause loss of HFE protein function, the C282Y mutation being more detrimental due to concurrent disruption of the association of HFE with β_2-microglobulin, which is important for proper function [33].

HH initially presents with relatively non-specific symptoms, such as fatigue, joint and abdominal pain and palpitations, leading to frequent misdiagnosis. Later complications of iron deposition include cirrhosis, diabetes mellitus (DM) cardiomyopathy and primary hepatocellular carcinoma, which are also relatively common primary disorders. Symptoms generally appear between 40 and 60 years, with later onset in females attributed to iron loss through menstrual cycles, pregnancy and lactation [33, 35]. Management of HH is by periodic venesection or phlebotomy to remove blood and, consequently, excess iron; this treatment is effective, safe and inexpensive [33].

Although inheritance of *HFE* mutations is simple Mendelian, incomplete penetrance makes predictive testing difficult and provides an argument against population screening, which may inevitably have psychosocial consequences. On the other hand, it meets most of the WHO criteria which justify population screening (Table 2). Cascade screening for *HFE* is in fact implemented in many countries throughout Europe (Table 3).

2.5 Familial Hypercholesterolaemia

Familial hypercholesterolaemia (FH) is also rather common, with an estimated prevalence of 1/500 (0.2%) in Caucasians. Some populations show yet higher frequencies attributed to founder effects [36]. FH is characterised by abnormally high plasma levels of low-density lipoproteins (LDL) and total cholesterol, with predisposition to early-onset coronary heart disease (CHD) due to the formation of atherosclerotic plaques. FH is mostly caused by mutations in the LDL receptor (*LDLR*; >1000 mutations identified), apolipoprotein B (*APOB*; 9 mutations identified) or proprotein convertase stabilisin/kexin type 9 (*PCSK9*) genes, with autosomal dominant inheritance [37]. The latter implies that the inheritance of one mutation from just one parent will result in the disease phenotype; in fact, heterozygous patients constitute the vast majority of cases. Also, couples where one partner is affected (with a heterozygous genotype) have a 50% chance of disease transmission to the offspring [38]. The high degree of risk among family members makes cascade screening for FH a valuable tool in the identification of affected individuals (Table 2); early detection of FH and subsequent treatment with statins significantly reduce morbidity and mortality from CHD associated with FH [38, 39].

Despite international efforts, an estimated 80% of FH patients are still not being diagnosed [40]. An important pitfall is the phenotypic and genotypic heterogeneity of the disease. Until recently, diagnosis was mostly made by means of LDL measurements, with specific cut-off points according to age and family history (lower cut-offs are used for those having an affected first-degree relative than those having an affected second-degree relative, for example) [41]. However, LDL and total cholesterol levels are highly variable in FH patients, even after adjustment for gender, age and body mass index (BMI), and may also overlap with those of the general population, resulting in reduced sensitivity and specificity [42].

The inclusion of mutation analysis has been proved to increase the specificity and sensitivity of the diagnosis of FH [36]. In fact, European criteria for FH developed by the Simon Broome Register Group (UK) and the Dutch Lipid Clinic Network, include the presence of a functional FH mutation, even in the absence of other criteria, as diagnostic of "definite" FH [43, 44]. This is due to the dominant nature of the genotype and the high penetrance of mutations, which is close to 100% [38]. Nonetheless, a wide genotypic variation is also observed: apart from populations with founder effects, which are characterised by a few mutations responsible for most cases, the situation is generally that of a large number of mutations giving rise to a highly heterogeneous population, such as in the UK, Italy and Germany [42]. Thus, each population must define the genotypic characteristics of its FH patients before a successful screening programme can be implemented, and even at this stage negative mutational analysis results do not necessarily exclude the presence of FH, since there might be mutations which are not included in the testing panel [38].

Table 2 describes how the Wilson and Jungner criteria apply to the monogenic disorders discussed above, for all of which some type of screening programme is currently implemented in most European countries (Table 3). It may be observed that the majority of the listed disorders do not satisfy the complete list of criteria,

Table 2 The Wilson and Jungner criteria as applied to a selected group of monogenic disorders

	Inborn errors of metabolism	Cystic fibrosis	Thalassaemia	Hereditary haemochromatosis [5]	Familial hyper-cholesterolaemia
Important burden	Yes, collectively	Yes	Yes	Yes	Yes
Accepted & specific treatment	Yes, for most	Yes	Yes (blood transfusions)	Yes (phlebotomy)	Yes (statin administration)
Facilities for diagnosis & treatment	Yes	Not yet evaluated	Yes	Not yet evaluated	Not yet evaluated
Early stage	Yes	Yes	Yes	Yes	Yes
Suitable test	Yes	Yes (in 90% of cases)	Yes	Uncertain predictive value	Yes (dominant inheritance)
Test is Acceptable (Health benefits outweigh psychosocial risks)	Uncertain	Uncertain (yes when limited to NBS by IRT?)	Yes	Uncertain	Uncertain
Known natural history	Yes	No	Yes	No	No
Agreement about when to treat	Yes (as early as possible)	No	Yes (CBC cut-offs)	No	No
Acceptable cost of care	Yes	Uncertain	Yes	Uncertain	Uncertain
Ongoing process	Yes, in diseases with established programmes (PKU, MSUD, etc.)	Yes	Yes	Cannot yet be addressed	Cannot yet be addressed

Table 3 Showing the number of European countries with established newborn, population-wide and cascade screening programmes for the conditions described in the text. Adapted from Javaher et al. (2010) [45].

Screening strategy	Inborn errors of metabolism	CF (*CFTR)*	Thalassaemia/ Haemoglobinopathies	HH (*HFE*)	FH (*LDLR*)
NBS	26	15	8	–	–
Population-wide	–	7	9	–	–
Cascade	–	16	10	11	8

and that the most important criteria, which may have had the highest impact on the decision-making process, are the burden of the disease, the availability of a specific and acceptable treatment (or rather management, as no definitive treatment yet exists for any of the disorders), and the presence of an early stage during which the condition can be diagnosed (or predicted) and treatment initiated.

As can be observed in Table 3, all 26 European countries reviewed by Javaher et al. (2010) have taken up one form or another of newborn screening [45]. All states have NBS programmes for congenital hypothyroidism, and all except Malta for PKU.

It is evident that several factors played an important part in the decision-making process to set up the screening programmes, highlighting the importance of the previously described criteria. PKU can be defined as the classical type of disease ideal for NBS: although classified as a rare disease, it is of early onset (symptoms developing in the first few months of life), it is relatively easy to diagnose before symptoms develop and also easy to treat, and it has severe complications if left untreated. The same can be applied to congenital hypothyroidism, although it is estimated that only around 15 % of cases are genetic and 85 % are due to thyroid dysgenesis [46]; thus this disorder was not addressed in detail in this chapter. Newborn screening for other inborn errors of metabolism which are very rare has been made feasible through MS/MS, which is currently being applied in 11 European countries. It is important to note, however, that despite the ability of MS/MS to detect up to 45 disorders, most countries choose to report only a few of these disorders, mostly due to ethical and psychosocial reasons.

In the case of CF, NBS is generally by an established algorithm, involving a primary screening test for immunoreactive trypsinogen (IRT), followed by either repeated IRT testing or DNA testing. The second tier depends on whether repeat samples are taken from the newborns; where only one blood sample is available, a positive IRT is followed by DNA testing. The latter will defer in detection rate according to the number of mutations screened for, due to the large number of possible *CFTR* mutations causative of CF. Population-wide testing for carriers and couples at risk of having affected children are also dependent on mutation panels and thus include the inevitable degree of false-negative findings [47]. As has already been mentioned, psychosocial risks involved in genetic testing for *CFTR* mutations are also significant [11]. Cascade screening plays an important role since the mutations are narrowed down to those carried by the proband.

Currently, only cascade screening programmes are in place for HH and FH. This is due to the importance of family history in these disorders as well as the later onset, which makes screening targeted only at high-risk individuals more feasible. The screening process is generally initiated when the first case showing the symptoms and carrying a causative mutation, or proband, is found, and his/her relatives are followed-up to find whether they are carriers of the same mutation, in order to prevent the consequences of the disease.

The HH and FH cascade screening programmes provide valuable arguments in the implication of screening for monogenic subsets of common, multifactorial conditions. Being highly penetrant and autosomal dominant, *LDLR* mutations do not just confer a risk for FH but justify preventive measures, i.e. statin administration to

prevent hypercholesterolaemia. Furthermore, FH in turn confers an increased risk of heart disease such as myocardial infarction. However, the low penetrance of HH mutations, which are very common especially in Northern Europe, makes screening for such mutations a risk status assessment, similar to that in multifactorial disorders. Thus, no population-wide screening programmes to detect *HFE* mutations are in place; rather, probands present with elevated iron levels in serum and their relatives are followed up to determine the risk of having inherited the same disorder. It is only at this level that genotyping of symptomless individuals take place [5].

An important observation to make is that there are significant discrepancies in the screening programmes present between European countries, and no consensus exists as yet as to which disorders should be screened for or, as in the case of MS/MS, reported [48].

Even more diverse are the European policies on population-based carrier screening programmes, targeting either pre-conception or prenatal individuals. In the case of such programmes, the epidemiology of the targeted disorders probably plays the most important part in their selection. A good model to illustrate this diversity are the population-wide carrier screening programmes for thalassaemia and other haemoglobinopathies established in several countries. Population-wide haemoglobinopathy screening is present in countries having higher prevalences, such as in the Mediterranean region, with ethnic-specific screening being preferred in countries such as Germany. Furthermore, in countries with high prevalence such as Cyprus, the population screening programme is run in parallel to a newborn screening programme, to ensure maximum coverage of affected individuals [45]. Increased public awareness and uptake of prenatal screening have largely contributed to the success of these programmes [49]. Other country specific differences involve pre-conception versus prenatal screening. While in Cyprus pre-conception screening of β-thalassaemia is carried out in all cases (followed by prenatal diagnosis in those cases that are at risk), in other countries such as the UK, screening is carried out prenatally.

3 The Way Forward—Public Health Genomics Perspectives on use of Next-Generation Sequencing for Newborn Screening

NBS started with the Guthrie test in the 1960s and rapidly expanded around the globe. It has been possible to test for a handful of disorders with this method. In the late 1990s, early 2000s, we have seen the introduction of MS/MS, which has been replacing the Guthrie test in many countries. With MS/MS, it is possible to expand the number of screened disorders extensively, without significantly increasing the overall costs.

In the transition from the Guthrie test to MS/MS, the major question was not the detectability of the disorders with this technology, but how to decide on which disorders to include in the NBS programmes. Although there has been a general

consensus on screening criteria, such as the Wilson and Jungner criteria [3], various countries interpreted them differently or used modified criteria when selecting the disorders to be included in the NBS programmes. For example, Germany has been using three criteria [50], whereas the UK has been using 22 criteria, all of which must be fulfilled before a disorder is included in the NBS programme [51]. Additionally, various countries have different stakeholders and technology-push vs. market-pull dynamics for NBS, as well as different values, structures and processes in health technology assessment, all of which are rooted in differences in their health care systems [52]. These lead to different lists of disorders covered in NBS programmes, as seen in Table 3.

We are approaching another shift in NBS with the upcoming next-generation sequencing (NGS) technologies. Also termed as 'massively parallel sequencing' or 'second generation sequencing' [53], NGS has been reducing both the cost and time required to accomplish whole genome sequencing. With the rapidly decreasing cost of this technology, soon it will be possible to sequence the whole genome of an individual for less than 1000 US dollars. Once DNA sequencing technology is sufficiently robust and affordable, it will be possible for all babies to have their genomes sequenced at birth, replacing both newborn bloodspot screening and additional genetic tests required later in life [54]. This means that it will be possible to screen for a virtually unlimited number of disorders in NBS with almost no additional costs per disorder. However, it is not the technologic capacity to sequence entire genomes, but the analysis and interpretation of the generated data, that is the main bottleneck for the application of NGS for whole genome or exome sequencing [54].

The data generated from whole genome or exome sequencing in the newborn phase can be used not only to screen for monogenic disorders or monogenic subset of complex disorders, but also for pharmacogenetics (potential response to drugs), nutrigenetics (response to nutrients) and risk assessment programmes for major complex disorders, such as cardiovascular diseases or type 2 diabetes. Therefore, implementation of this technology will have an impact on the whole health care system, beyond the NBS programme.

There are several issues that need to be resolved when preparing for using NGS in the newborn phase. Some of these are presented very briefly below:

3.1 Human Resources

The knowledge, attitude and skills of health professionals to use genome-based knowledge are presently very limited. This shortcoming needs to be addressed effectively with professional education and training programmes. However, the effect of such training might be limited on health professionals that have already been practising in traditional ways for many years. The main target should be the under- and postgraduate education of health professionals.

Besides, to develop the tools for data analysis, bioinformaticians will be a crucial professional group required both in central levels and in local clinical services [54].

3.2 Informatics Capacity to Store and Process Data

Sequencing the whole genome or exome of large population groups will create massive amounts of data which need to be stored, retrieved and analysed. This requires data storage and processing infrastructures. Additionally, analytical tools to analyse and interpret the whole genome or exome must be developed.

3.3 Clinical Health Services

The systems must be in place to integrate the generated genome-based information in clinical health services. For example, in the first place, genome-based data can be used in the prescription of drugs which have significant pharmacogenetic interactions, such as warfarin. Nevertheless, the infrastructure to access such data and clinical work flows making use of the data must be in place, for NGS to be applicable to the healthcare setting.

3.4 Data

A recurring issue that arises when genome-based data are discussed is whether genetic data are different than other data concerning health. 'Genetic exceptionalism' claims that all genetic and genomic samples and data merit special protection, regardless of their medical sensitivity or predictive power. This claim has been rejected by various groups and reports, including the Public Health Genomics European Network [55], the Ickworth Group (an international group consisting of experts from multiple disciplines which came together in Ickworth, UK) [56] and others [57]. The main underlying idea is that genetic data deserve no separate status; they must satisfy equally high standards of data protection and confidentiality as other types of health data.

In the future paradigm of health care, the line dividing health care provision from health research will likely get thinner. In particular, in the context of the 'big data' approach, datasets created from the regular health care data and various other data collected from individuals are envisioned to be used by data mining to provide insights to health and diseases. Whole genome or exome sequence data of large populations will provide a great opportunity for such research and to discover the genetic basis of various diseases.

For this future vision, having longitudinal (long-data) and large sets of data (big-data) is required, but not enough. For the development of new forms of prevention, diagnosis and treatment of complex diseases, abundant and intricate health data must be combined with innovative analysis strategies in a cross-disciplinary environment [57]. However, recent developments in the EU legislation on data protection impede use of health care data for research, due to the restrictions proposed on the use of health data, even for research purposes [58–60]. This is an important regulatory bottleneck that needs to be overcome.

Genetic data hold a very promising future. However, precautions are required to protect the owner of the data, i.e. the individual. Regulatory mechanisms must be in place to prevent any discrimination that may arise due to genetic characteristics of the individual. GINA—Genetic Information Non-Discrimination Act in the USA, which came into force in 2009, is one of the major examples of a legislation that protects individuals from being discriminated by employers or insurance companies based on their genetic data.

3.5 Ethical Issues

An important issue that needs to be considered for whole genome or exome sequencing in the newborn phase is that the cost of sequencing the whole genome will soon practically be not more expensive than sequencing targeted genes. Therefore, sequencing the whole genome (or exome) at once seems to be the most practical solution. However, this brings ethical discussions on issues such as protection of the future autonomy of the screened infant, the right not to know, and the issues around incidental findings.

Incidental findings are any findings which are outside the scope of the clinical enquiry [54]. They are "the results of a deliberate search for pathogenic or likely pathogenic alterations in genes that are not apparently relevant to a diagnostic indication for which the sequencing test was ordered" [61]. In the context of whole genome or exome sequencing, the issues that may arise from incidental findings span multiple clinical, genetic and social dimensions, such as racial ancestry, misattributed parentage, consanguinity, disease susceptibility, and reproductive risks [54]. Several strategies are discussed to manage the issue of incidental findings, such as classification of genes and disorders according to net benefits [54] and providing lists of conditions that must be reported when found [61].

4 Complex Disorders

Common complex diseases can be defined as a group of disorders with similar symptoms but having a variable aetiology. Though the genetic component within complex diseases is inherited in a similar way to the inheritance in monogenic or Mendelian disorders, the main difference is that, while in Mendelian disorders a single gene variant is compulsory to reach the critical threshold in developing the disease, with modifier genes and environmental factors modifying the phenotype, in complex disorders both the threshold to develop the disease as well as its severity, are modified by complex aetiological factors. In between these two ends of the genetic spectrum, one finds a group of disorders in which alterations in susceptibility genes would bring one close to the required threshold for the condition, but other genetic, environmental and lifestyle factors are required for the disease to establish itself. This group can be considered as that of 'monogenic subtypes' and includes such genes as *BRCA1, BRCA2, MSH2* and *MLH1*.

Common complex disorders include cardiovascular disease (CVD), diabetes, cancer, dementia, auto-inflammatory and auto-immune diseases, amongst others. Preventive genetics in multifactorial disorders require an in-depth understanding of the genetic variability associated with the disorder, in order to define the complex disease into subtypes of variable aetiology. Genome-wide association studies (GWAS) have been used extensively to identify genetic variants associated with multifactorial diseases. Whereas current testing programmes include inherited monogenic diseases (Table 2), taken individually, the contributing genetic variants in multifactorial diseases still lack a high predictive value and hence genetic results are inconclusive and very difficult to interpret.

Of interest is the characterisation of monogenic subtypes within complex diseases that can be easily taken up into genetic testing programmes (Fig. 1). The term 'monogenic subtype' needs some clarification since the gene involved requires other contributing factors to cause the disease. The mutated gene in the monogenic subtypes within complex disorders, provides a cellular programme that is susceptible to the initiation of disease. The effect of the mutant will be exerted on tissues that normally express the gene. The resulting loss or gain of function will set a molecular threshold, requiring the contribution of additional factors, to initiate a specific disease. These susceptibility genes are masked by the presence of other variations that might occur during the disease state. Hence, low frequency susceptibility genes can only be identified through high penetrance in family studies.

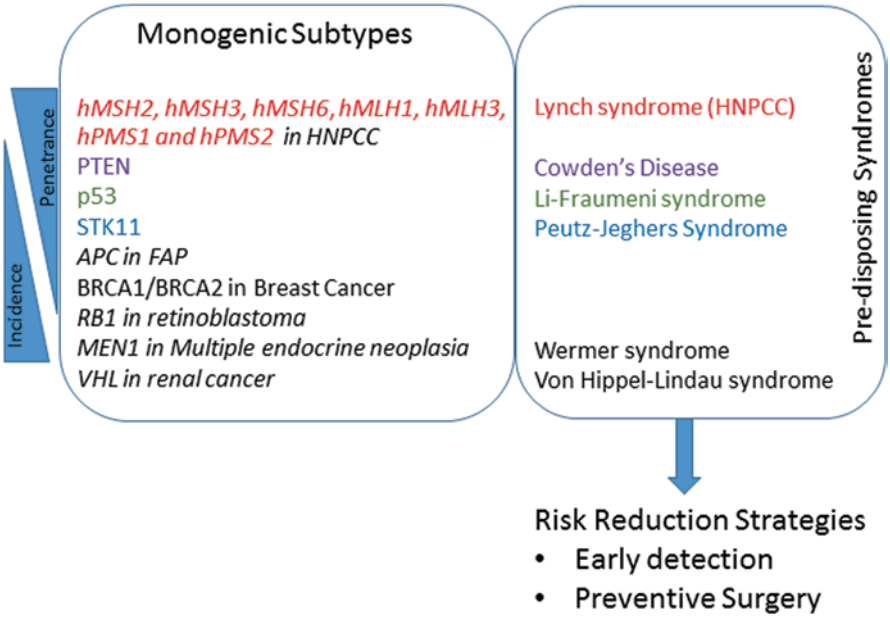

Fig. 1 Inherited susceptibility mutations in cancer. (Genes in red: mismatch repair genes; violet: phosphatases; green: apoptosis induction)

Major contributing genes in complex disorders have been identified due to their high penetrance, exemplified by *BRCA1* and *BRCA2* [62, 63] with increased risk of breast and ovarian cancers, *hMSH2* with increased risk of hereditary nonpolyposis colorectal cancer (HNPCC) [64], and α-synuclein in Parkinson disease [65].

The genetic basis of colorectal cancer (CRC) is well defined, and is used as a model to describe the molecular events that promote the progression of disease in cooperation with the defects in inherited susceptibility genes. The risk to develop CRC has been associated with inherited mutations in the mismatch repair genes [66]. Similarly, DNA repair genes predispose for breast cancer, including *BRCA1* and the Fanconi Anaemia (FA) genes *BRCA2 (FANCD1), FANCJ (BRIP1), FANCN (PALB2)* and *FANCO (RAD51C)* [67–69]. In addition, Fanconi genes are associated with other cancers, including acute myeloid leukaemia [70].

Inherited mutations in *PTEN, p53, RB1, MEN1* and *VHL* give rise to predisposing syndromes, namely Cowden's Disease [71], Li-Fraumeni syndrome [72], Retinoblastoma [73], Wermer syndrome [74], and von Hippel-Lindau syndrome [75], respectively. These inherited susceptibility genes have a low frequency with a high penetrance. Hence, these genes are candidates for a testing programme in specialised clinics, screening family members of patients, to identify risk and initiate preventive monitoring or discuss possible clinical solutions to reduce the risk significantly [76].

4.1 Familial Cancer Predisposing Syndromes

4.1.1 Familial Adenomatous Polyposis

Polyposis syndromes are exemplified by Familial adenomatous polyposis (FAP) characterised by *APC* gene mutations and a lifetime risk of CRC close to 100 %. APC is a tumour suppressor regulating the degradation of the transcription factor β-catenin, the effector molecule of the Wnt pathway. APC stabilises a protein complex that sequesters β-catenin in the cytoplasm, leading to proteosomal degradation. FAP is characterised by the presence of more than 100 colorectal adenomatous polyps prevalence [71]. Rectal bleeding indicates enlarged and numerous adenomas, a condition which is rare in children and adolescents. If untreated, the condition will develop into colorectal adenocarcinoma with an early age of onset. Surveillance, chemoprevention [77] and improved endoscopic treatment provide opportunities for better treatment of FAP [78] and decrease dependency on prophylactic cancer-preventive colorectal surgery [79].

4.1.2 Li-Fraumeni Syndrome

Li-Fraumeni Syndrome (LFS; OMIM 151623) is an autosomal dominant cancer predisposition syndrome associated with *p53* germline mutations [80]. Family

studies show a high penetrance with early onset of sarcomas, breast cancer and other non-therapy-related neoplasms. The age of primary cancer onset ranges from 4 months to 49 years with a mean age of 25 years [81]. The variety of neoplasms and the early age of onset is attributed to mutant *p53*, a major gatekeeper of apoptosis in response to DNA damage. Of interest, mutations that result in truncation of the p53 protein are associated with higher cancer risk and earlier age of onset [82]. Defective p53 function results in the accumulation of mutations in proliferative tissues. The population frequency of germline *p53* mutations in Europe and United States is around 1:5000 individuals [83]. Eligibility for genetic screening is determined by established clinical criteria that classify individuals with LFS. The classic LFS classification scheme requires a proband with sarcoma diagnosed before the age of 45, a first-degree relative with any cancer before 45 years of age and another first- or second-degree relative with any cancer diagnosed at under 45 years or with a sarcoma at any age [84]. Other classification schemes were designed based on further family studies and the Chompret criteria [85] enhances the predictive value of the classic LFS classification, resulting in a testing sensitivity of 95% [81]. The Chompret criteria consider any proband with adrenocortical carcinoma at any age of onset eligible to *p53* mutation analysis, irrespective of family history. In addition, the criteria include other proband neoplasms such as breast cancer and brain tumour diagnosed at an early age of 36 years. Presymptomatic testing for germline *p53* mutations predicts the susceptibility to various neoplasms, imposing ethical issues due to lack of complete clinical surveillance, preventive measures and treatment recommendations. The high penetrance of germline *p53* mutations in familial breast cancer patients predicts an average age of onset of 31 years [86], and hence provides eligibility for breast cancer screening in p53-mutant women who are in their mid-20s, followed by implementation of risk reduction strategies.

4.1.3 Cowden Syndrome

Cowden Syndrome (CS; MIM 158350) is a rare autosomal dominant cancer predisposition syndrome with a prevalence of 1 in 200,000 [87] and an age-related penetrance of around 80% [88]. The susceptibility gene in CS is *PTEN*, predisposing individuals to breast, endometrial and thyroid cancer [89]. PTEN is a ubiquitously expressed phosphatase involved in the attenuation of the PI3K pathway, hence acting as a proliferation suppressor [90]. The clinical symptoms of CS include multiorgan hamartomatous polyps in the majority of the affected subjects [91]. In addition to Cowden Syndrome in adults, germline *PTEN* mutations result in Bannayan-Riley-Ruvalcaba syndrome (BRRS; MIM 153480) in children [92], collectively known as the *PTEN* hamartoma tumour syndrome (PHTS). The highest age-adjusted standardised incidence ratio of germline *PTEN* mutants occurs in thyroid cancer, followed by endometrial, kidney, breast and colorectal cancer and melanoma (Table 4). Of interest, promoter mutations in the *PTEN* gene were associated with

Table 4 Age-adjusted estimated lifetime risk for cancers in some examples of cancer-predisposing syndromes

Syndrome	Mutant genes	Tumour	Estimated lifetime risk (70 years) (%)
Cowden syndrome Tan et al. 2012 [93]	*PTEN*	Breast	85.2 (71.4–99.1%)
		Thyroid	35.2 (19.7–50.7%)
		Endometrium	28.2 (17.1–39.3%)
		Colorectal	9.0 (3.8–14.1%)
		Kidney	33.6 (10.4–56.9%)
		Melanoma	6 (1.6–9.4%)
Lynch syndrome Bonadona et al. 2011 [95]	*MLH1*	Colorectal	41 (25–75%)
		Endometrium	54 (20–80%)
		Ovarian	20 (1–65%)
	MSH2	Colorectal	48 (30–77%)
		Endometrium	21 (8–77%)
		Ovarian	24 (3–52%)
	MSH6	Colorectal	12 (8–22%)
		Endometrium	16 (8–32%)
		Ovarian	1 (0–3%)
Peutz-Jeghers syndrome Hearle et al. 2006 [105]	*STK11/LKB1*	Gastrointestinal	57 (39–76%)
		Breast	45 (27–68%)
		Gynaecological	18 (9–34%)
		Pancreas	11 (5–24%)
		Lungs	17 (8–36%)

breast cancer, while nonsense mutants were associated with colorectal cancers [93]. The age of onset of any of the core cancers within the family will establish the age for clinical observations, taken as 5 years less than the age of the youngest family member affected.

4.1.4 Lynch Syndrome

Lynch Syndrome (MIM 120435) is an autosomal dominant cancer predisposing syndrome caused by germline mutations in the mismatch repair (MMR) genes, including *MLH1, MSH2, MSH6 and PMS2* [94]. Lynch Syndrome is also known as hereditary nonpolyposis colorectal cancer (HNPCC) syndrome and confers a high risk to early-onset colorectal and endometrial cancer [95]. In contrast to polyposis syndromes, HNPCC lacks characteristic diagnostic features and clinics depend on standardised criteria, such as the Amsterdam criteria, to establish diagnosis. Eligibility for carrier screening requires a family history characterised by immunohistochemistry-verified CRC cases in at least three relatives, including a first-degree relative, the presence of the disease in at least two successive generations, and one of the relatives diagnosed with CRC with an age of onset less than 50 years, with absence of polyposis syndrome [96]. In addition, the inheritance of syndrome-associated cancers within the family and testing for microsatellite instability are used to predict patients with HNPCC [96]. Lynch syndrome accounts for 2–5 % of the total CRC cases, as reflected in the high estimated lifetime risk of *MLH1* and *MSH2* germline mutation carriers (Table 4). Of interest, 10–15 % of CRCs have microsatellite instability, a useful marker of mismatch repair genes loss of function [97]. The majority of cases with no familial predisposition are caused by sporadic hypermethylation of the *MLH1* gene promoter, supporting the high risk of inherited *MLH1* mutants in the development of carcinoma. Lynch syndrome patients are also at risk of developing other cancers, including stomach, ovarian, renal, pancreatic, small intestinal, brain and skin tumours; it can thus be included in the subset of cancer-predisposing syndromes [98].

4.1.5 Peutz-Jeghers Syndrome

Peutz-Jeghers syndrome (PJS; MIM 175200) is an autosomal dominant condition arising from germline mutations in the serine/threonine kinase gene (*STK11/LKB1*) [99]. Since PJS condition is rare, estimates of frequency within populations vary significantly. Patients present with mucocutaneous pigmentation and gastrointestinal polyposis [100] and have an increased risk of gastrointestinal cancers (as in most polyposis syndromes), breast ovarian, uterine, cervical, lung and testicular cancers [101, 102] (Table 4). Loss of heterozygosity (LOH) of the normal *STK11* allele in 70 % of PJS patients, followed by the progression of hamartomas to

adenocarcinomas, suggest that *LKB1* is a tumour suppressor gene [103]. Interestingly, somatic mutations in *LKB1* are common in lung cancer [104], supporting the involvement of inherited *LKB1* mutants in the progression of disease.

4.2 Tools in Predictive (& Preventive) Genetics

Unlike in monogenic disorders, screening for complex diseases presents a greater challenge since the aim is that of risk assessment rather than presymptomatic diagnosis. Furthermore, the concept of personalised (genomic) medicine seems to conflict with the aim of public health, that is, *to improve health from a population perspective* [1]. However, predictive genetics reduces the burden imposed on the health care system by admission of fewer patients with advanced stages of disease; thus, its importance is being increasingly recognised. Family history and personal genomics (the assessment of individual genetic variations at multiple loci) are two important predictive genetics tools. These have a recognised value in the diagnosis of monogenic conditions (as observed in cascade screening programmes such as for HH); however, their value in the management of risk of complex diseases remains to be established [106]. Although assessment of family history provides information on the inheritance of a phenotype [107], the likelihood of an individual carrying a mutant susceptibility gene to develop cancer depends on other genetic variations (modifier genes), as well as on dietary, lifestyle and environmental factors influencing the age of onset and severity of the disease. The routine use of family history or personal genomics alone as a measure of risk of common complex conditions does not generate sufficient evidence in the primary care setting; however, when used together and in conjunction with evidence-based medicine (EBM-the application of population-derived data), they comprise a very useful tool in the improvement of disease prevention [106]. Public health genomics shall play an essential role in designing more effective genetic screening programmes, by applying data derived from personal genomics to public health. This becomes especially important with the advent of cost-effective services for whole genome sequencing or microarrays detecting large panels of mutations, which may undoubtedly lead to over-diagnosis.

Cascade screening for genetic predisposition to cancer is a form of "systematic predictive testing", where asymptomatic individuals at an increased risk due to their genotypic inheritance may benefit from surveillance programmes to ensure early detection of tumours.

Colorectal cancer is a major contributor to cancer morbidity and mortality. 15% of CRCs are familial with 2–5% caused by HNPCC and less than 1% associated with polyposis syndromes (FAP and PJS). Following diagnosis of Lynch disease, family members benefit from clinical screening by colonoscopy [108]. Recommendations include offering a colonoscopy every 1 or 2 years starting at the age of 25 years or at 5 years before the youngest diagnosed member of the family, whichever

is the earlier [109]. In the case of FAP families, individuals with an APC mutation should strongly consider a prophylactic colectomy before the age of 25 years. Referral of genetic testing for *APC* mutations will provide confirmation of familial adenomatous polyposis (FAP) or, in the case of family members not exhibiting adenomatous polyps, will evaluate relatives at risk and initiation of surveillance programmes. Surveillance should be offered for *APC* mutant members that defer surgery [110]. For patients diagnosed with PJS, risk reduction strategies include upper and lower endoscopy, breast examination, endoscopic ultrasound and CA19–9 tumour marker testing for pancreatic tumour surveillance and ultrasound, cervical cytology and CA125 testing tumour marker testing for ovarian cancer [111].

Familial cases account for 10% of breast cancer in Western countries. Susceptibility genes are inherited in an autosomal dominant pattern, but with limited penetrance. High risk families testing positive for the *BRCA1* and *BRCA2* mutant genes are associated with a four or more times higher incidence of the disease in close relatives. The use of surveillance programmes and/or risk reduction strategies in healthy *BRCA1* and *BRCA2* mutation carriers are instrumental to ensure a positive impact of genetic screening on the health care system. Risk-reducing mastectomy (RRM) supresses breast cancer development by 90% [112]. Inherited mutations in *p53* (Li-Fraumeni) and *PTEN* (Cowden syndrome) have a high incidence of breast cancer development, but the syndromes are very rare. Testing for mutations within these genes requires the use of further diagnostic criteria as detailed below.

Risk assessment tools for complex diseases take into consideration various contributing factors, including the incidence of disease in first-degree relatives; previous diagnostic test results; monitoring results (if any); and presence of genetic mutations/polymorphisms associated with the disease of interest. In the case of breast cancer, risk assessment includes age at first live birth, the use of hormone replacement therapy and other risk factors specifically associated with the disease. The breast cancer risk assessment tool (GAIL model) is used to measure contribution of variants to the calculated risk [113]. The selection of the genetic contribution to multifactorial diseases is not a simple task. Also, the interrogation of genetic variation through screening programmes or referral for testing depends on the minor allele frequency within a population and also on the penetrance within a family having a history of the phenotype. Less penetrant genes with higher prevalence are more significant from a public health point of view. For instance, *the factor V Leiden mutation*, with increased risk of thrombotic events [114], is integrated in the testing regime of health care genetic clinics.

Most of the common chronic disorders, such as asthma, cancer, cardiovascular diseases, obesity, diabetes, hypertension, psychiatric disorders, arthritis, Parkinson's and Alzheimer's, have a complex aetiology and pathophysiology. The current view is that these disorders are due to numerous small, additive genetic defects compounded with environmental and lifestyle causes. Considering that these conditions constitute a major health and economic burden and are the cause of substantial morbidity and mortality, a concerted action is required to elucidate the pathophysiology

and thus open the road for successful treatments and preventive strategies. One major drawback in the determination of aetiological factors that singly confer a small increase in risk is the need of a large number of affected and unaffected individuals (‘cases’ and ‘controls’), so as to achieve statistically significant results. It is very difficult for single clinical and research centres to obtain such large numbers in a relatively short time and with reasonable budgets. For this reason, biobanks and their related databanks have become an important tool for the elucidation of the pathophysiology of these disorders.

5 Biobanks and Preventive Genetics

The Organisation for Economic Co-operation and Development (OECD) definition of a Biobank is “A collection of biological material and the associated data and information stored in an organised system, for a population or a large subset of a population.” This definition brings about the need to consider a number of terms, basically “biological material”, “associated data and information,” “stored in an organised system,” and “population or a large subset of a population.”

It is current practice, if not a legal requisite, for hospitals and laboratories to collect and store whole organs and tissues that have been excised for diagnostic or therapeutic aims. In addition to these archival banks, since the early 70s specialised collections have been initiated, targeting cells and their products including DNA, RNA and proteins. Collections of biological material, without any associated data, have very limited usefulness as biobanks. The collection of data, including medical histories, lifestyle, social and environmental information, increases the research value of these biobanks as the samples can now be separated into different case and control categories. The next important step in the establishing of a useful biobank is the establishment of associated databases that results in an easier and more efficient search and classification tool as compared to pen and paper processes. Finally, to be considered as a biobank, the samples have to be collected either from the whole population or from a subset of the population that has a particular disorder. In each case, the information collected can either be retrospective, transvers or prospective, depending on the final aim and use of the biobank.

5.1 Future Biobanks

The networking of biobanks from different countries or centres, in particular those where both the samples and data have been collected with standardised protocols, has the potential of becoming an ideal platform to collect the necessary data of thousands of individuals with the same medical condition. This has been the main impetus behind various international initiatives in forming large, virtual biobanks through

the networking of individual databases corresponding to individual biobanks. Apart from data on demographic, environmental risk factors, health, lifestyle, nutrition and socioeconomic variables, these biobanks might also hold 'omics' (genomics, transcriptomics, proteomics and metabolomics) data. These data and related bioinformatics software offer advanced possibilities in understanding the disease pathophysiology, thus paving the way for therapeutic and prevention programmes for a number of chronic diseases.

6 Conclusion and Recommendations

Consensus is being sought as to which genetic diseases should be included in population screening programmes and new criteria are being defined to achieve this aim [115]. These emerging criteria include important psychosocial aspects of screening, which become especially important when the test is only an assessment of risk rather than a definitive prediction, as well as ethical procedures such as informed consent [5]. Probably, the most effective way of reaching a suitable conclusion is to organise the established and emerging criteria into a whole process of policy-making, involving a thorough assessment of public health requirements, evaluation of the involved tests and interventions, and the actual development of the policy and implementation of the screening programme (Fig. 2). It is only through rigorous planning and organisation that a screening programme may be truly efficacious and cost-effective. In addition, the decision to integrate genetic testing for the identity of carriers at pre-conception, prenatal or cascade screening is important for autosomal recessive and low penetrance disorders. Furthermore, adverse results in both types of conditions lead to decision-making which involves many psychological and ethical issues [49, 116, 117]. This is especially because, in screening for complex or untreatable conditions, the benefits conferred do not necessarily involve treatment/management, but other life-plans such as reproductive or lifestyle choices.

A global effort should be made to standardise the design of such screening programmes, since integration of preventive and predictive genetics into the diverse health care systems would always remain under the responsibility of national/regional health authorities. Hence, guidelines should be set-up and implemented by leading regulatory bodies, such as the European Medicines Agency and the US FDA, under the recommendation of global experts in the field. Finally, standardisation of screening programmes may be also achieved by setting up regional centres of expertise, for example across the European Union. Such centres would provide standardisation and cost-effectiveness by carrying out tests for rare genetic conditions for all the participating countries.

Fig. 2 Established [3] & emerging [5, 115] criteria for screening programmes, with criteria which specifically apply to genetic screening programmes in italics

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Roadmap to Drug Development Enabled by Pharmacogenetics

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Abstract The primary goal of the pharmaceutical industry is to develop safe and effective medications. As the industry matures and the existing arsenal of marketed therapeutics grows, novel drugs must exhibit greater efficacy and safety to achieve registration and favorable reimbursement. Furthermore, gaining market-share has become extremely competitive, in terms of both meaningful clinical effects and tolerated safety profiles. As a result, the pharmaceutical industry has experienced a steady decline in productivity in recent decades. However, the achievement of regulatory approvals for targeted therapeutics may reverse this drop in productivity. The convergence of high-throughput genetic analysis technologies and the exponentially expanding biological and genomic knowledgebase have provided many clear examples that genetic variation can affect both disease risk and drug response. Therefore, evaluation of genetic variation in clinical trial populations should be considered essential and routine from the earliest phases of drug development. Pharmacogenetics (PGx) in particular has gained considerable attention from drug developers, regulators and payers over the past decade as a means to achieving safer, efficacious and more cost-effective drugs. While PGx science has great potential to impact positively the success of developing a new medicine, the integration of PGx into the decision making processes of the drug development pipeline has been difficult. The goal of this chapter is to describe the principles and requirements of an efficient

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and valuable PGx strategy that makes use of every opportunity during the course of developing innovative medicines. This strategy combines a proven methodology with rigorous genetic science to create a "*Pipeline Pharmacogenetic Program*".

Keywords Novel drugs · Companion diagnostics · Pipeline pharmacogenetics · Clinical trials · Drug development · Project management methodology

1 Pharmacogenetics in Today's Market-Place

Consumer demand for customized products and services is well established and evident in mainstream retail markets as well as emerging technologies. Gone are the days of "one-size fits all" and if a product or service is mass produced, then the available combinations, flavors and add-ons are so numerous that most consumer experiences can be, or at least feel, truly personalized. Similar pressures exist in healthcare markets. In fact, personalized medicine has the potential to benefit the consumer more than most retail products. The complexities of health and disease, underlined by each patient's specific environmental and genetic factors, call for a truly personalized approach given the suboptimal performance of standard therapy (most drugs exhibit response rates lower than 60%) [1]. Recognizing this growing need for individualized healthcare, many USA healthcare providers and hospitals offer services through "Centers of Personalized Medicine", like the Duke University Health System, MD Anderson Cancer Center, and Cleveland Clinic. Similarly, clinical pharmacologists and medical laboratories throughout Europe provide Personalized Medicine services, including the Karolinska Medical System and the Erasmus University-Rotterdam. Furthermore, international collaborative networks on personalized medicine are quickly forming to enhance knowledge acquisition and leverage capabilities. The Personalized Medicine Coalition, for instance, consists of over 200 academic, industry, healthcare provider and payer groups "seeking to advance the understanding and adoption of personalized medicine concepts and products for the benefit of patients" [2]. The European Commission is dedicating considerable investment in Horizon 2020 for innovation across European member countries with an emphasis on personalized medicine and systems medicine [3]. A key element of Personalized Medicine concepts and products has been and will likely continue to be in the area of PGx. There are currently 128 FDA-approved drugs that contain pharmacogenomic information in their label [4]. Indeed, regulatory agencies promote using genetic information in the drug development process in order to improve safety and efficacy by using pharmacogenomics information to decrease adverse events and to identify non-responders [5]. The general public also appears to have considerable interest and willingness in PGx testing to predict side effects, guide dosing and assist with drug selection [6]. The growing genetic testing market, estimated at $ 5.9 billion in 2016, and numerous direct-to-consumer and physician-provided genetic test companies are evidence of the economic forces driving the industry [7, 8]. The need for tailored medicines and the favorable regulatory environment to facilitate their development is driving increased availability of genetic testing services, thus creating market forces that reduce the cost of acquiring individual genetic information. For example, cur-

rently the cost for whole genome sequencing is $ 5800 per sample [9]. It is therefore an unquestionable fact that Personalized Medicine has arrived and its utilization and effects on healthcare is growing. For instance, in a recent McKinsey report the authors indicate that already over a third of the drugs currently in clinical studies are associated with a companion biomarker, indicating that newly approved drugs in the coming years will increasingly be dominated by targeted therapeutics [10]. However, the process of investigating, validation and qualifying companion PGx tests is challenging. It requires early investments in scientific infrastructure, and it hinges on clear *a priori* commercial and regulatory strategies to ensure the timely and cost-effective launch of the two end-products (i.e. the drug (Rx) and the diagnostic (Dx)). In addition to the principles of an efficient and valuable PGx strategy, we outline below the requirements, advantages and challenges associated with integrating PGx investigations into the drug research and development (R&D) pipeline. The information reported is based on our deep expertise in "Pipeline Pharmacogenetics" acquired over cumulative decades of application across diverse therapeutic areas and several global pharmaceutical companies.

2 Pharmacogenetics-by-Design: the R&D Environment

The application of PGx to currently marketed drugs as a method to predict safety and efficacy is of significant value to patients, physicians, regulators, payers and industry (some examples include warfarin, abacavir and multiple oncologic agents). The inability to predict the risk of adverse drug reactions (ADR) each time a patient is exposed to a new medicine continues to dramatically affect patients' morbidity and mortality. For example, in two separate studies, researchers reported that ADRs are estimated to be the 7th most common cause of death in a 2001 Swedish population based study [11] and that the incidence of serious ADRs was estimated to be 6.7% of hospitalized patients in the US [12]. In addition, most medicines display significant inter-individual variability in efficacy, but the current clinical practice approach addresses this problem by passive and reactive empirical methodology: treatment is administered according to standard protocols and outcome is assessed during later visits to determine efficacy. This "trial and error" practice is usually followed by either dose adjustments or triage onto other medicines if the patient poorly responds or fails treatment. This practice also results in prolonged procedures, including delay of efficacious treatment (sometimes over the course of months and years in the case of immunomodulatory treatments), risk of exposure to unnecessary drugs (which are always associated with a host of side effects), protracted suffering of patients and their caregivers, and, finally, additional cost to payers.

The development of PGx tests for registered medicines aims to identify optimum benefit-risk ratios and allow prospective testing prior to administration of drug. The availability of PGx testing also permits a differentiation strategy that guides the pharma industry to develop medicines tailored specifically for non-responder populations, thus addressing true unmet medical needs. Regulatory approved PGx safety tests prospectively predict who is at risk of considerable harm and provide high value as a warning to healthcare providers and patients regarding drugs about to be launched or

currently on the market. Equally important, predicting specifically who is going to be at risk of ADRs and excluding them from treatment prevents valuable medicines from being withdrawn from the marketplace. The PGx test thus serves to identify those patients who should be administered a drug and expect meaningful efficacy and safety.

For formularies such as Australia's pharmaceutical benefits scheme (PBS) as well as commercial payers such as those in the United States who are looking at drugs and their value to the public to whom they are responsible, PGx testing allows identification of subpopulations for whom there is an unmet need and greatest benefit [13, 14]. However, little progress has been made on the pharmacoeconomics of the prospective use of genomic biomarkers in the prediction of the benefit-risk ratio for patients. Notwithstanding, there are many examples of genetic variation being significantly associated with ADRs and effective as Dx in clinical practice, such as hepatotoxicity and hypersensitivity reactions [15]. Case studies teach us that genetic variation in the drug target (e.g., receptor) and signal transduction pathway of the majority of drugs accounts for much of the variability in response to medicines [13]. Variation in genes associated with immunological reactions and pathways can also be implicated in drug safety, most notably the MHC/HLA system. One such example is in the use of a PGx test for HLA-B*5701 prior to the administration of abacavir has resulted in the complete mitigation of cases of serious hypersensitivity reaction to the drug. Subjects who are HLA-B*5701 negative almost never develop immunologically-confirmed hypersensitivity reaction upon secondary administration of abacavir, on the other hand, HLA-B*5701 positive subjects (5% of the Caucasian population) have a 70% chance of developing a serious hypersensitivity reaction leading to hospitalization and possibly death if untreated [16].

The FDA and EMEA in addition to other regulatory agencies around the world, have experience with PGx integration into drug development. The FDA has substantial experience with how PGx may be used and there are now several FDA-approved drugs with PGx information in their labeling [5]. This illustrates that there is now a clear expectation that PGx data would be available on safety and efficacy and the FDA has published guidance on this [5]. The FDA has now seen PGx used where variability in response or exposure is observed, where adverse events are a concern, where drug dosage adjustment based on genotype is suggested and where known polymorphism at the target and or signal transduction pathway is evaluated. ADME gene variation involved in the metabolism of molecules has also been seen by the regulators and several molecules approved have ADME genotyping recommendations in the label (aripiprazole and CYP2D6 metabolizer status is an example).

3 The Roadmap to "Pipeline Pharmacogenetics"

3.1 Scientific Rationale

Pharmacogenetics, like any discipline employed for the purpose of improving the way drugs are designed and developed, is first and foremost a science. It is critical that during the course of PGx application this perspective remains the leading prin-

ciple during the selection of methodologies, technologies and analysis procedures. This is particularly true given the exponential growth seen in recent years in scientists' capability to sequence genomes, analyze Big Data and integrate complex phenotypic and medical information into clinically meaningful health management decisions. Still, one may ask—what is the advantage of embarking on the PGx process at early development phases, given limited power considerations associated with the size of these studies (often only a few hundred patients are collectively exposed to an investigational drug leading up to Phase III of its development)? After all, one could argue that postponing the investment would enable focusing efforts on drugs only after demonstrating favorable proof-of-concept (PoC) results and passing the initial safety hurdles. The counter argument lies in the very premise of the concept of "Pipeline PGx", and is well supported by positive, as well as negative, examples: the initial clinical development phases stand to benefit the most from the PGx methodology. PGx-enabled PoC design can maximize efficacy signals and exclude safety outliers so as to shift the overall benefit/risk ratio, resulting in increased probability of technical success early on for the entire program. *Post hoc* attempts to rescue development programs incur costs and waste valuable time depriving patients of effective treatments. History has repeatedly shown that only pre-emptive and systematic application of available scientific understanding of the mode-of-action of drugs and associated pathways can yield pharmaceutical successes that meet regulatory requirements. It is this mind-set and systematic approach that led to the development of a predictive test for abacavir hyper-sensitivity reaction described above [16, 17] or the positioning of prasugrel in a highly competitive landscape against clopidogrel [18, 19]. It is also thanks to this approach and adoption of emerging scientific discoveries that enabled the refocusing of the development of crizotinib from a c-Met-inhibitor to an anaplastic lymphoma kinase (ALK)-inhibitor, and thus formed the target of a co-developed diagnostic for defining patient eligibility [20].

3.2 Sample Collection Strategy

PGx research depends on the collection of DNA samples to generate data. In order to respond to the regulatory authorities' guidelines associated with genetic analysis, most pharmaceutical companies are now devoting resources within their clinical trial programs to enable the collection and storage of DNA samples. These DNA samples provide the pharmaceutical industry with the opportunity to investigate drug response, thereby increasing the likelihood of developing better therapies for patients and enhancing our understanding of the of disease context (e.g. progression and subtype characteristics compounding PGx outcomes) [21]. The collection rates of optional DNA samples, however, remain below the ideal target rate of 90–100 % which appropriately represents the PGx population out of the overall clinical trial ITT (intention to treat) dataset. This variable collection rate may be due to a variety of reasons as listed in Table 1.

Efforts should be made to mandate DNA sample acquisition across all programs where it is determined that DNA collection has a clear rationale and local laws/

Table 1 Common reasons for insufficient DNA sample collection rates in clinical trials and suggested mitigation plans [5, 22, 23]

	Issue	Mitigation plan
1	Insufficient understanding of the informed consent by clinical trial subjects	Ensure following best practices for informed consent writing [24], ensure site staff is knowledgeable and supportive (see 2 below)
2	Lack of support or interest by the site staff	Ensure communication to Principal Investigator clearly states the rationale and medical value of PGx testing in the study. Education program via Investigator Meeting, study newsletters as well as support and accessibility of knowledgeable PGx personnel
		Real-time. Incentivized DNA collection monitoring program Clear lab manual instructions that are easy to follow
		Mandatory DNA collection with clear underlying clinical justification is best practice.
		Incorporation of clear requirements in proficiency of DNA sample collection capabilities and attitudes should be incorporated a priori into site selection procedures
3	Reluctance of CROs to invest efforts in genetic study submission requirements	Select CROs experienced in DNA collection globally Include performance matrix of DNA collection as key elements of service contract Ensure communication to CRO clearly states the rationale and medical value of PGx testing in the study Mandate review by sponsor of country-specific submissions along with up-to-date regulatory guidelines in each recruiting country Establishing routine monitoring procedures for submission and sample collection
4	IRB/EC variation in interpretation of regulations	Clear protocol and ICF language on the purpose and rationale for DNA collection, adjusted to the specific requirements (in terms of detail and format) to each target country and recruiting center
5	Lack of logistical infrastructure	Select central labs with proven capabilities in collection and handling of samples intended for DNA collection (including tumor source) Consider providing refrigerators, centrifuges, dry ice, etc. as needed to ensure quality of samples maintained throughout the custody chain
6	Perception that DNA samples are associates with greater privacy violation risks than the collection of other types of samples during the clinical trial	Dialogue with Key Stakeholders regarding coding practices such that equal standards are applied to DNA and non DNA samples

regulations permit, and ideally from all mid and late phase programs as means for risk mitigation. DNA collection at baseline allows appropriate regulatory utility if and when needed [5]. The benefits of DNA sampling and storage are evident in drug labels, and contribute to internal decision making and regulatory filings [21]. Collecting DNA samples at >90% rate is key to successful and effective translation of findings into improved performance, given that otherwise any such attempt would be significantly compromised by the requirement to conduct new confirmatory prospective studies [21]. The underlying working assumption of PGx, in drug development terms, is valuable *only* when it is delivered in time for project team decision-making. Ultimately, timeliness of results is what facilitates achieving the objectives of each drug development program [14].

A DNA sample collection strategy requires the following key elements:

DNA Sampling Strategy Senior management within the company must provide explicit support that will allow for a clearly defined process to collect DNA samples within clinical trials to address clinical, scientific and regulatory issues in drug development [23]. Ensuring open communications and responsiveness to IRBs, ECs and other Regulatory bodies in the collection process will help to fully utilize the value of PGx research [22]. Funding will also be required in order to create the appropriate infrastructure to not only collect the sample, but to track the collected DNA samples to allow for timely and complete reconciliation (i.e. matching signatures on consent forms with acquired samples at the storage site). An integrated sample management process ensures efficient access to the samples to support the PGx analysis as well as ensures a method to keep the samples secure and private, allow for the tracking of the DNA sample from collection through to genotyping, storage, utility, destruction throughout the chain of custody to support the PGx analysis.

Training Education and training on the value of PGx and why there is the need to achieve optimal DNA collection rates must be provided to both key internal stakeholders (clinical project teams and their operationally focused colleagues) and external collaborators (such as contracted clinical research organizations (CROs) and clinical trial site staff) [14]. A patient's level of understanding of how these samples will be used can be influenced by the level of the investigator's enthusiasm for genetic research.

Informed Consent To be able to use a DNA sample collected in a clinical trial there needs to be a consent form that pre-defines the genomic objective prior to sample acquisition. These objectives can include pre-planned analysis around known factors that are likely to influence the safety, efficacy and/or dosing of the drug [5]. These types of analyses often require access to individual clinical information, particularly in cases of safety investigations. Broader investigations of an open-ended nature can also be considered as long as the sponsor clearly states that intended research will be limited to PGx purposes, i.e. understanding the response profile of the drug. Sponsors wishing to engage in further unspecified broad research which is beyond the scope of PGx would need to separate this research objective from the

PGx objective, placed under strictly voluntary basis, and often commit to anonymizing samples before analysis.

There are some special considerations to take into account when developing the consent form. Regulations around the informed consent vary both globally and locally. To allow for the main study to move forward without any delays, many pharmaceutical companies have created a separate genetic consent form from the main study consent, due to the additional approvals that may be required for collection of genetic samples and PGx research [25]. There are other special considerations that may need to be addressed in the consent form, such as possible ethical implications of the collected data, security and privacy terms of the acquired genetic information and under what circumstances research results might be returned back to the study participants [25].

Sample Collection PGx samples should be collected from all subjects randomized to treatment in all cohorts and in all phases of clinical trials to ensure samples are collected from subjects who have the potential to have a variation in response to the drug [5]. Collection of these samples at the time of enrollment will ensure minimal bias (avoiding lack of representation of DNA samples from subjects who withdrew from the study for any reason) and importantly ensure coverage of sampling from subjects subsequently experiencing ADR during the course of the study. The sample set also needs to be representative of the targeted population for the therapy to cover genetic variation among individuals from different geographic locations [22]. The voluntary and incomplete nature of many exploratory genetic studies conducted in prior years has often raised concerns about potential bias and statistical power, which could compromise the scientific rigor of such studies [5]. There are multiple sample types that can be used for DNA analysis additional to blood (e.g. buccal swabs, hair follicles, etc.) and are particularly relevant to pediatric or other special populations. Furthermore, in oncology studies tumor source DNA and/or circulating tumor cells (CRC) are also required to fully capture the PGx associated variation source that can affect the studied endpoints. When considering DNA samples from sources other than blood a robust quality assurance and quality control programs must be put in place to ensure sufficient yield and DNA quality [14]. This is particularly important when considering tumor source DNA sampling, including aspiration, formalin fixed paraffin-embedded tissue (archived versus fresh), fresh frozen biopsy, etc. and likely to differ from one cancer type to another. In these cases it is beneficial to collect tumor DNA at treatment failure so as to investigate mechanisms of resistance to therapy which are often underlined by the tumor's rescue mutations.

Sample Retention The retention of the DNA sample allows for the opportunity to perform investigations that may occur after the completion of the studies. Samples should be retained for a time period that will permit post marketed analysis should the need arise (e.g., at least 15 years) [5]. Long term sample storage will allow for the investigation of not only observations that emerge during the trial, but also any observations that may occur in subsequent trials and in the first several years after the drug has been on the market. These can be used to investigate external claims

generated by other groups once the drug is marketed, and may facilitate study of additional indications as part of the life cycle management of the product.

3.3 Fit-for-Purpose Genetic Interrogation

Traditionally, PGx studies were performed using a candidate-gene approach, often with genetic variants of the molecular drug target itself, or key polymorphic genes up or downstream in the drug target biological pathway. While candidate-gene hypotheses are statistically powerful, testing discrete genetic drug response hypotheses with a small number of variants, hypothesis-free approaches offer the opportunity for discovering novel genetic markers of drug response and revealing novel biological pathways. These genome-wide methodologies can be performed with custom or commercially available SNP arrays (genome-wide association studies/GWAS), and more recently have incorporated genome-wide sequencing technologies (whole genome sequencing/WGS or whole-genome exome sequencing/WGES). The shift to genome-wide genetic investigations has evolved as a consequence of several factors including lower costs for genotyping or sequencing, better statistical analysis methods and improved design of PGx clinical studies.

Historically, genome-wide association analyses of disease susceptibility have identified common sequence variants that impart modest, 10–20 % increases in disease risk. In contrast, the genetic risk attributed to variants associated with drug response (safety or efficacy) has been much larger (300–2000 %) [26, 27]. One explanation for this large difference in disease vs. drug-response genetic risk ratio could theoretically be attributed to the shorter period of evolutionary time that humans have been exposed to drugs, resulting in decreased selection pressure [28]. Leveraging this interaction of a patient's genome with drug response provides the potential to prescribe the right drug to the right patient (and at the right time for the right cost!). It should be noted that even though PGx science may lead to improvements in drug development, registration and patient health, its implementation has been hampered by the opinion that it might not be cost-effective [29]. However, this argument is becoming less relevant as costs of genotyping technologies drop and as central labs and medical centers increase their investment in genetic testing. Coupled with this is a robust improvement in the technology and breadth of gene tests available in a point-of-care instrumentation format that can provide the clinician with immediately actionable genetic information for personalized prescribing.

3.3.1 Technology of Choice, Genotyping and Sequencing

Candidate gene studies, utilizing either small number of often functionally significant SNPs in a key gene or a few genes (e.g., drug target or critical gene in drug target biological pathway) provide concise answers to specific gene association questions. They are usually employed if there is *a priori* genetic evidence that implicates a particular gene in drug disposition (ADME genes) or drug-response for efficacy/

safety purposes. Targeted gene variant assay panels are widely available from commercial sources and validated for use in diagnostic applications [30, 31]. Candidate gene studies have the advantage of being technically robust and are generally used to confirm a genetic hypothesis derived from a preceding study or reported finding. The original study(ies) is thus referred to as "hypothesis-generating", and often relies on approaches like customized, therapeutically- or disease- focused arrays or GWAS. The results of later confirmatory candidate gene studies often form the basis for development of a genetic companion diagnostic(s) co-development program, temporally synchronized with registration studies for a specific therapeutic.

In contrast, larger customized-array approaches or genome scans are undertaken when little or no genetic information exists, linking the clinical phenotype of interest to specific gene(s). Until recently, whole genome genotyping was usually more expensive than a candidate gene/SNP approach and results were limited to fairly common genetic variants that were selected for coverage across the entire human genome. Recently however, high-density arrays with tagging SNPs capable of assaying genetic variation down to ~1 % minor allele frequency (MAF) have been combined with custom arrays allowing the examination of groups of genetic variants with particular functional significance (e.g. exome arrays, ADME arrays, HLA arrays) [32].

Whole-genome sequencing (WGS) [33, 34] and whole-genome exome-sequencing (WGES) [35–37] costs are also plummeting and these technologies will ultimately replace array-based genotyping approaches in the near future. Advantageously, cheaper WGS and WGES [38] will permit transition away from GWAS-common variants to inclusion of rare genetic variants with potentially greater clinical effects. While accounting for a lower number of patients per specific variant, phylogenetic and coalescence methods are enabling the clustering of evolutionary-related variants into powerful genomic associations [39]. These WGS off-the-shelf products now widely validated for accuracy and coverage, also possess the advantage of condensed order-to-result timelines, since customized array solutions typically require 12–16 weeks for array design and manufacturing. These timelines are often incompatible with clinical development deadlines and force pharmaceutical companies to revert to pre-designed solutions in many cases. In fact, the high cost and complex logistics of obtaining properly consented DNA samples from well-phenotyped clinical trial subjects coupled with the ever decreasing costs of genotyping or sequencing on a genome-wide scale mean that GWAS or WGS/WGES is often cheaper than a candidate gene approach. Thus in practice a large database can be created of genetic variation across the genomes of the entire clinical trial cohort and then sequentially queried *in silico*, starting with a concise candidate gene analysis (hypothesis testing) and ending with a genome-wide screen for genetic variants with large effect (hypothesis-generation) [40, 41].

3.3.2 Statistical Analysis Considerations

The major objective of PGx analysis is to identify genetic marker(s) that can differentiate distinct subgroups of patients in a clinical trial based upon their drug response. Additionally, the pharmaceutical industry is also interested in discovering

genetic variants that are prognostic of a specific disease state or rate of progression of a pathological phenotype. Analytical models for predictive genetic markers include an interaction effect between genotype and treatment while prognostic markers are generally a main effect; where "response" is independent of drug therapy [42].

Early exploratory PGx studies generally analyze many potential genetic variants (candidates) or even scan entire genomes (GWAS, WGS, WES) to identify genetic markers, but small sample size/power, multiple testing, and a high false discovery rate can constrain the ability to discern valid, statistically significant results [43–47]. One key approach to screen out false positive results is to replicate results from the initial exploratory study in a separate clinical trial with similar patients and treatment. Lastly, a prospective, confirmatory study is necessary to test hypotheses related to specific genetic effects and evaluation of the clinical utility of the genetic markers (e.g. specificity, sensitivity, positive predictive value and negative predictive value), establishing the qualifying performance characteristics of the genetic diagnostic (Dx) as a basis for its regulatory approval. Therefore, three separate clinical trials (exploratory, replication, confirmatory) are necessary to go from discovery of a genetic marker to a companion diagnostic, reinforcing the need to start a PGx strategy early in the drug development pipeline.

Study design considerations are important at all steps of the PGx pipeline process. For confirmatory studies, consideration of targeted, enriched or stratified trial designs can be advantageous [48], but are usually only employed when there is an abundance of *a priori* information on a particular genetic marker. Adaptive studies or "gated" approaches permit the analysis of particular genetically defined subgroups when a study fails to meet its primary objective(s), and statistical concerns about multiple testing can be controlled by judicious "alpha-spend" [49]. For exploratory studies, weaker genetic effects can be revealed by using an extreme-phenotype approach that accentuates the differences between subgroups (e.g. super-responders vs. non-response) [50], and variations of this approach may be of particular importance for the study of genetic markers related to serious adverse events (SAEs). Lastly, improvements in the integration of genetic, genomic and clinical information, coupled with newer analytical techniques like Bayesian approaches, multivariate analysis of genetic "features" (SNPs, CNVs, SNVs, etc.) [51, 52] or phylogenetic analysis of sequence data [53], will create new ways to evaluate PGx study data and discover and develop more robust genetic markers of disease and drug-response.

The cost of functional validation can be high if a large number of gene associations emerge from GWAS or sequencing studies, and predefined lists of candidate genes in biological pathways of interest are often chosen for follow-up association studies. Approaches that combine GES with functional genomic bioinformatics filters (e.g. protein folding, gain/loss-of function predictions) or systems biology approaches (genetic, genomic, proteomic, metabolomics, etc.) [54] can also be used to prioritize results for wet-lab functional validation and may uncover novel pathways of biological relevance that are missed in pre-determined analyses.

In conclusion, drug trials of the future will be focused on genomically-targeted patients; identifying those most likely to respond to treatment and least likely to have an adverse event [55–57]. Synergistic effects of high-resolution genomic data (e.g. DNA/RNA sequence), better statistical analysis methods, rapid testing, as well as cheaper genomic analyses will translate into substantial savings in drug development cost and greater patient benefit.

3.4 Integrated Execution Methodology

Opportunities for PGx and the value to the portfolio exist throughout the development process from preclinical through Marketing/Pharmacovigilence, as long as PGx is in lock step with discovery and clinical development milestones. For this value to be realized, PGx objectives must be integrated into study protocols from early drafting to ensure that the proper support framework and budget are in place for sample collection, data management, and statistical analysis. In addition, experienced PGx personnel should be fully integrated into the clinical development teams from their inception point. The PGx team should be led by a scientist and consist of contract and vendor manager, genomic data manager, statistician, bioinformatician, and PGx project manager.

3.5 Communicating with Stakeholders

Managing the exchange of information and expectations across and outside the organization is challenging, though essential, for a successful drug development program. The internal and external stakeholders for PGx information are similar to other elements of the Clinical program, though some specific considerations are noteworthy for a PGx program.

Internal Stakeholders

Drug Discovery teams:
Disease genetics can be critically important in target and lead identification and validation, making PGx involvement at the earliest stages of discovery highly valuable.

Clinical trial design teams:
Integrating clinical objectives (primary, secondary, exploratory, gated) in clinical studies is the key to generating both retrospective, as well as prospective, actionable genomics results, tailored specifically for the enrolled population.

Clinical operations teams:
Once PGx is built into the clinical program, managing sample collection and clinical data availability is necessary.

Drug program/management teams:
Overarching program teams defining the overall strategy for the compound and evaluating novel indication or combination strategies for the compound, need to

be informed of the PGx progress and results, especially if and when unfavorable safety and efficacy results emerge in a study. If an integrated, prospective approach is taken, PGx information can be used to save some programs in light of results that would initially seem to kill a program.

Senior management (technical and non-technical):
Decisions of funding and ultimately the fate of programs facing unfavorable results are generally in the hands of senior managers that may not have specific and technical PGx background. Keeping management informed of the PGx strategy and value proposition, as well as current results is essential.

External Stakeholders
Regulatory Agencies:
As with much of the work companies plan and execute to develop drugs, communicating PGx plans and results to regulatory agencies in a timely manner is critical, especially at key clinical milestones. Agencies endorse the use of PGx information to increase the understanding of patient safety and drug efficacy as part of the benefit-risk assessment [58]. Furthermore, several communication routes are possible to convey PGx related information and should be chosen as appropriate, including for instance in the FDA the voluntary exploratory data submission (VXDS) route (non-trial specific), the "conventional" submission route to CDER, and co-development route to CDER and CDRH simultaneously.

Academic collaborators:
Trial recruitment rate is often better when key opinion leaders in the relevant therapeutic areas are involved in research and development of drugs. Including participation of academic collaborators in the PGx aspects of projects can often provide added benefit. This is also key for smooth introduction into the clinic and correlates well with market adoption at commercialization.

Payers:
Optimizing the health outcomes of patients is the primary goal of payers. Understanding payer's willingness and overall market drivers for drugs with PGx opportunities and label information will aid in developing a realistic value proposition, especially with companion diagnostic opportunities.

Physicians:
Beyond the physician's involvement in clinical trials, increasing the physician-wide knowledge of PGx and drug safety and efficacy will ultimately lead to better adoption by patients.

Patients:
Patients demand personalized approaches to many projects and services, and have increased willingness to provide genetic information when participating in clinical trials. Reaching out to patients or advocacy groups with regards to the opportunities to improve health and wellness through PGx is essentials. Recently patient advocacy groups have shown to be instrumental in targeted therapeutic approaches to drug development, for instance in the case of Cystic Fibrosis and Vertex's Kalydeco.

4 Pipeline Pharmacogenetic: Practical Application

For PGx to be successful, the objectives, tasks, and supporting roles (internally and externally) must be managed with a systematic methodology. Employing the established framework of formal Program and Project Management will maximize the delivered value of PGx. Since new molecular entities and drug candidates are considered *Program-level* effort due to the long timeframe (> 10–15 years from candidate selection to end of patent protection, plus possible product line extension), the corresponding PGx effort integrated in the development of these assets should be managed as a Program. The key deliverable emerging from a properly managed PGx Program is a PGx Strategy that is fully integrated and aligned with the asset development program. In a similar manner, individual preclinical and clinical studies that support assets are considered projects since they are a "temporary endeavor undertaken to create a unique product, service or result" [59], so the corresponding PGx experiments and studies should be managed as projects that are arranged and executed to secure the goals set within the PGx Strategy.

4.1 PGx Program Stages

The ideal PGx program would start very early in the asset life cycle, possibly pre-candidate selection or even at or as part of biological target identification. However, even mature assets with established clinical programs in Phase 1–3, possibly even approved and marketed assets, can initiate a PGx program. There are 3 key stages in the life cycle of a PGx Program illustrated in Table 2 [14].

Confirm When PGx is first considered for a drug candidate, the PGx team should be gathering and evaluating information related to disease biology, existing genomic factors for the biological target and potential patient populations, competitive landscape, early safety signals, available information on ADME, and other information useful to start formulating a PGx strategy. This early exploratory program stage results in the confirmation that there is indeed a PGx opportunity for a particular asset.

Integrate The chief purpose of this program phase is to establish the initial integrated PGx strategy, and to convey the value that PGx will bring to the particular asset and overall portfolio. It is recommended that this guiding information be recorded in the PGx Strategy and Value Proposition document (SVP) at this phase. The SVP is an overarching, "living" document that would serve as a reference point for all tactical decisions related individual PGx projects (Sect. 3.5.2). Stakeholders and funding sources (e.g. clinical teams, senior management) should be in agreement with the PGx strategy at this point.

Implement and Refine Once the PGx Strategy is established, this final Program phase is essentially the PGx program at "steady state" and is the longest phase,

Table 2 Pipeline Pharmacogenetics (PGx) Program Methodology

I. Confirm	II. Integrate	III. Implement and refine
Purpose:	*Purpose:*	*Purpose:*
Understand molecule, gather information and confirm PGx opportunity	Integrate with development team, create initial PGx strategy, identify value proposition	Implement PGx strategy, execute PGx experimental projects and deliver refined PGx strategy
Activities:	*Activities:*	*Activities:*
Molecule investigation via review of: - Preclinical data - Intended therapy - Target and pathway - External literature - Portfolio priority - Existing Clinical data, if available	Engage clinical team via: - Detailed molecule investigation - Review safety signals - Understand label, differentiation goals, development plan	Operationalize PGx via: - Protocol development, regulatory planning, and trial execution - Experimental project execution - Results interpretation - Strategy refinement
Key deliverable:	*Key deliverable:*	*Key deliverables:*
PGx molecule assessment	PGx strategy and value proposition	Experimental data and interpretation PGx strategy refinement

where individual projects are executed in alignment with the strategy. New information is gathered from external sources and results from implemented PGx projects, and the PGx strategy and corresponding SVP document are updated and refined to adapt to the changing situation of the asset.

4.2 PGx Project Stages

Once the PGx strategy is developed and clinical integration points are established, PGx projects should be implemented within the program by using the following 5 stages illustrated in Table 3 [14].

Scope A considerable part of "scoping" a project is in the gathering of specific clinical trial information from which genomic samples will be used along with the available clinical data. If the clinical team incorporated PGx objectives in the protocol prospectively and clinical samples and data were collected in preparation for PGx analysis, then this stage will largely be focused determining specific genomic assay platforms, vendor selection, and cost estimates. Also during this stage, the PGx lead should have specific engagements with the clinical team and funding sponsors (e.g. senior management) to reacquaint internal stakeholders to the purpose of the project and secure funding support. The final objective of this stage is to clearly delineate and document the objectives and boundaries of the project. This is critical to prevent "project creep" without deliberate and controlled scope revision,

Table 3 Pipeline Pharmacogenetics (PGx) Project Methodology

I. Scope	II. Plan	III. Execute	IV. Interpret	V. Close
Purpose:	*Purpose:*	*Purpose:*	*Purpose:*	*Purpose:*
Determine if a project will contribute to the PGx strategy, is feasible, and will be timely	Develop the project plan, identify deliverables, acquire resources, and create work breakdown and schedule	Execute the defined work to meet the project deliverables	Interpret the results of execution and recommend necessary next steps	Actively close the project, archive records, and perform post-project assessment
Activities:	*Activities:*	*Activities:*	*Activities:*	*Activities:*
Investigate: - Samples - Phenotype - Genotype - Statistical power - Technical feasibility - Strategy alignment	- Select deliverables - Create Project plan - Select vendor and technology - Work breakdown - Create schedule	- Genotyping - Genotyping data delivery - Statistical and power analyses	Statistical analyses results interpretation	Perform document quality checks, collate project archive, and represent findings in updated PGx strategy
Key deliverables:	*Key deliverables:*	*Key deliverables:*	*Key deliverables:*	*Key deliverables:*
Scope summary Project charter	Deliverables list Genetic variant list Genotyping contract PGx statistical analyses plan Project schedule	Genetic data and QA results Statistical requirements, output and report	PGx results interpretation and recommendation	Project archive binder Refined PGx strategy and value proposition

which is inevitable when clinical results emerge, organization priorities shift, and new genomic techniques/approaches are considered.

Plan Once the scope of the PGx project is finalized and approved, the planning of the project is initiated. The detailed project schedule is established and the overall operations and expectations of the project, including expected activities, deliverables and special considerations are documented in a Project Charter.

Execute Most of the expected activities defined during planning occur during the execute stage of the PGx project, usually starting with the planned genomic assays, including sample shipment and vendor management (if applicable). Other activities may include genomic data QC, development of statistical analysis plan and defining the expected table/lists/figures, genomic data transmission and merging with clinical data, and performing statistical analysis.

Interpret After the statistical analyses evaluating genetic associations with clinical responses/outcomes have been completed, the interpretation of the data and development of a recommended next step occurs. This important stage is led by the PGx

Scientist in consultation with the PGx statistician and clinician/clinical team, where necessary. The output of this stage is usually the results interpretation and recommendation document or report that can be summarized and incorporated into regulatory submission documents, manuscripts, etc.

Close In the final stage of the project, all PGx related documentation is stored and archived to retain the necessary information for regulatory review and future projects as part of the same program or for other programs with similar strategy and implementation.

5 Specific Examples in Early-Development

5.1 OPRM1 PGx and Alcohol Dependence

Pharmacotherapy of alcohol dependence shows widely divergent responses both within and between patients, and part of this variability can be attributed to the underlying genotype. Recently, treatment response to the opioid receptor antagonist naltrexone was shown to be predicted by a genetic variant of the OPRM1 gene (rs1799971) [60]. In a recent study, the effect of two genetic variants in OPRM1 and a variable-nucleotide tandem repeat (VNTR) in the dopamine receptor gene (DRD4) were evaluated for association with the clinical efficacy of a novel opioid receptor antagonist for the treatment of alcohol dependence [61]. Asp-carriers of the OPRM1/rs1799971 genetic variant did not demonstrate an enhanced response to LY2196044 treatment when evaluated by changes in % heavy drinking days (HDD), % days abstinent, or drinks per day. Surprisingly, however, placebo-treated Asp-carriers demonstrated a blunted response to standard medical management versus Asp-non-carriers by all efficacy measures. This Asp-carrier dependent "placebo-effect" reached statistical significance for change in % days abstinent and drinks per day ($p=0.0202$ and $p=0.0093$, respectively) but not change in % HDD ($p=0.1261$). Val-carriers of the OPRM1/rs1799972 variant treated with LY2196044 consistently had greater reduction in % HDD, % days abstinent, and drinks per day, but none of these reached statistical significance ($p=0.0653$, 0.8895 and 0.1073. LY2196044-treated patients who were DRD4-VNTR L-carriers had greater reductions in % HDD ($p=0.0565$), increased % days abstinent ($p=0.0496$), and reduced drinks per day ($p=0.0069$) than placebo-treated L-carriers.

In this study, Asp-carriers did not show a greater response to LY2196044 treatment, but instead had a blunted response to medical management in the placebo group. The difference between this result and earlier reports may be due to the differences in pharmacological profiles between LY2196044 and naltrexone, trial designs, definition of clinical endpoints and/or response, or unknown phenotypic differences within this trial population. The DRD4 L-carriers comprised >39% of the trial participants and showed statistically significantly superior treatment response. DRD4 L-carriers have demonstrated better response to other treatments for

alcohol consumption including olanzapine [62] and naltrexone [63]. Thus, DRD4-L may represent a common, robust genetic marker of opioid receptor antagonist response and form the basis for a potential tailored drug development program and companion diagnostic.

5.2 Oncology and Rare Diseases/Early Phase

One of the most vibrant and successful areas for implementation of PGx has been oncology. Recent years have seen development of novel therapeutics that is almost exclusively a "targeted therapeutic" approach, requiring a co-developed test to identify the target responder population. The greatest successes in this realm over the last couple of years encompass the ALK-inhibitor, crizotinib, and the B-Raf inhibitor, vemurafenib. Already at early phase I studies was a beneficial effect demonstrated in marker-positive carriers, which formed the basis for development decisions and study design for each of these molecules. Competitors are now developing second-generation BRAF and ALK inhibitors, benchmarked by the first-to-market compounds, both in terms of efficacy, as well as in terms of diagnostics and combination therapy.

Another immediate application for PGx early on relates to the growing clinical development field of rare diseases. Increasing in-depth characterization of the molecular biology of inherited disorders, fueled by financial incentives in the form of the Orphan Drug Act and expedited regulatory review processes, such as Fast Track and the Breakthrough Therapy designation, have led many biopharmaceutical companies to focus efforts on these ailments. Some of the successes in this field have revolutionized the care and life-expectancy of subjects with diseases such as Fabry disease (Fabrazyme), Pompe disease (Myozyme) and Cystic Fibrosis (Kalydeco) [64]. In these cases, the development is targeted for carriers of specific mutations and may employ comprehensive genetic and molecular screening already at early phases, followed by limited to no requirement for late stage registration studies prior to marketing approval.

6 Late Stage Drug Development and Pharmacogenetically-Enabled Clinical Trials: Rx/Dx co-Development

A drug development plan accompanied by pre-emptive Pipeline PGx approach from the get-go should culminate in late, Phase III clinical trials with a focused, well designed PGx component. It is not to claim that all drugs should be guided by a PGx designation, rather that by the time a drug is tested for registration purposes, the PGx characteristics of its efficacy and safety profile should be embedded into the program. The translation of this statement could mean a range of possibilities, depending on the specific drug and indication, starting with screening subjects for

eligibility based on carrier status of a particular genetic variant (i.e. the genetic predictor will become a required biomarker for prescription purposes), through to exploratory study of potential findings as no large PGx effects are anticipated based on pre-clinical and early development studies. In the latter case, exploratory analyses (and integral sample collection) are pursued to account for unexpected adverse drug reaction and other unexpected findings, such as high PK variability. The recently published draft guidance from FDA on enrichment strategies in clinical trials is the agency's response to recent development programs that employed genetic and other biomarkers in order to demonstrate favorable and safe benefit-risk balance [48]. One of the fields that have seen most innovation and creativity in this aspect has been Alzheimer's disease (AD) clinical research. The first such late-phase trial employed genotype of the apolipoprotein E (APOE) epsilon 4 (E4) gene as stratification biomarker toward development of rosiglitazone for the indication of mild-to-moderate AD treatment. The design was based on a prior Phase II trial that showed efficacy in an exploratory PGx analysis in APOE E4 non-carriers. The main Phase III study failed to reach its co-primary endpoints. Unfortunately, the result does not necessarily reflect lack of efficacy in this target indication as a high proportion of the study participants were of Asian ancestry, unknown at the time to possess a genetic signature that is different than that possessed by Caucasians and other ancestries. To this end, the study was likely underpowered to detect the clinical effect. It did indicate potential efficacy in the low dose arm in APOE E4 non-carriers.

Another set of studies employed APOE E4 carrier status as a patient selection criterion into clinical trials testing the efficacy of bapinuzumab, a humanized monoclonal antibody targeted against extra-cellular amyloid plaques, for the treatment of mild-to-moderate AD. The biomarker was considered to be predictive of drug response based on exploratory analyses of Phase II data, which did not reach statistical significance for its primary endpoint. As a result, treatment response in patients with the APOE E4 genotype versus patients without the APOE E4 genotype, was assessed in two phase 3, multicenter, randomized, double-blind, placebo-controlled studies, which were completed in April and June 2012, each with > 1,100 participants. The initial plan included two active doses in each trial, with the higher dose discontinued in the two APOE E4 carrier studies due to increased risk of amyloid-related imaging abnormalities (ARIA). Neither one of these studies reached statistical significance for clinical endpoints.

It is, however, by now generally accepted by field experts that treating AD at the mild-to-moderate clinical stages is simply too late, as the overt cell death and overall brain damage accumulated exceeds the potential for recovery. Given that neurons do not regenerate, it is unlikely that disease could be reversed once it has passed a critical severity threshold. Instead, efforts are now invested in preserving neuronal capacity at early disease stages (terms Mild Cognitive Impairment, MCI) or, better yet, to delay the onset of first symptoms and possibly prevent AD altogether. However, the feasibility of conducting disease prevention studies in this highly prevalent, yet highly heterogeneous disease in terms of age of onset, progression and clinical course, is very low. It is therefore necessary to employ an enrichment strategy that can pinpoint individuals at high-risk of developing the first symptoms within a short time frame of several years. Furthermore, it is critical to demonstrate

that the potential benefit (i.e. delay of onset) outweighs the risks (adverse events) in a cognitively normal elderly population. Thus, the clinical study design should randomize high-risk individuals into active versus placebo treatment, while the low-risk individuals (expected to live several years before potentially converting their risk status to the high level) should be administered placebo only, in a blinded fashion. This scheme allows for full evaluation of the treatment effects, parallel to qualification of the biomarker in a prospective, unbiased manner.

Other therapeutic areas are employing PGx at the registration phase for various purposes. One important goal is to ensure characterization of already-known biomarkers in the context of novel investigational drugs since, for the first time in the development process, large populations of patients are being exposed to these compounds. To this end, the FDA publishes a list of Pharmacogenomic Biomarkers in Drug Labels mentioned also above. Some, but not all, of these labels include specific actions to be taken based on genetic information, and the scope of biomarker type ranges between genetic sequence variation to expression changes and others. For those genes with known functional relevance to protein activity or/and to clinical outcomes, regulators require and/or encourage developers to evaluate them in the course of clinical development of investigational drugs.

7 Pipeline Pharmacogenetics: Summary

7.1 Barriers

The use of PGx is now fairly common within the pharmaceutical industry. Therefore it is not unreasonable to expect the delivery of tailored therapeutics across many disease areas. However, while PGx has had a dramatic effect on new personalized medicines for oncology, most of the other therapeutic areas seem to be lagging behind. One reason is the lack of organized, therapy-wide PGx strategies for assets at all stages of drug development carried out by skilled PGx scientists and project managers using a comprehensive Pipeline PGx methodology. As described in the sections and examples above, a valuable R&D PGx strategy starts with DNA collections from every subject in every clinical trial and integrates well-designed PGx scientific hypotheses into clinical study protocols. Delivery of time-driven PGx results permits R&D leaders to make key decisions and develop safe and effective tailored medicines.

Unfortunately, many barriers exist to successful implementation of the Pipeline PGx approach within the pharmaceutical industry. First, many argue that statistical significance of PGx effects are impossible to attain in phase 1 or 2 studies. Additionally, some contend that the size of drug-response genetic effects are too small and current studies will be unable to detect them. Both of these opinions are based on a confused understanding of the difference between disease genetics and PGx. There are many examples of very large genetic effects on both efficacy and AE's. In addition, specific genetic variants effects on disease are often quite distinct from

those on drug response. Secondly, anecdotal organizational "opinions" can hinder the implementation of PGx. Many clinical project teams erroneously believe that collecting DNA samples during the course of a clinical trial will impede recruitment, but this has consistently been shown to not be true. Thirdly, many are concerned on how various global drug regulatory agencies will interpret PGx data, and the belief that PGx results may lead to label restrictions and a restricted commercial potential. In fact, regulators have published guidelines on how PGx approaches (therapeutic coupled with a companion diagnostic) can lead to faster regulatory approval, focused labels and safer, more efficacious treatments, personalized for specific patient subgroups.

7.2 Outlook and Recommendations

The application of PGx tools, technologies and strategies to understanding the genetic contribution to pathophysiology and therapeutic response has been successful, and key stakeholders (patients, physicians, regulators, payers) have recognized these achievements. Recent progress in understanding the science of the genome, technological developments and bioinformatic/analytical approaches demonstrate that we can identify genetic markers that contribute to the safe and efficacious use of therapeutics. The evolving regulatory and business climate is placing greater value on increasing specificity and certainty around therapeutic choice.

However, the high attrition rates and reduced productivity of the pharma industry R&D is unsustainable and new strategies for tailoring medicines are needed. Currently, pharmaceutical companies are rarely, and/or inefficiently, leveraging the value inherent in the science of PGx to assist with critical decision making during drug development. One of the reasons is the lack of a systematic approach to incorporate PGx into the standard drug development process. This chapter has described a coherent Pipeline PGx methodology, described the tactical elements of this method, and provided successful examples of its application to drug development. In addition, some of the organizational and conceptual barriers that exist within and outside the pharmaceutical industry have been described. Therefore we recommend routine implementation of the PGx methodology throughout the drug development continuum that will deliver safer, efficacious and valuable tailored therapies for the benefit of patients, healthcare providers, payers, and the pharmaceutical industry.

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Pharmacogenetics—Statistical Considerations

Aiden Flynn, Craig Ledgerwood and Caroline O'Hare

Abstract The growth of Pharmacogenetics (PGx), using biomarkers to diagnose, prognose and identify patient subgroups most responsive to clinical intervention, heralds the possibility of more effectively targeted therapies and personalised medicine. Whilst demonstrating clinical significance in a number of studies, greater use of PGx has been limited by the need for further technological/methodological advancement together with a more integrated approach in study design and data analysis at the outset of clinical studies. Consideration of the statistical factors to be examined over the course of biomarker studies at the planning stage, instead of the current trend for retrospective analysis, will ensure that studies will be suitably powered to address specific questions and that subsequent data analysis will account appropriately for sources of variability. This will improve confidence levels in the conclusions drawn and the overall utility of PGx research. Greater use of PGx in the development of personalised medicine will require more guidance by statisticians and quantitative biologists in the handling and extraction of information derived from the data produced from large studies within the multidisciplinary network of researchers involved. This chapter highlights the key limiting statistical factors to be considered when embarking upon investigations using PGx, affecting the quality of information obtained from clinical data generated in personalised medicine research.

Keywords Pharmacogenetics (PGx) · Biomarkers · Data analysis · Statistics · Study design optimisation · Simulation · Modelling · Personalised medicine · Bioinformatics

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1 Introduction

The development of Pharmacogenetics (PGx) using biological markers (biomarkers) to identify patient groups responsive to treatment during clinical trials promises a new era in personalised medicine. Its application within recent clinical development programmes has grown considerably as both healthcare providers and drug developers have recognised its importance in directing treatments to those most likely to benefit. Where PGx has been implemented it can be used to guide decision making in clinical studies. It offers additional options over the course of drug development by helping explain unexpected variability in safety and efficacy outcomes in clinical interventions. Previously, such variability would have resulted in the termination of costly research programmes. However, as PGx can identify patient subgroups which are most responsive to treatment it can be used to focus further studies within these subsets.

Despite the potential value of PGx in improving the benefits and reducing the risks of some drugs in development by targeting treatments more effectively, so far its successes within research have been limited. This may be due in part to its predominant use, at present, as a tool to re-evaluate development plans when study outcomes are negative or ambiguous rather than being integrated at the outset in a personalised medicine approach. However, although PGx is still an evolving strategy requiring further technological and methodological development to optimise its use in data analysis and study design, its uptake in research programmes at the prospective planning stage, aiding study design and data analysis, is likely to improve its utility. To this end, this chapter identifies and quantifies the key limiting statistical factors commonly encountered when using PGx within a research study, which affect the quality of the information derived from personalised medicine research.

2 Types of Biomarkers

The aim of PGx analysis is to identify and characterise clinical responses occurring in patients subject to a given clinical intervention. These effects can be traced through data sets acquired from a variety of biomarkers. The use of biomarkers to track disease and its treatment offers the future possibility of individualised therapies providing personalised medicine for each patient. The biomarkers observed are biological characteristics that may be detected and measured objectively and used as an indicator of normal biological, pathogenic or pharmacologic processes in response to therapeutic intervention. Identification of individual biomarkers in the form of chemical, physical or biological parameters can be used either to measure progress of a disease or the efficacy of its treatment. As a result, biomarkers may be used to diagnose or predict treatment or disease outcome.

There are different types of biomarkers, with each type requiring the application of distinct statistical methods depending on their relationship to the observed

Table 1 Examples of predictive and prognostic biomarkers in current use

Biomarker	Type	Associated biological process/ function	Indication
EGFR (ErbB-1)	Predictive	Signal transduction, cell proliferation, regulation of DNA replication/repair, stress response, cell adhesion, cell migration	Advanced non-small cell lung cancer, anal cancer glioblastoma multiforme
HER2/neu (ErbB-2)	Predictive	Transcriptional regulation, signal transduction, cell proliferation	Breast cancer
BluePrint ®	Predictive	80 gene panel for assessing molecular subtype of breast cancer	Breast cancer
MammaPrint®	Predictive	70 gene panel to categorise lymph node negative breast cancer	Breast cancer
OncoTypDX®	Predictive/ prognostic	21 gene panel for assessing response to chemotherapy of estrogen receptor (ER) positive tumours	Breast/colon cancer
HLA-B*5701	Predictive	Immune regulation	Hypersensitivity reaction to Abacavir
K-RAS	Predictive/ prognostic	Ras protein signal transduction, cell proliferation, gene expression regulation	Colorectal cancer
AB1-42	Prognostic	Protein component isoform of amyloid deposits associated with Alzheimer's Disease (AD)	Alzheimer's disease

treatment response. Therefore, the objective of a biomarker's use and its characteristics should be clear at the outset of analysis to ensure that the correct statistical approach is applied to the data. Some examples of biomarker types and their uses are given in Table 1. In this chapter, two types of biomarkers are considered, prognostic and predictive markers. For statistical purposes there is an important difference between these two marker types. Prognostic biomarkers, such as AB1-42, are linked to the prognosis or likely disease outcome in a defined patient group independent to the treatment given. As a consequence, they are usually identified with models where the biomarker is fixed as the main effect. In contrast, predictive biomarkers, including HER2, are able to help identify patients likely to respond to a given treatment but not to a comparator where response may be measured as efficacy or safety. Their identification requires the application of a statistical model which allows interaction between biomarker and treatment. In some instances, however, biomarkers may be both prognostic and predictive. An example of this is mutant K-ras which expressed in non-small cell lung tumours and can be used to predict responsiveness to EGFR Tyrosine Kinase Inhibitors.

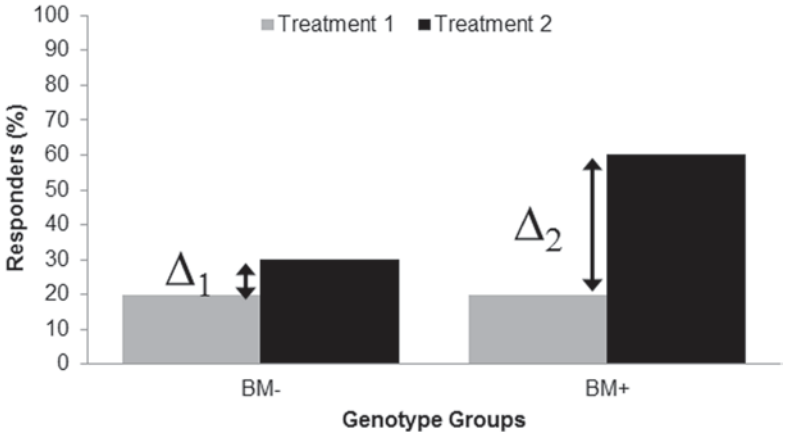

Fig. 1 An example of a predictive biomarker that is able to distinguish between groups of patients. In this case, the difference in the response rate in the BM+ group between treatment 2 and treatment 1 is greater than the equivalent difference in the BM− group. In other words, Δ_2 is greater than Δ_1

Identification of prognostic and predictive marker types is proving extremely useful in the development of personalised medicine. Prognostic markers can be used to segment populations by setting inclusion criteria at the start of a clinical study. This results in a reduction in the overall variability in the measure of response. In contrast, predictive markers are used to target treatments to patients more likely to derive benefit and are frequently further investigated as diagnostics for identifying responsive patient groups. An example of the use of a predictive biomarker to distinguish patient subgroups is shown in Fig. 1.

2.1 Biomarker Platforms

There are many different methods or platforms employed to measure biomarkers. These include a wide variety of technologies that can be used to produce biomarker data ranging from imaging modalities to the measurement of molecular biomarkers indicating gene expression, RNA expression, protein concentrations, single nucleotide polymorphisms (SNPs) or metabolites. Biomarker data can also take the form of continuous measurements, categories and ordinal scores. Different platforms may measure single markers or many thousands of markers simultaneously, generating data with specific attributes which will need to be accounted for in any statistical analysis. Data generated from some platforms may also require pre-processing steps such as scaling or normalisation [1] which must be considered prior to analysis. Consequently, it is important that the properties of the data obtained from each type of platform are factored into any statistical analysis.

2.2 Variability and Data Quality

Despite the accuracy of many of the biomarker platforms used, biomarker data can be prone to variability and bias. This can result in any subsequent analysis being subject to greater levels of statistical uncertainty leading to increased study failure rates. There are many factors which cause the observed variability and bias. These include the handling methods used for the tissue sample, when the sample was taken, patient factors such as drop-out rates, as well as inter-laboratory variation. If such factors are not addressed, inconsistent results are produced for the same biomarker across different biomarker studies [2, 3]. Therefore, it is critical that possible sources of variability should be evaluated during the development of a biomarker and suitable strategies for handling these sources and minimising their effect implemented.

2.3 Sources of Missing Data

Biomarker data often has a higher proportion of missing values than clinical data. These can arise as a result of numerous factors such as low consent rates for optional samples, patient drop-outs due to non-response or toxicity and measurements below the limits of detection of the biomarker assay. One key problem with these missing data is that the data are not missing at random. Indeed, the patients with missing values can be more likely to differ in their response to treatment compared to those with non-missing values. Therefore, it is important that missing biomarker data is not ignored as they are often informative. During any analysis involving missing biomarker data, it is important to compare key variables (e.g. those likely to impact response) between patients with and without biomarker data in order to understand differences between the missing group and the remainder of the study population. In addition, it is useful to understand reasons for missing data and take appropriate action. Where the pattern of missing data is understood, the implementation of models or imputation methods can help to recover the true underlying population statistics in the presence of missing data. On the latter point, information relating to the reason for missing data (e.g. below the limit of quantification) is often not recorded within the data set. This illustrates the need to improve on data standards and management practices relating to biomarker data.

2.4 Dimensionality and False Positives

Biomarker studies often involve the evaluation of numerous biomarkers in order to generate new hypotheses relating to the association between biomarker and response to treatment. This type of repetitive analysis results in a high number of false positive associations if the appropriate methods for controlling for the false

positive rate are not used. Methods for adjusting for multiple testing have been reviewed elsewhere [4]. However, it should be noted the strategy for controlling false positives should be consistent with the aims of the experiment and the proposed use of the results. In hypothesis generating studies, it does not make sense to apply an overly conservative strategy that limits the likelihood of identifying plausible markers. Furthermore, exploratory studies do not end when a statistically significant p-value is generated. Indeed, there are often further steps in the evaluation process that will remove further spurious associations leaving those markers which are biologically plausible and have a clinically meaningful application.

3 Study Design Options

Good study design improves significantly the probability of meeting research objectives whilst minimising known sources of variability and bias. In PGx, the study design options depend on how and when PGx is being applied. At present, the early stages of PGx research is usually exploratory whereby many biomarkers are investigated, often using data collected as part of a study designed for another purpose. This is usually followed by confirmatory research where PGx becomes the primary objective in a prospectively designed study. To date, most methodological research into study designs for PGx has focused on the prospective, confirmatory applications.

3.1 Confirmatory Studies

Confirmatory studies are designed primarily to test a hypothesis based on observable pre-specified biological effects. Such studies are designed prospectively and measure markers of relatively known function which have previously been shown to explain variability in patient response. Several study designs that use prognostic and predictive markers to stratify the study population have been suggested and evaluated [5–8]. Three common designs used in confirmatory studies for predictive markers are an enriched design, a stratification design and an adaptive design, as shown in Fig. 2. The merits of each of these are discussed in the following sections.

In the targeted or enriched design (Fig. 2a) patients are selected for the study based on their biomarker status in a pre-screening step. This allows patients with the negative status to be excluded from the study. Positive status patients are then randomised to one of the treatment groups. The main advantage of this design is that a treatment effect can be observed within smaller studies. The disadvantage of this approach is that it does not provide information on the effect of treatment in the excluded population. As a result, it can only be used when there is already prior knowledge of the impact of a single biomarker.

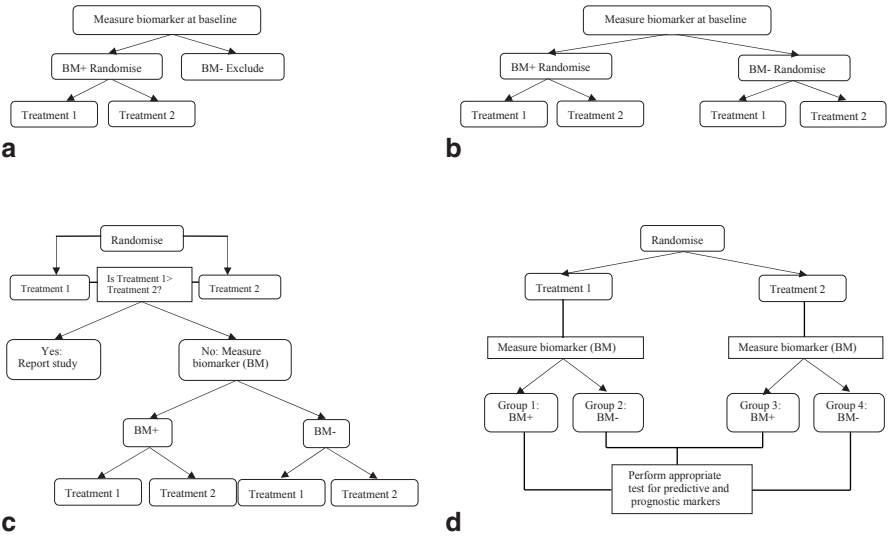

Fig. 2 Examples of study designs for Pharmacogenetics: Enriched design (**a**), Stratification design (**b**), Adaptive design (**c**), Retrospective design (**d**)

In contrast, the stratification design is less restrictive in its remit (Fig. 2b). It also has a pre-screening step whereby all the study subjects are stratified according to biomarker status and then randomised to treatment. The advantage of this design is that information can be collected on the treatment effect in the negative biomarker status group. Moreover, the performance characteristics of a diagnostic test, for example its sensitivity and specificity, can be estimated. However, as with enriched design, considerable prior knowledge about the biomarker is also required.

Both the enriched and the stratification designs are useful when studies are designed to test a single hypothesis relating to a given biomarker. However, more often studies have multiple objectives and involve evaluating a treatment effect in the entire study population as well as within sub-populations. In this instance an adaptive design is useful (Fig. 2c) [9]. With this design, patients are randomised to treatment groups and the treatments are compared. If there is no difference in the treatments, patients are stratified by biomarker status and a comparison of treatments is performed within these strata. This approach leads to a higher false-positive error rate, as multiple statistical tests are performed. Controlling for false-positives will result in larger studies. However, this design is more flexible than the targeted or stratified design as it allows the testing of multiple objectives and can be modified to include the evaluation of multiple biomarkers.

3.2 Exploratory Studies

PGx is used in exploratory studies for identifying useful biomarkers and to generate hypotheses for testing in further studies. Exploratory studies can range from

evaluating small groups of candidate markers to large scale biomarker arrays, depending on prior knowledge of their biological function and relationships to treatment response.

Currently, clinical studies often collect blood samples with a view to using them for exploratory PGx research [10]. If a PGx study is initiated, the study population is stratified retrospectively and iteratively using the biomarkers under investigation (Fig. 2d). The advantage of this approach is its flexibility, as it does not compete with the original study objective and many biomarkers can be evaluated retrospectively. However, it has a limited ability to detect biomarker effects due to the restricted sample size and the need to control for the high false-positive rate. Moreover, bias and imbalance are introduced into the strata as patients within them have not been randomised to treatment [11]. Consequently, biomarkers identified using retrospective analysis may require further support from data derived in prospectively designed studies [12].

As a result of the limitations of the retrospective approach in exploratory studies, PGx has not provided the breakthroughs anticipated. Notable exceptions to this have been studies with drugs associated with large genetic effects, such as Abacavir [13] and Panitumumab [14]. This has highlighted the key challenge in PGx research. Where insufficient patient data has been available, it has been hard to detect more moderately sized effects and thus identify biomarkers with clinical utility. Nevertheless, retrospective PGx approaches will continue to play an important exploratory role. However, to improve the likelihood of successful exploratory PGx studies, a more integrated approach is required in research programmes at the outset of clinical study design and data analysis. Indeed, recent research using computer simulation to design studies addressing multiple objectives, including PGx investigations [15], showed that prospective planning is vital. This is particularly important when studies are designed for another purpose, so that useful PGx data can be generated without impacting the primary objectives of the study.

3.3 Data Analysis Methods

The main objective for PGx analysis is to identify and/or characterise genetic effects. Whilst there are too many methods to review adequately in this article, there are some general principles that are broadly followed in basic analyses, as shown in Fig. 3. Current approaches to biomarker or feature discovery involve a multistep process whereby biomarkers are selected for further investigation based on the strength of association with an outcome; typically by setting an arbitrary limit on the likelihood of detecting false positives (e.g. p value <0.05). Evaluation of biomarkers involves the application of a statistical model comprising the factors that are thought to contribute to the observed variability in response. These models can include two types of effects: main effects where factors make a sole contribution to

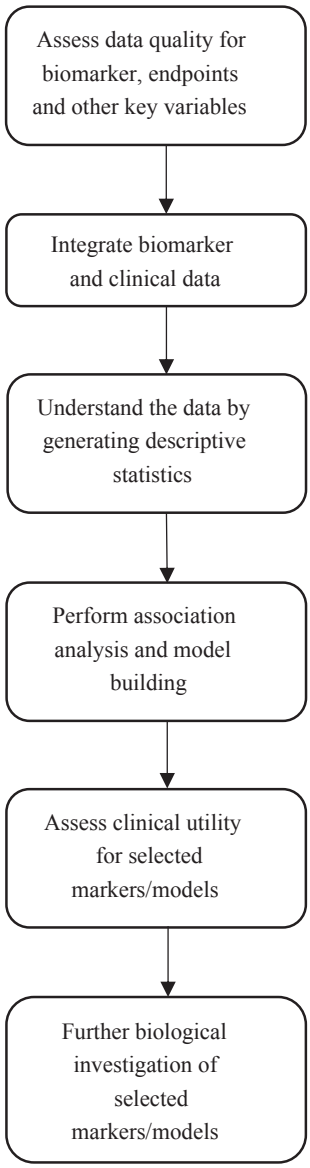

Fig. 3 An example of a statistical analysis strategy for a personalised medicine study. The analysis typically involves multiple steps that integrate different sources of information

the observed variability; and interaction effects where two or more factors make a combined contribution. This model can be written in the form of

$$R = B + T + B \times T$$

where R is the response to treatment, B is the biomarker measurement and T is the treatment group.

The choice of model is important as it will determine the utility and application of selected markers. Models with genotype as a main effect are useful for identifying markers that are associated with response, regardless of treatment (prognostic markers) whilst markers that are associated with response in the presence of treatment (predictive markers) can be identified using models with an interaction between genotype and treatment. Genetic markers are then selected on the basis of their prognostic or predictive utility.

3.4 Alternative Analysis Methods

The analysis methods described above relate in general to linear regression models. However, these can be limited in terms of their ability to incorporate complex relationships between different predictive and prognostic factors. In addition, linear modelling tends to require the pre-specification of the structure of the model. Clearly, this can lead to an over-simplification of the form of the relationships amongst predictive factors and outcomes. There are numerous other approaches that do not make the same assumptions and are more flexible in terms of enabling complex relationships to be modelled. There are too many to cover in any detail but neural networks [16], support vector machines [17] and random forests [18, 19] are regularly used to develop predictive models with some success. All modelling approaches need to take account of the study design and the biomarker utility and type.

High dimensional biomarker data sets are often sparse, in the sense that the model fitting process may have a limited number of observations that can be used to estimate the model parameters. There are a few useful methods that can be used to handle low density data including exact methods, lasso, elastic nets and others [20].

4 Model Building and Validation

The development of predictive and prognostic models generally involves the evaluation of biomarkers in the context of many other factors, such as demographics, baseline measures and environmental factors. As a result, these models include a combination of many factors that are additive in terms of their association with outcome. The development of these models is a multi-step process comprising variable selection and model evaluation followed by model validation [20].

Approaches to variable selection and model evaluation are generally well established. Typically, variable selection and model evaluation is an iterative process whereby variables are added or removed from a model following an evaluation of the contributions of those variables to the performance of the model. Following the model building process, the final model is the one that is considered to be the best performer.

One major problem with using high-dimensional data to build a predictive model is over-fitting of the data. In this instance, many variables are shown to have strong

relationships with the outcome as a result of random selection. Consequently, any model that is based on these random relationships will not generalise to unseen data or an independent data set, highlighting the importance of model validation.

There are numerous ways to perform model validation [20]. A common approach is to train the model on data from one study and then use an independent dataset to validate the model by assessing its performance in the second dataset. One problem with this method is the lack of availability of a relevant independent dataset. An alternative approach is to split the data from one study into a training and validation set. The robustness of the model may be evaluated using an iterative procedure for selecting the test and validation set. This, however, relies on the availability of enough observations (patients) as splitting the data will reduce the power to identify useful markers. Where sample size is limited, another useful strategy is to use leave-one out cross-validation. In this case, the model is trained on all but one observation and the ability of the model to predict the outstanding observation is evaluated. This evaluation is performed repeatedly by randomly selecting the observation that is left out of the model building step.

Recent work has shown that the best approach to model building is to integrate the variable selection and the validation steps into one large iterative process [21]. The benefit of this method is that the performance of many models can be assessed at once whilst controlling for false positives. For all the cross-validation approaches described above, the model building and performance characterisation is performed in the same dataset using data that were collated under study-specific conditions. The most robust form of validation involves the use of completely independent data (external validation) to assess the performance of a model.

5 Diagnostic Development

The use of statistics and modelling is vital in demonstrating the utility of companion diagnostics, prior to regulatory approval. The sensitivity and specificity of a diagnostic in its target population, as well as its positive and negative predictive value need to be identified under the original conditions in which it has been evaluated and developed. There are also a range of criteria that need to be set [22], such as defining the optimal threshold for biomarkers on a continuous scale and evaluating the repeatability and reproducibility of the biomarker assay. In addition, the diagnostic development process can be validated by understanding and quantifying the factors that may impact its performance. When a diagnostic is being co-developed with a drug for regulatory approval, good coordination between these processes is critical. Diagnostics development may often fall behind that of its associated drug, due to identification of biomarkers over the course of a research programme. This can cause delays in drug approval unless both development programmes are well synchronised.

6 Visualisation and Presentation

A key component of any analysis in a personalised medicine study is a clear and simple visualisation of the results. The use of good graphical outputs able to display relevant information simply, help to place the results in a suitable context facilitating the interpretation of large data sets. Well-designed graphical displays can integrate information on the clinical utility of biomarkers along with biological information, such as the functional annotation of the gene region, by overlaying both sources of information on the same plot. Another important aspect of visualisation is the presentation of high dimensional data. In this case, the use of multi-panel plots, heat maps, contour and surface plots are extremely useful. In addition, it is common to reduce the dimensions of data using methods such as multi-dimensional scaling, principal components analysis and clustering. This enables the data to be displayed on standard plots in two or three dimensions and can also uncover hidden structures in the data.

The presentation of simple summary statistics can often mask effects and responses that are notably different to those of the broader population. Therefore, it is important to be able to distinguish those observations that differ in order to understand variability in the data and identify patients that derive benefit. Consequently, any analysis of personalised medicine research should include graphical displays that enable the visualisation of individual data points. It also presents an opportunity for the observations obtained from a biomarker study to be visualised alongside information derived from other sources, placing it within a wider biological context.

7 Bioinformatics and Biological Interpretation

Since the completion of the Human Genome Project (HGP) and the arrival of next generation sequencing (NGS), technological advances in genomic sequencing have increased the speed at which entire genomes can now be sequenced. In addition, the use of microarray gene chip technology to screen patient tissue samples for the presence of genetic biomarkers associated with some disease processes has become increasingly commonplace. These advances in the area of medical genetics have resulted in the generation of unprecedented volumes of raw biological data. The need to analyse this data in order to understand it and how it might be used for clinical applications has required the capabilities provided by the expanding field of bioinformatics. Bioinformatics combines the mathematics, computer sciences and statistics required for the collection, banking, deciphering, analysing and modelling that is necessary to analyse large amounts of biological information. Indeed, bioinformaticians continue to seek to address the pressing need for data analysis through the development of analytical tools that can be utilised on desktop systems to analyse and interpret the data collected.

The recent era of next generation sequencing and multiplex microarray platforms has allowed a vast expansion in the number of sequences able to be analysed in each experiment. Prior to the emergence of these technologies, the focus of molecular biology was on known sequences previously identified and attributed to a given protein and/or function. Complementary probes were used to identify the presence and abundance of those target sequences and determine differences between treated/non-treated or resistant/responsive groups. Performed initially in singleplex assays (PCR), this quickly progressed to multiplex microarrays which could simultaneously measure thousands of targets (genes, single nucleotide polymorphisms (SNP) or messenger RNA transcripts (mRNA)) thanks to the technologies developed by Affymetrix, Agilent and Illumina. However, whilst this has increased the number of sequences that can be analysed it has also raised problems in their analysis due to the high number of dimensions in the data produced and the relatively low number of observations in studies.

Bioinformaticians have played a key role in implementing these technologies and addressing the difficulty in dealing with high dimensional data. The pre-processing of data has become critical to the utility of high-throughput systems, with several normalisation techniques, such as Robust Multi-array Average (RMA) and the current Affymetrix algorithm MAS5, being developed and used routinely in both the proprietary software provided by the instrument manufacturers and in open source packages, such as that available on the Bioconductor software repository (http://www.bioconductor.org/). Following normalisation, the next problem is dealing with the high dimensional data and correcting for the false discovery rate in hypothesis testing. Both commercial and open source packages use standard statistical methods to make comparisons between groups. P-values are typically adjusted to correct for the number of tests being performed through methods such as Benjamini–Hochberg [23].

Whilst many of the current tools for analysing and interpreting microarray and next generation sequencing data are useful, the huge quantities of data they produce nevertheless continue to create new challenges in data analysis. The technical process of sequencing an entire genome may have become routine, however analysis of the data it generates remains problematic as it is very computationally intensive with over 3 billion base pairs and 50 million variations to consider. Although sequencing and microarray platforms have been around for some time, the ability to process the volume of data produced in a routine setting at an affordable cost has only become a relatively recent possibility due to the ability to store and process the terabytes of data produced.

Currently, a number of software tools exist which facilitate the process of interpreting sequencing and microarray data. Alignment tools, such as BLAST, have been used for years to identify proteins or genes from short amino acid or nucleotide sequences or to compare the similarity between two or more sequences. The concept of the algorithms used by FASTA/BLAST and software for NGS sequence alignment are similar, however, the alignment of hundreds of millions of short sequences (FASTQ) from the entire genome takes a lot longer to perform even with accelerated algorithms. The Bowtie sequence alignment tool is one of the most

widely used aligners, due to its speed and the fact it is freely available to use. Even this can take several hours per sample to process and many researchers choose to run these on cloud-based platforms such as Amazon's Web Services. Other tools for analysing these alignments to identify variations, splice variants and differential expression of genes and isoforms are also freely available, such as the web-based application, Galaxy. However, these are still being actively developed and there are no clearly defined procedures as yet for the analysis and interpretation of alignments. Indeed, there are a plethora of tools both commercial and open-source for visualising, analysing and interpreting the large volume of information produced by each experiment.

Although the development of sequencing, storage and processing techniques has evolved concurrently, the ability to interpret the biological relevance of the information generated is still lagging. Some understanding of the underlying biological processes may be obtained through the mining of large gene sequence databases. These repositories of information encompass the knowledge gained to date regarding the biological relevance of genes and variations in sequences. Further biological context for biomarker studies might also be obtained through the use of data banked in public databases, such as ArrayExpress a functional genomics database containing data from both microarray and high-throughput sequencing studies. These databases now play a fundamental role in biological research and development, acting as a warehouse for storing, organising and providing large data sets relating to the occurrence and consequences of many biological processes, including gene variation, drug transport, drug targets, and other proteins of importance for drug response or toxicity [24–27]. Amongst the large number of databases generally available, some have become important bioinformatics tools within pharmacogenetics, such as the Human Genome Project (HGP) [28], Ensembl [29], the SNP databases dbSNP and JSNP [30], and HapMap [31]. These databases are rapidly expanding as they are continually updated with new submissions of genetic information, especially regarding the variation across the population.

The HGP demonstrated that the 20,000-plus genes expressed in humans only accounts for 1.5 % of the genome, with very little known about the function of the remaining 98.5 %. This is now being addressed through global collaborative projects such as ENCODE, which aims to completely annotate the non-protein encoding regions of the genome. Moreover, large sequencing projects like HapMap phase 3 and the 1000 Genomes Project, aim to give a clearer picture regarding the intrinsic genetic variation present within the human population. These projects in particular will provide a valuable resource for bioinformaticians, giving an important insight into the range of variation inherent in the human genome in general across multiple ethnicities. This will no doubt raise more considerations for analysis and interpretation of genomic data.

Possibly the most influential sequence database to date has been GenBank, an open access database storing known gene sequences from over 100,000 distinct species, along with their protein translations. It is run and maintained by the National Center for Biotechnology Information (NCBI) which plays an active and collaborative role in the development of computational biology. Their bioinformatics

resources can be used to annotate and analyse an abundance of disparate data. Access to this important and expanding resource allows researchers to derive possible connections between the different aspects of biomarker data and thereby shape a more biologically meaningful view of it [32, 33].

The Ensembl database also provides genomic information with a rich source of gene variant data from humans and other species, including single nucleotide polymorphisms (SNPs). Alongside the HGP and Ensembl, dbSNP, Japan's JSNP and HapMap are another three of the more widely accessible and utilised bioinformatics resources. The Single Nucleotide Polymorphism Database, (dbSNP), created to supplement GenBank, is a public access archive for genetic variation within and between organisms developed by the NCBI. It comprises information on over 64 million distinct SNP variants in 55 species, including *Homo sapiens* [34]. Meanwhile, the HapMap project provides an alternative platform of information designed to enable researchers to carry out large scale studies to link genetic variants to the risk of specific diseases [31].

Other useful databases storing genetic data are the Gene Expression Omnibus (GEO), the Kyoto Encyclopaedia of Genes and Genomes (KEGG) [35] and PharmGKB [36]. The analysis of the data contained in these databases is now an integral element of the bioinformatics process. Many of the data storage facilities have analytical applications bolted on as add-ons, whilst others are standalone warehouses that store the information that is used in other analytical applications. As with genetic databases, there are many bioinformatics tools available for dissecting, analysing and visualising the data, a selection of these have been listed in Table 2. This is also an evolving field within bioinformatics with new software tools under development.

Another recent tool useful in understanding the biological complexity of differentially expressed genes and proteins and identifying statistically significant sets of genes, is Gene Set Enrichment Analysis (GSEA) [37]. This is a powerful analytical method for interpreting gene expression data, deriving its power by focusing on groups of genes or gene sets that share common biological function, chromosomal location or regulation. Whilst single-gene analysis is useful it can miss important effects on biological pathways which are distributed across large networks of genes and are hard to detect at individual gene level. In contrast, GSEA which examines sets of related genes can identify many common biological pathways as cellular processes often affect networks of interacting genes. The advantage of GSEA is that it facilitates interpretation of genome-wide expression data as it focuses on gene sets which give more reproducible and therefore interpretable data.

Undoubtedly, constructing biological meaning from lists of statistically significant markers remains a challenge to bioinformaticians. However the development of complex data warehouses and other resources, that detail the structures and processes and interactions where individual genes/proteins exist, have helped to overcome such difficulties through the grouping of long lists into smaller sets of related genes or proteins that share a similar physiological/pathological function, cellular localisation, position in the genome or can be defined by similar gene ontology terms. There is a plethora of gene set databases that use examples found in the

Table 2 A non-exhaustive list of bioinformatics tools available with a brief description of the tool and an external link

Tool	Description	Link
ArrayExpress	Functional genomics database containing data from microarray and high-throughput sequencing studies	http://www.ebi.ac.uk/arrayexpress/
BioMart	Search engine allowing generation of tables of terms linked to genes and SNPs	http://www.ensembl.org/biomart
BLAST	Database allowing searching and alignment of sequences to the RefSeq genome	http://www.blast.ncbi.nlm.nih.gov/Blast.cgi
GeneMania	Search engine to find related genes through linkage of association information	http://www.genemania.org/
GSEA	Tool to perform enrichment analysis on gene sets provided in MSigDB database	http://www.broadinstitute.org.gsea/index.jsp
Haploview	Range of tools for analysing linkage disequilibrium and haplotype patterns	http://www.broad.mit.edu/mpg/haploview/
KEGG mapper	Collection for mapping gene sets to the KEGG pathways	http://www.kegg.jp/kegg/kegg1b.html
KEGG pathway	R package for analysis and visualisation of expression data within KEGG pathway	http://www.bioconductor.org/packages/release/bioc/html/KEGGprofile.html
PathNet	Tool that performs pathway analysis using topological information from pathways	http://www.bioconductor.org/packages/release/bioc/html/PathNet.html
Pathway browser	Tool for visualising pathways	http://www.reactome.org/PathwayBrowser/
SNAP	SNP annotation/proxy search tool using linkage disequilibrium and physical distance	http://www.broadinstitute.org/mpg/snap/
Stitch	Tool for exploration of known/predicted molecular interactions	http://www.stitch.embl.de/
Sweep	Tool for large scale haplotype analysis	http://www.broadinstitute.org/mpg/sweep/index.html
topGO	Compares GO term representation in an expression set accounting for GO topology	http://www.bioconductor.org/packages/2.12/bioc/html/topGO.html
UCSC genome browser	Tool providing interactive graphical interface to visualise genome annotation and chromosomal position	http://www.genome.ucsc.edu/

literature and gene sets that have been computationally derived. Databases such as, MSigDB and ConsensusPathDB, have brought together a large collection of gene sets comprising gene regulation, protein interactions, genetic interactions, biochemical reactions, drug-target interactions, pathways, gene ontology, disease regu-

lation and many more. As differences in database structures and terminology exist, moving towards large warehouses of this information and development of advanced tools for mining the information are crucial to their implementation. Tools have been developed as stand-alone applications or as add-ons in other packages such as R (PGSEA). The principles of these tools are largely similar, in that they comprise an annotation database, a process that can assign those annotations to a given gene list, a further process that performs a statistical test to identify annotations that are significantly represented in the gene list and a method to interpret this graphically.

Examples of statistical approaches used in gene enrichment analysis are over-representation analysis (ORA), functional class scoring and pathway topology. Using a statistical test, for example the hypergeometric or binomial, ORA evaluates whether a specified functionally defined group of genes/proteins is represented within a gene list, or if it occurs merely by chance [38]. One drawback, however, is that ORA treats each gene equally, losing any possible correlation and interaction between genes. Functional class scoring overcomes some of the limitations of ORA as it treats the genes differently depending on the strength of the individual raw microarray values [38]. The pathway topology approach has advantages over both of these methods, in that it does not only consider the number of genes in a pathway to identify significant pathways, but also utilises information about inter-pathway connectivity. GSEA utilises its own novel method for performing the analysis, this calculates an enrichment score using a weight Kolmogorov-Smirnov-like test. The enrichment score generally reflects the degree of over representation at the top or bottom of a ranked gene list [37].

Whilst it remains challenging, bioinformatics has made some progress in understanding and interpreting large data sets by exploiting alternative data resources. The effort to understand in more depth the implications of the data collected and analysed within an experiment has required input from other data sources enabling a fuller understanding of its meaning. It is becoming clear that results from primary biomarker analyses might need augmentation with additional information from other studies to provide a greater biological context for the role of the biomarker in question. Further biomarker context could be given by its characterisation as well as by pathway analysis. Additional data in this form would help authenticate the patient subgroups identified from biomarker analysis by providing further supporting evidence. Alternatively, longitudinal data would allow further characterisation of subgroups using variables that change over time. This would require the pre-selection of those variables, with the selection process used described.

8 Future Opportunities

There are a number of opportunities within statistics and modelling to improve the application and implementation of pharmacogenetics in future studies. Four key areas to be considered are improving study design, integration of analysis methods, use of disparate data sources to provide biological context and better multi-

disciplinary collaboration involving quantitative scientists. Addressing these factors will develop PGx by increasing the success rate of exploratory studies to identify new biomarkers. Additional use of computer simulation will enable the application of smarter clinical trials that optimise the likelihood of success of a study without prohibitively increasing its size.

At present integrative analysis methods, such as Bayesian methods, are based on the idea of obtaining a consensus by combining prior information and current opinion, thereby providing a statistical framework that enables the quantitative (probabilistic) integration of information across multiple analysis steps [39]. This approach can limit biomarker discovery as such studies may be underpowered to detect small to moderate (but biologically important) effects; it filters potentially useful information in variables that fail to reach significance and it ignores the additional control of false positives that naturally occurs in the subsequent analysis steps. To progress, there is a need to develop methods that do not filter out useful information and that enable the quantitative integration of information from additional analysis steps, such as clinical and biological pathway analysis and comparisons with literature. Furthermore, there is enormous scope for developing and applying statistical models that more closely reflect the underlying biology and patterns of response; using models that better describe the data will increase the power to detect genetic markers. The development of these capabilities will require extensive methodological research and development for the integration and application of disparate data sources.

It is clear that statisticians and quantitative biologists are of increasing importance in the multidisciplinary network of researchers involved in the development of personalised medicine. Bioinformatics has helped develop new algorithms and software to facilitate the analysis of complex data sets. The development of new computational data and analytical solutions are crucial to handling and extracting the information derived from large clinical studies, improving the understanding of disease progression and treatment. Nevertheless, a knowledge gap still exists between the exploratory world of bioinformatics and the rigour and regulation of clinical statistics. Closer collaboration between quantitative scientists will break down the barriers in communication that exist between the disciplines and will enable scientists to gain experience, knowledge and an appreciation of the skills and capabilities that exist in other fields. Deeper understanding of other capabilities and technologies will lead to new innovations that make use of the extensive information available and improve the application of Pharmacogenetics in the quest for personalised medicine.

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Pharmacokinetics and Pharmacogenetics: Bringing the Magic Bullet Closer to Reality

Janet Mifsud and Marc Maliepaard

Abstract Why do some patients respond positively to some drugs, while others may experience adverse effects? Can we predict which patients will react in which way? Does a magic bullet exist? The trend towards pharmacogenetics and personalised medicine in the last few years has somewhat sidelined the relevance of the traditional pharmaceutical sciences, such as pharmacokinetics and pharmacodynamics. Yet these actually are part and parcel of pharmacogenetics. Indeed understanding pharmacokinetics and pharmacodynamics in pharmacogenetics is essential in assessing the risk of new chemical entities (NCEs) in populations and individuals. Clinical pharmacokinetics, in fact, can be understood to have been a precursor to the implementation of pharmacogenetic understanding in the clinical setting. In this chapter, examples will be given of the strong interrelation between pharmacogenetics and the various pharmacokinetics processes i.e. absorption, distribution, metabolism and elimination. Reference will be made to studies which have shown how pharmacogenetics can be reinterpreted into pharmacokinetic principles, thus leading to the individualisation of drug therapy in the individual patient. The impact on recent regulatory guidelines published on the role of pharmacokinetics in pharmacogenetics and their impact on regulation of new medicinal drug development will also be discussed.

Keywords Pharmacokinetics · Pharmacodynamics · Pharmacogenetics · ADME · Drug transporters · Therapeutic drug monitoring · Individualised therapy · Drug development

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1 Introduction: What is PK and What is its Role in PGx?

Drugs are, fundamentally, chemical compounds and drug therapy is a dynamic process. Thus the body handles the drug as it would any other chemical compound as it moves in the body. This fate of the drug proceeds over a certain time interval and the various, so called 'pharmacokinetic' processes (from Ancient Greek *pharmakon* "drug" and *kinetikos* "to do with motion") determine the time course of the drugs: drugs are *liberated* from the formulation; *absorbed* through the administration site; *distributed* through the body; *metabolised* mostly in the liver (but not only); *eliminated* mostly in the kidneys (but not only), often given the acronym of ADME [1].

Pharmacokinetics was first conceived as a term in 1950s by the German Professor Dost. Coincidently it was around the same time that Vogel Friedrich in 1959 published his key paper *Moderne probleme der Humangenetik*—the influence of genetic factors on the response to drug [2].

Pharmacokinetics was popularised by Holford in 1982 with his use of the aphorism 'pharmacokinetics is what the body does to the drug while pharmacodynamics is what the drug does to the body' [3]. Pharmacokinetic/pharmacodynamic (PK/PD) relationships are important in the drug therapy because they are predictive sciences (Fig. 1). They are essential to determine drug plasma/response relationships and thus the dose and dosage regimen needed in an individual patient, predict drug-drug and drug-food interactions and, in fact, form the basis of what has become more colloquially as *personalised medic*ine. It is mandatory for regulatory purposes that new chemical entities (NCEs) in various stages of drug development have their pharmacokinetic parameters well characterised with the use of software such as WINNONLIN® and NONMEM®, prior to marketing authorisation.

The *FDA Critical Path Initiative* and *NIH Roadmap* in 2004 changed the focus in innovation in drug development towards one based on translational science. It also led to a renewed understanding of the importance of PK/PD as quantitative pharmacology and led to terms such as "pharmacometrics", and "model-based drug development" [4]. This led to a shift from traditional paradigms, such as the ef-

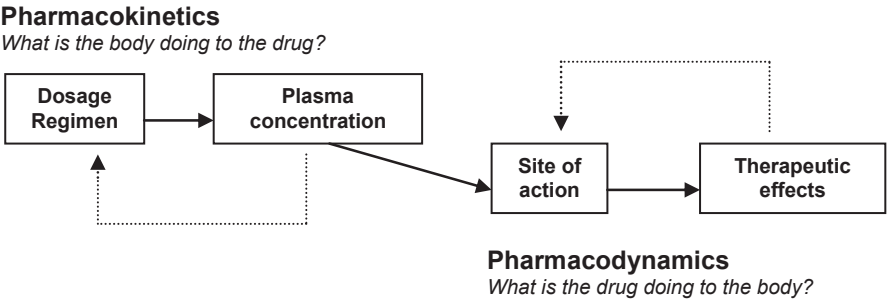

Fig. 1 The relationship between PK and PD in determining dosage regimens. The plasma–drug concentration data or effects produced are used via PK as a feedback (*dashed lines*) to modify dosage regimen to achieve optimal therapy

ficacy models in anticancer drug development area, to one where there is a truly scientific relation between preclinical and clinical pharmacology. The result thus expected would be one where there would be a reduction in the number of drugs being withdrawn from the market, due to unexpected and unacceptable adverse effects not picked up during drug development (cf. COX-II inhibitor class suits) [5].

At the same time, there was a parallel exponential shift in the understanding and role of genetics in pharmacology and the emergence of pharmacogenetics with the availability of cheaper and more rapid genetic analytical tests. This led to a new understanding that PK/PD differences may be due, in specific instances, to genetic variance [6]. Pharmacogenetics (PGx) was originally defined as the use of biological markers (DNA, RNA or protein) to predict the efficacy of a drug and the likelihood of the occurrence of an adverse event in individual patients. New definitions have emerged in recent years, yet the essential principles remain the same.

1.1 Succinylcholine: The First PK/PGx Study?

This parallel emergence of PK/PGx could not be better exemplified than in the emergence of the understanding of the differing mode of action of the muscle relaxant succinylcholine in some patients. In the 1950s it was clinically observed that patients differed in their response to this drug. It became known that the duration of this drug's action is determined by its enzymatic hydrolysis. It was only at a later stage that the genetic basis was discovered i.e. subjects homozygous for a gene encoding an atypical form of the hydrolysing enzyme, have a prolonged drug-induced muscle paralysis [7].

Other drugs followed suit. In the 1960s, it was discovered that a defect in N-acetylation metabolism of the drugs isoniazid (used for the treatment of tuberculosis), the antihypertensive drug hydralazine and the anti-arrythmic drug procainamide, could lead to prolonged half-life of these drugs in some patients, and this too was genetically determined [8].

The discovery in the mid-1970s, of the key role by the family of cytochromes, P450 system in drug metabolism, especially in hydroxylation, led, at the end of the 1980s, to an understanding of the molecular basis for slow metabolism in some patients, especially for those drugs which have a narrow therapeutic window [9].

Yet these developments remained only interesting academically. One of the key turning point in regulatory terms, however, was the discovery that irinotecan, which is used to treat bowel cancer, is metabolised by UDP-glucuronosyltransferase (UGT1A1). The *UGT1A1* promoter has a wild and variant copy (denoted *UGT1A1**28). This *UGT1A1**28 polymorphism is characterised by the presence of an additional TA repeat in the TATA sequence of the *UGT1A1* promoter, ((TA)7TAA, instead of (TA)6TAA). The latter causes a reduced UGT1A1 expression which leads to irinotecan toxicity. Today *UGT1A1* genotyping is mandatory in determining irinotecan dosage according to the FDA label for irinotecan, however in the EU the limitations of data available added to the reluctance to add a warning in several EU Member

States, i.e., it was not known what dose is the optimal one, in the *UGT1A1* WT as well as the *UGT1A1**28 [10].

More recently also for drug transporters the first genetic polymorphism was described which resulted in clinically relevant effects on the pharmacokinetics and adverse effects of some drugs, mainly statins, i.e., the *SLCO1B1* gene, encoding the OATP1B1 transporter protein [11].

1.2 Why Bother with PK/PGx?

Pharmacokinetic processes, like any aspect of human physiology, are inherently variable, and subject to both environmental and host-determined influences such as physiological and genetic factors. Genetic polymorphism (the occurrence in the same population of multiple allelic states) is responsible for a major proportion of the observed interindividual variability. These thus play a major role in PK/PD and therefore in dose response. Pharmacokinetic variation due to genetic factors can have the following consequences in any of the ADME processes such as:

- altered absorption/clearance
- difference in formation of active metabolites
- changes in drug interactions
- ethnic variation in drug response [12].

It is thus important to rationalise pharmacokinetic variation as exhibited by differing plasma concentration time curves in individual patients, on the basis of PGx effects and the interpretation of allelic changes. These need to be put into the context of PGx effect upon function e.g. one needs to be aware of how much an individual enzyme like CYP2D6 which is responsible for the metabolism of many drugs, contributes to the overall drug clearance. It is also important to consider what is the active moiety, the pathways affected and whether competing pathways could be present. In fact, PK/PGx studies would be key for those drugs which have a high inter-individual variation in efficacy and/or potency and have a narrow therapeutic range [12].

Thus, the ultimate goal of PK/PGx is to identify the contribution of genetic variability to differences in PK/PD, which in turn lead to individual differences in drug responses. This would enable prescribers to utilise a patient's PGx profile in order to select the drug which would exhibit the greatest efficacy and the least adverse effects in that specific patient and/or prescribe the drug at a dose which is appropriate for that patient i.e. personalised medicine [13].

In this review, a systematic discussion will be given on the PGx effect on the various PK processes i.e. ADME (absorption, metabolism, distribution and elimination). It will be shown how the development of a better understanding of PK/PGx guided principles, provide a crucial basis for the development of personalised medicines in individuals or specific subpopulations, optimising risk/benefit relationships, by maximising therapeutic efficacy with minimal adverse effects. It should

be kept in mind, however, that the fulcrum of PK/PGx relationships would be the clinical availability of reliable PGx test, and a strong relationship between genotype and phenotype. To date not all studies provide confirmatory evidence in this regard.

2 PK/PGx Concepts in the Absorption of Drugs

Absorption is no longer considered to be a passive mechanism and is now known to be the summation of extremely complex processes. Several membrane bound drug transporters, such as P-glycoprotein (P-gp, MDR1) and multidrug resistance (MDR) transporters, encoded by the *ABC* genes, have been identified as being responsible for the transport of drugs across membranes, especially those in the gastrointestinal tract following oral administration [14, 15].

These mechanisms have an important bearing on a drug's systemic bioavailability (F) which is used as a measure of how much drug eventually reaches the circulation after oral and any other non IV administration. Since the bioavailability of an intravenous drug dose is assumed to be 100 %, F is best calculated as the ratio of drug concentrations after giving the drug by the route of interest (usually oral) compared with the same dose given intravenously [15].

Sequencing of the *ABCB1* gene (which encodes P-gp) has shown that there are more than 50 single nucleotide polymorphisms (SNPs) for this gene, which vary in frequency according to ethnicity [16]. Wild-type *ABCB1* alleles have been associated with increased tissue expression of P-gp, and it has been suggested that the haplotype of three specific SNPs (1236C > T in exon 12; 3435C > T in exon 26 and SNP 2677G > (T, A) in exon 21) are more predictive of phenotype (i.e., reduced transport activity) than the individual SNP genotype.

A 3435C > T mutation rs1045642 linked to one of the other mutations has been found to result in a changed protein folding, which can change substrate binding[17]. However, the robustness of *ABCB1* genotype/phenotype association, has not yet been established despite many studies e.g. no direct influence has been found of the effect of MDR1 C3435T polymorphism on digoxin pharmacokinetics [18]. This greatly limits, to date, the use of *ABCB1* in PK/PGx.

3 PK/PGx Concepts in Distribution

Following administration, a drug is distributed into all of the body compartments and tissues that it is able to enter taking into account physical-chemical properties. The drug is said to distribute into an imaginary volume, called its volume of distribution, or Vd. This volume is imaginary because it is based on sampling drug concentrations in some reference fluid (usually serum or plasma) immediately after dosing, with the assumption that the entire dose of drug is uniformly distributed throughout the body. For drugs which partition into lipids, e.g. general anaesthetics,

plasma concentrations immediately after dosing will be quite low and the volume of distribution may appear to be many times larger than the volume of an average human being. Vd is essential for understanding where the drug goes and for estimating key parameters such as dose [12].

Vd is normally understood to be dependent on physiological parameters such as body mass index and fat deposits which may not have immediate PGx relations. However Vd may be PGx dependent in that distribution to certain body compartments, such as the brain across the blood brain barrier (BBB) and breast milk, may be dependent on transporters dependent on *ABC* genes, as outlined above. Overexpression of these *ABC* genes in certain patients may lead to drug efflux and what is clinically described as drug resistance [12].

Some studies suggest that the *ABCB1* variant 3435C>T rs1045642 affects plasma drug levels and drug resistance for drugs such as phenytoin, by-inhibiting transport [19]. In a study of British persons with epilepsy, the rs1045642 CC genotype was associated with drug resistance. In addition, a study of Egyptian persons with epilepsy showed increased likelihood of resistance to phenytoin in C allele carriers [20]. However, a meta-analysis failed to replicate the association with rs1045642, although many of these studies comprised patients on a variety of AEDs rather than phenytoin alone [21].

Also for the *SLCO1B1* gene, encoding OATP1B1, polymorphisms were demonstrated to result in clinically relevant effects on the pharmacokinetics, and more specifically the distribution, and adverse effects of some drugs, mainly statins. The *SLCO1B1**15 variant, 521T>C (Val174Ala) rs4149056 significantly affects the pharmacokinetics and adverse effects of many statins, and in a GWAS study indeed appeared to be associated with simvastatin-induced myopathy in patients treated for hypercholesteraemia. The prevalence of the *SLCO1B1**15 allele in the Caucasian population is 18% [11, 22].

Another key pharmacokinetic parameter in PK distribution is related to protein binding. Most drugs bind to plasma proteins to some extent and it may play a significant role in pharmacokinetics if it exceeds 80% (e.g. warfarin or phenytoin) as it is only the free (non-bound) drug which can exert a therapeutic effect. However to date while protein binding is an important pharmacokinetic parameter, at present no examples exists which point at PG affecting protein binding [15].

4 PK/PGx Concepts in Metabolism

There are over 170 genes known or expected to have a role in drug disposition, with more than half known to be polymorphic [23]. In pharmacokinetics, the highest level of polymorphism is found in genes involved in drug metabolism, especially cytochrome (CYP) P450 enzymes. These, in fact, account for over 80% of current PGx drug labelling requirements.

Several CYPs have been shown to be polymorphic as a consequence of single nucleotide polymorphisms (SNPs), gene deletions and gene duplications. Perhaps

the most studied is CYP2D6, which is involved in metabolism of approximately 100 drugs. More than 80 variants of CYP2D6 have been identified (http://www.cypalleles.ki.se), resulting in CYP enzymes with varying activities [24].

Examples abound in the literature of studies carried out on CYP2D6. For example it is responsible for the metabolism of codeine to the active metabolite, morphine. Thus the pharmacological activity of codeine is regulated by *CYP2D6* polymorphisms. In fact codeine has little therapeutic effect in patients who are CYP2D6 poor metabolisers, whereas due to excessive prodrug activation, CYP2D6 ultrarapid metabolisers suffer from adverse events due to increased levels of active metabolites. Thus *CYP2D6* genotype test results can be used to guide the dosing of codeine [25], and recently, information regarding the consequences of *CYP2D6* polymorphism have been included in the labelling of codeine.

However, even within the same drug class, genotype does not always predict drug metabolism. As an example, CYP2C19 is important in the metabolism of drugs such as protein pump inhibitors and an apparent gene–dose effect has been shown for the *CYP2C19**17 allele for pantoprazole, which predicts the plasma elimination rate constant, but this was not found for omeprazole. This difference could be to the difference in the contributions of CYP2C19 and CYP3A4 in the respective metabolism of the two drugs [26]. In fact, dependence on CYP2C19 metabolism is now seen by some as an undesirable property for NCEs in developments [6].

Polymorphisms in *CYP2D6* and *CYP2C19* have also been found to impact the metabolism of tricyclic antidepressents, such as amitriptyline and imipramine. These are demethylated by CYP2C19 to pharmacologically active metabolites, but then undergo further hydroxylation by CYP2D6 to less active metabolites. Thus polymorphisms in *CYP2D6* and *CYP2C19* may change the drug clearance or the ratio of parent drug to metabolites and dose adjustments can be estimated from the metaboliser status [27].

In oncology, the use of tamoxifen has for long been a mainstay in the adjuvant treatment of oestrogen receptor-positive breast cancer. Activity of tamoxifen is generally acknowledged to be mediated by the active metabolite endoxifen, which formation is catalysed by CYP2D6 [28–30]. Though the formation of endoxifen in CYP2D6 poor metaboliser patients is shown to be reduced, the consequences of the polymorphic status of *CYP2D6* for the success rate of tamoxifen treatment in relation to breast cancer recurrence or survival is not settled yet. In most cases these important clinical parameters have been investigated in fairly small studies, with only a small proportion of the known *CYP2D6* polymorphisms taken into account, whereas in some cases tumoral *CYP2D6* variations were assessed instead of germline variations, leading to a lack of Hardy–Weinberg equilibrium [31]. Though the totality of data are suggestive for a relationship between breast cancer recurrence and *CYP2D6* polymorphic status, more confirmative studies are needed, in particular with respect to the relationship between *CYP2D6* polymorphism and survival.

For clopidogrel, being a prodrug needing activation to an active metabolite mediated by CYP2C19, the efficacy may vary depending on the presence of specific functional allelic variants in patients. The conversion of the clopidogrel prodrug to active drug is strongly reduced in about 20 % of Asian patients being CYP2C19

poor metaboliser. This reduced metabolism results in less anti-coagulation and less protection against cardiovascular events [32, 33].

On the other hand, *CYP2C9* has two common variant alleles (*2 and *3); which, unlike CYP2D6 and CYP2C19, retain enzymic activity albeit at a reduced rate [19]. Thus *CYP2C9* polymorphisms only have a minimal impact on pharmacokinetics and thus generally no significant effect on therapeutic outcome. However, in the case of a drug with a narrow therapeutic index, such as warfarin, *CYP2C9* genotype has been shown to correlate with the titrated dose in a population of 200 patients [34]. It was found that the highest titrated dose, was in patients homozygous for the wild-type *1 allele, which has the highest activity, whilst the lowest titrated dose was in patients with *3 homozygotes, which have the lowest enzyme activity.

There may also be key ethnic differences in these CYP variants. For phenytoin, *CYP2C9**3 (rs1057910 A>C) is associated with decreased metabolism of this drug. However, the *CYP2C9**2 variant was found to be associated with decreased metabolism in patients with epilepsy, but not with phenytoin dose in a study of white persons with epilepsy. There are different *CYP2C9* variants (*CYP2C9**5, *6, *8 and *11) in black populations, which are linked with a decreased phenytoin metabolism. In Asian Indians, increased free phenytoin was found in *CYP2C9**3 carriers which led to an increased risk for concentration-dependent toxicity compared with *1 homozygotes [19].

Furthermore, in white populations, the frequency of carriers of the wild-type *CYP3A5**1 allele (showing CYP3A5 activity) is only about 15 %, whereas it is up to 50 and 90 % in Asians and Blacks, respectively [35]. In a meta-analysis [36] a clear effect of CYP3A5 on rejection rates was indeed concluded after the first month of the treatment with the immunosuppressant tacrolimus.

Hundreds of studies were carried out in this area in recent years. This has led to pharmaceutical companies screening out compounds, in drug development, to assess whether they are substrates solely for a known polymorphic enzyme in order to avoid the wider intersubject variability in exposure [26]. However, by doing this, it may be argued that one may end up relatively often with drugs in development which may be substrates for less studied genetic polymorphisms. Therefore, the EMA advocates that the involvement of known polymorphic enzymes and transporters should not prohibit further development of the drug, but instead should be taken into account during this clinical development, in order to provide satisfactory efficacy and safety in genetic subpopulations that have variable systemic exposure of active [37].

5 PK/PGx Concepts in Elimination and Clearance

Immediately after a dose of drug is administered, the body begins to eliminate or clear it. Most drug elimination follows first-order kinetics. That is, a constant fraction of drug is eliminated from the body during each unit of time and it assumes the

drug is uniformly distributed in a single body compartment with most of the drug eliminated from the body after four or five half-lives [12].

Clearance describes the rate at which the drug is eliminated from its volume of distribution, and its units are volume/time. Another important noncompartmental PK term is AUC, the area under the concentration-time curve. This term can be used to calculate overall clearance and half-life values for a drug. In addition, AUC is frequently used to compare drug exposures achieved with different drug doses, or to compare pharmacokinetics in the presence or absence of a drug with the potential to produce a PK drug interaction. It is to be pointed that drug systematic clearance is a summation of all the various organ clearances such as hepatic clearance renal clearance, salivary clearance, biliary clearance.

Renal clearance can be influenced by PGx differences especially for drugs which are eliminated mostly unchanged in urine. One such example is memantine, a frequently prescribed anti-dementia drug, which is mainly eliminated unchanged by the kidneys, partly via tubular secretion. Considerable inter-individual variability in plasma concentrations has been reported. A population pharmacokinetic study was performed in 108 patients who were genotyped for common polymorphisms in renal cation transporters (SLC22A1/2/5, SLC47A1, ABCB1). A SNP in NR1I2 (encoding the pregnane X receptor PXR) rs1523130 was identified as the unique significant genetic covariate for memantine clearance ($p = 0.006$), with carriers of the NR1I2 rs1523130 CT/TT genotypes presenting a 16% slower memantine elimination than carriers of the CC genotype [38].

6 PK and PGx in dose prediction

As has been described in the previous sections, several genetic polymorphisms have been identified in drug targets, drug-metabolising enzymes and drug transporters. Thus individual patients could theoretically be screened for specific polymorphisms, effectively acting as biomarkers, facilitating more specific and individualised choice of drug and dose (see Fig. 2). This strategy may enable therapeutic concentrations to be attained more quickly. However, it should be kept in mind that for a PGx test to be useful, the genotype must have a major influence on the PK/PD of a drug with a narrow therapeutic index.

As discussed in previous sections, genetic polymorphisms can result in changes to functional activity and PK through changes in expression of enzymes such as CYP3A5, UGT1A3, UGT2B17 and CYP2D6. This may result in important changes in clinical outcome which need to be noted.

Such genetic tests may be particularly useful in certain patients, such as renal transplant patients where PK/PD are hard to predict. PGx can be used to reduce the wide interindividual variation in the dose of immunosuppressive drugs required to achieve target blood concentrations, since PGx can be used to predict metabolism of these drugs, improving graft outcome. Several clinically useful strategies have

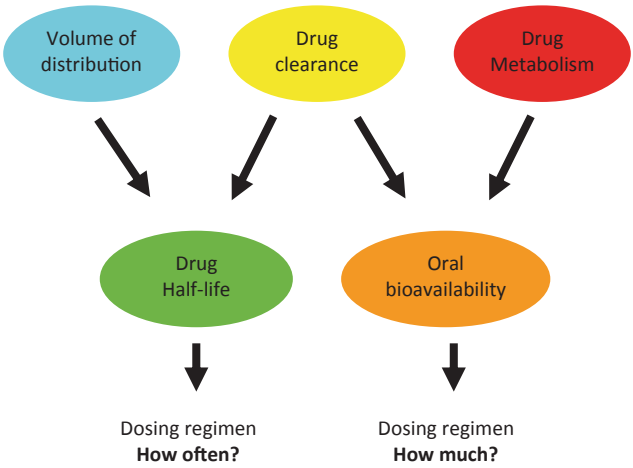

Fig. 2 The key pharmacokinetic parameters and their importance for designing dose regimen and dose size

emerged such as the use of the cytochrome P450 (CYP)3A5 (*CYP3A5*) genotype to predict the optimal initial dose for the immunosuppressant, tacrolimus [36, 39].

Warfarin has a narrow therapeutic index and a high dose variability. Thirty percent of this variance can be explained by SNPs in the warfarin drug target *VKORC1* and 12% by two non-synonymous SNPs (*2, *3) in CYP2C9. Affected individuals require, on average, lower doses of warfarin to maintain a therapeutic INR and more time to achieve stable dosing. A PK/PD model for warfarin, with *CYP2C9* and *VKORC1* genotype, age and target international normalised ratio (INR) as dose predictors has been developed [40]. Such a dosing algorithm may yield a more rapid dosing at the appropriate level, which is expected to reduce mortality of warfarin treatment. The actual effect of genotype-based dosing of warfarin during the initiation of therapy in patients with atrial fibrillation or venous thromboembolism has recently been tested in prospective clinical trials. In these cases warfarin was either prescribed according to a *CYP2C9*, *VKORC1* based dosing algorithm or the standard dosing regimen. The percentage of time that patients were in the therapeutic range for the international normalised ratio (INR) during the first weeks after warfarin initiation was measured. Results of these prospective studies however were not consistent, with some studies showing that genotype guided dosing was associated with a higher percentage of time in the therapeutic INR range than was standard dosing, whereas other reported that genotype-guided dosing of warfarin did not improve anticoagulation control during the first weeks of therapy during [41, 42]. Currently, EU drug regulatory agencies do not require genotyping before initiation of warfarin therapy, however, the warfarin drug label in the USA (Coumadin, FDA) [43] presents dosing information on the combined *VKORC1* and *CYP2C9* status that should be considered if this genotype is known prior to treatment.

7 What is the Role of PK/PGx Relationship in the Development of New Drugs?

As a result of the large output of high through put screening (HTS) in the evaluation of a new chemical entities (NCE), PK/PD relationships are established early on the drug development. PK/PD data are also used in the evaluation of preclinical studies and in the prediction of these parameters in actual patients. This optimises drug screening and reduces the risk of late stage attrition due to poor pharmacokinetics (see Fig. 3).

In fact, *in vitro* screening of a broad panel of *in vitro* metabolic or transport pathway evaluations for NCEs generally is determined early in preclinical evaluations. Such PK/PGx evaluations may trigger subsequent clinical PG-related investigations, e.g. by the inclusion of various PG variant patients in the clinical studies, in order to obtain an appropriate dose advice for the different important phenotypes for a certain polymorphic enzyme shown to be important in the pharmacokinetics of the drug. The PK/PGx evaluations are also important in the evaluation of drug interactions and dosing paradigms for desirable agents [4].

Recently the characterisation and development of pharmacophore template model for many CYPs such as for the active site of CYP2D6 has taken large steps forward. For example, this model has in fact been used in drug development of a novel

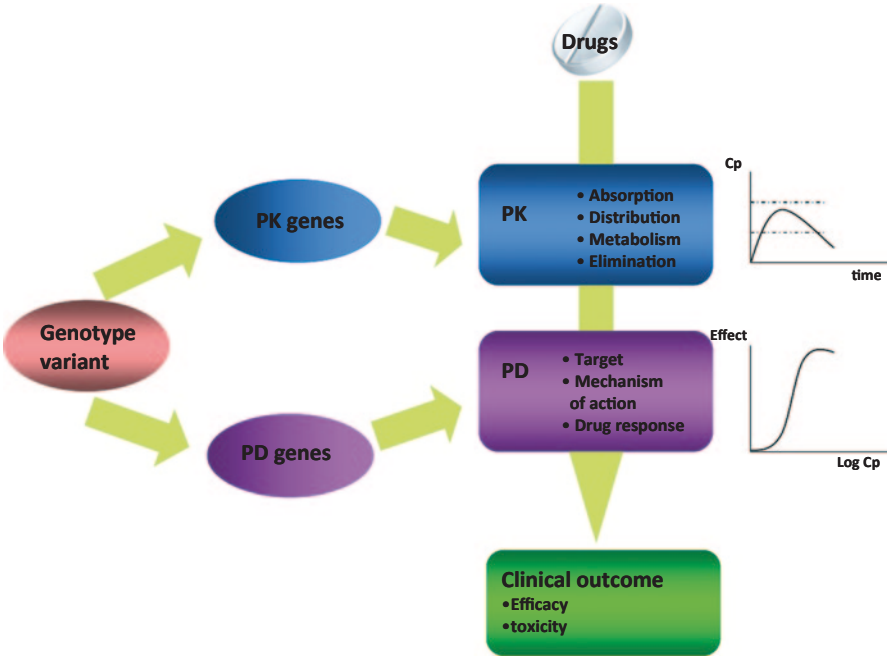

Fig. 3 Correlation of PK/PD and PGx in clinical outcome. By including PGx regarding PK and PD genes, PK and PD, and thus efficacy and safety of the drug may be optimised

calcium channel antagonist, together with in vitro data. These models indicated that the metabolism of this NCE was dependent on CYP2D6, and thus volunteers in the first in human study were genotyped for *CYP2D6*. Two volunteers were identified as poor metabolisers for CYP2D6 and were included in the study. The resulting PK data showed that the half-life of the drug was much higher in these individuals and it was decided to stop further development of the compound [6]. The current situation is that this is not considered a desirable approach anymore. Instead, a drug should be developed taking into account the various subpopulations. Indeed, stopping development because CYP2D6 is involved may seem wise on the short term, but on the longer term it may lead to the discovery that other, as yet unidentified polymorphic enzymes and transporters are important, which may appear only later in drug development, and was not anticipated. In that case, one is better off with a very well known polymorphic enzyme, to take into account just from the beginning of the development programme.

Go/no go decisions using such information from polymorphic enzymes, involves several approaches, many of them involving complex databases, drug–disease–trial models and simulation for the integration of information. Physiology-based pharmacokinetic (PBPK) models have also been developed which provide a very useful mechanistic approach to drug development. This plethora of information may result in a number of conflicting information which may determine issues such as when it may be best to stop the development especially if there are back ups without the potential issue? While data can be generated on different doses, this greatly confounds the drug development program. In addition, the decision to terminate often considers multiple aspects such as the known frequency of polymorphism, the fraction metabolised through pathway, regulatory and commercial pressures, therapeutic window of the drug, the indication and unmet medical needs, alternative current treatment options.

Moreover even if 2D6 is identified, other polymorphic pathways may play key roles in the development of that compound. It may be valid for some time that it would be best to develop a drug with a well known polymorphism but this can only be done in conjunction with all the other factors as discussed in previous sections.

8 PK/PGx Concepts in Drug Regulatory Guidelines

Drug regulatory authorities, such as the Food and Drug Administration (FDA) through the *Interdisciplinary Pharmacogenomics Review Group IPRG* and European Medicines Agency (EMA) through the *Pharmacogenomics Working Party PGWP* have for some time been establishing guidelines for submission of pharmacogenetic data on therapeutic drugs to assist in the tailoring of drug therapy to individual patients.

These agencies now request PK/PGx information in the labelling for several drugs. For example, in 2007, FDA (but to date not EMA) issued a labelling change advising physicians to consider the use of “genetic tests to improve their initial

estimate" of the dose of anticoagulant drug warfarin, which is widely prescribed for reducing the risk of thrombosis and its complications [44]. This recommendation has set a precedent for the use of genetic technologies in clinical practice and now several bodies are pushing for such novel technology to enhance personalised medicine. Likewise, recently, information regarding the consequences of *CYP2D6* polymorphism have been included in the labelling of codeine. Presently, there are over 70 licensed drugs with PGx labels, where the polymorphic *CYP2C9, CYP2C19* and *CYP2D6* account for the majority of these labels. In fact IPRG and PGWP now liaise together with industry for combined VGDS—Voluntary Genomic Data Submissions/Pharmacogenomic briefing meetings in order to streamline regulatory requests in this fast developing area.

Further, the EMA PGWP published a guideline on the role of pharmacogenetics in PK in 2012 [37] soon followed by a guidance on this topic by the FDA (Guidance on Clinical Pharmacogenomics: Premarketing Evaluation in Early Phase Clinical Studies) [44]. These guidelines request the drug developers to identify those pharmacogenomic factors that may affect safety and/or efficacy of drugs that are currently being developed. For that purpose, the consequences of pharmacogenomic variation should be investigated if *in vitro* and/or clinical (*in vivo*) studies indicate that a known functionally polymorphic enzyme or drug transporter is likely to be important in the disposition of the drug, or if these represent an important factor in the formation, elimination or distribution of a pharmacologically active or toxic metabolite. Pharmacogenomic investigations are also required when clinical studies indicate that major interindividual differences in the pharmacokinetic properties (that cannot be explained by other intrinsic or extrinsic factors) are likely to influence the efficacy or safety of the drug in a genetically variable subpopulation. When looking at a global level, there appears broad agreement on the requirements with respect to pharmacogenomics related to pharmacokinetics in the EU, USA and Japan, though some divergence still exists on some areas, like the actual cut-off which would trigger the need for *in vivo* pharmacogenomic investigations, and the stringency by which banking of DNA samples from ongoing clinical studies is required [45]. Overall, however, it is clear that in the future, for new drugs, more pharmacogenomic data is expected to become available which will enable appropriate dosing in e.g. patients with a different metaboliser status, than has been in the past.

9 Outlook and Recommendations

The better understanding of pharmacogenetics on PK/PD inter-individual variability of drug disposition might be beneficial in the context of individual dose optimisation in personalised medicine. The greatest understanding has been in that of metabolic phenotyping especially of metabolising enzymes. The application of PGx to predict other PK processes and thus dosage regimens depends, however, on various other cofounding factors such as disease and co-administered drugs which limits the feasibility of clinical applications to date. This will determine how much

a genetic variant contributes to a clinically significant pharmacokinetic variability overall.

There is now a growing recognition that the future of the pharmaceutical industry will depend a great deal on the integration of PGx and PK/PD data to guide drug decision-making. Novel PGx biomarkers are important to fill in gaps of uncertainty about therapeutic targets, variability in drug response; algorithm-based dose determination; response monitoring; early indicator/predictor of toxicity/adverse reactions. The debate still remains on the adoption of PGx assays in clinical examinations and the implications of reimbursement. Recent data on warfarin and clopidogrel have identified barriers to successful implementation [46]. The data available for such technology varies a great deal but used with agreed clinical guidelines, appears to be the strongest predictor of reimbursement. However bringing better clinical evidence is needed.

It important to note, however, that other factors can also influence PK/PD processes which may impact the predictability of outcomes in these patients, such as co morbid medical conditions, smoking, diet, drug interactions, race and frailty.

Integrating PK/PD with PGx can be our magic ball in the determination of drug doses, dosing intervals, titration regimens in order to decrease the risk of drug adverse events and toxicity and ensure successful outcomes in patients. Further knowledge is likely to add to our understanding of differences in sub-populations, but the potential limitations of these approaches should be recognised in order that they can be applied beneficially [6].

The availability of open access on-line resources such as PharmGKB, the pharmacogenomics database, and simulation models such as SIMCYP® have greatly facilitated the availability of resources which systematically assess the vast information now available on the impact of genetic variation on drug response for clinicians and researchers [47].

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Pharmacogenetics of Adverse Drug Reactions

Richard Myles Turner and Munir Pirmohamed

Abstract A large variation in drug response exists between patients, with susceptible individuals being at risk of experiencing an adverse drug reaction (ADR). This susceptibility is attributable to environmental, clinical and genetic factors although the contribution of each varies with the drug, ADR and ethnicity. The variation in drug response makes personalisation of pharmacological therapy appealing to minimise ADRs whilst promoting efficacy. Pharmacogenetics seeks to contribute through genetic-guided drug and dose selection strategies. ADR pharmacogenetics was first highlighted in the 1950s, but it is only in the last decade that it has seen a rapid expansion, aided by significant advances in our knowledge of the human genome and improved genotyping technologies. ADRs can be classified according to whether the dominant mechanism is immune- or nonimmune-mediated. Several ADRs have been strongly associated with specific human leukocyte antigen (*HLA*) alleles. There is growing evidence for a central role of these alleles in the pathogenesis of immune-mediated delayed hypersensitivity ADRs through facilitation of 'off-target' interactions that lead to the presentation of 'altered self,' drugs and/or their metabolites to the T-cell receptor in an HLA-restricted fashion. Genetic variation can also predispose to nonimmune-mediated ADRs through perturbing drug pharmacokinetics or by altering nonimmune pharmacodynamic processes. In particular, genetic variants of phase I and phase II biotransformation enzymes and drug transporters alter the availability of a drug at the site(s) responsible for the ADR. Depending on the drug and ADR, these sites may be the therapeutic target site, the same molecular site in another tissue or distinct off-target sites. A prominent example of pharmacogenetics improving drug safety and enhancing the cost-effective use of limited healthcare resources is the reduction in the incidence of the abacavir hypersensitivity syndrome. It is apparent though that the success of ameliorating the abacavir hypersensitivity syndrome by genetic screening is proving difficult to emulate for other drug-ADR combinations. This highlights the considerable hurdles

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encountered in translating a pharmacogenetic association into a clinical test that benefits patient safety. The development of international consortia alongside the potential of next generation sequencing technologies and other innovations offer tantalising prospects for future advances in pharmacogenetics to reduce the burden of ADRs.

Keywords Adverse drug reaction · Pharmacogenetics · Predictive genotyping · Translation · Abacavir · Hypersensitivity · Malignant hyperthermia · Codeine · Warfarin · Statin

1 Introduction

For many drugs, substantial evidence exists at the population level to advocate their use. However there is considerable inter-individual variability in drug response, affecting both drug efficacy and safety [1]. Over 961.5 million prescription items were dispensed in England in 2011 [2]. This high drug usage and the individuality of drug response contribute to the high frequency of adverse drug reactions (ADRs). A prospective study in England estimated that 6.5 % of hospital admissions for patients >16 years old were related to an ADR, with a median inpatient stay of 8 days [3]. This was contextualised through extrapolation to the entire hospital bed base of England to suggest that the equivalent of up to seven 800 bed hospitals in England could be occupied at any one time with patients admitted with ADRs [3]. Studies from other countries have reported similar ADR-related hospitalisation rates [4–8]. Clearly, ADRs pose a significant international challenge to the health and safety of individual patients and to the efficient use of limited resources by healthcare services.

An ADR, as defined by the World Health Organisation (WHO), is ‘a response to a drug that is noxious and unintended and occurs at doses normally used in man for prophylaxis, diagnosis or therapy of disease or for the modification of physiologic function’ [9]. Table 1 provides definitions and examples of the related pharmacovigilance terms [9–12]. In essence, an adverse event (AE) is an umbrella term for any harm occurring to a patient temporally associated with but not necessarily directly attributable to a therapeutic intervention [10, 13]. A subdivision of AE is an adverse drug event, which describes maleficence associated with the use of a drug and includes overdoses, medication error and ADRs [11, 14, 15].

There is considerable variability between ADRs in terms of presentation and level of current aetiological understanding. This poses a challenge to their accurate categorisation and so, different classifications have been developed. The most well-known system delineates ADRs into types A and B. Type A (‘augmented’) ADRs constitute over 80 % of ADRs; they are dose-dependent and predictable from the main pharmacological action of a drug [13, 16]. This is because Type A ADRs are ‘on-target’ and manifest through excessive drug action at the therapeutic target site. Type B (‘bizarre’) ADRs are dose-independent and are not predictable from a drug’s conventional pharmacology [13, 16] as they represent idiosyncratic ‘off-target’ drug

Table 1 Pharmacovigilance terminology for adverse effects

Adverse effect term	Definition	Example(s)
Adverse event	Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment [10].	In a clinical trial for a topical emollient for psoriasis a road traffic accident could be a serious, unexpected, not study related adverse event
Adverse drug event	An injury resulting from the use of a drug [11].	i) Intentional overdose
		ii) Medication error
		iii) Adverse drug reaction
Medication error	A medication error is any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the health care professional, patient, or consumer [12].	Decrease in consciousness following accidental insulin overdose due to a prescribing or administration error
Adverse drug reaction	A response to a drug that is noxious and unintended and occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for the modification of physiologic function [9].	Hypersensitivity reaction to allopurinol through standard clinical use of the drug

effects. This classification was first defined in 1977 [17] and has been variously extended subsequently to include additional categories as shown in Table 2 [13, 18]. However, it can prove difficult to categorise ADRs using this system. For example, some type B ADRs, including statin-induced muscle toxicity, are clearly dose related and other type B ADRs, such as hypersensitivity to abacavir, are now predictable. A second system is the DoTS classification, which categorises ADRs according to dose relatedness, timing and patient susceptibility factors [19]. This descriptive system improves the accuracy of ADR classification, but its complexity makes it more difficult to use.

In this chapter, ADRs will be classified as immune- or nonimmune-mediated. Immune-mediated ADRs result principally from a deleterious immune reaction mounted following drug exposure. Nonimmune-mediated ADRs encompass all other ADRs and as a point of clarification, include infections that result from predictable immunosuppression by biologics and disease-modifying agents. This is a simple classification, but it reflects the clinical presentation and predominant pathogenic processes of many ADRs and is helpful when considering pharmacogenetics.

The reasons for the heterogeneity in inter-individual drug response are often not known but there are a trilogy of implicated factors: environmental (e.g. drug-drug and drug-food interactions), clinical (e.g. age, co-morbidities, body mass index (BMI), pregnancy) and genetic [20]. The contribution of each postulated factor

Table 2 Adverse drug reaction pharmacovigilance classification and characteristics [13, 18]

ADR classification	Characteristics	Example
Major types		
A	*Augmented*	Hypotension with iloprost therapy
	Due to main pharmacological action of a drug	
	Common	
	Dose-related	
	Predictable from conventional pharmacology	
	Severity variable, but usually mild	
B	*Bizarre*	Achilles tendonitis with quinolone therapy
	Associated with off-target drug effects	
	Uncommon	
	No clear dose relationship	
	Unpredictable from conventional pharmacology	
	Variable severity; proportionately more serious than type A	
Supplemental Types		
C	*Continuing*; time-related; ADR persistence for a long duration	Osteonecrosis of the jaw with bisphosphonate therapy
D	*Delayed*; time-related; ADR of slow onset	Tardive dyskinesia with antipsychotic therapy
E	*End-of-treatment*; associated with dose reduction or therapy discontinuation	Benzodiazepine withdrawal syndrome after abrupt drug cessation
F	*Failure of therapy*; inadequate therapeutic drug action so it does not achieve its intended purpose	Ischaemic stroke second to atrial fibrillation whilst on warfarin

likely varies with the drug, ADR and patient ethnicity [21]. A genetic basis for specific ADRs was first suggested in the 1950s when perturbed drug metabolism was associated with abnormal drug responses, such as butyrylcholinesterase deficiency and prolonged apnoea after succinylcholine administration. Over the last decade there has been a rapid growth in our understanding of ADR pharmacogenetics. This has been facilitated by an increased knowledge of the human genome and its variation through the Human Genome Project and HapMap Projects. Further, advances in genetic technologies and a reduction in processing costs have increased the volume of pharmacogenetic research conducted and its capacity to yield associations. At present, disproportionately more is known about associations of strong individual effect size with specific ADRs. However for some ADRs it may be that

the genetic contribution is polygenic with distinct loci of individual low effect size collectively contributing. Despite the advances of the last decade, elucidation of potential complex interplays within and between different biological systems is proving challenging.

The rest of this chapter explores further the pharmacogenetics of immune- and nonimmune-mediated ADRs. A discussion about all known genetic associations is beyond the scope of this chapter and so a range of genetic associations with distinct ADRs have been selected, although the tables included provide additional examples. The selected associations facilitate expansion on the following key themes:

- the effect of specific genetic mutations on protein function,
- the variable extent of genetic contribution to ADRs,
- the pathogenesis of ADRs,
- the clinical application of specific genetic-ADR associations through predictive genotyping and
- the current variable evidence base supporting their use.

Lastly, the many challenges faced by pharmacogenetics in translating an observed genetic-ADR association from the 'bench' to the 'bedside' will be highlighted and contemporary strategies and future possibilities to overcome these obstacles and deepen our understanding of pharmacogenetics will be outlined.

2 Immune-Mediated Adverse Drug Reactions

Immune-mediated ADRs are off-target ADRs and more specifically, represent a form of hypersensitivity reaction. Hypersensitivity reactions can be classified according to the Gell and Coombs system into types I-IV representing IgE-mediated allergic reactions (type I), direct antibody-mediated (type II), immune complex-mediated (type III) and delayed-type hypersensitivity (DTH) reactions (type IV). At the time of writing, comparatively less is known about the pharmacogenetics of type I-III hypersensitivity reactions and therefore this section will concentrate on DTH reactions.

Over the last decade, the increasing use of genome-wide association studies (GWAS) in pharmacogenetic research has identified a growing number of ADRs that are strongly associated with specific human leukocyte antigen (*HLA*) haplotypes, genes and/or alleles. Table 3 provides an overview of *HLA*-ADR associations [22–59].

The *HLA* class I and II genes, located on chromosome 6, are the most polymorphic of the human genome and over 7000 classical alleles have been identified between them [60]. There is strong linkage disequilibrium between the alleles [61]. Classical HLA class I molecules (encoded on 3 loci: *HLA-A, -B, -C*) are expressed on the surface of most nucleated cells and present peptide antigen to the T-cell receptor (TCR) of CD8+ T-cells [62]. The peptides presented by HLA class I molecules are mostly derived from the degradation of intracellular proteins, although

Table 3 Examples of *HLA* associations to hypersensitivity adverse drug reactions

Reaction	Drug	HLA- association(s)	Reference(s)
Hypersensitivity syndrome/DRESS/DIHS	Abacavir[a]	*B*57:01*	[22, 23]
	Allopurinol[a]	*B*58:01*	[24, 25]
	Carbamazepine	*A*31:01*	[26, 27]
	Nevirapine	*C*08:02-B*14:02* (*Italian*), *C*08* (*Japanese*), *B*35:05* (*Thai*)	[28–30]
Stevens-Johnson syndrome/Toxic epidermal necrolysis	Allopurinol[a]	*B*58:01*	[24, 31]
	Carbamazepine[a]	*B*15:02*[a], *A*31:01*	[32–34]
	Lamotrigine	*B*38*	[35]
	Methazolamide	*B*59:01*	[36]
	Nevirapine	*C*04:01 (Malawian)*	[37]
	Oxicam NSAIDs	*B*73:01*	[35]
	Phenytoin	*B*15:02*	[33, 38]
	Sulfamethoxazole	*B*38*	[35]
Delayed exanthem without systemic features	Allopurinol	*B*58:01* (*Han Chinese*)	[39]
	Aminopenicillins	*A2, DRw52*	[40]
	Carbamazepine	*A*31:01*	[27, 41]
	Nevirapine	*DRB1*01:01* (*French*) *B*35:05* (*Thai*) *C*04* (*Thai*)	[42] [30] [43]
Drug-induced liver injury	Antituberculosis drug therapy	*DQB1*02:01*	[44]
	Co-amoxiclav	*DRB1*15:01-DQB1*06:02, A*02:01*	[45, 46]
	Flucloxacillin	*B*57:01*	[47]
	Lapatinib	*DQA1*02:01*	[48]
	Lumiracoxib	*DQA1*01:02*	[49]
	Nevirapine	*DRB1*01*	[50]
	Ticlopidine	*A*33:03* *A*33:03* with *CYP2B6*1H or *1J*[b]	[51, 52]
	Ximelagatran	*DRB1*07, DQA1*02*	[53]
Agranulocytosis	Clozapine	*DQB1* 6672G > C	[54]
	Levamisole	*B*27*	[55]
Asthma	Aspirin	*DPB1*03:01*	[56, 57]
Pneumonitis	Gold	*B*40, DRB1*01*	[58]
Proteinuria, Thrombocytopaenia	Gold	*DRB1*03*	[59]
Urticaria	Aspirin	*DRB1*13:02-DQB1*06:09*	[56]

DRESS drug reaction with eosinophilia and systemic symptoms, *DIHS* drug-induced hypersensitivity syndrome, *NSAID* non-steroidal anti-inflammatory drug

[a] odds ratio > 50 and reproduced in > 1 study. Adapted from Phillips et al. [78]

[b] *CYP2B6* is not an *HLA* gene

HLA class I molecules on specific dendritic cell subsets are additionally capable of presenting extracellular peptides through 'cross-presentation' [63]. Classical HLA class II molecule expression (encoded on 3 loci: *HLA-DP, -DQ, -DR*) is restricted to professional antigen-presenting cells (e.g. dendritic cells, macrophages, B-cells) and they present extracellular-derived peptides to the TCR of CD4+ T-cells [62]. *HLA* polymorphisms localise to the sequence motifs that encode residues of the peptide-binding groove [60, 64]. These polymorphisms alter the stereochemistry of pockets within the groove, creating individual HLA allotypes with distinct peptide-binding portfolios [62, 65]. The HLA system is integral to the development of T-cell tolerance to 'self' and to the development of adaptive immunity in response to 'non-self' peptide. *HLA* incompatibility is also known to be important in the pathogenesis of allogeneic transplant rejection and several HLA associations have been previously reported for autoimmune diseases including ankylosing spondylitis (with *HLA-B27*) and rheumatoid arthritis (e.g. with *HLA-DRB1* alleles [66]).

Most of the hypersensitivity ADRs with *HLA* associations, including the specific reactions to abacavir, carbamazepine, allopurinol and flucloxacillin discussed below, are considered DTH reactions. In keeping with DTH reactions, they normally present $\geq$72 h after drug exposure, may resolve with drug cessation and often re-present more rapidly and with a more severe phenotype following drug re-exposure. A T-cell mediated immunopathogenesis is thought to underlie this temporal pattern. Analogous to the development of pathogen-induced adaptive immune responses, it is thought that a T-cell clone(s) can be primed by presentation of culprit antigen on an HLA molecule during primary drug exposure and effector memory T-cells are rapidly activated with secondary exposure [62, 67, 68]. The isolation of drug-specific T-cells from patients that have suffered DTH ADRs supports T-cell involvement [69, 70].

Two hypotheses have conventionally been proposed to describe potential off-target pharmacodynamic processes that may lead to the neo-antigen formation necessary for DTH drug-specific T-cell development: the hapten (or pro-hapten) model and the pharmacologic interaction with immune-receptors (p-i) model [71]. The hapten model proposes that drugs and their metabolites are too small to be independently immunogenic and so covalently bind to self-protein and the resulting *de novo* hapten-self peptide adduct is antigenic [71, 72]. The p-i hypothesis proposes that drugs may interact directly with HLA molecules, without specific self-peptides, to elicit a T-cell response [73]. Regardless of the mechanism of neo-antigen formation, it is widely assumed that additional 'danger' signals are required to overcome the immune system's default tolerance and permit generation of an adaptive immune response. This concept is referred to as the 'danger hypothesis' [74]. Amongst the other key themes of this chapter, the following ADR examples illustrate how prior understanding of genetic susceptibility can facilitate elucidation of underlying mechanisms of antigen formation and presentation.

2.1 *HLA-B*57:01* and Abacavir Hypersensitivity Syndrome

Abacavir represents the epitome of translational pharmacogenetics as the loop from laboratory observation to improved patient care for the genetic association between *HLA-B*57:01* and the abacavir hypersensitivity syndrome (AHS) has been closed [75]. Abacavir is a nucleoside reverse transcriptase inhibitor indicated to treat HIV and is prescribed as a constituent of highly active antiretroviral treatment (HAART). AHS occurs in 2.3–9% of patients [76] with a median time to onset of 8 days therapy [77]. The clinical diagnostic criteria require ≥2 of: fever, rash, nausea, vomiting, arthralgia, myalgia, headache, lethargy or gastrointestinal symptoms and importantly, onset must occur within 6 weeks of commencing therapy and remit within 72 h of abacavir cessation [76]. Unlike other drug hypersensitivity reactions, the mild to moderate rash is not a consistent feature [67] and eosinophilia is unusual [78]. Although the initial reaction is unpleasant, the significant morbidity and mortality occurs upon rechallenge [67, 78], consistent with a DTH reaction.

In 2002, two groups independently reported an association between AHS and *HLA-B*57:01* [22, 23] and subsequent further observational research confirmed the association [79, 80]. The Prospective Randomised Evaluation of DNA Screening in a Clinical Trial (PREDICT-1) study was a multicentre, double-blind randomised controlled trial (RCT) that demonstrated pre-therapy *HLA-B*57:01* screening significantly decreased the incidence of AHS [77]. Briefly, 1956 patients were enrolled and randomised on a 1:1 basis. The interventional group received pre-therapy *HLA-B*57:01* genotyping and either HAART with abacavir for *HLA-B*57:01* negative patients or HAART without abacavir for *HLA-B*57:01* positive patients. The control group received HAART with abacavir and retrospective *HLA-B*57:01* genotyping from blood samples taken pre-therapy. All participants with clinically diagnosed hypersensitivity reactions underwent skin patch testing for immunological corroboration to improve the specificity for the hypersensitivity phenotype. The study demonstrated that avoiding abacavir in *HLA-B*57:01* positive patients in the prospective screening interventional group eliminated immunologically confirmed hypersensitivity reactions (0 vs. 2.7% in control group, $p < 0.001$) with positive and negative predictive values of 47.9% (PPV) and 100% (NPV), respectively [77]. An estimate of the number needed to screen (NNS) to prevent one case of AHS, given an *HLA-B*57:01* carriage prevalence of 6%, was ~25 [77]. However 84% of participants were Caucasian, limiting generalisation. The Study of Hypersensitivity to Abacavir and Pharmacogenetic Evaluation (SHAPE) was a retrospective case-control study that addressed this and demonstrated that *HLA-B*57:01* has 100% sensitivity for immunologically confirmed AHS in both US White and Black patients [81]. Pharmacoeconomic evaluations have demonstrated a cost effectiveness to pre-prescription *HLA-B*57:01* screening [80, 82, 83]. Observational data from open-screening studies has addressed practical matters of implementation [84–86] and shown genotyping to reduce the frequency of abacavir discontinuation due to clinically suspected as well as true immunological hypersensitivity reactions [85, 87]. This is most likely because the former clinician strategy of over-diagnos-

ing AHS to ensure high sensitivity to avoid AHS maleficence at the expense of lower specificity [78] is no longer required given the exclusivity of the association between *HLA-B*57:01* and AHS. In accordance with the substantial evidence base, the drug label has been updated and clinical guidelines either mandate or strongly recommend prospective screening [76]. To summarise, abacavir represents a pioneering example of ADR translational pharmacogenetics and has charted a course that other genetic-ADR associations might follow from initial observations to a RCT to studies that address generalisation, pharmacoeconomics and applicability in widespread clinical practice.

Identifying the genetic basis for AHS has directly benefitted patient care but until recently, insight into the underlying immunopathogenesis has been limited. However 3 recent independent studies have begun to expose the pharmacodynamic off-target molecular mechanisms [88–90]. Native abacavir can bind non-covalently with exquisite specificity to *HLA-B*57:01* at the base of its peptide-binding groove, extending into the deep F pocket [88, 89]. The specificity for the interaction is accounted for by the F-pocket architecture and in particular residue 116 [88]. In the absence of abacavir, the C-terminus of peptides that bind to the F-pocket of *HLA-B*57:01* have large hydrophobic residues, such as tryptophan and less commonly phenylalanine [89]. In the presence of abacavir, the peptide repertoire of *HLA-B*57:01* shifts, so that around 20–25% of recoverable peptides are novel [88] and have alternative residues including isoleucine or leucine at their C-terminus [88, 90]. In essence, abacavir alters the stereochemistry of *HLA-B*57:01* to create an HLA neo-allotype with a novel peptide portfolio. This model of antigen presentation is distinct from both the hapten and conventional p-i models. It is proposed that T-cells will not have been exposed to the novel range of *HLA-B*57:01*-restricted peptides during thymic maturation and so will lack tolerance. The formation of memory T-cells will lead to systemic AHS that is more deleterious upon abacavir re-exposure. In support of the large peptide shift and subsequent large array of 'altered immunological self', the observed CD8+ T-cell response is polyclonal [65]. Further, the effector T-memory cells from *HLA-B*57:01* positive patients with a clinical history of AHS respond preferentially in the presence of specific peptide and abacavir together rather than to peptide or abacavir alone [89]. This indicates that the memory T-cell response to self-peptide requires abacavir for efficient presentation.

Although the exact intracellular site(s) where abacavir associates with HLA-B*57:01-peptide complexes is currently unclear, there is evidence to suggest the endoplasmic reticulum [65]. However, a minority of T-cell clones *in vitro* appear to react to abacavir too quickly to be explained by *de novo* HLA-B*57:01-novel peptide assembly [91]. This suggests that abacavir may additionally bind to HLA-B*57:01-native peptide complexes already present on the cell surface, possibly distorting their stereochemistry [65]. This mechanism is in keeping with the conventional p-i hypothesis. An inadequately resolved question is why the PPV of *HLA-B*57:01* for AHS is <50% [77]. Postulated mechanisms to account for this include (a) the inter-individual polygenic influence on the novel peptide portfolio itself [89]; and (b) heterologous immunity as a result of pre-existing viral infections, in keeping

with data which show that hypersensitivity reactions are often associated with reactivation of viruses such as Epstein-Barr virus [92].

2.2 *HLA-B*15:02, HLA-A*31:01 and Carbamazepine Hypersensitivity*

Carbamazepine is indicated in the treatment of epilepsy, trigeminal neuralgia and bipolar affective disorder but in up to 10% of patients, it can provoke a cutaneous ADR [93]. Drug-induced skin injury (DISI) encompasses a spectrum of manifestations and can be caused by a diverse range of drugs including anticonvulsants, allopurinol and β-lactam antibiotics [94]; Table 3 lists drugs with known associations between DISI and genetic variants. There exists both inter- and intra-drug heterogeneity in DISI presentation but fortunately most reactions are mild [94]. Standardising phenotypic definitions for serious DISI conditions has been challenging and required an international collaborative approach. Carbamazepine itself can cause DISI ranging from mild maculopapular exanthema (MPE) of increasing severity to the hypersensitivity syndrome (HSS), also referred to as drug reaction with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity syndrome (DIHS) [94], to the distinct Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [95].

HSS/DRESS/DIHS (herein referred to as HSS) and SJS-TEN represent severe cutaneous adverse reactions (SCARs) [96]. HSS is a multisystem disorder that carries a mortality rate of 10% [96]. HSS can be diagnosed by the presence of at least 3 of: cutaneous involvement, internal organ involvement, fever, lymphadenopathy and eosinophilia (and/or atypical lymphocytes) with at least 1 of the first 2 criteria listed being present [94]. The skin manifestation is most commonly an exanthematous eruption and the internal organ involvement includes hepatic dysfunction and interstitial nephritis [94, 97]. SJS and TEN represent different severities along a spectrum of the same disease and are characterised by epidermal detachment involving the skin and mucous membranes with systemic manifestations including fever, intestinal and pulmonary involvement [94, 98]. SJS is diagnosed when epidermal detachment affects ≤10% of the body surface area, TEN is diagnosed when >30% is affected and an overlap syndrome exists for 10–30% epidermal detachment [99]. SJS and TEN have estimated mortality rates of up to 5 and 50%, respectively [98].

Genetic association studies have shown *HLA-B*15:02* to be a susceptibility factor for carbamazepine-induced SJS-TEN in people of Han Chinese descent [32] and certain other Asian ethnicities including Thai, Malaysian and Indian [33, 100–102]; a meta-analysis of studies with Asian patients derived a pooled odds ratio (OR) of 113.4 (95% confidence interval (CI) 51.2–251.0) [34]. A recent open-label prospective study in Taiwan has demonstrated the beneficence of pre-therapy *HLA-B*15:02* screening in Han Chinese patients and reported no cases of SJS-TEN in both the carbamazepine-taking *HLA-B*15:02* negative cohort and *HLA-B*15:02* positive cohort administered alternative medication or advised to continue their pre-

study medication [103]. Due to ethical and sample size considerations, an estimated historical annual incidence of carbamazepine SJS-TEN (0.23%) was used as the comparator rather than a prospective non-screened control group (with appropriate blinding), unlike the PREDICT-1 study for AHS [77]. Furthermore this genetic correlation is both phenotypically restricted to SJS-TEN [34, 41] and ethnically restricted: it has not been reproduced in Europeans [104, 105] or other specific Asian populations including South Koreans [26] and Japanese [106–108]. The reason(s) for the latter are not clear but allelic frequency should be considered since *HLA-B*15:02* is present in 8.6% of the Han-Chinese population but in <1% of Europeans, Koreans and Japanese [26, 109], reducing the power of studies in these populations to detect statistically significant associations [109].

A more recently reported carbamazepine DISI association is with *HLA-A*31:01* and importantly, it is associated with MPE, HSS and SJS-TEN and is present in multiple diverse ethnicities, including Han Chinese [41], Koreans [26], Japanese [107] and Europeans [27]. Interestingly, the allele frequency of *HLA-A*31:01* for these ethnic groups is 1.8, 10.3, 9.1 and 2–5%, respectively [26, 109]. The magnitude of the association though is smaller than with *HLA-B*15:02*, with a meta-analysis pooled OR for *HLA-A*31:01* of 9.5 (95% CI 6.4–13.9) [34]. However, the estimated NNS to prevent one case of carbamazepine DISI with *HLA-A*31:01* is lower and ranges from 47–67 depending on ethnicity. This is in contrast to the estimated NNS of 461 Asian patients with *HLA-B*15:02* to specifically prevent one case of SJS-TEN [34]. This difference in NNS is largely attributable to the higher incidence of ADRs (up to 10% [93]) that are associated with *HLA-A*31:01* compared to *HLA*B15:02* (circa 0.23% [103]), due to the relationship of *HLA-A*31:01* with a broader range of phenotypes. These findings form a credible foundation for a future prospective study to assess the clinical benefit of pre-therapy *HLA-A*31:01* screening.

It has been shown that carbamazepine can non-covalently associate with HLA-B*15:02 molecules and in the presence of carbamazepine, there is a shift in the peptide repertoire of HLA-B*15:02 with a novel preference for smaller residues at the 4th and 6th peptide positions and an increase in hydrophobic residues at several positions [88]. The scale of this peptide shift is smaller (approximately 15%) than for abacavir [88]. This off-target pharmacodynamic effect does resonate with the novel model proposed for abacavir and *HLA-B*57:*01, but overall the mechanisms leading to carbamazepine hypersensitivity ADRs are likely to be more complex. This is because firstly, it has been shown that carbamazepine and its metabolite, carbamazepine-10,11-epoxide, can associate with other structurally related *HLA-B75* family members [110]. Secondly, the availability of a restricted range of T-cell clonotypes with specific TCR rearrangements has been demonstrated to be an important determinant in the pathogenesis of *HLA-B*15:02*-associated carbamazepine-induced SJS-TEN [111]. This indicates that both the TCR repertoire and *HLA* genotype modulate the risk of carbamazepine-induced SJS-TEN and likely explains why some *HLA-B*15:02* carriers tolerate carbamazepine [109]. Further research is still required to understand other complicating observations. These include how two seemingly disparate *HLA* alleles, *HLA-B*15:02* and *HLA-A*31:01*, are linked to

carbamazepine ADRs and furthermore, how *HLA-A*31:01* can be associated with multiple phenotypes.

2.3 HLA-B*58:01 and Allopurinol Hypersensitivity

Allopurinol, an analogue of hypoxanthine, inhibits xanthine oxidase (XO) and is indicated in the management of gout and other hyperuricaemic conditions including tumour lysis syndrome. Although generally well tolerated, ~2–3% of patients suffer mild hypersensitivity reactions including MPE [39, 112] and crucially, ~0.1–0.4% of patients develop HSS or SJS-TEN [112]. The SCARs, HSS and SJS-TEN, normally present within weeks to months of commencing allopurinol but may take considerably longer [112]. Allopurinol is a major cause of SCARs [113, 114] and the combined mortality from allopurinol-induced SCARs approaches 25% [112].

In 2005, a strong genetic association between *HLA-B*58:01* and allopurinol-induced SCARs (HSS/SJS-TEN) was described in the Taiwan Han-Chinese population (OR 580.3, 95% CI 34.4–9780.9); all 51 cases (100%) carried *HLA-B*58:01* in comparison to only 20 of 135 allopurinol-tolerant controls (15%) [24]. This association has been replicated in Thai [31], Korean [25], European [35] and Japanese patients [115], although its magnitude was more modest for the latter 3 ethnic groups, possibly reflecting the lower prevalence of *HLA-B*58:01* in these populations. A meta-analysis has confirmed the association between allopurinol-induced SJS-TEN and *HLA-B*58:01* in both Asian and non-Asian patients compared to allopurinol-tolerant controls (combined OR 96.6, 95% CI 24.5–381.0) [116]. Based on data from the Han Chinese and Thai populations, current estimates for the PPV and NPV for *HLA-B*58:01* are ~1.5 and 100%, respectively [112], although these values will be lower for other ethnic groups. An exciting development is the ongoing prospective study in Taiwan to assess the clinical benefit of pre-therapy genotyping for *HLA-B*58:01* prior to commencing allopurinol [112]. Similarly to the prospective study discussed above for *HLA-B*15:02* screening prior to initiating carbamazepine [103], an estimated historical ADR incidence is being used for the control. At the time of writing, no results from this study have been published.

Interestingly, unlike carbamazepine and *HLA-A*31:01*, it is less clear at the current time whether *HLA-B*58:01* also predisposes to MPE in patients taking allopurinol. A study in Australia demonstrated no association between *HLA-B*58:01* and MPE [117], but a study of Han-Chinese patients in mainland China reported *HLA-B*58:01* as a risk factor for both allopurinol-induced MPE and SCARs [39]. More research into this area is required, but one hypothesis from the available literature is that the risk of MPE with *HLA-B*58:01* may be ethnically-restricted. If this association is confirmed, it will increase the PPV further for the affected ethnic groups and so augment the potential utility of pre-therapy *HLA-B*58:01* screening in these groups to reduce the burden of allopurinol-induced ADRs.

The exact underlying mechanism(s) by which allopurinol, or its long-circulating active metabolite oxypurinol, interact with *HLA-B*58:01* for the generation

of drug-specific T-cells has yet to be elucidated. However, as the PPV of *HLA-B*58:01* for SCARs is low (~1.5%) [112], this alludes to other contributing factors in their pathogenesis. It has long been thought that viruses play a role in drug hypersensitivity and there is increasing recognition that the reactivation of herpes viridae is important in the aetiology of HSS [92, 118]. However, any interaction(s) between allopurinol and/or oxypurinol and viruses is poorly understood. Prior to the discovery of *HLA-B*58:01*, several non-genetic risk factors were espoused including renal dysfunction, higher allopurinol doses, diuretic use and concomitant antibiotic therapy [112]. Although verification of these variables is difficult as SCARs are fortunately rare events, patients on allopurinol with renal insufficiency have been shown to be almost 5 times more likely to develop SCARs [24]. In addition, patients on a daily dose of ≥200 mg allopurinol seem to be at an increased risk of SJS-TEN compared to lower doses [113]. By assimilation of these 2 observations, it can be hypothesised that increasing the plasma concentration of allopurinol and/or oxypurinol increases the risk of drug-specific T-cell development [109]. To mitigate the risk of ADRs the dose of allopurinol could be reduced, but it is well established that the most commonly used doses of allopurinol (≤300 mg daily) are frequently ineffective already for the long term treatment of hyperuricaemia in gout [119].

Nevertheless, *HLA-B*58:01* is the single largest predictor of allopurinol-induced SCARs and this makes genetic screening appealing to directly prevent *HLA-B*58:01*-associated SCARs. In addition, it is conceivable that a successful genetic screening programme may indirectly improve the overall benefit: harm ratio of allopurinol further. This is because genotyping may empower clinicians to titrate allopurinol doses up to optimise efficacy in *HLA-B*58:01* negative patients with normal renal function. However, any benefit derived from genetic screening in the ongoing Taiwan study will require follow up studies to determine the extent of generalisation. This is because for other ethnic groups and especially Europeans, allopurinol-induced SCARs also occur in *HLA-B*58:01* negative patients. Furthermore, the identification of other (non)-genetic risk factors may be required to improve the PPV of the test, as currently many *HLA-B*58:01* patients will be unnecessarily denied allopurinol in place of other urate-lowering therapies, with unmeasured effects as yet on cost-effectiveness and treatment efficacy.

*2.4 HLA-B*57:01 and Flucloxacillin-Induced Liver Injury*

Flucloxacillin is a narrow-spectrum beta-lactam antibiotic indicated in Gram-positive bacterial infections and in particular, is used to treat non-methicillin resistant *Staphylococcus aureus* infections. In approximately 8.5 per 100,000 patients treated with flucloxacillin, cholestatic liver injury occurs [120]. Drug-induced liver injury (DILI) is associated with a structurally disparate range of drugs but notably these include non-steroidal anti-inflammatory drugs (NSAIDs) and certain antimicrobials including flucloxacillin [121]. Although rare, drug-induced liver injury (DILI) can be severe and accounts for up to 15% of all cases of acute hepatic failure [122–124].

Analogous to DISI, the type and severity of DILI vary between causative drugs and for a given drug, presentation is variable [121]. Consequently, standardising the DILI phenotype is not straightforward but the diagnosis can be made from clinical, biochemical and histopathological parameters [125].

The aetiology of DILI can be divided into immune- and nonimmune-mediated processes [121]. Pharmacogenetic associations with DILI have now been identified for several drugs and the associated genetic variants reflect both immune and non-immune aetiologies (see Tables 3 and 4, respectively for examples). However, DILI can be difficult to categorise by this means. This is because, although recognition of clinically suggestive features of hypersensitivity is relatively easy, the absence of such features, such as eosinophilia, does not preclude immune system involvement [126].

To date, the strongest DILI genetic association described is between flucloxacillin and *HLA-B*57:01* with an OR of 80.6 (95% CI 22.8–284.9) [47]. This is intriguing as the *HLA-B*57:01* allele is also strongly associated with AHS, yet AHS rarely involves hepatitis [78].

Unlike AHS, it is improbable that this association will lead to a screening test for clinical practice because, despite an adequate estimated sensitivity and specificity (84 and 94%, respectively) [47], the rarity of flucloxacillin-induced liver injury diminishes the PPV to 0.12% [127]. An estimate of the NNS to prevent one flucloxacillin-induced liver injury case is 13,513 and the screening approach would unnecessarily deny almost 7% of patients first line flucloxacillin therapy, with unmeasured adverse effects on infectious disease treatment efficacy and cost effectiveness [127]. However genetic testing may help establish the diagnosis of flucloxacillin-induced liver injury when the underlying cause of liver dysfunction is unclear [47].

The off-target pharmacodynamics that underpin flucloxacillin-induced liver injury are being unravelled. Flucloxacillin can adduct covalently to proteins to form neo-antigen drug-protein conjugates and specific flucloxacillin-modifiable lysine residues on albumin, the major circulating protein, have been identified [128]. It is predicted that several albumin-derived peptides containing flucloxacillin-modifiable lysine residues have high-affinity for HLA-B*57:01 [129] and could be presented on HLA-B*57:01 by professional antigen presenting cells through cross-presentation. Flucloxacillin-responsive CD4+ and CD8+ T-cells have been characterised *in vitro* from patients who have previously suffered flucloxacillin cholestatic liver injury and their activation is dependent on peptide processing. *In vitro*, the CD8+ T-cell activation is restricted to HLA-B*57:01 and the very similar allotype, HLA-B*58:01 [129]. The proposed model, which aligns with the hapten hypothesis, suggests that immunogenic peptide neo-antigens are derived from natural processing of flucloxacillin-protein conjugates and can be presented on HLA-B*57:01 to generate an adaptive immune response [129]. Interestingly, besides this proposed alternative mechanism of neo-antigen formation, this immunopathogenesis differs to that of abacavir in at least 2 ways. Firstly, CD4+ as well as CD8+ flucloxacillin-responsive T-cells have been cloned from patients and secondly, the T-cell clones

Table 4 Examples of associations between adverse drug reactions and nonimmune-related genetic variants

Reaction	Drug	Gene association(s)	Variant(s)	Reference(s)
i) Drug metabolizing enzyme and drug transporter variants				
Increased risk of bleeding	Clopidogrel	*CYP2C19*	*17	[130, 131]
	Warfarin	*CYP2C9*	*3	[132–135]
Increased risk of opioid toxicity	Codeine	*CYP2D6*	Ultrarapid metabolisers	[136, 137]
	Tramadol	*CYP2D6*	Ultrarapid metabolisers	[138, 139]
Drug-induced liver injury	Antituberculosis drug therapy	*NAT2*	Slow acetylator	[140–142]
		GSTM1	null/null	
		CYP2E1 (East Asians)	*1A/*1A	
	Diclofenac	*UGT2B7*	*2	[143]
		ABCC2	rs717620	
		CYP2C8	Different haplotypes	
	Tacrine	*GST T1* *GST M1*	Double null/null	[144]
	Troglitazone	*GST T1* *GST M1*	Double null/null	[145]
Diarrhoea, neutropaenia	Irinotecan	*UGT1A*	*Poor metabolisers*	[146]
Drug discontinuation	Risperidone	*CYP2D6*	Poor metabolisers	[147]
Muscle toxicity	Simvastatin	*SLCO1B1*	rs4149056	[148]
Myelosuppression	Azathioprine, 6-mercaptopurine, thioguanine	*TPMT*	Poor metabolisers	[149]
Peptic ulcer disease	NSAIDs	*CYP2C19*	*17	[150]
Prolonged apnoea	Succinylcholine, mivacurium	*BCHE*	rs1799807, other variants	[151]
Stent thrombosis	Clopidogrel	*CYP2C19*	*2	[152]
Therapy-induced toxicity[a]	5-fluorouracil/ capecitabine	*DPD*	rs3918290, rs55886062, rs67376798	[153]
ii) Other variants				
Drug-induced liver injury	Methotrexate	*MTHFR*	rs1801133	[154]
Malignant hyperthermia	Halogenated inhalation anaesthetics	*RYR1*	rs118192163 >30 other variants	[155, 156]
		CACNA1S	rs1800559 rs80338782	[157, 158]

Table 4 (continued)

Reaction	Drug	Gene association(s)	Variant(s)	Reference(s)
i) Drug metabolizing enzyme and drug transporter variants				
Metabolic syndrome	Clozapine, risperidone	*5HTR2C*	rs1414334	[159]
Nonimmune haemolytic anaemia	Primaquine, dapsone, methylene blue, others	*G6PD*	Mediterranean, A-(202A), >150 other variants	[160, 161]
Therapy-induced toxicity[a]	5-fluorouracil/ capecitabine	*TYMS*	rs45445694	[162]

NSAID non-steroidal anti-inflammatory drug

[a] Toxicity from 5-fluorouracil-based therapy includes diarrhoea, mucositis, nausea, neutropaenia

show cross-reactivity *in vitro* with other commonly prescribed beta-lactam antibiotics including amoxicillin and piperacillin [129].

In summary, this section illustrates that immune-mediated DTH reactions are an emerging prominent type of off-target ADR with the potential for significant morbidity and mortality. However, pharmacogenetics has been pivotal in reducing the healthcare burden associated with abacavir, may have important future roles in the prevention of carbamazepine and allopurinol DISI and is facilitating elucidation of underlying immune-mediated aetiologies.

3 Nonimmune-mediated Adverse Drug Reactions

Nonimmune-mediated ADRs are a heterogeneous group in aetiology and presentation. However, over the last decade it has been increasingly recognised that susceptibility to many nonimmune ADRs is associated with gene variants of drug metabolising enzymes (DMEs) and less frequently, with drug transporters. It is thought that perturbed pharmacokinetics increases the availability of drug/metabolite(s) at the target site(s), increasing the likelihood of developing an ADR. The sites that mediate nonimmune ADRs include both on-target and off-target sites. On-target ADRs manifest through excessive drug/metabolite(s) action either at the therapeutic target site or at the same molecular site located in other tissues. The latter occurs for instance with NSAID-induced upper gastrointestinal ADRs.

It is important to note that, although the majority of ADRs with a genetically-influenced pharmacokinetic-mediated susceptibility found to date are nonimmune ADRs, perturbed pharmacokinetics is also relevant in the genesis of a few immune-mediated ADRs. This was described earlier for the case of allopurinol-induced SCARs and non-genetic pharmacokinetic factors. Furthermore, genetic susceptibility to ticlopidine-induced hepatotoxicity has been demonstrated to be greatest in patients with *HLA-A*33:03* in combination with variants of a DME (Table 3).

In the following section, the effects of gene variants of phase I and phase II biotransformation enzymes on susceptibility to ADRs will be discussed in the context of codeine/warfarin and azathioprine, respectively. Then, the effects of gene variation for a drug transporter will be illustrated for statin-induced muscle toxicity. However as the first example of malignant hyperthermia shows, genetic susceptibility to nonimmune-mediated ADRs can occur through plausible pharmacodynamic mechanisms too. Table 4 lists examples of ADRs associated with nonimmune-related genetic variants.

3.1 RYR1 and Anaesthesia-Induced Malignant Hyperthermia

In 1962, a paper was published about a pedigree that contained 10 relatives who had unfortunately and unexpectedly died during or shortly following general anaesthesia [163]. The deaths were associated with core body temperatures, when measured, in excess of 41 °C and followed an autosomal dominant inheritance pattern [163]. Other pedigrees have since been described [164, 165] and over 500 cases of malignant hyperthermia (MH) have now been reported in the medical literature [166].

MH is precipitated by volatile anaesthetics in genetically susceptible individuals. All halogenated inhalation anaesthetics have been implicated including halothane, isoflurane, sevoflurane and desflurane [167]. The depolarising neuromuscular blocker, succinylcholine, augments the adverse response to these potent inhalation anaesthetics but its role as an independent precipitant of fulminant MH is controversial [167, 168]. Rarely, non-pharmacological stressors including environmental heat [169, 170], infections [170] and severe exercise or emotional strain [171] have been implicated in MH-like episodes.

The incidence of anaesthetic-induced MH is approximately 1 per 50,000 adults and 1 per 15,000 paediatric patients [172] and it occurs in all ethnic groups [173]. The basis of MH is hypermetabolism which can present as tachypnoea, a rise in end-tidal carbon dioxide exhalation, tachycardia, cyanosis, cardiac arrhythmias, skeletal muscle rigidity, hyperthermia [174], convulsions and eventual death [163]. Associated electrolyte complications include acidosis, hyperkalaemia, elevated creatine kinase (CK) and acute kidney injury (AKI) [174]. Timely intervention improves prognosis [175]. However, an early diagnosis of MH can be challenging as the initial clinical signs are nonspecific and variable in their time course, making them easily mistaken for other pathologies (e.g. sepsis, thyrotoxic crisis) [176]. Nevertheless, the mortality from MH has dramatically fallen from 70 % in the 1970s [169] to < 5 % today [173]. This reduction has been aided by the introduction of the muscle relaxant dantrolene for treatment of suspected MH [166] and testing for susceptible relatives (see later) [169]. A clinical grading scale has been introduced to help researchers retrospectively assess the likelihood of MH following an adverse anaesthetic event, which enables accurate phenotyping and determination of future susceptibility [177].

RYR1 is located on chromosome 19 and encodes ryanodine receptor 1 (RyR1). There are 3 RyRs isoforms (RyR1–3) and each forms a homotetrameric assembly within the endoplasmic (or sarcoplasmic) reticulum and functions as a Ca^{2+} channel [178]. They have evolved into the largest ion channels found to date (~2.2MDa) [179]. This is undoubtedly to facilitate tight channel regulation through interaction with numerous regulatory small molecules and proteins, which is important as Ca^{2+} is a potent intracellular mediator of several cell processes [179]. RyR1 is widely expressed in skeletal muscle and is pivotal to excitation-contraction coupling [180]. RyR1 opens in response to nerve impulses and releases Ca^{2+}, from the sarcoplasmic reticulum where it has been sequestered, into the cytoplasm to drive muscle contraction. It is thought that a direct physical connection exists between the voltage-gated Ca^{2+} channel $Ca_V1.1$ (the skeletal dihydropyridine receptor) in the transverse tubule and RyR1 [181, 182], which induces conformational changes that open RyR1 when the wave of depolarisation from the neuromuscular endplate is detected by $Ca_V1.1$ [183].

Approximately 70% of MH susceptible families carry *RYR1* variants [156]. A nonsynonymous mutation of *RYR1* was found to cause the porcine stress syndrome in inbred pigs, which is an animal model of MH [184]. The analogous C1843T mutation in humans was subsequently identified in an analysis of 1 of 35 MH susceptible pedigrees [185]. Currently, over 200 *RYR1* mutants have been described and most are single nucleotide polymorphisms (SNPs), but only 31 have been designated as causative of MH according to the specific criteria set out by the European Malignant Hyperthermia Group (EMHG) [155].

Impaired Ca^{2+} homeostasis underlies the pathogenesis of MH [173]. Gain-of-function *RYR1* mutations have been shown *in vitro* to lead to RyR1 hyperactivation [186, 187]. The increase in intracellular Ca^{2+} concentration results in sustained muscle contraction and heat generation [173]. Attempts to restore the Ca^{2+} balance and the contracting muscle filaments deplete the cell of adenosine triphosphate resulting in muscle rigidity, loss of integrity to the sarcolemma and leakage of intracellular contents (e.g. K^+, myoglobin) out into the extracellular fluid predisposing to systemic sequelae [172]. However, the exact mechanism(s) by which volatile anaesthetics precipitate this potentially fatal cascade has not been clearly elucidated [188].

Interestingly, ~20% of patients have undergone previous uneventful general anaesthesia with potent inhalation agents before experiencing MH [166]. The reasons for this incomplete penetrance are not fully understood but hypotheses include dose and/or duration dependency effects of the volatile anaesthetic agents [167], the ambient temperature and the simultaneous use of possible mitigating drugs [173].

The majority of MH occurs in asymptomatic individuals and they are considered to have a genetically-determined subclinical myopathy [176]. However, there are at least 3 rare clinical myopathies likely associated with MH susceptibility: central core disease (CCD), multiminicore disease (MmD) and King-Denborough syndrome [189, 190]. Within each syndrome there is clinical, genetic and histological variability and considerable overlap exists, in particular, between CCD and MmD [189]. Importantly, the majority of CCD cases are associated with *RYR1* variants

and furthermore, *RYR1* mutations have also been found in cases of MmD [189] and King-Denborough syndrome [191], although these links are less certain [192]. Of the 200 *RYR1* variants, ≥150 are associated with MH alone (subclinical myopathy), ~100 with CCD and ≥20 with both MH and CCD [192]. *RYR1* alleles are also implicated in instances of exercise-induced rhabdomyolysis [193, 194]. Clearly, *RYR1* is involved in a spectrum of muscle disorders, but at the present time the degree of genotype to phenotype concordance is incompletely understood.

The gold standard for MH diagnosis in patients and unaffected relatives is the *in vitro* muscle biopsy contracture test (IVCT), which assesses muscle contraction in response to caffeine and halothane [195]. However, the IVCT is invasive, costly and confined to specialist centres. Therefore, genetic testing has been increasingly used since 2001 [156] to determine MH susceptibility in family members of MH patients that have been shown to carry a causative *RYR1* mutation, as classified by the EMHG [196]. A relative not carrying the familial *RYR1* mutation should still undergo an IVCT though as the absence of a *RYR1* mutation does not exclude MH susceptibility [176].

~75% of MH events occur in patients with no reported family history [166] and therefore universal pre-anaesthetic genetic screening is appealing. However, genetic screening for MH is currently untenable, due to the heterogeneous and incompletely understood genetics underpinning MH susceptibility. The complexity of the RyR1 molecule makes structural and functional predictions of *RYR1* variants challenging [197] and regardless, 30% of MH cases are not associated with *RYR1*. At least 5 other genetic loci have been implicated [172] but of these to date, only nonsynonymous SNPs in *CACNA1S*, the gene encoding the α1 subunit of $Ca_V1.1$, have been linked to MH and in only 1% of cases [157, 158, 172].

In summary MH is a potentially fatal disorder with a strong genetic predisposition, although the full spectrum of genetic risk variants and associated genotype-phenotype correlations are incompletely characterised. However, this strong genetic susceptibility lends itself to the future prospect of successful genetic screening to reduce the incidence of drug-induced MH.

3.2 CYP2D6 and Codeine Analgesia and Safety

Codeine is a weak opioid that is indicated for analgesia in mild to moderately severe pain and as an antitussive and anti-diarrhoeal agent. Although it has been used for many years, recent concerns are mounting over its variable efficacy and safety.

Figure 1 shows the principal pharmacokinetic pathways for codeine. Codeine is considered a prodrug whose function is derived from conversion into 2 active metabolites: morphine and morphine-6-glucuronide (M6G). Both are agonists for the widespread μ-opioid receptor, which is largely responsible for the therapeutic effects and opioidergic ADRs [198, 199]. The affinity of morphine for μ-opioid receptors is 200-fold stronger than compared to codeine [200]. The polymorphic cytochrome 2D6 enzyme (CYP2D6) catalyses the O-demethylation of codeine into

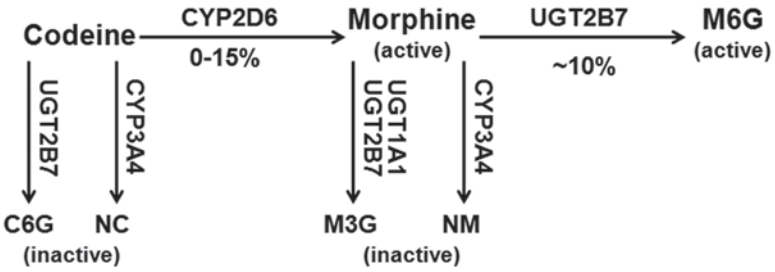

Fig. 1 Codeine metabolism. *C6G* codeine-6-glucuronide, *NC* norcodeine, *M6G* morphine-6-glucuronide, *M3G* morphine-3-glucuronide, *NM* normorphine, *CYP2D6* cytochrome P450 2D6, *CYP3A4* cytochrome P450 3A4, *UGT2B7* uridine diphosphate glucuronosyltransferase 2B7, *UGT1A1* uridine diphosphate glucuronosyltransferase 1A1

morphine. Uridine diphosphate glucuronosyltransferase 2B7 (UGT2B7) catalyses morphine into M6G and, in conjunction with UGT1A isoforms, into inactive morphine-3-glucuronide (M3G). Only a minority of codeine biotransformation (≤15%) [201] is via CYP2D6; the majority of codeine is converted directly into the inactive metabolites codeine-6-glucuronide and norcodeine by UGT2B7 and CYP3A4, respectively. The major codeine metabolites, including morphine and M6G, are excreted renally [201].

CYP2D6 belongs to the superfamily of cytochrome P450 (*CYP*) genes. They encode haemoproteins that catalyse oxidative, phase I metabolism [202] and account for ~75% of all drug metabolism reactions [203]. Although 57 *CYP* genes have been identified, ~95% of these reactions are catalysed by just 5 isoenzymes including CYP2D6 [203]. Direct clinical measurement of CYP2D6 phenotypic activity is unfeasible as it is primarily expressed in the liver and indirect measurements of CYP2D6 metabolites in the plasma or urine are susceptible to other factors including renal dysfunction and drug interference. Consequently, *CYP2D6* genotyping as a phenotype surrogate is appealing for clinical practice.

CYP2D6 is located on chromosome 22 and over 80 alleles have been identified [204]. They are formed by a range of genetic alterations including SNPs, insertions and deletions and can be grouped functionally into increased, normal, reduced and non-functional alleles [137]. An individual's *CYP2D6* genotype can in turn be categorised into 1 of 4 predicted phenotype classes based on the combination of *CYP2D6* alleles they carry: an extensive, intermediate, poor or ultrarapid metaboliser (EM, IM, PM and UM, respectively) [205]. The EM is the wild-type CYP2D6 phenotype, IMs have reduced activity and PMs have no enzymatic activity as they carry no functional alleles. If multiple copies of functional alleles are detected this is denoted the UM phenotype as high enzymatic activity is expected [137]. There is considerable variability in the prevalence of *CYP2D6* alleles and in the prevalence of the extreme phenotypes in different ethnic groups (0–10 and 0–29% for PMs and UMs, respectively) [137].

It has been shown that following codeine administration, PMs have significantly lower plasma morphine concentrations, reduced urinary active metabolite excretion

and decreased analgesia compared to EMs [206, 207]. Conversely, plasma morphine concentrations and urinary active metabolite excretion are significantly higher in UMs compared to EMs [208]. Furthermore although there is no definitive study, a growing series of case reports are documenting severe ADRs after standard codeine use associated with the UM phenotype [136, 209–213]. These case reports are from neonatal [209], paediatric [210–212] and adult populations [136, 213] and the documented on-target (opioidergic) ADRs include: severe epigastric pain, euphoria and dizziness [213], central nervous system/respiratory depression [136, 211] and death [209, 210, 212]. One especially poignant case was the death of a 13-day old neonate who was breastfed by a mother taking codeine (and paracetamol) for episiotomy pain [209]. The autopsy found an extremely high level of morphine in the neonate's blood and a sample of stored maternal breast milk from day 10 showed an elevated morphine concentration. The mother was found to have a C*YP2D6* gene duplication indicative of the UM phenotype [209]. Following this report, the US Food and Drug Administration (FDA) issued a warning on codeine use by nursing mothers [214].

Although there is increasing concern regarding the efficacy and safety of codeine, several barriers exist that hamper the translation of *CYP2D6* genotyping into widespread clinical practice. Firstly, the ADR profile of PMs is incompletely understood [137]. Secondly, when compared to the prevalence of the UM phenotype (0–10%), the documented case reports of severe ADRs are rare, suggesting that there are additional genetic and non-genetic susceptibility factors. The pharmacogenetic influence of *UGT2B7* is controversial at present [201]. Other risk factors may include renal dysfunction [136, 201, 215], drug inhibitors of CYP3A4 [136, 201], ontogeny [215, 216] and repeated episodes of hypoxia [215]. The paediatric case reports are from children receiving codeine after adeno(tonsillectomy) for recurrent tonsillitis and obstructive sleep apnoea (OSA) [210–212]. OSA leads to intermittent sleep hypoxia and it has been shown that opioid analgesia sensitivity increases in children after recurrent hypoxia [217]. Another factor is potential publication bias favouring selection of case reports documenting extreme but fortunately uncommon ADRs with codeine. 10 of 11 UM participants in a pharmacokinetics study felt sedation (91%) compared to 6 of 12 (50%) EMs ($p=0.03$) suggesting that ADRs in UMs may occur more frequently than is reported [208]. Other potential barriers include the absence of prospective studies that demonstrate clinical benefit of *CYP2D6* genotyping, scarce cost-effectiveness data, lack of clinician knowledge and no clear guidelines on what constitutes a suitable substitute for codeine in CYP2D6 PMs and UMs. This is important because CYP2D6 is involved in the metabolism of other opioid drugs including oxycodone, hydrocodone and tramadol. There is evidence at least for tramadol that CYP2D6 PMs experience reduced analgesia [218] and UMs a higher risk of nausea [138] when compared to EMs. There is also a case report of respiratory depression following tramadol in a UM patient with renal dysfunction [139]. Tramadol and codeine are step 2 'weak' opioid drugs on the WHO analgesia ladder [219] and are often used interchangeably in clinical practice for a patient that does not tolerate one. However if tramadol is also undesirable in CYP2D6 PMs and UMs, clinical guidance regarding suitable alternative analgesic agents is warranted.

3.3 *CYP2C9 and the Risk of Haemorrhage with Warfarin*

Warfarin is the most frequently prescribed oral anticoagulant worldwide [220] and is indicated in the prophylaxis and treatment of venous thromboembolism (VTE) and in the prophylaxis of systemic embolism in predisposing conditions such as atrial fibrillation and following mechanical heart valve insertion [221]. It is a coumarin-derived therapeutic that is administered as a racemic mixture; the S-warfarin enantiomer is more potent than R-warfarin [222]. They disrupt the vitamin K cycle by antagonising vitamin K epoxide reductase, resulting in a decrease in vitamin K-dependent post-translational γ-carboxylation of protein glutamate residues [223, 224]. This notably diminishes the activity of clotting cascade proteins including the procoagulant factors II, VII, IX and X and anticoagulant molecules protein C and protein S [225]. The overall anticoagulant effect is quantified by the prothrombin time-derived international normalised ratio (INR); the usual desired therapeutic INR is 2.5 [226]. However, certain high thrombotic risk conditions such as recurrent VTE(s) on warfarin and mechanical heart valves warrant higher anticoagulation levels (e.g. a desired INR range of 3.0–4.0) [226].

Epidemiological evidence has implicated warfarin as a major cause of ADRs; it is the therapeutic associated with the greatest number of preventable ADRs in Sweden [227] and the third most common cause of ADR-related hospitalisations in the UK [3]. Haemorrhage is an on-target ADR and is the predominant ADR associated with warfarin [221], especially during therapy initiation [228]. It is highly correlated to the intensity of anticoagulation [229, 230] and the risk of clinically significant bleeding increases when the desired INR range is higher [221]. The safe management of warfarin therapy is notoriously challenging because of the wide inter-individual range of optimal dose requirements (0.6–15.5 mg/day) and its narrow therapeutic index [231]. It is worth noting also that there is evidence to suggest a pharmacogenetic association between *CYP2C19*17* carriage and increased bleeding risk in patients taking clopidogrel (Table 4) [130, 131], although for now, the genetic susceptibility to haemorrhage on warfarin will be outlined.

CYP2C9, like CYP2D6, is 1 of the 5 main human CYP DMEs [203]. CYP2C9 is the principal enzyme involved in the metabolism of the potent S-warfarin stereoisomer, while R-warfarin is cleared via CYP1A1/CYP1A2/CYP3A4 [228]. Over 30 allelic variants of *CYP2C9* are known, but their relative prevalence varies with ethnicity [220]. The *CYP2C9* reference genotype **1/*1* produces the normal (EM) phenotype [220] and a resultant estimated warfarin half-life of 30–37 h [232]. The 2 most frequent reduction-of-function minor alleles amongst people with European ancestry are *CYP2C9*2* (rs1799853) and *CYP2C*3* (rs1057910) [202]. Both are characterised by one nonsynonymous SNP, prolong the half-life of warfarin (up to 92–203 h in **3/*3* homozygotes [233, 234]) and are associated with reduced maintenance warfarin dose requirements [235].

A recent meta-analysis has reported hazard ratios for the risk of bleeding in patients on warfarin with **1/*3* or **3/*3* genotypes, compared to **1/*1* patients, to be 2.05 (95% CI 1.36–3.10) and 4.87 (95% CI 1.38–17.14), respectively, suggestive of a gene-dose effect [135]. Although *CYP2C9*2* was also significantly associated

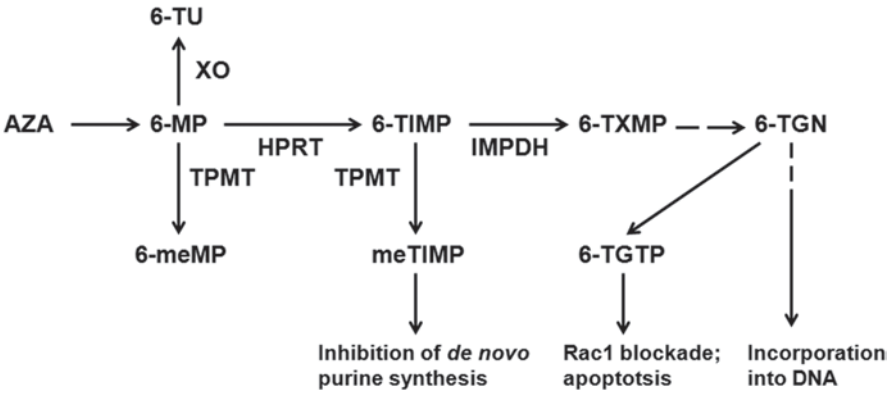

Fig. 2 Azathioprine (*AZA*) and 6-mercaptopurine (6-MP) metabolism (simplified). *me prefix* methyl, *XO* xanthine oxidase, *TU* thiouric acid, *TPMT* thiopurine methyltransferase, *HPRT* hypoxanthine phosphoribosyltransferase, *TIMP* thioinosine monophosphate, *IMPDH* inosine monophosphate dehydrogenase, *TXMP* thioxanthosine monophosphate, *TGN* thioguanine nucleotides, *TGTP* thioguanine triphosphate

with bleeding, albeit with a lower pooled effect size than *CYP2C9*3*, after stratification into **1/*2* and **2/*2* genotypes through synthesis of studies that reported these individual genotypes, neither genotype was significantly associated with bleeding. Overall, *CYP2C9*3* is the main risk factor for bleeding on warfarin, which is biologically plausible as the **3* allele has a more deleterious effect than **2* on CYP2C9 enzyme function [135]. For a more comprehensive account of overall warfarin pharmacogenetics, dosing strategies to incorporate multiple environmental, clinical and genetic factors and a discussion regarding the recently published prospective warfarin pharmacogenetic RCTs, the reader at this point is referred to Chap. 11.

3.4 TPMT, Azathioprine- and 6-Mercaptopurine-Induced Myelosuppression

The immunosuppressive agent azathioprine (AZA) is a pro-drug of 6-mercaptopurine (6-MP). AZA is indicated in both the prophylaxis of transplant rejection and in the treatment of many autoimmune conditions including inflammatory bowel disease (IBD), rheumatoid arthritis and severe eczema [236]. 6-MP is conventionally used with haematological malignancies and in particular acute lymphoblastic leukaemia [237], although it also has a role in IBD [238]. AZA/6-MP can induce several ADRs including myelosuppression (predisposing to neutropaenic sepsis), DILI, pancreatitis, nausea and vomiting [239]. Although ADRs occur in 10–28 % of patients [240], the rate of fatal ADRs among AZA users is estimated at 1 in 10,000 [241].

Approximately 90 % of AZA is converted to 6-MP by ubiquitous non-enzymatic processes [242, 243]. Figure 2 depicts the 3 main competing enzyme pathways for

metabolism of 6-MP: thiopurine methyltransferase (TPMT), XO and the main anabolic pathway via hypoxanthine phosphoribosyltransferase (HPRT) [244]. Both therapeutics are subject to extensive intestinal and hepatic first pass metabolism following oral dosing [244, 245]. Although there is an incomplete understanding of the modes of action of AZA/6-MP [246], the accumulation of 6-thioguanine nucleotide (6-TGN) metabolites formed *in vivo* via HPRT is thought to contribute to both their efficacy [247] and when in relative excess, the increased risk of myelosuppression [242, 248]. The immunosuppressive mechanisms include incorporation of 6-TGNs into DNA inhibiting leukocyte DNA synthesis [244, 249] and blockade of Rac1 protein by the 6-TGN derivative, 6-thioguanine triphosphate (6-TGTP), inducing T-cell apoptosis [246]. TPMT can methylate both 6-MP and the intermediate metabolite, 6-thioinosine monophosphate (6-TIMP), to give 6-methylmercaptopurine (6-meMP) and methyl-TIMP (meTIMP), respectively. meTIMP may be efficacious through *de novo* purine synthesis inhibition [240, 250] whilst high levels of TPMT methylated thiopurine metabolites (and further phosphorylated metabolites) may be associated with DILI [251–255].

TPMT is a phase II biotransformation enzyme, encoded by *TPMT* on chromosome 6 [243], and is a major pharmacokinetic determinant for active 6-TGN metabolite levels [240], which are inversely related to TPMT activity [244, 256, 257]. It is variably expressed in several tissues; the highest levels of TPMT are present in the liver and the lowest in the brain and lung [240]. Erythrocyte TPMT activity correlates with hepatic TPMT activity [258] permitting direct TPMT phenotypic assessment of patients in clinical practice, which is unusual for a DME [259]. TPMT enzymatic activity follows a trimodal distribution; ~90% of individuals have high activity, ~10% intermediate and 0.3% low/undetectable enzyme activity [260, 261].

Around 30 allelic variants of *TPMT* have been reported [20] and despite ethnic variability, 3 account for >90% of the minor alleles: *TPMT*2*, *TPMT*3A* and *TPMT*3C* [254]. They are caused by one (*TPMT*2*, *TPMT*3C*) or two (*TPMT*3A*) nonsynonymous SNPs that reduce enzymatic activity through enhancing the rate that the TPMT variant is catabolised [262–264]. Analogous to *CYP2D6* and *CYP2C9*, *TPMT* genotype correlates with the variable TPMT enzymatic activity levels: heterozygotes have intermediate activity (IM) and individuals carrying no normally functioning alleles have low/absent activity (PM) [254]. Like *CYP2D6* and *CYP2C9*, homozygous deficient individuals include both those homozygous for 1 variant allele and compound heterozygotes with 2 distinct inactivating alleles [243]. *TPMT *1/*1* individuals have normal phenotypic activity (EM).

Clinically, ~27% of AZA/6-MP-induced myelosuppression cases are explained by inactivating *TPMT* alleles [265], although little correlation exists with other specific ADRs including DILI [239, 266]. A meta-analysis of patients with chronic inflammatory diseases has reported a gene-dose effect for this on-target ADR: homozygous deficient individuals carry a higher risk of leukopaenia (OR 20.84, 95% CI 3.42–126.89) than heterozygotes (OR 4.29, 95% CI 2.67–6.89) when compared with **1/*1* individuals [149] and in general the myelosuppression onset is earlier [265, 267] and more severe [267]. A second systematic review, not limited to a

specific class of disease, has reported that 86% of *TPMT* homozygous deficient patients develop myelosuppression and the pooled OR for patients with intermediate TPMT activity or one *TPMT* variant allele, compared with wild-type, was 4.19 (95% CI 3.20–5.48) [268]. For both studies, their results were primarily derived from synthesis of observational studies.

As clinical evidence has grown, consensus national clinical guidelines have been published that recommend and interpret pre-therapy TPMT testing, including the UK dermatology [269] and rheumatology guidelines [270]. In patients identified as TPMT deficient (by either genotyping of homozygous deficiency or TMPT phenotypic analysis of low/absent activity), guidance advises selection of alternative immunosuppressive therapy in non-malignant conditions and a reduction in starting dose to 10% of normal when treating malignancy [254]. For heterozygous variant/intermediate activity patients commencing AZA/6-MP therapy, a dose reduction of 30–70% is suggested [254]. TPMT analysis has been adopted into clinical practice and a national survey reported that 94% of dermatologists, 60% of gastroenterologists and 47% of rheumatologists in England requested TPMT testing [271].

Despite the relatively high, albeit variable, clinical uptake of TPMT testing, outstanding issues remain. Firstly, there is a lack of robust prospective randomised evidence assessing the utility of pre-therapy TPMT analysis in reducing myelosuppression. An RCT ($n=333$) was undertaken but the recruitment target ($n=1000$) was not met due to guideline-driven pre-existing routine TPMT testing at some centres adversely impacting study recruitment [272]. The one patient in the non-genotyped arm found at study completion to be *TPMT* homozygous deficient developed severe, early onset neutropaenia. However overall, the study found no difference in the rates of AZA cessation due to ADRs between the *TPMT* genotyped arm (with recommended AZA dose reduction and avoidance in heterozygous and homozygous *TPMT* deficient patients, respectively) and the non-genotyped arm, and no increase in AZA cessation in *TPMT* heterozygous patients compared to wild-type patients [272].

Secondly, whilst the evidence and recommendations for *TPMT* homozygous deficient individuals are relatively clear, the optimal management strategy for heterozygous patients is less certain. Although overall they appear to be at a modest increased risk of myelosuppression [149, 268], complicating factors include the observation that only ~30–60% of heterozygous patients do not tolerate full doses of AZA/6-MP [254, 257, 273] and the benefit: harm ratio attributable to different thiopurine starting doses for heterozygotes likely varies depending on the disease-specific necessity for rapid therapeutic action. A higher risk of myelosuppression with a higher starting dose in a heterozygote might be justifiable for treating malignancy, but not chronic, stable immunological disease.

Thirdly, *TPMT* can be analysed by phenotype or genotype and the screening test protocol remains incompletely standardised. Erythrocyte TPMT activity is predominantly offered to clinicians in the UK, but it can be affected by patient ethnicity, concurrent use of interacting drugs (e.g. mesalazine, sulfasalazine, allopurinol), allogeneic erythrocyte transfusions during the preceding 120 days, and in haematological malignancies, it can be affected by disease-related influences [274]. Whilst

the overall genotype to phenotype test concordance is 98.4% in healthy volunteers, it decreases to 86% in the intermediate TPMT activity range, attributable to both non-genetic influences on TPMT activity, as described above, and to a lesser extent, novel mutations [275]. Therefore, neither test is 100% sensitive to correctly identify TPMT deficiency, but research from a National Centre suggests that genotyping is more accurate and should be used as the primary test, in contrast to current UK practice [276].

Therefore, a pharmacogenetic association exists between *TPMT* and myelosuppression and there is strong evidence, affirmed by clinical guidelines, for avoiding thiopurine drugs or significantly reducing their dose in *TPMT* homozygous deficient patients, given their near universal experience of myelosuppression at conventional doses [254]. Further research is required to clarify optimal management for heterozygous patients. However, it is already cost-effective to routinely test TPMT status to identify homozygous deficient patients alone [274]. Pre-therapy TPMT testing is not a substitute for routine on-therapy blood test monitoring, given that several thiopurine ADRs are not associated with TPMT and the majority of myelosuppression cases are still not accounted for by *TPMT* variants [265]. Finally, in addition to TPMT testing, there is also a growing role for thiopurine metabolite level monitoring (e.g. 6-TGNs) to individualise thiopurine doses soon after starting treatment; prospective studies to evaluate this proactive approach are ongoing [277].

3.5 SLCO1B1 and Statin-Induced Muscle Toxicity

Statins are the most commonly prescribed class of medication worldwide [278] and are highly efficacious in the primary and secondary prevention of cardiovascular disease [1]. They reduce plasma low-density lipoprotein (LDL) cholesterol through competitive inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in *de novo* cholesterol synthesis. This in turn leads to an upregulation of hepatic LDL receptors, increasing cholesterol influx into hepatocytes and reducing the plasma burden [279].

The currently licensed statins have a good safety profile, but carry a small risk of skeletal muscle toxicity [280]. The spectrum of muscle pathology varies from the most common manifestation of asymptomatic elevations in plasma CK level, to myopathies with pain and high plasma CK levels through to rhabdomyolysis with the potential sequelae of AKI and death. Alternatively, statin therapy can cause myalgias with no detectable plasma CK rise [21]. Depending on precise definitions, myopathy and rhabdomyolysis occur at frequencies of ~1/1000 and ~1/100,000, respectively [281], although this is modulated by other risk factors including higher statin dose, female gender, older age, low BMI, untreated hypothyroidism and other drug therapies, for example concomitant use of gemfibrozil [281].

The solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) belongs to the superfamily of solute carrier (*SLC*) influx transporter genes and encodes the organic anion-transporting polypeptide 1B1 (OATP1B1) [282]. OATP1B1

is one of the most highly expressed influx transporters within the human liver [283]. It facilitates hepatic uptake of a variety of xenobiotic compounds and endogenous substances [284] and so affects the level of exposure of substrate drugs to intracellular hepatic DMEs [285].

Although the effects of statins on the off-target muscle tissue are incompletely defined at present [286], there exists a significant association between gene variants of *SLCO1B1* and the risk of statin-induced muscle ADRs. A seminal statin GWAS used data from the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) RCT in the UK; 85 cases of definite or incipient myopathy were contrasted with 90 controls [148]. Both the cases and controls for the GWAS had been prescribed 80 mg simvastatin daily. Only an intronic SNP variant, rs4363657, was strongly correlated with myopathy and further regional genetic analysis showed it to be in near complete linkage disequilibrium with the nonsynonymous SNP, rs4149056, in exon 6 (*SLCO1B1*5*; 521T > C; V174A). Further, a gene-dose relationship was demonstrated for rs4149056: the OR for myopathy in heterozygotes and homozygotes for the minor C allele was 4.5 (95% CI 2.6–7.7) and 16.9 (95% CI 4.7–61.1), respectively, when compared to the ancestral TT genotype. Overall, greater than 60% of the myopathy cases in this study were attributable to the C variant [148]. The association with rs4149056 has been replicated [148, 287, 288] but the incidence of severe myopathy and the magnitude of correlation were lower in a second UK randomised trial population [289], attributable to the smaller 40 mg daily simvastatin dose used [148]. The rs4149056 variant has been subsequently associated with more mild, statin-induced muscle ADRs [290], reduced simvastatin adherence [290] and general intolerance to simvastatin defined as a composite endpoint of prescribing +/− mild biochemical changes [291]. The weight of evidence to date for rs4149056 is with simvastatin and the evidence with other statins is less compelling [287, 290, 292], suggesting that rs4149056 may represent a simvastatin-specific effect.

Mechanistically, rs4149056 may interfere with localisation of the transporter to the hepatic plasma membrane reducing its activity [284]. It is associated with higher statin, and especially simvastatin acid, plasma concentrations [293–295] that conceivably increase skeletal muscle drug exposure. However, the relationship between plasma simvastatin acid concentration and muscle toxicity is not straightforward. Clinically, current FDA guidance recommends against the 80 mg simvastatin dose unless a patient has tolerated the higher dose for over 12 months [296].

Overall, the rs4149056 variant is a plausible candidate for a predictive test to reduce simvastatin-induced skeletal muscle ADRs. Current guidance suggests that when initiating simvastatin therapy in CT or CC genotype patients, simvastatin 20 mg daily is selected rather than the normal 40 mg daily dose, possible routine CK surveillance is utilised and alternative statin therapy is commenced rather than increasing the dose of simvastatin if lipid goals are not reached. However, the effects of these recommendations on the incidence of simvastatin ADRs and adherence are currently unknown [281].

4 Outlook and Recommendations

The aspiration of pharmacogenetics is to individualise drug treatment to minimise harm and promote efficacy. Pre-therapy predictive genetic testing seeks to tailor therapy to reduce ADRs primarily through guiding drug or dose selection and has impacted positively upon clinical practice, notably with abacavir. Genetic screening may also find a role in identifying patients for whom regular biomarker surveillance may be indicated to minimise the incidence of severe ADRs. In addition to the direct patient benefit of reducing ADRs, there are at least 3 other potentially favourable spin-offs from understanding the pharmacogenetics of ADRs. Firstly, genetic-ADR associations provide novel insights that facilitate investigation into underlying pathological processes and the extrapolation of new knowledge regarding hypersensitivity reactions may have implications for cancer, autoimmune and infectious disease management. Secondly, the safety profile of new therapeutics may be improved through screening of drug candidates for affinity to high risk *HLA* alleles, for example *HLA-B*57:01* and *HLA-B*58:01* [71]. Thirdly, the beneficial side effects of some drugs have resulted in new therapeutic indications, for example with sidenafil (Viagra) and its fortuitous alleviation of erectile dysfunction. Pharmacogenetics has the potential to increase this 'drug repositioning' through identifying novel off target pharmacodynamic sites.

Abacavir has provided a blueprint for translational pharmacogenetics, but it has yet to be emulated. This is partly due to certain 'favourable' characteristics of AHS including: the high relative prevalence of AHS [76], the exclusivity of the association between *HLA-B*57:01* and immunologically-mediated AHS, the reduction of false-positive clinical diagnoses mediated by the screening programme [78], the vocal patient lobby, and a physician community who were relatively amenable to changing their prescribing and clinical behaviour. It is also because there are multiple obstacles encountered when attempting translation. It is important to first understand these hurdles, and then to have a systematic approach to both developing the ADR-genotype evidence base and to implementing it in clinical practice [297].

Many ADRs are rare and some, such as the HSS, consist of varying constellations of non-specific features. As a result, international consortia using standardised definitions for these ADRs are advisable so patient samples of sufficient size with well demarcated phenotypes that are generalisable across ethnic groups can be pooled together. The 'International Serious Advent Consortium' and their 'Phenotype Standardisation Project' are both steps in the right direction [298]. These coordinated efforts are a prerequisite to reducing the risk of type I and type II errors in genetic association studies of rare and variable ADRs.

Pharmacogenetics has traditionally harnessed the candidate gene approach, whereby genes predicted to be relevant, typically through knowledge of a drug's pharmacology, are selectively studied. However, this approach is limited to contemporary knowledge and so has largely been superseded by GWAS, which has no stipulation for *a priori* hypotheses [20] and can test at least 10^6 SNPs concurrently. However GWAS increases sample size requirements and data capture, increasing

the complexity of study data management and statistical processes and potentiates the threat of selective publication reporting. Further, the lack of a preformed hypothesis augments the importance of confirming biological causality for GWAS putative associations.

Nevertheless, GWAS is a valuable asset: it can confirm in a 'blinded' fashion the results of previous candidate gene studies [20] and offer a novel foothold into the idiosyncratic processes of off-target ADRs. For polygenic ADRs, GWAS may detect new loci of individual small effect size and assess genotype-phenotype associations of larger haplotype signatures. The '1000 Genomes Project,' which has recently described the genomes of 1092 individuals, is in turn increasing the resolution of GWAS [299]. The 1000 Genomes Project should additionally provide a baseline reference for normal human genetic variation, enable fine mapping of existing GWAS associations and aid discovery of new genetic associations, partly through its detailed identification of indels and larger deletions as well as contemporary SNPs [299]. In the near future, next generation sequencing technologies that provide high throughput whole genome capability will offer the pinnacle of DNA resolution whilst advances in our understanding of epigenetic imprinting and microRNA regulation promise new directions for the study of ADR pharmacogenetics. As genetic variation does not usually account for all of the inter-individual variation in drug response, incorporation of data from transcriptomics, metabolomics and proteomics may further improve predictive values [127].

After identification and validation of a statistically significant genetic association(s) for an ADR, several hurdles still bar adoption into clinical practice. Large, well-conducted prospective studies represent the gold standard to confirm clinical outcome benefit, although given the rarity of some ADRs these are not always practical. For other ADRs, genetic sub-studies of clinical trials and registries will likely offer the highest attainable level of evidence [300]. Subsequent pharmacoeconomic studies should base their analyses on this high quality data rather than expert opinion and retrospective data [301].

Logistical and knowledge barriers to the implementation of ADR pharmacogenetics also exist. On-demand genotyping, where the treating physician requests a specific pharmacogenetic test for a patient when seeking to prescribe a drug with a clinically established ADR-genotype association, relies on both a physician's knowledge of pharmacogenetics and a system for following-up and acting on the pharmacogenetic test result. Robust and validated point-of-care genotyping tests may be necessary. An alternative proposed method is pre-emptive genotyping, where multiple relevant SNPs are routinely genotyped together and this genetic data is incorporated into a patient's electronic medical record, with subsequent access by automated clinical decision support (CDS) algorithms to provide a clinically relevant alert regarding a potential drug-genotype interaction specific to the individual patient, at the point in time when the physician is seeking to prescribe the drug of interest. This approach provides the pharmacogenetic information at the most pertinent time and secondly, the CDS approach is likely better suited to keep up with our rapidly expanding understanding of

ADR pharmacogenetics. However, the associated computational challenges are considerable [302].

Finally, a genetic test should be ethically acceptable to patients, clinicians and society. The emphasis of pharmacogenetics is for the beneficial personalisation of medicine, yet paradoxically the realisation of this goal requires not only very large international research collaborations but also active engagement with society as a whole. This is not least because genetic information harbours potential adverse implications, such as individual discrimination by insurance firms based on high risk genotype carriage and neglect of ethnic minorities by pharmaceuticals opting to segregate research initiatives to benefit the majority to maximise profit margins [303]. Open dialogue between patients, healthcare services, insurance providers, pharmaceuticals and the wider public is required to address these risks. If society chooses pharmacogenetics, it must safeguard against encroachment on the rights of individuals and minority groups. Ultimately, the widespread application of pharmacogenetics throughout clinical practice to ameliorate ADRs remains far off, but the examples in this chapter and the promises inherent in the new technologies foreshadow a future potential.

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Pharmacogenomics for Haemoglobinopathies Therapeutics

Aikaterini Gravia, Vasiliki Chondrou, Theodora Katsila and George P. Patrinos

Abstract Individual genetic composition has a fundamental role in the variations observed in drug response and tolerance. Pharmacogenomics aims to delineate the individual genetic profiles and drug response/toxicity. Nowadays, there are several medical disciplines where pharmacogenomics is readily applicable, while in others its usefulness is yet to be shown. Recent experimental evidence suggest that single nucleotide polymorphisms (SNPs) in modifier genes residing outside the human β-globin cluster are significantly associated with response to hydroxyurea (HU) treatment in β-type haemoglobinopathies patients, deducted from the increase in foetal haemoglobin levels. This chapter aims to provide an update and to discuss future challenges on the application of pharmacogenomics for β-type haemoglobinopathies therapeutics in relation to the current pharmacological treatment modalities for those disorders and the complexity of their pathophysiology.

Keywords β-thalassaemia · Sickle cell disease · Pharmacogenomics · Biomarkers · Single nucleotide polymorphisms · Hydroxyurea treatment

1 Haemoglobinopathies

Being easily isolated from blood, the study of haemoglobin has shed light on the understanding of the fundamental principles of gene regulation, gene function and the molecular basis of human genetic disorders. Haemoglobin is the key tetramer oxygen transport protein of red blood cells, consisting of two α-like and two β-like globin polypeptide chains [1–4]. Notably, various types of haemoglobins are found at different developmental stages; just before birth, foetal haemoglobin (HbF) represents the bulk of haemoglobin production, while ten months after birth it gradually declines to reach almost 1% of the total haemoglobin production, being restricted to a distinct erythrocyte population, also known as F-cells [4]. In adults, both HbF and the number of F-cells share a genetic determination, and the latter varies among populations. The

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primary adult and children haemoglobin is the HbA ($\alpha_2\beta_2$), while HbA_2 ($\alpha_2\delta_2$) and foetal haemoglobin HbF ($\alpha_2\gamma_2$) are found in amounts of less than 3 %, respectively.

Haemoglobinopathies are divided into two main categories: thalassaemia syndromes and structural haemoglobin variants, both resulting from genomic variations that can be found in cis and/or in trans of the globin gene cluster. Thalassaemia syndromes (namely, α-thalassaemia and β-thalassaemia) are inherited autosomal recessive disorders with defects in globin synthesis and not in haemoglobin structure. The structural haemoglobin variants (or abnormal haemoglobins) are disorders characterised by defects in haemoglobin structure. The majority of haemoglobin variants are extremely rare, with the exception of HbS, HbC and HbE, that are found in certain populations, presumably due to positive natural selection. Abnormal haemoglobins can cause: (a) sickle cell disease (SCD) that results from red cell membrane deformation, (b) haemolytic anaemia, as a result of unstable haemoglobins, (c) methaemoglobinemia owing to rapid oxidation of haemoglobin, and (d) erythrocytosis due to unnatural oxygen affinity [3–7]. Particularly, SCD and β-thalassaemia are one of the commonest single gene disorders and at the same time one of the most serious health problems worldwide. These disorders are most prevalent in Asian and African populations, as well as those residing in the Mediterranean basin. Nowadays, however, due to the international migration, haemoglobinopathies extend worldwide. These genetic disorders are caused by genetic quantitative and qualitative defects in haemoglobin production [4].

Nowadays, more than 1000 Hb variants have been discovered and characterised [8], being a milestone in the history of haemoglobin research, where T.H.J. Huisman could not be omitted. A registry of these Hb variants and related information has been available online, at HbVar database (http://globin.bx.psu.edu/hbvar). During the last century, major developments in Hb research have been made using physical, chemical, physiological and genetic methods, impacting our understanding and management of the thalassaemias and sickle cell disease.

2 Therapeutic Approaches

The β-thalassaemias are considered as one of a few clinical conditions in which a mutant gene that is normally expressed later in development can be functionally replaced by a gene, which is transiently expressed during foetal life [9]. Foetal γ globin expression can be re-activated, being an appealing therapeutic approach as the foetal globin genes are universally present and noteworthy, appropriately contextually integrated in the β-globin locus in human haematopoietic stem cells. However, anaemia in β-thalassaemia syndromes can be also due to the rapid cellular apoptosis (α-globin chain precipitation) and/or the relatively low levels of endogenous erythropoietin (EPO) [10]. Thus, the ultimate goal of transfusion independence for thalassaemia patients should be approached via the stimulation of both foetal globin gene expression and erythropoiesis. In this context, chemotherapeutic agents, erythropoietin (EPO) preparations and short chain fatty acid derivatives (SCFADs) have demonstrated proof-of-principle in animal models and clinical trials.

Focusing on the pharmacological reactivation of HbF to compensate for the loss of HbA, the only pharmacological agent recognised from FDA used to increase the HbF in adults is hydroxycarbamine (or hydroxyurea, HU). Even though the mechanism underlying the HU action still remains elusive, it has been shown to inhibit cellular ribonucleotide reductase, whose role is critical for the DNA synthesis in the dividing late progenitor cells. HU is broadly used for ameliorating SCD symptoms and to a lesser extend in β-thalassaemia patients, while it also serves as a chemotherapeutic agent for many myeloproliferative conditions [11]. Nevertheless, HU is cytotoxic and can lead to cytopenia, hyperpigmentation, weight gain, hypomagnesemia or it may have a teratogenic effect [5]. The patient response to HU as well as the HU toxicity incidents vary [4, 12–14]. Thus, the discrimination between responders and non-responders is of fundamental importance towards patient stratification.

Collins and colleagues have observed that haematologic responses to the foetal globin inducer sodium phenylbutyrate occurred only in those subjects who had high endogenous EPO levels, being unrelated to any particular pattern of globin gene mutation [15]. Hence, a red cell survival advantage of increased endogenous EPO in β-thalassaemia has been suggested that may facilitate an effective γ-globin induction. In agreement to the above, a subset of subjects with inappropriately low levels of endogenous EPO has responded to combined therapy with butyrate plus EPO, whereas each agent alone had a lesser or minimal effect in the same time frame [10]. It seems, therefore, that the exogenously administered EPO acts both as a survival factor and an erythropoietic stimulant. Prolonging erythroid precursor cell survival could be beneficial, allowing a foetal globin inducer to act towards the correction of the pro-apoptotic chain imbalance and hence, improving the anaemia found in β^+- thalassaemia patients. In thalassaemia intermedia and major, EPO has been combined with HU or arginine butyrate. Combinations of EPO with stem cell factor (SCF) to stimulate proliferation and transforming growth factor-β (TGF-β) to induce premature differentiation (like HU) could be also considered for more severe phenotypes, although pricing could be an obstacle for long-term use.

In other studies, SCFADs and hydroxamic acids have been shown to induce foetal globin gene expression, stimulate erythroid proliferation and prolong erythroblast survival [10, 16, 17]. In particular, they activate the $^{A}\gamma$ globin gene promoter in cells cultured from β-thalassaemia patients, without inhibiting the erythroid cell growth. Sodium 2,2-dimethylbutyrate and α-methyl-hydrocinnamate stimulate erythroid colony formation more than the optimal haematopoietic growth factors alone and at the same time, signal through STAT-5 phosphorylation that is common to EPO and IL-3 signalling pathways. Most importantly, some of these agents hold the promise of oral administration, being more tolerable for long-term treatments, providing two therapeutic effects via one tolerable agent [10, 17].

Although the development of drugs to increase foetal haemoglobin has been the major therapeutic strategy in the treatment of both disorders, SCD and thalassaemias, and new foetal haemoglobin–modulating agents have been studied, only HU has shown long-term benefit. To this end, the gradual elucidation of the pathophysiology of the disease(s) has led to alternative strategies, treating the associated complications (decreasing the iron overload and reducing the oxidative stress). More recently, novel agents have been developed targeting the multiple pathways causing

vascular injury in haemoglobinopathies; the increased adhesion of cells to the vascular endothelium, the NO dysregulation, inflammation, oxidative injury and the altered iron metabolism. Such agents (propranolol, statins, niacin, curcuminoids, hepcidin agonists/antagonists) have reached phase 1 and 2 clinical trials [18].

3 Pathophysiological Features and Obstacles

The reduction of the globin chain imbalance has been well accepted as the way to improve red cell survival and blood counts in β-thalassaemia. Nevertheless, a number of factors seem to collectively contribute to anaemia, such as ineffective erythropoiesis and erythroid precursor apoptosis. Thus, combination therapy is required with more than one agent acting at various molecular levels to achieve a tolerable and long-term therapeutic response. Moreover, different magnitudes of therapeutic effect for different thalassaemia patients are required to achieve functional clinical endpoints that could result in abolishing or decreasing needs for regular red blood cell transfusion. In thalassaemia intermedia patients with basal total haemoglobin levels of 6–8 g/dL, a 1–2 g/dL increase would be quite adequate to prevent the need for a regular transfusion program, being highly beneficial, whereas thalassaemia major patients having baseline haemoglobin levels below 5 g/dL would require higher levels of foetal globin induction.

The paediatric pathophysiology of both SCD and thalassaemia should also be considered when haemoglobinopathies' therapeutics are in question. In SCD, acute chest syndrome, resulting from pulmonary microvascular occlusion and being a common cause of death, occurs in all age groups, but is most common in childhood. In children, acute sequestration of sickled cells in the spleen may also occur, exacerbating anaemia. Chronic spleen damage increases susceptibility to pneumococcal and *Salmonella* infections (including *Salmonella* osteomyelitis) that are especially common in early childhood and can be rapidly fatal [19]. Children with thalassaemia intermedia (mild to severe anaemia) or thalassaemia major need blood transfusions coupled to chelation therapy. Children with β-thalassaemia have elevated plasma levels of conjugated dienes and thiobarbituric acid-reactive substances (markers of lipid oxidation), while the RBC glutathione levels are much reduced [20]. There is evident oxidant injury to RBC haemoglobin and lipids.

Considering the overall complexity in the pathophysiology of haemoglobinopathies as well as the still unresolved pathophysiological issues in thalassaemias [20], patient stratification is critical towards effective cure or mitigation of the disease. In this context, pharmacogenomics are expected to have a fundamental role.

4 Pharmacogenomics for Haemoglobinopathies

Pharmacogenomics aims to determine how the genetic background of a patient influences his response to a drug or the probability to develop adverse drug reactions, via the correlation of gene expression or Single Nucleotide Polymorphisms

(SNPs) with drug efficacy and toxicity [21]. The application of pharmacogenomics in haemoglobinopathies is particularly appealing due to the limitation of the therapeutic approaches and the complexity of disease pathophysiology. So far, the role of HU treatment towards the cure or mitigation of the disease has been vital. HU increases the HbF levels mainly in SCD, but also in compound heterozygous SCD/β-thalassaemia patients, ameliorating their clinical manifestations. Additionally, β-thalassaemia intermedia patients have been also shown to respond to HU treatment. Herein, we summarise the current knowledge regarding the genetic factors that have been reported to influence HbF expression levels in relation to HU treatment, including a large number of genomic variations residing inside or outside the human β-globin gene cluster (Tables 1, 2).

Table 1 Summary of the various studies attempting to correlate genomic variations in genes located within the human globin gene cluster with HU treatment efficacy

Patient sample	No of patients	Origin	Gene involved	Association with HU treatment response	References
SCD	150	NA	*HBG2* (XmnI polymorphism)	YES	[23]
β-thalassaemia major	34	NA	*HBG2* (XmnI polymorphism)	YES	[25]
β-thalassaemia major	133	Iranian	*HBG2* (XmnI polymorphism); *HBB* framework 2	YES	[26]
β-thalassaemia major	143	NA	*HBG2* (XmnI polymorphism)	YES	[27]
β-thalassaemia major	54	Algerian	*HBG2* (XmnI polymorphism)	YES	[28]
β-thalassaemia major	18	NA	*HBG2* (XmnI polymorphism)	YES	[29]
β-thalassaemia intermedia	37	NA	*HBG2* (XmnI polymorphism)	YES	[30]
β-thalassaemia intermedia	16	NA	*HBG2* (XmnI polymorphism)	NO	[31]
β-thalassaemia major/ intermedia	38/41	Western Indian	*HBG2* (XmnI polymorphism); α-thalassaemia	YES	[13]
β-thalassaemia major	81	Iranian	*HBG2* (XmnI polymorphism)	YES	[38]
HbE/β-thalassaemia	13	Indian	*HBG2* (XmnI polymorphism); *HBB* genotype	NO	[32]
β-thalassaemia	232	Iranian	Hetero- and homozygous*HBB* null (β^0) mutation	YES	[33]
			HBG2 (XmnI polymorphism)	NO	
β-thalassaemia intermedia	24	NA	Hb Lepore; δβ-thalassaemia	YES	[34]

Table 2 Summary of the currently available studies aiming to correlate genomic variations located in genes outside the human globin gene cluster with HU treatment efficacy

Sample	No of patients	Origin	Chromo-some	Gene and markers involved	Association with HU treatment response	References
SCD	137	NA	13	*FLT1* (rs2182008, rs9319428, rs8002446)	YES	[35]
			6	*MAP3K5*(rs9376230,rs9483947)		
			6	*PDE7B*(rs2327669, rs11154849, rs9376173, rs1480642, rs487278)		
			9	*ASS* (rs7860909, rs10793902, rs10901080)		
			8	*TOX* (rs826729, rs765587, rs9693712, rs172652, rs380620)		
			6	*ARG1* (rs17599586, rs2781667)		
			14	*ARG2*(rs10483801, rs10483802)		
			17	*NOS2A*(rs1137933, rs944725)		
			12	*NOS1* (rs816361, rs7977109)		
β-thalassaemia and SCD/β-thalassaemia compound heterozygotes	38	Western Greek	6	*MAP3K5*(rs9483947, rs9376230)	YES	[37]
			6	*PDE7B*(rs2327669)	NO	
β-thalassaemia major	81	Iranian	2	*BCL11A*(rs766432,rs4671393)	YES	[38]
			6	*HBS1 L-MYB* (rs9399137, rs4895441)	NO	
SCD children	93	NA	2	*BCL11A*(rs1186868, rs1427407)	NO	[39]
β-thalassaemia and SCD/β-thalassaemia compound heterozygotes	38	Western Greek	8	*KLF10* (rs3191333)	YES	[40]
SCD	386	NA	10	*SAR1A* (rs2310991, g.-809 C > T, g.-502G > T, g.-385 C > A, rs4282891)	YES	[41]

4.1 Genomic Biomarkers Linked to the Human β-Globin Gene Cluster

Patients' response to HU varies in terms of amplitude and velocity, leading to the in depth investigation of the determinants of this differential response. This investigation began years ago and continues until today, revealing several genetic factors including SNPs in various genes that are linked (or not) to the β-globin gene cluster. These genes are believed to act by modulating HbF levels.

Steinberg et al. [23] studied 150 HbS homozygous patients treated with HU. A group of them showed almost a 40% HU-induced HbF levels, compared to the remaining of the study group. In order to define the genetic factors that may have influenced these responses, they focused on genetic factors that are believed to influence the foetal globin (*HBG1* and *HBG2*) gene expression, including the haplotypes of α- and β-globin gene clusters and the X-linked F-cell expression locus (FCP) [22]. As concluded, the FCP and the HbF levels before the treatment were not correlated with the HU-induced HbF response. Notably, the absence of a particular β-globin gene haplotype (namely, the Central African Republic-CAR) was related with higher HbF response [23].

Although HU is established to ameliorate the clinical manifestations of SCD, many patients die of this disease, mostly by acute chest syndrome (as it has been described earlier in the text, it occurs in all age groups, but it is most common in childhood), even upon HU treatment. Bakanay et al. [24] compared the β-globin gene cluster haplotype distributions (BAN, BEN, CAM, SEN) between the deceased and surviving patients treated with HU, concluding that homozygosity in the BAN haplotype or heterozygosity in the CAM haplotype were more likely to be observed in the deceased patient group [24].

In β-thalassaemia major, the increase of HbF expression by HU administration is not as effective as in the case of SCD. This may be due to the heterogeneity of the disease and also due to the complexity of the genetic elements involved in the HbF expression patterns of patients. Alebouyeh et al. [25] attempted to correlate the HU response in two different groups of β-thalassaemia major patients, from whom, 25 were blood transfusion-dependent and nine were non-dependent, in order to explore candidate genetic markers for the pharmacological HbF reactivation by HU. They have demonstrated that the XmnI polymorphism (*HBG2*: g.-158C > T) and the IVSII-1G > A mutation, both at the homozygous state, were found in the responders of both groups. On the contrary, these markers were either not present or in a heterozygous state in the non-responders groups tested. Nevertheless, the small number of patients included in the study as well as the fact that two siblings (responders group) were found to bear the common allele for both the XmnI polymorphism and the IVSII-1G > A mutation suggest that further research must be carried out in order to identify and elucidate the genetic modifiers for the HbF upregulation upon HU treatment [25].

Yavarian et al. [26] studied 133 Iranian transfusion-dependent β-thalassaemia patients, treated with HU in order to determine their response to the drug and the

associated genetic background; 61% of the patients became blood transfusion-independent, after 4 months of HU administration (good responders), 23% of the patients, remained blood transfusion-dependent, albeit at a less frequent rate (moderate responders) than before HU treatment, whereas in the remaining 16% of the patients HU, even after a year of administration, had no effect in their clinical manifestation and the frequency of blood transfusion. The authors examined the genetic aetiology of β-thalassaemia in these patients, the human β-gene cluster haplotype and their molecular background in the promoter region of the globin genes as well as at the HS2 hypersensitive site of the β-globin Locus Control Region (LCR). They concluded that the XmnI polymorphism was the most important genetic factor correlated with HU response and that its linkage with the human β-globin gene cluster haplotype I and with the *HBB* gene framework 2 is the "favourable genetic background" for good response to HU [26]. Also, Ansari et al. [27], studied 143 β-thalassaemia patients, treated with HU, confirming that XmnI polymorphism in homozygous or heterozygous state is a genomic marker to predict HU response. This finding was also demonstrated in two other studies including 54 Algerian β-thalassaemia patients [28] and 18 homozygous β-thalassaemia patients treated with HU for a period of 4 years [29].

From a number of studies, it is evident that HU is more promising treating β-thalassaemia intermedia due to the lesser imbalance of α/β-globin chain. Dixit et al. [30] studied the response of 37 β-thalassaemia intermedia patients to HU, from whom almost 70% were categorised as responders. The response to HU was not associated with the β-thalassaemia mutation. On the other hand, a statistically insignificant correlation of HU response and the XmnI polymorphism was observed, suggesting that the combination of other genetic elements can possibly influence the final response to HU treatment [30]. In a similar survey of 18 homozygous β-thalassaemia patients, 11 of who were transfusion-dependent were treated with HU for 50 months in order to correlate their response to HU and their genetic background. The results showed that 82% of transfusion-dependent patients who were treated with HU turned into transfusion-independent, while 78% of them were found homozygous or heterozygous for the XmnI polymorphism. Interestingly, this genomic variation was not present in the HU non-responders. Nevertheless, there were two responding patients, who were negative for the XmnI polymorphism. In total, these data suggest that there may be other genetic elements, which could determine the HU response. In the same study, no correlation between response to HU and the nature of β-thalassaemia mutation or α-thalassaemia deletion was observed [29]. However, in a similar study of 16 transfusion-independent Iranian patients, treated with HU for 6 months, Ehsani et al. [31] could not establish any correlation between the XmnI polymorphism and the response to HU treatment. This may be due to the fact that in this study, as with the previous one, the number of patients was too small to reach any significant conclusion.

Similarly, Italia et al. [13] attempted to correlate the response to HU of 79 β-thalassaemia patients of western Indian origin, from which 38 were β-thalassaemia intermedia and 41 were β-thalassaemia major, treated with HU for almost a year. The correlation (if any) of the HU response to the genetic factors residing within the human β-globin gene cluster was investigated. As it was shown, in the presence

of α-thalassaemia, β-thalassaemia patients showed a better HU response, and the presence of the XmnI polymorphism in homozygosity also resulted in a better clinical response to HU [13]. In a subsequent study of 13 Indian HbE/β-thalassaemia patients with severe clinical manifestations, from whom 36.3 % of the patients were good responders, 36.3 % were partial responders and 27.2 % showed no response, Italia et al. [32] failed to correlate the HU response with specific genetic factors, focusing mainly to the *HBB* genotype and the human β-globin cluster haplotype and XmnI polymorphism, most likely due to the small number of patients [32].

Karimi et al. [33] attempted to correlate the response to HU with *HBB* gene mutations and the XmnI polymorphism in a much larger patient sample, consisting of 232 β-thalassaemia patients of Iranian origin, upon HU treatment for a 13-year period. These authors showed that β-thalassaemia patients with homozygous or heterozygous for a β^0 mutation were better HU responders compared to patients who were homozygous for a β^+ mutation. Interestingly, though, these authors could not establish any correlation between the XmnI polymorphic site and HU response [33].

Finally, Rigano et al. [34] studied the HU efficiency in a long and short term treatment of 24 β-thalassaemia intermedia patients and concluded that the presence of Hb Lepore and δβ-thalassaemia genotypes were indicators of a better HU response [34].

All these studies are summarised in Table 1.

4.2 Genomic Biomarkers Non-Linked to the Human β-Globin Gene Cluster

Apart from the numerous studies presented above with the aim to delineate the response to HU and the genomic markers present in the human β-globin gene cluster, a number of studies have been recently conducted, attempting to implicate the genomic loci residing on other chromosomes with HU response. Some of these genes, particularly *BCL11A*, have been shown to be directly related with increasing HbF levels and as such, these genes might constitute excellent candidates for pharmacogenomic biomarkers to predict HU response (Table 2).

In the most comprehensive study so far, Ma et al. [35] studied 137 SCD patients in an effort to correlate the HU response to several genomic biomarkers that are linked trait loci (QTLs), located on chromosomes 6 and 8 as well as the X-chromosome (these genes have been previously linked to HU metabolism and to erythroid progenitor proliferation). In particular, the authors investigated the association of 327 tagSNPs within these loci to the HU response of patients, using HapMap data. It was concluded that the rs2182008 variation in the *FLT1* gene, either in homozygosity or heterozygosity, was correlated with an almost 6-fold increase in HbF expression levels, following HU treatment. Moreover, two other *FLT1* gene variants, namely the rs9319428 and rs8002446, were found to be associated with the HU response. Overall, there were various genomic variations residing in the *MAP3K5*, *PDE7B*, *ASS*, *TOX*, *ARG1*, *ARG2*, *NOS2A* and *NOS1* genes, found to be correlated

with the HU influence to the HbF expression. Notably, the majority of the above mentioned SNPs were located in intronic or in untranslated regions of the candidate genes [35].

Similarly, Tafrali et al. [36] attempted to elucidate a probable association between the genetic variations in the *MAP3K5* and *PDE7B* gene with the β-thalassaemia disease severity and response to HU in two groups of 38 β-thalassaemia homozygous and SCD/β-thalassaemia compound heterozygous patients of western Greek origin. The authors showed that there is a significant correlation between two single nucleotide polymorphisms residing in the region of *MAP3K5* intron 1 (rs9483947, rs9376230) and improved HU response. Also, by comparative whole-transcriptome analysis in erythroid progenitor cell cultures from normal Maltese adults and Maltese HPFH haploinsufficient cases, bearing the *KLF1*:p.K288X nonsense mutation [37], before and after HU treatment, *MAP3K5* gene expression was increased upon HU treatment [36].

A retrospective association study was conducted with the purpose of detecting genetic determinants of the HU response in 81 transfusion-dependent β-thalassaemia patients from Iran. Genomic variants, located in three QTLs that have been previously shown to have an effect on HbF and F-cell levels, namely the XmnI polymorphism (rs7482144), two SNPs in the intron 2 of the *BCL11A* gene (rs766432, rs4671393) and two SNPs in the intergenic region of *HBS1L-MYB* gene (rs9399137, rs4895441) were investigated. The authors failed to obtain a correlation between the *HBSL1L-MYB* SNPs and HU response. On the contrary, the presence of the XmnI polymorphism, as well as the minor alleles of the *BCL11A* SNPs, namely rs766432C and rs4671393A, were significantly associated with good response to HU treatment [38]. Similarly, Flanagan et al. [39] attempted to elucidate the effect of HU on the erythroid gene expression in 93 children suffering from SCD, in order to explore how HU can influence both the red cell development and the HbF reactivation. Although the existence of the rs1186868 or rs1427407 SNPs in the *BCL11A* gene, in either homo- or heterozygosity, led to the down-regulation of the *BCL11A* expression and higher HbF levels, the authors failed to observe any difference in HbF levels, subject to HU treatment, between the patients who were homozygous or heterozygous for the above SNPs and the wild type ones [39].

Borg et al. [40] conducted a pharmacogenomic study on the HU effect on HbF levels of Hellenic SCD/β-thalassaemia compound heterozygotes, the first to be carried out using a whole transcriptome analysis approach. The authors have comparatively analysed, using whole transcriptome analysis, human erythroid progenitor cells, treated with HU, derived from SCD/β-thalassaemia patients that responded or not to HU. They also studied the effect of the HU on erythroid progenitor cells of healthy and *KLF1*-haploinsufficient Maltese adult patients ex vivo, expressing low and high HbF levels, respectively, aiming to reveal differential expression profiles in genes implicated in augmenting HbF levels. *KLF10* was shown to be the strongest candidate, among 43 identified genes, in both analyses [40]. Subsequently, the authors used an independent cohort of SCD/β-thalassaemia compound heterozygotes so as to corroborate their results. Their genotyping analysis demonstrated that the presence of the rs3191333 SNP in the 3′ UTR of the *KLF10* gene can be

correlated with the severity of β-thalassaemia, as well as with efficacy to the HU therapy. In conclusion, *KLF10* has not only been shown to be a pharmacogenomic marker to predict β-thalassaemia patient response to HU, but has also been implicated for the first time in erythropoiesis [40].

Finally, Kumkhaek et al. [41] examined 386 SCD patients in an effort to correlate polymorphisms in the *SAR1A* gene promoter region with differential response to HU and differences in HbF levels among different patients. It was concluded that 5 SNPs in the *SAR1A* regulatory region were correlated with patients' response to HU and with different HbF levels, after a 2 year treatment with HU [41].

5 Concluding Remarks

In this chapter, we (i) provided an succinct overview of haemoglobinopathies, (ii) presented the challenges of their pathophysiology and the limitations of their therapeutics and (iii) summarised our current knowledge regarding the genetic factors that have been reported to influence HbF expression levels in relation to HU treatment, including a large number of genomic variations residing inside or outside the human β-globin gene cluster (Tables 1, 2).

Contrary to other medical specialties, such as oncology, and treatments, such as anticoagulation therapies, experimental data supporting the use of pharmacogenomics for haemoglobinopathies therapeutics using HU are currently very limited, and clearly, more pharmacogenomic studies are needed, not only in larger, but also in ethnically diverse β-thalassaemia and SCD patients groups. In this way, a better picture will be obtained as to whether it is possible to stratify those patients who are likely to benefit from HU therapy. In addition, similar studies may be also conducted for more pharmacological agents and different treatment modalities, such as decitabine and/or butyrate, although presently at experimental stage. However, although drug-induced augmentation therapies towards HbF levels have been demonstrated as a therapeutic modality for β-type haemoglobinopathies patients, it should be clarified that these cannot correct *per se* the numerous events that underlie the pathophysiology of this group of disorders. In addition, one should bear in mind that no straightforward correlation between HbF increase and clinical improvements in β-type haemoglobinopathies patients has been demonstrated. Therefore, all possible phenotype and clinical indicators should be determined to categorise the "responder" and the "non-responder" patient groups for pharmacogenomic studies, which do not necessarily have to be correlated with HbF increment alone, particularly in the case of SCD. Similar complexities also exist for other thalassaemia-related treatments, such as the use of iron chelators.

It should be also noted that not all genomic loci that have been shown to increase HbF levels can be also considered as pharmacogenomic markers for HU response. *KLF1*, one of the key players participating in *HBB* gene activation that is recently shown to be also indirectly involved in human foetal globin gene silencing [36] is not correlated with increased HbF levels upon HU treatment (Kaimakis and Patrinos,

unpublished). The same is true for genomic variations in the *HBBP1* pseudogene and *PDE7B* gene that although recently shown to be related with β-thalassaemia disease severity [37, 42], genomic alterations in these genes cannot be correlated with response to HU treatment.

Whole genome association and whole transcriptome pharmacogenomic studies are only beginning and there are only few reports in the field [35, 37, 40]. Such studies may identify novel gene candidates that participate in different pathways related to HU treatment, such as stage-specific transcription factors, novel erythroid genes and/or genes involved in HbF-inducing HU metabolism. Also, the scarcity of β-thalassaemia intermedia patients and the need to stratify these patients not only according to their response status, but also, and most importantly, according to their *HBB* genotype, makes the formation of large multi-center consortia more than ever urging to better orientate pharmacogenomic marker identification in good and poor responders to HbF-inducing therapy. This will in turn facilitate the design of customised high throughput pharmacogenomic tests for β-type haemoglobinopathies.

Pharmacogenomic studies may be also extended to other therapeutic modalities for β-thalassaemia, such as iron chelation therapy. In particular, a fraction of β-thalassaemia patients present a number of adverse effects to iron chelators, which result in early death [43]. The correlation of genomic variations located in genes that influence, e.g. iron homeostasis with tolerance or response to iron chelation treatment would potentially better stratify patients for iron chelation therapies and enable the emergence of new and improved iron chelators. Similarly, as with β-thalassaemia and SCD patients, whole genome pharmacogenomic studies in these patient groups can also establish genes involved in iron chelators' metabolism pathways, hence allowing identifying putatively useful pharmacogenomic markers for iron chelation therapies, leading to the individual tailoring of chelation therapy to maximise iron excretion.

Pharmacogenomics in children bring on additional challenges. It is well established that there are differences in drug response among children and adults [44], especially in drug metabolism and gene expression, as the latter is a highly dynamic process functioning from the neonatal period over childhood and the adult life later on. Thus, the data quality and its analysis/ interpretation is challenging *per se*. Ethical and legal aspects also accompany this, since the child in question is incapable of giving informed consent himself [44]. Data interpretation difficulties and ethical considerations are clearly needed to be addressed.

In essence, although pharmacogenomics for β-type haemoglobinopathies is currently in its infancy, there is definitely a big potential to determine whether genomic biomarkers can be exploited in the clinic to stratify β-thalassaemia and SCD patients that are likely to benefit from therapy.

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Pharmacogenetics of Neurodegenerative Disorders

Ramón Cacabelos, Clara Torrellas, Pablo Cacabelos, María J. Villanueva, Sergio Piñeiro and Cristina Solveira

Abstract Neurodegenerative disorders (NDDs) (Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, Huntington's disease) represent a major problem of health in developed countries, with an important repercussion in disability and health economics. NDDs pose several challenges to our society and the scientific community: they represent an epidemiological problem and a socio-economic, psychological and family burden; most of them have an obscure/complex pathogenesis; their diagnosis is not easy and lacks specific biomarkers; and their treatment is difficult and inefficient. Most NDDs share some common features: they are polygenic disorders in which genetic, epigenetic and environmental factors are involved; some of them follow a general rule in genomics related to disease onset, clinical course and prognosis; multifactorial dysfunctions in several metabolomic networks lead to functional damage to specific brain circuits; accumulation of toxic proteins (i.e. conformational changes) in the nervous tissue is involved in many cases of NDDs; all of them are costly for society, deteriorating the quality of life of sufferers and increasing disability; and although NDDs do not have a curative treatment, in practice available therapeutics is susceptible to pharmacogenomic intervention.

The genes involved in the pharmacogenomics of drugs to treat NDDs fall into five categories: (i) genes associated with disease pathogenesis (pathogenic genes); (ii) genes associated with the mechanism of action of drugs (mechanistic genes);

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(iii) genes associated with drug metabolism; (iv) genes associated with drug transporters; and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions. Pharmacogenomics accounts for 30–90 % variability in pharmacokinetics and pharmacodynamics. Only 20–30 % of the Caucasian population processes normally approximately 60 % of the current drugs which are metabolised via cytochromes CYP2D6, CYP2C9 and CYP2C19. Clinical pharmacogenomics may contribute to personalising pharmacological treatment, predicting patient/drug-dose selection, minimising drug interactions, increasing drug efficacy, and reducing unnecessary costs.

Keywords Neurodegenerative disorders · Alzheimer's disease · Parkinson's disease · Pharmacogenomics · CYP2D6 · CYP2C9 · CYP2C19 · CYP3A4/5 · APOE · Transporters

1 Introduction

Neurodegenerative disorders (NDDs) represent relatively frequent forms of central nervous system (CNS) disorders which can be classified according to their phenotypic expression affecting cognition, movement, strength, motor coordination or other activities/functions of the CNS (Table 1). Together with stroke and neuropsychiatric disorders, NDDs are the third greatest problem of health in developed countries, representing 10–15 % of deaths after cardiovascular disorders (25 %) and cancer (20 %) [11]. CNS disorders pose several challenges to our society and the scientific community: they represent an epidemiological problem and a socio-economic, psychological and family burden; most of them have an obscure/complex pathogenesis; their diagnosis is not easy and lacks specific biomarkers; and their treatment is difficult and inefficient. In terms of economic burden, approximately 10–20 % of direct costs are associated with their pharmacological treatment, with a gradual increase in parallel with the severity of the disease [11].

The pharmacological management of NDDs is an issue of special concern due to its complexity, poor cost-effectiveness, lack of specificity, and also the polymedication required to modulate the bulk of symptoms accompanying their clinical manifestations. A growing body of fresh knowledge on the pathogenesis of NDDs, together with data on neurogenomics and pharmacogenomics, is emerging in recent times. The incorporation of this new armamentarium of molecular pathology and genomic medicine to daily medical practice, together with educational programmes for the correct use of drugs, must help to: understand brain pathogenesis, establish an early diagnosis, and optimize therapeutics either as a preventive strategy or as a formal symptomatic treatment [12-16].

Table 1 Neurodegenerative disorders [1-10]

Disease	Subtypes	Disturbance					Prevalence
		Cognition	Movement	Strength	Coordination	Myelin	
Acute Disseminated Encephalomyelitis (ADEM)		+	+			+	N/A
Alzheimer disease (AD)	Alzheimer disease, early onset Alzheimer disease, late onset Dementia in Alzheimer disease, atypical or mixed type	+					5,000–7,000 per 100,000 individuals
Amyotrophic lateral sclerosis (ALS)	Sporadic ALS Familial ALS Western Pacific ALS Hiramaya disease		+	+			1–9 per 100,000 individuals
Ataxia with oculomotor apraxia (AOA)	Ataxia with oculomotor apraxia type 1 Ataxia with oculomotor apraxia type 2		+		+		0.4 per 100,000 individuals
Ataxia-telangiectasia (A-T) or Louis-Barr disease		+	+		+		0.1–0.9 per 100,000 individuals
Central pontine myelinolysis (CPM)		+	+	+	+	+	N/A
Cerebellar ataxia	Early-onset cerebellar ataxia Late-onset cerebellar ataxia		+		+		8.9 per 100,000 individuals
Cerebral amyloid angiopathy (CAA)	Icelandic type Dutch variant British type Familial Danish dementia Finnish type amyloidosis	+					N/A

Table 1 (continued)

Disease	Subtypes	Disturbance					Prevalence
		Cognition	Movement	Strength	Coordination	Myelin	
Charcot-Marie-Tooth (CMT)	Charcot-Marie-Tooth type 1 Charcot-Marie-Tooth type 2			+			10–50 per 100,000 individuals
Corticobasal degeneration (CBD)		+	+				1–9 per 100,000 individuals
Creutzfeldt-Jakob disease (CJD)		+			+		0.1–0.9 per 100,000 individuals
Dementia with Lewy bodies (DLB)		+	+				60–90 per 100,000 individuals
Dentatorubralpallido-luysian atrophy (DRPLA)		+	+		+		0.1–0.9 per 100,000 individuals
Diffuse cerebral sclerosis of Schilder		+	+			+	N/A
Fatal familial insomnia (IFF)		+					< 0.1 per 100,000 individuals
Frontotemporal dementia (FTD)	Pick's disease Frontal dementia Primary progressive aphasia Semantic dementia	+					1–9 per 100,000 individuals
Gerstmann-Straüssler-Scheinker disorder (GSS)		+	+		+		0.001–0.01 per 100,000 individuals
Guillain-Barré syndrome (GBS)			+	+		+	1–9 per 100,000 individuals

Table 1 (continued)

Disease	Subtypes	Disturbance					Prevalence
		Cognition	Movement	Strength	Coordination	Myelin	
Hereditary spactic paraplegia (HSP)	Hereditary spactic paraplegia uncomplicated		+	+			4.1 per 100,000 individuals
	Hereditary spactic paraplegia complicated	+	+	+	+		
Huntington disease (HD)		+	+				1–9 per 100,000 individuals
Kuru			+		+		N/A
Leukodystrophies	Adrenoleukodystrophy Adrenomyeloneuropathy Krabbe disease Pelizaeus-Merzbacher disease Leukoencephalopathy with vanishing white matter or VWM disease Canavan disease Alexander disease	+	+			+	N/A
Multiple sclerosis (MS)	Disseminated MS Generalised MS MS no specified MS of brain stem MS of cord	+	+	+	+	+	60–90 per 100,000 individuals
Multiple system atrophy (MSA)		+	+	+	+		1–9 per 100,000 individuals
Neuromyelitis optica or Devic's disease (NMO)			+	+		+	1–9 per 100,000 individuals

Table 1 (continued)

Disease	Subtypes	Disturbance					Prevalence
		Cognition	Movement	Strength	Coordination	Myelin	
Parkinson's disease (PD)	PD no specified PD idiopathic PD primary	+	+	+	+		120 per 100,000 individuals
Progressive multifocal leukoencephalopathy (PML)		+		+	+		N/A
Progressive supranuclear palsy (PSP)	Richardson syndrome Progressive supranuclear palsy-parkinsonism pure-akinesia-with-gait-freezing (PAGF)	+	+		+		N/A
Spinal muscular atrophy (SMA)	SMA type I, or Werdnig-Hoffman disease), SMA type II, chronic infantile SMA type III chronic juvenile or Kugelberg-Welander disease SMA type IV Adult onset			+			11 per 100,000 individuals
Subacute sclerosing panencephalitis (SSPE) or Dawson Disease		+	+	+	+		<0.1 per 100,000 individuals
Transthyretin-related hereditary amyloidosis (TTR) (Familial amyloid polyneuropathy)			+	+			N/A

+ main pathologic features

Most NDDs share some common features: they are polygenic disorders in which genetic, epigenetic and environmental factors are involved; some of them follow a golden rule: the higher the number of genes affected, the earlier the onset of the disease, with a faster progression, and a poorer therapeutic response to conventional drugs; and the smaller the number of genes disturbed, the later the onset, with a slower progression and a more favourable therapeutic response to current treatments; multifactorial dysfunctions in several metabolomic networks lead to functional damage to specific brain circuits; accumulation of toxic proteins (i.e., conformational changes) in the nervous tissue is involved in many cases of NDDs; all of them are costly for society, deteriorating the quality of life of sufferers and increasing disability; and although NDDs do not have a curative treatment, in practice available therapeutics is susceptible to pharmacogenomic intervention [17-19].

The introduction of novel procedures into an integral genomic medicine protocol in CNS disorders is an imperative requirement for clinical practice and drug development in order to improve diagnostic accuracy (disease-specific biomarkers) and to optimise therapeutics (pharmacogenomics) [20-26]. Drug treatment has made remarkable strides, with the introduction of many new drugs; however, improvement in terms of clinical outcome has fallen short of expectations, with up to one third of the patients continuing to experience clinical relapse or unacceptable medication-related side-effects in spite of efforts to identify optimal treatment regimens. Potential reasons to explain this historical setback might be that: the molecular pathology of most NDDs is still poorly understood; drug targets are inappropriate, not fitting into the real aetiology of the disease; most treatments are symptomatic, but not anti-pathogenic; the genetic component of NDDs is poorly defined; and the understanding of genome-drug interactions is very limited [12, 15, 16, 24].

2 Pharmacogenomics

The genes involved in the pharmacogenomic response to drugs in CNS disorders may fall into five major categories: (i) genes associated with CNS pathogenesis (disease-specific genes) (Tables 2, 3, 4, 5, 6); (ii) genes associated with the mechanism of action of drugs; (iii) genes associated with drug metabolism; (iv) genes associated with drug transporters; and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions [11, 16, 18, 19] (Tables 7–12). The therapeutic outcome (efficacy and safety) is the result of the interplay of drugs with these different categories of gene products and epigenetic factors to reverse or modify the phenotypic expression of a given disease [57]. Pharmacogenomics account for 30–90 % variability in pharmacokinetics and pharmacodynamics.

2.1 Pathogenic Genes

Over 6,000 genes distributed across the human genome have been screened for associations with CNS disorders during the past 30 years. Studies of many candidate genes potentially associated with a particular NDD could not be replicated in different settings, cohorts, and geographical contexts due to methodological problems, sample selection and multi-ethnic genetic variation. Prototypical examples of NDDs are Alzheimer's disease (Table 2), Parkinson's disease (Table 3), amyotrophic lateral sclerosis (Table 4), multiple sclerosis (Table 5), and Huntington's disease (Table 6).

2.2 Genes Involved in the Mechanism of Action of CNS Drugs

Most genes associated with the mechanism of action of CNS drugs encode receptors, proteins, enzymes, and neurotransmitters on which these drugs act as ligands (agonists, antagonists), enzyme modulators (substrates, inhibitors, inducers) or neurotransmitter regulators (releasers, reuptake inhibitors) [17] (Tables 7, 8, 9, 10, 11, 12).

2.3 Genes Involved in Drug Metabolism

Drug metabolism includes phase I reactions (i.e. oxidation, reduction, hydrolysis) and phase II conjugation reactions (i.e. acetylation, glucuronidation, sulphation, methylation). The principal enzymes with polymorphic variants involved in phase I reactions are the following: Cytochrome P450 monooxygenases (CYP3A4/5/7, CYP2E1, CYP2D6, CYP2C19, CYP2C9, CYP2C8, CYP2B6, CYP2A6, CYP1B1, CYP1A1/2), EPHX1/EPHX2 (epoxide hydrolases), esterases, NQO1 (NADPH-quinone oxidoreductase), DPD (dihydropyrimidine dehydrogenase), ADH (alcohol dehydrogenase), and ALDH (aldehyde dehydrogenase); and major enzymes involved in phase II reactions include UGTs (uridine 5′-triphosphate glucuronosyl transferases), TPMT (thiopurine methyltransferase), COMT (catechol-O-methyltransferase), HMT (histamine methyl-transferase), STs (sulfotransferases), GST-A (glutathione S-transferase A), GST-P (glutathione S-transferase P), GST-T (glutathione S-transferase T), GST-M (glutathione S-transferase M), NAT1 (N-acetyltransferase 1), NAT2 (N-acetyltransferase 2), and others.

Patients with NDDs are chronic users of both specific pharmacotherapy and drugs for comorbid symptomatology (psychotropic agents) whose metabolism is mainly via the cytochrome P450 (CYP) gene superfamily (Tables 7, 8, 9, 10, 11, 12, 13). P450 enzymes convert xenobiotics into electrophilic intermediates which are then conjugated by phase II enzymes to hydrophilic derivatives that can be excreted, according to the database of the World Guide for Drug Use and Pharma-

Table 2 Alzheimer disease-related genes [15]

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Alzheimer disease 1, familial	104300	Amyloid beta (A4) precursor protein	APP	21q21.3	104760
		Granulin precursor	GRN	17q21.31	138945
		Microtubule-associated protein tau	MAPT	17q21.31	157140
Alzheimer disease 2	104310	Apolipoprotein E	APOE	19q13.32	107741
Alzheimer disease 3	607822	Presenilin 1	PSEN1	14q24.2	104311
Alzheimer disease 4	606889	Presenilin 2	PSEN2	1q42.13	600759
Alzheimer disease 5	104300	Alzheimer disease 5	AD5	12p11.23-q13.12	602096
Alzheimer disease 6	104300	Alzheimer disease 6	AD6	10q24	605526
Alzheimer disease 7	606187	Alzheimer disease 7	AD7	10p13	606187
Alzheimer disease 8	104300	Alzheimer disease 8	AD8	20p	607116
Alzheimer disease 9, late onset, susceptibility to	104300	Alzheimer disease 9	AD9	19p13.2	608907
Alzheimer disease 10	104300	Alzheimer disease 10	AD10	7q36	609636
Alzheimer disease 11	609790	Alzheimer disease 11	AD11	9p22.1	609790
Alzheimer disease 12	611073	Alzheimer disease 12	AD12	8p12-q22	611073
Alzheimer disease 13	611152	Alzheimer disease 13	AD13	1q21	611152
Alzheimer disease 14	611154	Alzheimer disease 14	AD14	1q25	611154
Alzheimer disease 15	611155	Alzheimer disease 15	AD15	3q22-q24	611155
Alzheimer disease 16	300756	Alzheimer disease 16	AD16	Xq21.3	300756
Alzheimer disease, late-onset	104300	Amyloid beta (A4) precursor protein-binding, family B, member 2	APBB2	4p14-p13	602710

Table 2 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Alzheimer disease, late-onset, susceptibility to	104300	Nitric oxide synthase 3	NOS3	7q36.1	163729
		Plasminogen activator, urinary	PLAU	10q22.2	191840
Alzheimer disease, pathogenesis, association with	104300	Sortilin-related receptor	SORL1	11q24.1	602005
Alzheimer disease, susceptibility to	104300	Alpha-2-macroglobulin	A2M	12p13.31	103950
		ATP-binding cassette, subfamily A, member 7	ABCB7	19p13.3	605414
		Angiotensin I-converting enzyme	ACE	17q23.3	106180
		ADAM metallopeptidase domain 12	ADAM12	10q26.2	602714
		ADAM metallopeptidase domain 7	ADAM17	2p25	603639
		Advanced glycosylation end product-specific receptor	AGER	6p21.32	600214
		Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	AGT	1q42-q43	106150
		Arachidonate 5-lipoxygenase	ALOX5	10q11.21	152390
		Amyloid beta (A4) precursor protein-binding, family A, member 1	APBA1	9q21.11-q21.12	602414
		Amyloid beta (A4) precursor protein-binding, family A, member 2	APBA2	15q13.1	602712
		Amyloid beta (A4) precursor protein-binding, family A, member 3	APBA3	19p13.3	604262
		Amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	APBB1	11p15.4	602709
		Amyloid beta (A4) precursor protein-Binding ,family B, member 2	APBB2	15q22.2	602710

Table 2 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Alzheimer disease, susceptibility to	104300	Amyloid beta (A4) precursor protein-binding, family B, member 3	APBB3	19q13.12	602711
		Amyloid P component, serum	APCS	1q21-q23	104770
		Anterior pharynx defective 1 homolog B (C. elegans)	APH1B	15q22.2	607630
		Amyloid beta (A4) precursor-like protein 1	APLP1	19q13.1	104775
		Amyloid beta (A4) precursor-like protein 2	APLP2	11q24	104776
		Apolipoprotein D	APOD	2p24-p23	107730
		Apolipoprotein C-I	APOC1	19q13.2	107710
		Amyloid beta precursor protein (cytoplasmic tail) binding protein 2	APPBP2	17q23.2	605324
		Ataxin 1	ATXN1	6p23	601556
		Beta-site APP-cleaving enzyme 1	BACE1	11q23.2-q23.3	604252
		Beta-site APP-cleaving enzyme 2	BACE2	21q22.3	605668
		Butyrylcholinesterase	BCHE	3q26.1-q26.2	177400
		Brain-derived neurotrophic factor	BDNF	11p13	113505
		Bridging integrator	BIN1	2q14	601248
		Bleomycin hydrolase	BLMH	17q11.2	602403
		Biogenesis of lysosomal organelles complex-1, subunit 3	BLOC1S3	19q13.32	609762
		Bromodomain PHD finger transcription factor	BPTF	17q24.3	601819
		Calcium homeostasis modulator 1	CALHM1	10q24.33	612234
		Calpastatin	CAST	5q15	114090
		Cholesteryl ester transfer protein, plasma	CETP	16q21	118470
		CD2-Associated protein	CD2AP	6p12	604241

Table 2 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Alzheimer disease, susceptibility to	104300	CD33 antigen	CD33	19q13.3	159590
		Cholesterol 25-hydroxylase	CH25H	10q23	604551
		Cholinergic receptor, nicotinic, bet 2 (neuronal)	CHRNB2	1q21.3	118507
		Clusterin	CLU	8p21-p12	185430
		Catechol-O methyltransferase	COMT	22q11.21	116790
		COX10 homolog, cytochrome C oxidase assembly protein, heme A: farnesyltransferase (yeast)	COX10	17p12	602125
		COX15 homolog, cytochrome c oxidase assembly protein (yeast)	COX15	10q24	603646
		Complement component (3b/4b)receptor 1 (Knops blood group	CR1	1q32	120620
		cAMP responsive element binding protein 1	CREB1	2q34	123810
		Cystatin C	CST3	20p11.21	604312
		Cathepsin B	CTSB	8p22	116810
		Cathepsin D	CTSD	11p15.5	116840
		Cytochrome P450, family 19, Subfamily A, polypeptide 1	CYP19A1	15q21.1	107910
		Cytochrome P450, family 46, subfamily A, Polypeptide 1	CYP46A1	14q32.1	604087
		D-amino acid oxidase activator	DAOA	13q34	607408
		Death-associated protein kinase 1	DAPK1	9q21.33	600831
		Drebrin 1	DBN1	5q35.3	126660
		24-dehydrocholesterol reductase	DHCR24	1p32.3	606418
		Endothelin-converting enzyme 1	ECE1	1p36.1	600423
		ERI 1 exoribonuclease family member 3	ERI3	1p32	609917

Table 2 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Alzheimer disease, susceptibility to	104300	Exocyst complex component 3-like 2	EXOC3L2	19q13.32	–
		FBJ murine osteosarcoma viral oncogene homolog	FOS	14q24.3	164810
		GRB2-associated binding protein 2	GAB2	11q14.1	606203
		Galanin-like peptide	GALP	19q13.43	611178
		Gliosis, familial progressive subcortical	GPSC	17q21-q22	–
		Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	GRIN3A	9q31.1	606650
		Glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	GRIN3B	19p13.3	606651
		HECT domain containing 2	HECTD2	10q23.32	–
		HFE gene	HFE	6p21.3	613609
		Major histocompatibility complex, class I, A	HLA-A	6p21.3	142800
		Heat-shock 70-kd protein 5	HSPA5	9q33.3	138120
		Islet amyloid polypeptide	IAPP	12p12.1	147940
		Intercellular adhesion molecule 1	ICAM	19p13.3-p13.2	147840
		Insulin-degrading enzyme	IDE	10q23-q25	146680
		Interleukin-1-Alpha	IL1A	2q14	147760
		Interleukin-6 (interferon, beta 2)	IL6	7p21	147620
		Interleukin-10	IL10	1q31-q32	124092
		Interleukin 33	IL33	9p24.1	608678
		Kv channel interactin protein 3, calsenilin	KCNIP3	2q21.1	604662
		Lamin A/C	LMNA	1q22	150330
		Low-density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)	LRP1	12q13.3	107770

Table 2 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Alzheimer disease, susceptibility to	104300	Low-density lipoprotein-related protein 1B (deleted in tumours)	LRP1B	2q21.2	608766
		Low-density lipoprotein-related protein 8, apolipoprotein E receptor	LRP8	1p34	602600
		MAP/microtubule affinity-regulating kinase 4	MARK4	19q13.3	606495
		MicroRNA 144	MIR144	17q11.2	612070
		Membrane metalloendopeptidase	MME	3q25.2	120520
		Myeloperoxidase	MPO	17q23.1	606989
		Membrane-spanning 4-domains, subfamily A, member 4E	MS4A4E	11q12.2	608401
		Membrane-spanning 4-domains, subfamily A, member 6A	MS4A6A	11q12.1	606548
		5,10-methylenetetrahydrofolate reductase (NADPH)	MTHFR	1p36.3	607093
		Myosin, heavy chain 13, skeletal muscle	MYH13	17p13	603487
		NEDD8 activating enzyme E1 subunit 1	NAE1	16q22	603385
		Nicastrin	NCSTN	1q22-q23	605254
		Notch homolog 3 (Drosophila)	NOTCH3	19p13.2-p13.1	600276
		Pax transcription activation domain-interacting protein 1	PAXIP1	7q36	608254
		Phosphoenolpyruvate carboxykinase 1 (soluble)	PCK1	20q13.31	614168
		Presenilin enhancer 2 homolog (C. elegans)	PSENEN	19q13.12	607632
		PiggyBac transposable element derived 1	PGBD1	6p22.1	–
		Phosphatidylinositol binding clathrin assembly protein	PICALM	11q14	603025
		Peptidylprolyl cis/trans isomerase, NIMA-interacting 1	PIN1	19p13	601052
		Pitrilysin metallopeptidase 1	PITRM1	10p15.2	–
		Paraoxonase 1	PON1	7q21.3	168820

Table 2 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Alzheimer disease, susceptibility to	104300	Prion protein	PRNP	20p13	176640
		Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	PTGS2	1q25.2-q25.3	600262
		Renal tumor antigen	RAGE	6p21.3	600214
		Reelin	RELN	7q22	600514
		Reticulon 4	RTN4	2p16.	604475
		Reticulon 4 receptor	RTN4R	22q11.21	605566
		S100-calcium-binding protein A1	S100A1	1q21	176940
		Serum amyloid A1	SAA1	11p15.1	104750
		Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	SERPINA3	14q32.1	107280
		SH3 and PX domains 2A	SH3PXD2A	10q24.33	–
		Sterol O-acyltransferase 1	SOAT1	1q25	102642
		Sortilin-related VPS10 domain containing receptor 1	SORCS1	10q23-q25	606283
		Sortilin	SORT1	1p21.3-p13.1	602458
		Somastostatin	SST	3q28	182450
		Saitohin	STH	17q21.1	607067
		TARD DNA-binding protein	TARDBP	1p36.22	605078
		TM2 domain containing 1	TM2D1	1p31.3	610080
		Transcription factor A, mitochondrial	TFAM	10q21	600438
		Tumor necrosis factor (TNF superfamily, member 2)	TNF	6p21.3	191160
		Tyrosine kinase, nonreceptor, 1	TNK1	17p13.1	608076

Table 2 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Alzheimer disease, susceptibility to	104300	Translocase of outer mitochondrial membrane 40 homolog (yeast)	TOMM40	19q13	608061
		Triggering receptor expressed on myeloid cells 2	TREM2	6p21.1	605086
		Transthyretin	TTR	18q12.1	176300
		Ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast)	UBE2I	16p13.3	601661
		Vascular endothelial growth factor	VEGFA	6p12	192240
		Zinc finger, FYVE domain containing 9	ZFYVE9	1p32.3	603755

Table 3 Parkinson disease-related genes

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Parkinson disease 1	168601	Synuclein, alpha (non A4 component of amyloid precursor)	SNCA	4q22.1	163890
Parkinson disease 2, juvenile	600116	Parkin	PARK2	6q26	602544
Parkinson disease 3	602404	Parkinson disease 3	PARK3	2p13	602404
Parkinson disease 4, autosomal dominant Lewy body	605543	Synuclein, alpha (non A4 component of amyloid precursor)	SNCA	4q22.1	163890
Parkinson disease 5, susceptibility to	613643	Ubiquitin carboxyl-terminal esterase L1	UCHL1	4p13	191342
Parkinson disease 6, early onset	605909	PTEN induced putative kinase 1	PINK1	1p36.12	608309
Parkinson disease 7, autosomal recessive early-onset	606324	Oncogene DJ1	DJ1	1p36.23	602533
Parkinson disease 8	607060	Leucine-rich repeat kinase 2 (dardarin)	LRRK2	12q12	609007
Parkinson disease 9	606693	ATPase type 13A2	ATP13A2	1p36.13	610513
Parkinson disease 10	606852	Parkinson disease 10	PARK10	1p32	606852
Parkinson disease 11	607688	GRB10-interacting GYF protein 2	GIGYF2	2q37.1	612003
Parkinson disease 12	300557	Parkinson disease-12	PARK12	Xq21-q25	300557
Parkinson disease 13	610297	HtrA serine peptidase 2	HTRA2	2p13.1	606441
Parkinson disease 14	612953	Phospholipase A2, group VI	PLA2G6	22q13.1	603604
Parkinson disease 15, autosomal recessive	260300	F-box only protein 7	FBOX7	22q12.3	605648
Parkinson disease 16	613164	Parkinson disease 16	PARK16	1q32	613164
Parkinson disease 17	614203	Vacuolar protein sorting 35, yeast, homolog of	VPS35	16q11.2	601501

Table 3 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Parkinson disease 18	614251	Eukaryotic translation initiation factor 4-gamma, 1	EIF4G1	3q27.1	600495
Parkinson disease, late-onset, susceptibility to	168600	Glucosidase, beta, acid	GBA	1q22	606463
Parkinson disease, susceptibility to	168600	Alcohol dehydrogenase 1C (Class I), gamma polypeptide	ADH1C	4q23	103730
		Microtubule-associated protein tau	MAPT	17q21.31	157140
		TATA box binding protein	TBP	6q27	600075

Table 4 Amyotrophic lateral sclerosis disease-related genes

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Amyotrophic lateral sclerosis, susceptibility to	105400	Dynactin 1 (p150, glued, Drosophila, homolog of)	DCTN1	2p13.1	601143
		Neurofilament protein, heavy polypeptide	NEFH	22q12.2	162230
		Peripherin	PRPH	12q13.12	170710
Amyotrophic lateral sclerosis 1	105400	Superoxide dismutase 1	SOD1	21q22.11	147450
Amyotrophic lateral sclerosis 2, juvenile	205100	Alsin	ALS2	2q33.1	606352
Amyotrophic lateral sclerosis 3	606640	Amyotrophic lateral sclerosis 3	ALS3	18q21	606640
Amyotrophic lateral sclerosis 4, juvenile	602433	Senataxin	SETX	9q34.13	608465
Amyotrophic lateral sclerosis 5, juvenile recessive	602099	Amyotrophic lateral sclerosis 5, juvenile recessive	ALS5	15q15.1-q21.1	602099
Amyotrophic lateral sclerosis 6, autosomal recessive, with or without frontotemporal dementia	608030	Fused in sarcoma	FUS	16p11.2	137070
Amyotrophic lateral sclerosis 7	608031	Amyotrophic lateral sclerosis 7	ALS7	20p13	608031
Amyotrophic lateral sclerosis 8	608627	Vesicle-associated membrane protein-associated protein B	VAPB	20q13.32	605704
Amyotrophic lateral sclerosis 9	611895	Angiogenin	ANG	14q11.2	105850
Amyotrophic lateral sclerosis 10, with or without FTD	612069	TAR DNA-binding protein	TARDBP	1p36.22	605078
Amyotrophic lateral sclerosis 11	612577	FIG4, S. Cerevisiae, homolog of	FIG4	6q21	609390
Amyotrophic lateral sclerosis 12	613435	Optineurin	OPTN	10p13	602432
Amyotrophic lateral sclerosis 13, susceptibility to	183090	Ataxin-2	ATXN2	12q24.12	601517

Table 4 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Amyotrophic lateral sclerosis 14, with or without frontotemporal dementia	613954	Valosin-containing protein	VCP	9p13.3	601023
Amyotrophic lateral sclerosis 15, with or without frontotemporal dementia	300857	Ubiquilin 2	UBQLN2	Xp11.21	300264
Amyotrophic lateral sclerosis 16, juvenile	614373	Sigma nonopioid intracellular receptor 1	SIGMAR1	9p13.3	601978
Amyotrophic lateral sclerosis 17	614696	CHMP family, member 2B	CHMP2B	3p11.2	609512
Amyotrophic lateral sclerosis 18	614808	Profilin 1	PFN1	17p13.2	176610
Amyotrophic lateral sclerosis-parkinsonism/dementia complex, susceptibility to	105500	Transient receptor potential cation channel, subfamily M, member 7	TRPM7	15q21.2	605692
Amyotrophic lateral sclerosis and/or frontotemporal dementia	105550	Chromosome 9 open reading frame 72	C9orf72	9p21.2	614260

Table 5 Multiple sclerosis disease-related genes [27]

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Multiple sclerosis, susceptibility to	126200	CD6 molecule	CD6	11q12.2	186720
		CD40 molecule, TNF receptor superfamily member 5	CD40	20q13.12	109535
		CD58 molecule	CD58	1p13.1	153420
		CD80 molecule	CD80	3q13.33	112203
		CD86 molecule	CD86	3q13.33	601020
		Cytochrome P450, family 27, subfamily B, polypeptide 1	CYP27B1	12q14.1	609506
		Major histocompatibility complex, class II, DP beta 1	HLA-DPB1	6p21.32	142858
		Major histocompatibility complex, class II, DQ beta-1	HLA-DQB1	6p21.32	604305
		Major histocompatibility complex, class II, DR beta-1	HLA-DRB1	6p21.32	142857
		Interleukin 2 receptor, alpha	IL2RA	10p15.1	147730
		Interleukin 7 receptor	IL7R	5p13.2	146661
		Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	IL12A	3q25.33	161560
		Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	IL12B	5q33.3	161561
		Interleukin 22 receptor, alpha 1	IL22RA1	1p36.11	605457
		SP140 nuclear body protein	SP140	2q37.1	608602
		Signal transducer and activator of transcription 3 (acute-phase response factor)	STAT3	17q21.2	102582

Table 5 (continued)

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Multiple sclerosis, susceptibility to	126200	Tyrosine kinase 2	TYK2	19p13.2	176941
		Multiple sclerosis, susceptibility to, 2	MS2	10p15.1	612594
		Multiple sclerosis, susceptibility to, 3	MS3	5p13.2	612595
		Multiple sclerosis, susceptibility to, 4	MS4	1p36	612596
		Tumor necrosis factor receptor superfamily, member 1A	TNFRSF1A	12p13.31	191190
Multiple sclerosis, disease progression, modifier of	126200	Programmed cell death 1	PDCD1	2q37.3	600244

Table 6 Huntington disease-related genes

Subtype		Genes			
	OMIM	Name	Symbol	Locus	OMIM
Huntington disease	143100	Huntingtin	HTT	4p16.3	613004
Huntington disease-like 1	603218	Prion protein	PRNP	20p13	176640
Huntington disease-like 2	606438	Junctophilin 3	JPH3	16q24.2	605268
Huntington disease-like 3	604802	Huntington-like neurodegenerative disorder 2	HDL3	4p15.3	604802
Huntington disease-like 4	607136	TATA box binding protein	TBP	6q27	600075

cogenomics [17]. Most CYP enzymes exhibit ontogenic-, age-, sex-, circadian-, and ethnic-related differences [58]. The practical consequence of this genetic variation is that the same drug can be differentially metabolised according to the genetic profile/expression during each subject's lifespan, and that knowing the pharmacogenomic profile of an individual, his/her pharmacodynamic response is potentially predictable to some extent.

As a general rule, the following phenotypes are differentiated: (a) extensive metabolisers (EMs) are individuals who respond normally to drugs at conventional doses; (b) intermediate metabolisers (IMs), carriers of a defective gene, which gives rise to an enzyme which is less efficient when metabolising drugs; (c) poor metabolisers (PMs), carriers of a defective gene whose mutation generates an enzyme with no drug-metabolising activity, or which simply has no enzyme; (d) ultra-rapid metabolisers (UMs), individuals whose gene is duplicated, triplicated or otherwise multiplied, bringing about an excessive enzymatic activity [17].

Among these enzymes, CYP2D6, CYP2C9, CYP2C19, and CYP3A4/5 are the most relevant in the pharmacogenetics of CNS drugs (Table 13).

2.3.1 *CYP2D6*

This drug metabolism gene is currently the most studied. Four RNA transcripts are expressed in the brain, liver, spleen and reproductive system, where 4 major proteins of 48–55 kDa (439–494 aa) are identified. This protein is a transport enzyme of the cytochrome P450 subfamily IID or multigenic cytochrome P450 superfamily of mixed-function monooxygenases which localises to the endoplasmic reticulum and is known to metabolise as many as 25% of commonly-prescribed drugs and over 60% of current psychotropics. This gene is highly polymorphic in the population. There are 141 *CYP2D6* allelic variants, of which the most important variants are listed in Table 13. 982 drugs are CYP2D6-related; 371 drugs are substrates, over 300 drugs are inhibitors, and 18 drugs are CYP2D6 inducers.

Table 7 Pharmacogenomics of Alzheimer's disease [17, 28-31].

Donepezil		
Category		Anti-Dementia agent; Cholinesterase inhibitor
Mechanism		Centrally active, reversible acetylcholinesterase inhibitor; increases the acetylcholine available for synaptic transmission in the CNS
Genes	Pathogenic	*APOE, CHAT*
	Mechanistic	*CHAT, ACHE, BCHE*
	Metabolism	
	Substrate	*CYP2D6* (major), *CYP3A4* (major), *UGTs, ACHE*
	Inhibitor	*ACHE, BCHE*
	Transporter	*ABCB1*
Galantamine		
Category		Anti-Dementia agent; Cholinesterase inhibitor
Mechanism		Reversible and competitive acetylcholinesterase inhibition leading to an increased concentration of acetylcholine at cholinergic synapses; modulates nicotinic acetylcholine receptor; may increase glutamate and serotonin levels
Genes	Pathogenic	*APOE, APP*
	Mechanistic	*ACHE, BCHE, CHRNA4, CHRNA7, CHRNB2*
	Metabolism	
	Substrate	*CYP2D6* (major), *CYP3A4* (major), *UGT1A1*
	Inhibitor	*ACHE, BCHE*
Memantine		
Category		Anti-Dementia drug; N-Methyl-D-Aspartate receptor antagonist
Mechanism		Binds preferentially to NMDA receptor-operated cation channels; may act by blocking actions of glutamate, mediated in part by NMDA receptors; Antagonist: GRIN2A, GRIN2B, GRIN3A, HTR3A, CHRFAM7A

Table 7 (continued)

Genes	Pathogenic	*APOE, PSEN1, MAPT*
	Mechanistic	*GRIN2A, GRIN2B, GRIN3A, HTR3A, CHRFAM7A*
	Metabolism nhibitor	
		CYP1A2 (weak), *CYP2A6* (weak), *CYP2B6* (strong), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (strong), *CYP2E1* (weak), *CYP3A4* (weak)
	Pleiotropic	*APOE, MAPT, MT-TK, PSEN1*
Rivastigmine		
Category		Anti-Dementia agent; Cholinesterase inhibitor
Mechanism		Increases acetylcholine in CNS through reversible inhibition of its hydrolysis by cholinesterase
Genes	Pathogenic	*APOE, APP, CHAT*
	Mechanistic	*ACHE, BCHE,CHAT, CHRNA4, CHRNB2*
	Metabolism Inhibitor	
		ACHE, BCHE
	Pleiotropic	*APOE, MAPT*
Tacrine		
Category		Anti-Dementia agent; Cholinesterase inhibitor
Mechanism		Elevates acetylcholine in cerebral cortex by slowing degradation of acetylcholine
Genes	Pathogenic	*APOE*

Table 7 (continued)

	Mechanistic Metabolism Substrate Inhibitor	*ACHE, BCHE,CHRNA4, CHRNB2*
		CYP1A2 (major), *CYP2D6* (minor), *CYP3A4* (major)
		ACHE, BCHE, CYP1A2 (weak)
	Transporter	*SCN1A*
	Pleiotropic	*APOE, MTHFR, CES1, LEPR, GSTM1, GSTT1*

ABCB1 ATP-binding cassette, sub-family B (MDR/TAP), member 1, *ACHE* Acetylcholinesterase (Yt blood group), *APOE* Apolipoprotein E, *APP* Amyloid beta (A4) precursor protein, *BCHE* Butyrylcholinesterase, *CES1* Carboxylesterase, *CHAT* Choline O-acetyltransferase, *CHRFAM7A* Cholinergic receptor, nicotinic, alpha 7, exons 5–10), *FAM7A* (family with sequence similarity 7A, exons A-E) fusion, *CHRNA4* Cholinergic receptor, nicotinic, alpha 4, *CHRNA7* Cholinergic receptor, nicotinic, alpha 7, *CHRNB2* Cholinergic receptor, nicotinic, beta 2 (neuronal), *CYP1A2* Cytochrome P450, family 1, subfamily A, polypeptide 2, *CYP2A6* Cytochrome P450, family 2, subfamily A, polypeptide 6, *CYP2B6* Cytochrome P450, family 2, subfamily B, polypeptide 6, *CYP2C19* Cytochrome P450, family 2, subfamily C, polypeptide 19, *CYP2C9* Cytochrome P450, family 2, subfamily C, polypeptide 9, *CYP2D6* Cytochrome P450, family 2, subfamily D, polypeptide 6, *CYP2E1* Cytochrome P450, family 2, subfamily E, polypeptide 1, *CYP3A4* Cytochrome P450, family 3, subfamily A, polypeptide 4, *GRIN2A* Glutamate receptor, ionotropic, N-methyl D-aspartate 2A, *GRIN2B* Glutamate receptor, ionotropic, N-methyl D-aspartate 2B, *GRIN3A* Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A, *GSTM1* Glutathione S-transferase mu 1, *GSTT1* Glutathione S-transferase theta 1, *HTR3A* 5-Hydroxytryptamine (serotonin) receptor 3A, *LEPR* Leptin receptor, *MAPT* Microtubule-associated protein tau, *MT-TK* Mitochondrially encoded tRNA lysine, *MTHFR* 5,10 methylenetetrahydrofolate reductase (NADPH), *PSEN1* Presenilin 1, *SCN1A* sodium channel, voltage-gated, type I, alpha subunit, *UGT1A1* UDP glucuronosyltransferase 1 family, polypeptide A1, *UGTs* UDP glucuronosyltransferase families

Table 8 Pharmacogenomics of Parkinson disease [17, 30].

Amantadine		
Category		Antiparkinsonian agent, Adamantane. Dopamine agonist
Mechanism		Inhibition of dopamine reuptake into presynaptic neurons increase of dopamine release from presynaptic fibers. Antagonist: GRIN3A. Agonist: DRD2
Genes	Pathogenic	*DDC, DRDs, GRIN3A, PARK2*
	Mechanistic	*DDC, DRDs, GRIN3A*
	Metabolism	No appreciable metabolism, although negligible amounts of an acetyl metabolite have been identified
Apomorphine		
Category		Antiparkinsonian agent; Dopamine receptor agonists; Nonergot-derivative Dopamine receptor agonist
Mechanism		Stimulates postsynaptic D2-type receptors within the caudate putamen in the brain. Agonist: DRD2, DRD3
Genes	Pathogenic	*PARK2*
	Mechanistic	*DRD2, DRD3*
	Metabolism Substrate Inhibitor	
		CYP1A2 (minor), *CYP2C9* (minor), *CYP2C19* (minor), *CYP3A4* (minor) *SULT1A1, SULT1E1*
		CYP1A2 (weak), *CYP2C19* (weak), *CYP3A4* (weak)
Benztropine		
Category		Antiparkinsonian agent; Anticholinergic agent
Mechanism		Possesses both anticholinergic and antihistaminic effects. Inhibits the reuptake and storage of dopamine. Antagonist: CHRM1, HRH1
Genes	Mechanistic	*CHRM1, HRH1*
	Metabolism Substrate Inhibitor	
		ABCB1, *CYP2C19* (minor), *CYP2D6* (major)
		SLC6A3
	Transporter	*ABCB1*

Table 8 (continued)

Biperiden		
Category		Antiparkinsonian agent; Anticholinergic agent
Mechanism		This antimuscarinic agent inhibits acetylcholine or other cholinergic stimulus at autonomic effectors innervated by post-ganglionic nerves. Inhibition of striatal cholinergic receptors. Antagonist: CHRM1, CHRNA2
Genes	Pathogenic	*PARK2*
	Mechanistic	*ACHE, CHRM1, CHRNA2*
	Metabolism Inhibitor	
		CYP2D6 (weak)
Bromocriptine		
Category		Antiparkinsonian agent; Dopamine receptor agonist; Ergot-derivative Dopamine receptor agonist
Mechanism		Activates postsynaptic dopamine receptors in the tuberoinfundibular and nigrostriatal pathways. Agonist: DRD1, DRD2, DRD3
Genes	Pathogenic	*PARK2*
	Mechanistic	*DRD1, DRD2, DRD3*
	Metabolism Substrate Inhibitor Inducer	
		ABCG2, CYP3A4 (major)
		ABCB1, CYP1A2 (weak), *CYP3A4* (moderate)
		ABCB1
	Transporter	*ABCB1, ABCG2*
	Pleiotropic	*DRD3, TGFB1, VEGFA*
Cabergoline		
Category		Antiparkinsonian agent; Dopamine receptor agonist; Ergot-derivative Dopamine receptor agonist
Mechanism		Long-acting dopamine receptor agonist with high binding affinity for dopamine D2 receptors and lesser affinity for D1, α1- and α2-adrenergic, and serotonin (5-HT1 and 5-HT2) receptors. Agonist: DRD1, DRD2, HTR1A, HTR1B, HTR2A, HTR2B, HTR2C. Antagonist: ADRA2A, ADRA2B, ADRA2C

Table 8 (continued)

Genes	Pathogenic	*PARK2*
	Mechanistic	*ADRA1s, ADRA2s, DRD1, DRD2, HTR1s, HTR2s*
	Metabolism Substrate	
		ABCB1, CYP3A4 (minor)
	Transporter	*ABCB1*
Carbidopa		
Category		Antiparkinsonian agent; Dopamine precursor
Mechanism		Inhibits the peripheral decarboxylation of levodopa to dopamine. Agonist: DRD2
Genes	Pathogenic	*PARK2*
	Mechanistic	*ACHE, COMT, DDC, DRD2*
	Pleiotropic	*ACE, BDNF, CHAT, OPRM1, PARK2*
Diphenhydramine		
Category		Antiparkinsonian agent; Anticholinergic agent
Mechanism		A potent antagonist to acetylcholine affecting muscarinic receptors; competes with free histamine for binding at HA-receptor sites. Antagonist: HRH1
Genes	Pathogenic	*PARK2*
	Mechanistic	*HRH1, CHRMs*
	Metabolism Substrate Inhibitor	
		CYP1A2 (minor), *CYP2C9* (minor), *CYP2C19* (minor), *CYP2D6* (major), *UGT1A3*, *UGT1A4*
		CYP2D6 (moderate)
Entacapone		
Category		Antiparkinsonian agent; Catechol-O-Methyltransferase (COMT) Inhibitor
Mechanism		Selective and reversible inhibitor of COMT. When entacapone is taken with levodopa, the pharmacokinetics is altered, resulting in more sustained levodopa serum levels compared to levodopa taken alone

Table 8 (continued)

Genes	Pathogenic	*PARK2*
	Mechanistic	*COMT*
	Metabolism Substrate Inhibitor	
		UGT1A1, UGT1A3, UGT1A4, UGT1A9, UGT1A10
		COMT, CYP1A2 (weak), *CYP2A6* (weak), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (weak), *CYP2E1* (weak), *CYP3A4* (weak)
Ethopropazine [32]		
Category		Antiparkinsonian agent; Anticholinergic agent
Mechanism		Partially blocks central cholinergic receptors, helping to balance cholinergic and dopaminergic activity in the basal ganglia. Ethopropazine 's local anesthetic effect is due to its antagonism of the NMDA glutamate receptor
Genes	Mechanistic	*CHRMs, NMDA*
	Metabolism Inhibitor	
		BCHE
Levodopa		
Category		Antiparkinsonian agent; Dopamine precursor
Mechanism		Levodopa circulates in the plasma to the blood-brain-barrier, where it crosses, to be converted by striatal enzymes to dopamine. Agonist: DRD1, DRD2, DRD3, DRD4, DRD5
Genes	Mechanistic	*DRD1, DRD2, DRD3, DRD4, DRD5*
	Metabolism Inhibitor	
		COMT
Lisuride		
Category		Antiparkinsonian agent; Ergot-derivative Dopamine receptor agonist
Mechanism		Displays dopaminergic and consequently prolactin-reducing properties. It has pronounced affinity for dopamine receptors in striatum and pituitary. Agonist: DRD2, HTR1A; Antagonist: DRD1

Table 8 (continued)

Genes	Mechanistic	*DRD1, DRD2, HTR1A*
	Metabolism Substrate	
		CYP2D6 (major), *CYP3A4* (major)
Pergolide		
Category		Antiparkinsonian agent; Ergot-derivative Dopamine receptor agonist
Mechanism		Stimulates postsynaptic dopamine receptors in corpus striatum, reduces serum prolactin concentrations, causes transient increases in serum somatotropin concentrations and decreases in serum luteinizing hormone concentrations. Agonist: *DRD1*, *DRD2*
Genes	Pathogenic	*PARK2*
	Mechanistic	*DRD1, DRD2, GH, LH, PRL*
	Metabolism Substrate Inhibitor	
		CYP3A4 (major)
		CYP2D6, CYP3A4
Pramipexole		
Category		Antiparkinsonian agent; Nonergot-derivative Dopamine receptor agonist
Mechanism		Binds to D2 subfamily dopamine receptor and to D3 and D4 receptors; Stimulates dopamine activity on nerves of striatum and substantia nigra. Agonist: DRD2, DRD3, DRD4
Genes	Pathogenic	*PARK2*
	Mechanistic	*DRD2, DRD3, DRD4*
	Metabolism Substrate	
		CYP3A4
Procyclidine		
Category		Antiparkinsonian agent; Anticholinergic agent

Table 8 (continued)

Mechanism		Might act blocking central cholinergic receptors, and thus balancing cholinergic and dopaminergic activity in the basal ganglia. Antagonist: CHRM1, CHRM2, CHRM4
Genes	Pathogenic	*PARK2*
	Mechanistic	*CHRM1, CHRM2, CHRM4*
Rasagiline		
Category		Antiparkinsonian agent; Monoamine Oxidase B inhibitor
Mechanism		MAO-B inhibitory activity; which causes an increase in extracellular levels of dopamine in the striatum
Genes	Pathogenic	*PARK2*
	Mechanistic	*MAO-B*
	Metabolism Substrate Inhibitor	
		CYP1A2 (major), *UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, UGT1A10, UGT2B7, UGT2B15*
		MAOB
Ropinirole		
Category		Antiparkinsonian agent; Nonergot-derivative Dopamine receptor agonist
Mechanism		Binds to dopamine receptors D2 (higher affinity), D3 and D4 in the striatum Agonist: *DRD2, DRD3, DRD4*
Genes	Pathogenic	*PARK2*
	Mechanistic	*DRD2, DRD3, DRD4*
	Metabolism Substrate Inhibitor	
		CYP1A2 (major), *CYP3A4* (minor)
		CYP1A2 (moderate), *CYP2D6* (moderate), *CYP3A4* (moderate)
Rotigotine		
Category		Antiparkinsonian agent; Nonergot-derivative Dopamine receptor agonist
Mechanism		Non-ergot dopamine agonist with specificity for D3, D2, and D1 dopamine receptors. Stimulation of postsynaptic dopamine D2-type auto-receptors within substantia nigra. Agonist: DRD1, DRD2, DRD3

Table 8 (continued)

Genes	Pathogenic	*PARK2*
	Mechanistic	*DRD1, DRD2, DRD3*
Selegiline [33]		
Category		Antiparkinsonian agent; Monoamine Oxidase B inhibitor
Mechanism		Binds to MAOB within the nigrostriatal pathways blocking microsomal metabolism of dopamine and enhancing the dopaminergic activity in the substantia nigra; at higher doses, it can also inhibit MAOA
Genes	Pathogenic	*PARK2*
	Mechanistic	*MAOA, MAOB*
	Metabolism Substrate Inhibitor	
		CYP1A2 (minor), *CYP2A6* (minor), *CYP2B6* (major), *CYP2C8* (minor), *CYP2C19* (major), *CYP2D6* (minor), *CYP2E1* (minor), *CYP3A4* (minor), *MAOA*
		CYP1A2 (weak), *CYP2A6* (weak), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (weak), *CYP2E1* (weak), *CYP3A4* (weak), *MAOB, MAOA*
	Transporter	*SCNA*
Tolcapone [34]		
Category		Antiparkinsonian agent; Catechol-O-Methyltransferase (COMT) inhibitor
Mechanism		A selective and reversible inhibitor of COMT. In presence of a decarboxylase inhibitor, COMT is the major degradation pathway for levodopa. Inhibition of COMT leads to more sustained plasma levels of levodopa and enhanced central dopaminergic activity
Genes	Pathogenic	*PARK2*
	Mechanistic	*COMT*
	Metabolism Substrate Inhibitor	
		UGT1A
		COMT

Table 8 (continued)

Trihexyphenidyl		
Category		Antiparkinsonian agent; Anticholinergic agent
Mechanism		Selective M1 muscarinic acetylcholine receptor antagonist. It is able to discriminate between the M1 and the peripheral muscarinic subtypes. It also partially blocks cholinergic activity in the CNS and it is thought to increase the availability of dopamine. Antagonist: CHRM1
Genes	Pathogenic	*PARK2*
	Mechanistic	*CHRM1*

ABCB1 ATP-binding cassette, sub-family B (MDR/TAP), member 1, *ABCG2* ATP-binding cassette, sub-family G (WHITE), member 2, *ACE* Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1, *ACHE* Acetylcholinesterase, *ADRA1s* Alpha 1 adrenergic receptors, *ADRA2s* Alpha 2 adrenergic receptors, *ADRA2A* Adrenoceptor alpha 2A, *ADRA2B* Adrenoceptor alpha 2B, *ADRA2C* Adrenoceptor alpha, *BCHE* Butyrylcholinesterase, *BDNF* Brain-derived neurotrophic factor, *CHAT* Choline O-acetyltransferase, *CHRM1* Cholinergic receptor, muscarinic 1, *CHRM2* Cholinergic receptor, muscarinic 2, *CHRM3* Cholinergic receptor, muscarinic 3, *CHRM4* Cholinergic receptor, muscarinic 4, *CHRM5* Cholinergic receptor, muscarinic 5, *CHRNA2* Cholinergic receptor, nicotinic, alpha 2 (neuronal), *COMT* Catechol-O-methyltransferase, *CYP1A2* Cytochrome P450, family 1, subfamily A, polypeptide 2, *CYP2A6* Cytochrome P450, family 2, subfamily A, polypeptide 6, *CYP2B6* Cytochrome P450, family 2, subfamily B, polypeptide 6, *CYP2C8* Cytochrome P450, family 2, subfamily C, polypeptide 8, *CYP2C19* Cytochrome P450, family 2, subfamily C, polypeptide 19, *CYP2C9* Cytochrome P450, family 2, subfamily C, polypeptide 9, *CYP2D6* Cytochrome P450, family 2, subfamily D, polypeptide 6, *CYP2E1* Cytochrome P450, family 2, subfamily E, polypeptide 1, *CYP3A4* Cytochrome P450, family 3, subfamily A, polypeptide 4, *DDC* Dopa decarboxylase (aromatic L-amino acid decarboxylase), *DRDs* Dopamine receptor, *DRD1* Dopamine receptor D1, *DRD2* Dopamine receptor D2, *DRD3* Dopamine receptor D3, *DRD4* Dopamine receptor D4, *DRD5* Dopamine receptor D5, *GRIN3A* Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A, *HRH1* Histamine receptor H1, *HTR1s* 5-Hydroxytryptamine type 1 receptors, *HTR1A* 5-Hydroxytryptamine (serotonin) receptor 1A, G protein-coupled, *HTR1B* 5-Hydroxytryptamine (serotonin) receptor 1B, G protein-coupled, *HTR2s* 5-Hydroxytryptamine (serotonin) type 2 receptors, *HTR2A* 5-Hydroxytryptamine (serotonin) receptor 2A, G protein-coupled, *HTR2B* 5-Hydroxytryptamine (serotonin) receptor 2B, G protein-coupled, *HTR2C* 5-Hydroxytryptamine (serotonin) receptor, G protein-coupled, *MAOA* Monoamine oxidase A, *MAOB* Monoamine oxidase B, *OPRM1* Opioid receptor, mu 1, *PARK2* Parkinson protein 2, E3 ubiquitin protein ligase (parkin), *SCNA* sodium channel alpha-subunit gene family, *SLC6A3* Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3, *SULT1A1* Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1, *SULT1E1* Sulfotransferase family 1E, estrogen-preferring, member 1, *TGFB1* Transforming growth factor, beta 1, *UGT1A* UDP glucuronosyltransferase 1 family, polypeptide A complex locus, *UGT1A1* UDP glucuronosyltransferase 1 family, polypeptide A1, *UGT1A3* UDP glucuronosyltransferase 1 family, polypeptide A3, *UGT1A4* UDP glucuronosyltransferase 1 family, polypeptide A4, *UGT1A6* UDP glucuronosyltransferase 1 family, polypeptide A6, *UGT1A7* UDP glucuronosyltransferase 1 family, polypeptide A7, *UGT1A9* UDP glucuronosyltransferase 1 family, polypeptide A9, *UGT1A10* UDP glucuronosyltransferase 1 family, polypeptide A10, *UGT2B7* UDP glucuronosyltransferase 2 family, polypeptide B7, *UGT2B15* UDP glucuronosyltransferase 2 family, polypeptide B15, *VEGFA* vascular endothelial growth factor A

Table 9 Pharmacogenomics of amyotrophic lateral sclerosis [17, 30].

Riluzole [35, 36]		
Category		Central nervous system agent, Glutamate inhibitor
Mechanism		Inhibition of glutamate release, inactivation of voltage-dependent sodium channels, and blocking the action of excitatory amino acid receptor agonists
Genes	Pathogenic	*GRIN3A*
	Mechanistic	*GRIN3A, SCN1A, SCN1B, SCN2A, SCN2B, SCN3A, SCN3B, SCN4A, SCN4B, SCN5A, SCN7A, SCN8A, SCN9A, SCN10A, SCN11A, SCNM1 SCNN1A, SCNN1B, SCNN1D, SCNN1G*
	Metabolism Substrate Inhibitor	
		ABCB1, CYP1A2 (major)
		CYP1A2 (major)
	Transporter	*ABCB1*

ABCB1 ATP-binding cassette, sub-family B (MDR/TAP), member 1, *CYP1A2* Cytochrome P450, family 1, subfamily A, polypeptide 2, *GRIN3A* Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A, *SCN1A* sodium channel, voltage-gated, type I, alpha subunit, *SCN1B* sodium channel, voltage-gated, type I, beta subunit, *SCN2A* sodium channel, voltage-gated, type II, alpha subunit, *SCN2B* sodium channel, voltage-gated, type II, beta, *SCN3A* sodium channel, voltage-gated, type III, alpha subunit, *SCN3B* sodium channel, voltage-gated, type III, beta subunit, *SCN4A* sodium channel, voltage-gated, type IV, alpha subunit, *SCN4B* sodium channel, voltage-gated, type IV, beta subunit, *SCN5A* sodium channel, voltage-gated, type V, alpha subunit, *SCN7A* sodium channel, voltage-gated, type VII, alpha, *SCN8A* sodium channel, voltage-gated, type VIII, alpha, *SCN9A* sodium channel, voltage-gated, type IX, alpha subunit, *SCN10A* sodium channel, voltage-gated, type X, alpha subunit, *SCN11A* sodium channel, voltage-gated, type XI, alpha, *SCNM1* sodium channel modifier 1, *SCNN1A* sodium channel, non-voltage-gated 1 alpha subunit, *SCNN1B* sodium channel, non-voltage-gated 1, beta subunit, *SCNN1D* sodium channel, non-voltage-gated 1, delta subunit, *SCNN1G* sodium channel, non-voltage-gated 1, gamma subunit

Table 10 Pharmacogenomics of multiple sclerosis [17, 30, 37]

Relapsing MS treatment		
Prednisone		
Category	Hormone	
Mechanism	Decreases inflammation by suppression of migration of polymorphonuclear leukocytes and reversal of increased capillary permeability, suppresses immune system by reducing activity and volume of lymphatic system	
Genes	Metabolism Substrate Inhibitor Inducer	
		ABCB1, CYP3A4 (major)
		CYP3A4 (weak)
		ABCB1, CYP3A4
	Transporter	*ABCB1*
	Pleiotropic	*AGT, AR, FKBP5, IL1B, IL4, IL6, IL8RB, IL10, MTHFR, NOS3, NPPA, NR1I2, NR3C1, SERPINA6*
Tecfidera (Dimethyl fumarate) [38-43]		
Category	Nervous system Drug; Methyl ester; Fumarate	
Mechanism	Primarily acts by triggering the activation of the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcriptional pathway	
Genes	Pathogenic	*CXCL11, TNF*
	Mechanistic	*CXCL2, CXCL11, HMOX1, MAPK1, MAPK3, NFKB1, NRF2, TNF*
	Pleiotropic	*TNF*
Drugs for slowing progression of MS		
Fingolimod [44]		
Category	Immunosuppressive agent; Sphingosine 1-phosphate receptor modulator	
Mechanism	The active metabolite (fingolimod phosphate) binds with high affinity to sphingosine 1-phosphate receptors 1, 3, 4, and 5. Blocks the capacity of lymphocytes to egress from lymph nodes, reducing the number of lymphocytes in peripheral blood. May involve reduction of lymphocyte migration into the central nervous system. Agonist: S1PR1, S1PR3, S1PR4, S1PR5. Antagonist: S1PR1	

Table 10 (continued)

Genes	Pathogenic	*CYP4F2*
	Mechanistic	*S1PR1, S1PR3, S1PR4, S1PR5, SPHK2*
	Metabolism Substrate	
		CYP2D6 (minor), CYP2E1 (minor), *CYP3A4* (minor), *CYP4F2* (major), *CYP4F12* (minor)
Glatiramer Acetate [45]		
Category	Biologic response modifier	
Mechanism	Binds to HLA-DRB1 variants and competes with various myelin antigens for their presentation to T cells; inhibition of dendritic cells and monocytes	
Genes	Pathogenic	*HLA-DRB1, MBP*
	Mechanistic	*HLA-DRB1, CD86, CTSS, IL1R1, IL12RB2, TRB@*
	Pleiotropic	*APP, FAS*
Interferon Beta-1a		
Category	Biologic response modifier	
Mechanism	Alters expression and response to surface antigens and can enhance immune cell activities. Agonist: IFNAR1	
Genes	Mechanistic	*IFNAR1, IFNAR2, PTGER3*
Interferon Beta-1b		
Category	Biologic response modifier	
Mechanism	Enhancement of suppressor T cell activity, reduction of proinflammatory cytokines, down-regulation of antigen presentation, and reduced trafficking of lymphocytes into CNS. Agonist: IFNAR1, IFNAR2	
Genes	Mechanistic	*IFNAR1, IFNAR2, PTGER3*
Mitoxantrone [46]		
Category	Antineoplastic agent; Anthracenedione	
Mechanism	Binds to nucleic acids and inhibits DNA and RNA synthesis by template disordering and steric obstruction	

Table 10 (continued)

Genes	Mechanistic	*ACSL3, CES2, CBR3*
	Metabolism Substrate Inhibitor	
		ABCC1, ABCG2 CYP2D6
		CYP3A4 (weak), *TOP2A.*
	Transporter	*ABCB1, ABCC1, ABCG2, ABCA1, ABCC6, TAP1*
	Pleiotropic	*ALDH2, GSTA1, GSTM1, GSTP1, MSH2, RGS2*
Natalizumab		
Category	Biologic response modifier; Selective adhesion-molecule inhibitor; Monoclonal antibody	
Mechanism	Blockade of T-lymphocyte migration into CNS	
Genes	Pathogenic	*FCGR2C*
	Mechanistic	*ITGA4*
Teriflunomide (Aubagio) [47-55]		
Category	Immunomodulatory agent; Pyrimidine Synthesis Inhibitor	
Mechanism	Inhibits the dihydroorotate dehydrogenase which inhibits the proliferation of activated T cells. Decreases the amount of activated CNS lymphocytes, which results in anti-inflammatory and antiproliferative effects	
Genes	Mechanistic	*DHODH, MAPKs, RAF1, TCR*
	Metabolism Substrate	
		ABCG2
	Transporter	*ABCG2*
	Pleiotropic	*RAF1*
Anti-symptomatic drugs		
Cyclophosphamide		
Category	Antineoplastic agent; Alkylating agents. Immunosuppressive agent	

Table 10 (continued)

Mechanism	Alkylating agent which prevents cell division by cross-linking DNA strands and decreasing DNA synthesis, also possesses potent immunosuppressive activity, has phosphorylating properties which enhance its cytotoxicity	
Genes	Metabolism Substrate Inhibitor Inducer	
		ALDH1A1, ALDH2, ALDH3A1, ABCC4, CYP2A6 (minor), *CYP2B6* (minor), *CYP2C9* (minor), *CYP2C19* (minor), *CYP3A4* (major), *CYP1A2, CYP1B1, CYP2D6, GSTA1, GSTM1, GSTP1*
		CYP3A4 (weak)
		ABCC4, CYP2B6, CYP2C8, CYP2C9, CYP3A4
	Transporter	*ABCB1, ABCG2, ABCC1, ABCC4, SLC5A5*
	Pleiotropic	*CBR3, CRHR1, CRHR2, EGFR, ERBB2, ERBB4, ERCC1, ERCC2, ESR1, ESR2, FOS, HTR3B, HTR3C, ICAM1, IL1B, IL1RN, IL4, IL6, IL10, IL12B, MAOA, MGMT, MMP3, MTHFR, MSH2, NQO1, PTGS2, SOD2, SLC5A5, TGFB1, TNF, TP53, VCAM1*
Dalfampridine (4-Aminopyridine)		
Category	Potassium channel blocker	
Mechanism	Increases conduction of action potentials in demyelinated axons through inhibition of potassium channels	
Genes	Mechanistic	*KCN*
	Metabolism Substrate	
		CYP2E1 (major)
Dantrolene		
Category	Direct-acting skeletal muscle relaxant	
Mechanism	Acts directly on skeletal muscle by interfering with release of calcium ion from sarcoplasmic reticulum, prevents or reduces increase in myoplasmic calcium ion concentration, which activates the acute catabolic processes associated with malignant hyperthermia	

Table 10 (continued)

Genes	Mechanistic	*RYR1*
	Metabolism Substrate	
		CYP3A4 (major)

ABCA1 ATP-binding cassette, sub-family A (ABC1), member 1, *ABCB1* ATP-binding cassette, sub-family B (MDR/TAP), member 1, *ABCC1* ATP-binding cassette, sub-family C (CFTR/MRP), member 1, *ABCC6 ALDH2* aldehyde dehydrogenase 2 family (mitochondrial), ATP-binding cassette, sub-family C (CFTR/MRP), member 6, *ABCG2* ATP-binding cassette, sub-family G (WHITE), member 2, *ACSL3* Acyl-CoA synthetase long-chain family member 3, *ALDH2* Aldehyde dehydrogenase 2 family (mitochondrial), *APP* Amyloid beta (A4) precursor protein, *CBR3* Carbonyl reductase 3, *CD86* CD86 molecule, *CE2* Carboxylesterase 2, *CTSS* Cathepsin S, *CXCL2* Chemokine (C-X-C motif) ligand 2, *CXCL11*: Chemokine (C-X-C motif) ligand 11, *CYP2D6* Cytochrome P450, family 2, subfamily D, polypeptide 6, *CYP2E1* Cytochrome P450, family 2, subfamily E, polypeptide 1, *CYP3A4* Cytochrome P450, family 3, subfamily A, polypeptide 4, *CYP4F2* Cytochrome P450, family 4, subfamily F, polypeptide 2, *CYP4F12* Cytochrome P450, family 4, subfamily F, polypeptide 12, *DHODH* Dihydroorotate dehydrogenase (quinone), *FAS* Fas (TNF receptor superfamily, member 6), *FCGR2C* Fc fragment of IgG, low affinity IIc, receptor for (CD32) (gene/pseudogene), *GSTA1* Glutathione S-transferase alpha 1, *GSTM1* Glutathione S-transferase mu 1, *GSTP1* Glutathione S-transferase pi 1, *HLA-DRB1* Major histocompatibility complex, class II, DR beta 1, *HMOX1* Heme oxygenase (decycling) 1, *IFNAR1* Interferon (alpha, beta and omega) receptor 1, *IFNAR2* Interferon (alpha, beta and omega) receptor 2, *IL12RB2* Interleukin 12 receptor, beta 2, *IL1R1* Interleukin 1 receptor, type I, *ITGA4* Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor), *MAPK1* Mitogen-activated protein kinase 1, *MAPK3* Mitogen-activated protein kinase 3, *MAPKs* Mitogen-activated protein kinases, *MBP* Myelin basic protein, *MSH2* MutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli), *NFKB1* Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, *NRF2* Nuclear factor (erythroid-derived 2)-like 2, *PTGER3* Prostaglandin E receptor 3 (subtype EP3), *RAF1* V-raf-1 murine leukemia viral oncogene homolog 1, *RGS2* Regulator of G-protein signaling 2, 24 kDa, *S1PR1* Sphingosine-1-phosphate receptor 1, *S1PR3* Sphingosine-1-phosphate receptor 3, *S1PR4* Sphingosine-1-phosphate receptor 4, *S1PR5* Sphingosine-1-phosphate receptor 5, *SPHK2* Sphingosine kinase 2, *TAP1* Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP), *TCR* T cell receptor, *TNF* Tumor necrosis factor, *TOP2A* Topoisomerase (DNA) II alpha 170 kDa, *TRB@* T cell receptor beta locus

Table 11 Pharmacogenomics of Huntington disease [17, 30]

Tetrabenazine [56]		
Category	Central monoamine-depleting agent	
Mechanism	Within basal ganglia, interferes with and depletes monoamine neurotransmitters (including dopamine, serotonin, and norepinephrine) in presynaptic vesicles (probably through actions on vesicle monoamine transporter), inhibits presynaptic dopamine release and also blocks CNS dopamine receptors	
Genes	Mechanistic	*VMAT2, DRD1, DRD2, DRD3, DRD4*
	Metabolism Substrate Inhibitor	
		CYP2D6 (major)
		VMAT2
	Transporter	*ABCB1, SLC18A1, SLC18A2, SLC6A3, SLC6A4*

ABCB1 ATP-binding cassette, sub-family B (MDR/TAP), member 1, *CYP2D6* Cytochrome P450, family 2, subfamily D, polypeptide 6, *DRD1* Dopamine receptor D1, *DRD2* Dopamine receptor D2, *DRD3* Dopamine receptor D3, *DRD4* Dopamine receptor D4, *SLC6A3* Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3, *SLC6A4* Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4, *SLC18A1* Solute carrier family 18 (vesicular monoamine), member 1, *SLC18A2* Solute carrier family 18 (vesicular monoamine), member 2, *VMAT2* Vesicle monoamine transporter type 2

Among the world healthy population, EMs account for 55.71 %, whereas IMs are 34.7 %, PMs 2.28 %, and UMs 7.31 %. Remarkable interethnic differences exist in the frequency of the PM and UM phenotypes among different societies all over the world [24, 59-61]. On average, approximately 6.28 % of the world population belongs to the PM category. Europeans (7.86 %), Polynesians (7.27 %), and Africans (6.73 %) exhibit the highest rate of PMs, whereas Orientals (0.94 %) show the lowest rate [61]. The frequency of PMs among Middle Eastern populations, Asians, and Americans is in the range of 2–3 %. *CYP2D6* gene duplications are relatively infrequent among Northern Europeans, but in East Africa the frequency of alleles with duplication of *CYP2D6* is as high as 29 % [62]. In Europe, there is a North-South gradient in the frequency of PMs (6–12 % of PMs in Southern European countries, and 2–3 % PMs in Northern latitudes) [17].

In Alzheimer's disease (AD), EMs, IMs, PMs, and UMs are 56.38, 27.66, 7.45, and 8.51 %, respectively, and in vascular dementia, 52.81, 34.83, 6.74, and 5.62 %, respectively. There is an accumulation of AD-related genes of risk in PMs and UMs. EMs and IMs are the best responders, and PMs and UMs are the worst responders to pharmacological treatment [63]. Patients with depression show significant differences in the genotypic and phenotypic profiles as compared to controls and also with respect to patients with psychosis, Parkinson's disease, or brain tumours. Patients with stroke show differences as compared to patients with brain tumours, and both patients with brain tumours or with cranial nerve neuropathies differ in their *CYP2D6* phenotype with regard to controls. These geno-phenotypic profiles might

Table 12 Pharmacogenomics of most frequently-used psychotropic drugs in NDDs [17, 30]

Antidepressants		
Amitriptyline		
Category	Antidepressant; Tricyclic and norepinephrine-reuptake Inhibitor	
Mechanism	Increases synaptic concentration of serotonin and/or norepinephrine in the central nervous system by inhibiting their reuptake in the presynaptic neuronal membrane. Agonist: NTRK1, NTRK2	
Genes	Pathogenic	*ABCB1, GNB3, HTRs, NTRK2, SLC6A4, TNF*
	Mechanistic	*ADRA1A, HTRs, NTRK1, NTRK2*
	Metabolism Substrate Inhibitor	
		ABCB1, CYP1A2 (minor), *CYP2B6* (minor), *CYP2C9* (minor), *CYP2C19* (minor), *CYP2D6* (major), *CYP3A4/5* (major), *GSTP1*
		ABCB1, ABCC2, ABCG2, CYP1A2 (moderate), *CYP2C9* (moderate), *CYP2C19* (moderate), *CYP2D6* (moderate), *CYP2E1* (weak)
	Transporter	*ABCB1, ABCC2, ABCG2, KCNE2, KCNH2, KCNQ1, SCN5A, LC6A4*
	Pleiotropic	*FABP1, GNAS, GNB3, NTRK1, TNF*
Citalopram		
Category	Antidepressant; Selective serotonin-reuptake inhibitor	
Mechanism	Selectively inhibits serotonin reuptake in the presynaptic neurons due to the S-enantiomer and enhances the actions of serotonin on 5HT1A autoreceptors. Displays little to no affinity for dopamine, adrenergic, histamine, GABA, or muscarinic receptor subtypes	
Genes	Pathogenic	*ABCB1, BDNF, CREB1, CRHR1, CRHR2, FKBP5, GRIA3, GRIK2, GRIK4, GSK3B, HTR1A, HTR1B, HTR2A, MAOA, SLC6A4, TPH1, TPH2*
	Mechanistic	*ADRs, CHRMs, DRDs, FKBP5, GABRs, GRIK4, HRHs, HTR1A, HTR1B, HTR1D, HTR2A, SLC6A4, TPH1*
	Metabolism Substrate Inhibitor	
		ABCC1, COMT, CYP2C19 (major), *CYP2D6* (minor), *CYP3A4* (major), *CYP3A5*
		ABCB1, CYP1A2 (weak), *CYP2B6* (weak), *CYP2C19* (weak), *CYP2D6* (weak), *MAOA, MAOB*
	Transporter	*ABCB1, SLC6A4*
	Pleiotropic	*BDNF*

Table 12 (continued)

Antidepressants		
Fluoxetine		
Category	Antidepressant; Selective Serotonin-reuptake Inhibitor	
Mechanism	Potentiates serotonergic activity in CNS inhibiting neuronal reuptake of serotonin (5-HT). Increases synaptic concentrations of serotonin in the CNS but has little or no effect on other neurotransmitters	
Genes	Pathogenic	*ABCB1, BDNF, CREB1, FKBP5, GSK3B, HTR1A, HTR2A, MAOA, NR3C1, NTRK2, SLC6A4, TBX21, TPH1, TPH2*
	Mechanistic	*BDNF, CHRMs, CREB1, DRD3, GSK3B, HTRs, MAOA, SLC6A4, TPH2*
	Metabolism Substrate Inhibitor	
		CYP1A2 (major), *CYP2B6* (major), *CYP2C8* (major), *CYP2C9* (major), *CYP2C19* (major), *CYP2D6* (major), *CYP2E1* (minor), *CYP3A4/5* (major), POR
		ABCB1, *CYP1A2* (moderate), *CYP2B6* (weak), *CYP2C8* (moderate), *CYP2C9* (weak), *CYP2C19* (moderate), *CYP2D6* (strong), *CYP3A4* (moderate), *MAOA*, *SLC6A4*
	Transporter	*ABCB1, KCNH2*
	Pleiotropic	*DRD3, FABP1, HTR2A, IFNA1, NTRK2, TPH1*
Maprotiline		
Category	Antidepressant; Tricyclic and norepinephrine-reuptake Inhibitor	
Mechanism	Inhibits the norepinephrine reuptake. Exhibits anticholinergic activity. Strong inhibitor of the histamine H1 receptor, which explains its sedative actions. Antagonist: ADRA2s, ADRA1s	
Genes	Pathogenic	*ABCB1*
	Mechanistic	*ADRA2s, ADRA1s, CHRM4, CHRM5, HRH1*
	Metabolism Substrate Inhibitor	
		CYP1A2 (minor), *CYP2C19*, *CYP2D6* (major), *CYP3A4,*
		MAOB
	Transporter	*ABCB1*, SLC6A2
Paroxetine		

Table 12 (continued)

Antidepressants		
Category	Antidepressant; Selective serotonin-reuptake inhibitor	
Mechanism	Acts by inhibiting serotonin reuptake from brain synapse stimulating its activity in the brain	
Genes	Pathogenic	*ABCB1, CREB1, HTR1B, HTR2A, HTR3B, MAOA, SLC6A3, SLC6A4, TNF, TPH1, TPH2*
	Mechanistic	*CREB1, HTR2A, HTR3A, STAT3, TNF*
	Metabolism Substrate Inhibitor	
		ABCB1, COMT, CYP1A2 (minor), CYP2C19 (minor), *CYP2D6* (major), *CYP3A4* (major), *MAOA, MAOB*
		ABCB1, *CYP1A2* (weak), *CYP2B6* (moderate), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (strong), *CYP3A4* (weak), *SLC6A3*, *SLC6A4*
	Transporter	*ABCB1, SLC6A3, SLC6A4*
	Pleiotropic	*HTR1D, HTR3C, HTR6, HTT, TPH1, TPH2*
Sertraline		
Category	Antidepressant; Selective Serotonin-reuptake Inhibitor	
Mechanism	Selective inhibitor of serotonin reuptake; very weak effects on norepinephrine and dopamine neuronal uptake	
Genes	Pathogenic	*ABCB1, CREB1, GNB3, HTR1B, MAOA, SIGMAR1, SLC6A4, TNF, TPH1, TPH2*
	Mechanistic	*HTR1B, HTR1D, SIGMAR1, SLC6A2, SLC6A3, SLC6A4, TNF*
	Metabolism Substrate Inhibitor	
		CYP2A6, CYP2B6 (minor), *CYP2C9* (minor), *CYP2C19* (major), *CYP2D6* (minor), *CYP3A4* (minor), *MAOA*, *MAOB*, *UGT1A1*, *UGT2B7*
		ABCB1, *ACHE*, *CYP1A2* (weak), *CYP2B6* (moderate), *CYP2C8* (weak), *CYP2C9* (weak), *CYP2C19* (moderate), *CYP2D6* (moderate), *CYP3A4* (moderate), *SLC6A4*
	Transporter	*ABCB1, SCL6A2, SLC6A3, SLC6A4*
	Pleiotropic	*FABP1, FOS, GNB3, TPH1, TPH2*
Trazodone		
Category	Antidepressant; Serotonin Modulator	

Table 12 (continued)

Antidepressants		
Mechanism	Inhibits the reuptake of serotonin, causes adrenoreceptor subsensitivity, and induces significant changes in 5-HT presynaptic receptor adrenoreceptors. Blocks histamine (H1), α1 and α2 adrenergic receptors	
Genes	Pathogenic	*ABCB1, GNB3, HTR1A, HTR2A, SLC6A4*
	Mechanistic	*ADRA1A, ADRA2s, HRH1, HTR2A, HTR2C*
	Metabolism Substrate Inhibitor Inducer	
		CYP1A2 (minor), *CYP2D6* (minor), *CYP3A4* (major), *GSTs*, *SOD2*
		CYP2D6 (moderate), *CYP3A4* (weak), *SLC6A4*
		ABCB1
	Transporter	*ABCB1, SLC6A4*
	Pleiotropic	*GNAS*, *GNB3*, *HTR2A*
Venlafaxine		
Category	Antidepressant; Selective serotonin and norepinephrine-reuptake inhibitor	
Mechanism	Venlafaxine and its active metabolite, O-desmethylvenlafaxine (ODV), are potent inhibitors of neuronal serotonin and norepinephrine reuptake and weak inhibitors of dopamine reuptake	
Genes	Pathogenic	*ABCB1, BDNF, CREB1, FKBP5, HTR1A, HTR2A* NR3C1, SLC6A3, SLC6A4, TPH2*
	Mechanistic	*BDNF*, *FKBP5*
	Metabolism Substrate Inhibitor	
		ABCB1, CYP2C9 (minor), *CYP2C19* (minor), *CYP2D6* (major), *CYP3A4* (major)
		ABCB1, *CYP1A2* (weak), *CYP2B6* (weak), *CYP2D6* (weak), *CYP3A4* (weak), *SLC6A2*, *SLC6A3*, *SLC6A4*
	Transporter	*ABCB1*, *ABCG2*, *SLC6A2*, *SLC6A3*
	Pleiotropic	*DRD2*, *HTR2A*, *TPH2*

Table 12 (continued)

Benzodiazepines		
Alprazolam		
Category	Anxiolytic, Sedative, and hypnotic; Benzodiazepine	
Mechanism	Binds to the GABA benzodiazepine receptor complex, particularly in the limbic system and reticular formation. The inhibitory effect of GABA on neuronal excitability increases the neuronal membrane permeability to chloride ions resulting in hyperpolarization and stabilization	
Genes	Pathogenic	*GABAARs*
	Mechanistic	*CLCNs, GABRAs, GABRBs, GABRD, GABRE, GABRGs, GABRP, GABRQ, GABRRs*
	Metabolism Substrate	
		CYP1A1 (minor), *CYP1A2* (minor), *CYP2C19* (minor), *CYP2D6* (minor), *CYP3A4/5* (major)
	Transporter	*CLCNs*
Diazepam		
Category	Anticonvulsant; Anxiolytic, Sedative, and Hypnotic; Benzodiazepine	
Mechanism	Binds to GABAA receptors enhancing the effects of GABA by increasing GABA affinity for its receptor. This action increases neuronal membrane permeability to chloride ions thus resulting in hyperpolarization and stabilization	
Genes	Pathogenic	*BDNF, CNR1, GABAARs*
	Mechanistic	*ACHE, BCHE, BDNF, CHRMS*, CLCNs, GABRAs, GABRBs, GABRD, GABRE, GABRGs, GABRP, GABRQ, GABRRs, TSPO*
	Metabolism Substrate Inhibitor	
		CYP1A2 (minor), *CYP2B6* (minor), *CYP2C9* (minor), *CYP2C19* (minor), *CYP3A4/5* (major), *UGTs*
		CYP2C19 (weak), *CYP3A4* (weak), *UGT2B7*
	Transporter	*CLCNs*
	Pleiotropic	*ACHE, FOS, IL6, SPG7*
Lorazepam		
Category	Anticonvulsant; Benzodiazepine; Anxiolytic, Sedative, and Hypnotic; Benzodiazepine	
Mechanism	Binds to GABAA receptors enhancing the effects of GABA by increasing GABA affinity for its receptor. This action increases neuronal membrane permeability to chloride ions thus resulting in hyperpolarization and stabilization. Antagonist: TSPO	

Table 12 (continued)

Benzodiazepines		
Genes	Pathogenic	*GABAARs*
	Mechanistic	*CLCNs, GABRAs, GABRBs, GABRD, GABRE, GABRGs, GABRP, GABRQ, GABRRs, TSPO*
	Metabolism Substrate	
		CYP3A4 (minor), *UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, UGT1A10, UGT2B7, UGT2B15*
	Transporter	*CLCNs*
Oxazepam		
Category	Anxiolytic, sedative, and hypnotic; benzodiazepine	
Mechanism	Binds to GABAA receptors enhancing the effects of GABA by increasing GABA affinity for its receptor. This action increases neuronal membrane permeability to chloride ions thus resulting in hyperpolarization and stabilization	
Genes	Pathogenic	*GABAARs*
	Mechanistic	*CLCNs, GABRAs, GABRBs, GABRD, GABRE, GABRGs, GABRP, GABRQ, GABRRs*
	Metabolism Substrate Inhibitor Inducer	
		CYP2D6, UGT2B7, UGT2B15
		UGT2B7
		CYP1A2
	Transporter	*CLCNs*
Temazepam		
Category	Anxiolytic, Sedative, and Hypnotic; Benzodiazepine	
Mechanism	Binds to GABAA receptors enhancing the effects of GABA by increasing GABA affinity for its receptor. This action increases neuronal membrane permeability to chloride ions thus resulting in hyperpolarization and stabilization. Antagonist: *TSPO*	
Genes	Mechanistic	*CLCNs, GABRAs, GABRBs, GABRD, GABRE, GABRGs, GABRP, GABRQ, GABRRs, TSPO*
	Metabolism Substrate	
		CYP2B6 (major), *CYP2C9* (major), *CYP2C19* (major), *CYP3A4* (major), *UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT1A10, UGT2B7, UGT2B15*
	Transporter	*CLCNs*

Table 12 (continued)

Neuroleptics		
Aripiprazole		
Category	Antimanic Agent; Atypical Antipsychotic	
Mechanism	Partial agonist of the D2 and 5-HT1A receptors and antagonist profile at the 5-HT2A receptor. Moderate affinity for histamine and alpha adrenergic receptors	
Genes	Pathogenic	*DRD2, DRD3, HTR2A*
	Mechanistic	*ADRA1A, DRD2, DRD3, HRHs, HTR1A, HTR2A, HTR2C*
	Metabolism Substrate	
		CYP2D6 (major), *CYP3A4* (major), *CYP3A5*
	Transporter	*ABCB1*
Haloperidol		
Category	Conventional Antipsychotic; First-Generation antipsychotic; Typical antipsychotic; Butyrophenone	
Mechanism	Blocks postsynaptic mesolimbic dopaminergic D1 and D2 receptors. Depresses release of hypothalamic and hypophyseal hormones. May depress reticular activating system. Antagonist: DRD2	
Genes	Pathogenic	*BDNF, DRD1, DRD2, DRD4, DTNBP1, GRIN2B, HTR2A*
	Mechanistic	*BDNF, DRD1, DRD2, DTNBP1*
	Metabolism Substrate Inhibitor	
		CBR, CYP1A1 (minor), *CYP1A2* (minor), *CYP2A6, CYP2C8* (minor), *CYP2C9* (minor), *CYP2C19* (minor), *CYP2D6* (major), *CYP3A4*/*5* (major), *UGTs*
		CYP2D6 (moderate), *CYP3A4* (moderate)
	Transporter	*ABCB1, ABCC1, KCNE1, KCNE2, KCNH2, KCNJ11, KCNQ1*
	Pleiotropic	*ADRA2A, CHRM2, FOS, GSK3B, GSTP1, HRH1, HTR2A, HTT, IL1RN*
Olanzapine		
Category	Atypical Antipsychotic. Antimanic agent	
Mechanism	Potent antagonism of serotonin 5-HT2A and 5-HT2C, dopamine D1–4, histamine H1 and α1-adrenergic receptors; moderate antagonism of 5-HT3 and muscarinic M1-5 receptors, and weak binding to GABA-A, BZD, and β-adrenergic receptors	

Table 12 (continued)

Neuroleptics		
Genes	Pathogenic	*COMT, DRD1, DRD2, DRD3, DRD4, GRM3, HTR2A, LPL*
	Mechanistic	*ADRA1A, ADRB3, CHRM1-5, COMT, DRD1, DRD2, DRD4, GABAARs, HRH1, HTR2A, HTR2C, HTR3A, LEP, RGS2, RGS7, STAT3*
	Metabolism Substrate Inhibitor	
		ABCB1, COMT, CYP1A2 (major), *CYP2D6* (major), *UGT1A4*
		ABCB1, CYP1A2 (weak), *CYP2C9* (weak), *CYP2C19* (weak), *CYP2D6* (weak), *CYP3A4* (weak), *FMO1, UGT1A4*
	Transporter	*KCNH2, SLC6A2*
	Pleiotropic	*APOA5, APOC3, GNB3, LEP, LEPR, LPL*
Quetiapine		
Category	Atypical Antipsychotic	
Mechanism	Antagonistic effect on dopaminergic (D1, D2), histaminergic (H1), sertoninergic (5-HT1A, 5HT2) and adrenergic (α1- and α2) receptors	
Genes	Pathogenic	*DRD1, DRD2, DRD4, HTR1A, HTR2A, RGS4*
	Mechanistic	*ADRA1s, ADRA2s, DRD1, DRD2, DRD4*, HRH1, HTR1A, HTR2A, HTR2B*
	Metabolism Substrate Inhibitor	
		ABCB1, CYP2D6 (minor), *CYP3A4/5* (major), *CYP3A7*, *CYP2C19*
		ABCB1
	Transporter	*ABCB1, KCNE1, KCNE2, KCNH2, KCNQ1, SCN5A*
Risperidone		
Category	Atypical Antipsychotic; Antimanic agent	
Mechanism	It has serotonin-dopamine antagonist activity. Antagonizes α1-, α2-adrenergic, and histaminergic receptors	

Table 12 (continued)

Neuroleptics		
Genes	Pathogenic	*BDNF, COMT, DRD1, DRD2, DRD3, DRD4, GRM3, HTR2A, PON1, RGS4*
	Mechanistic	*ADRA1A, ADRA1B, ADRA2s, DRD1, DRD2, DRD3, DRD4, FOS*, HTR2A, HTR2C, HTR3A, HTR3C, HTR6, STAT3*
	Metabolism Substrate Inhibitor	
		ABCB1, CYP2D6 (major), *CYP3A4/5* (minor), *COMT*
		ABCB1, CYP2D6 (weak), *CYP3A4* (weak)
	Transporter	*KCNH2, SLC6A4*
	Pleiotropic	*APOA5, BDNF, RGS2*

ABCB1 ATP-binding cassette, sub-family B (MDR/TAP), member 1, *ABCC1* ATP-binding cassette, sub-family C (CFTR/MRP), member 1, *ABCG2* ATP-binding cassette, sub-family G (WHITE), member 2, *ACHE* Acetylcholinesterase, *ADRA1A* Adrenoceptor alpha 1A, *ADRA1B* Adrenergic receptor, alpha 1b, *ADRA1s* Alpha 1 adrenoceptors, *ADRA2A* Adrenoceptor alpha 2A, *ADRA2s* Alpha 2 adrenoceptors, *ADRB3* Adrenoceptor beta 3, *ADRs* Adrenoceptors, *APOA5* Apolipoprotein A-V, *APOC3* Apolipoprotein C-III, *BCHE* Butyrylcholinesterase, *BDNF* Brain-derived neurotrophic factor, *CBR* NADH–cytochrome B5 reductase 1, *CHRM2* Cholinergic receptor, muscarinic 2, *CHRM4* Cholinergic receptor, muscarinic 4, *CHRM5* Cholinergic receptor, muscarinic 5, *CHRMs* Muscarinic cholinergic receptors, *CHRNs* Nicotinic cholinergic receptors, *CLCNs* Chloride channel, voltage-sensitive, *CNR1* Cannabinoid receptor 1 (brain), *COMT* Catechol-O-methyltransferase, *CREB1* cAMP responsive element binding protein 1, *CRHR1* Corticotropin releasing hormone receptor 1, *CRHR2* Corticotropin releasing hormone receptor 2, *CYP1A1* Cytochrome P450, family 1, subfamily A, polypeptide 1, *CYP1A2* Cytochrome P450, family 1, subfamily A, polypeptide 2, *CYP2A6* Cytochrome P450, family 2, subfamily A, polypeptide 6, *CYP2B6* Cytochrome P450, family 2, subfamily B, polypeptide 6, *CYP2C8* Cytochrome P450, family 2, subfamily C, polypeptide 8, *CYP2C9* Cytochrome P450, family 2, subfamily C, polypeptide 9, *CYP2C19* Cytochrome P450, family 2, subfamily C, polypeptide 19, *CYP2D6* Cytochrome P450, family 2, subfamily D, polypeptide 6, *CYP2E1* Cytochrome P450, family 2, subfamily E, polypeptide 1, *CYP3A4* Cytochrome P450, family 3, subfamily A, polypeptide 4, *CYP3A5* Cytochrome P450, family 3, subfamily A, polypeptide 5, *CYP3A7* Cytochrome P450, family 3, subfamily A, polypeptide 7, *DRD1* Dopamine receptor D1, *DRD2* Dopamine receptor D2, *DRD3* Dopamine receptor D3, *DRD4* Dopamine receptor D4, *DRDs* Dopamine receptors, *DTNBP1* Dystrobrevin binding protein 1, *FABP1* Fatty acid binding protein 1, *FKBP5* FK506 binding protein 5, *FMO1* Flavin containing monooxygenase 1, *FOS* FBJ murine osteosarcoma viral oncogene homolog, *GABAARs* Glutamate receptor, ionotropic, *AMPA 2, GABRAs* Gamma-aminobutyric acid (GABA) A receptors, *GABRBs* Gamma-aminobutyric acid (GABA) B receptors, *GABRs* Gamma-aminobutyric acid (GABA) receptors, *GABRD* Gamma-aminobutyric acid (GABA) A receptor, subunit delta, *GABRE* Gamma-aminobutyric acid (GABA) A receptor, epsilon, *GABRGs* Gamma-aminobutyric acid (GABA) A receptors, gamma, *GABRP* Gamma-aminobutyric acid (GABA) A receptor, pi, *GABRQ* Gamma-aminobutyric acid (GABA) A receptor, theta, *GABRRs* Gamma-aminobutyric acid (GABA) A receptors, rho subunits, *GNAS* GNAS complex locus, *GNB3* Guanine nucleotide binding protein (G protein), beta polypeptide 3, *GRIA3* Glutamate receptor, ionotropic, AMPA 3, *GRIK2* Glutamate receptor, ionotropic, kainate 2, *GRIK4* Glutamate receptor, ionotropic, kainate 4, *GRM3* Glutamate receptor, metabotropic 3, *GSK3B* Glycogen synthase kinase 3 beta, *GSTP1* Glutathione S-transferase

Table 12 (continued)

Neuroleptics
pi 1, *GSTs* Glutathione S-transferases, *HRH1* Histamine receptor H1, *HRHs* Histamine receptors, *HTR1A* 5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled, *HTR1B* 5-hydroxytryptamine (serotonin) receptor 1B, G protein-coupled, *HTR1D* 5-hydroxytryptamine (serotonin) receptor 1D, G protein-coupled, *HTR2A* 5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled, *HTR2B* 5-hydroxytryptamine (serotonin) receptor 2B, *HTR2C* 5-hydroxytryptamine (serotonin) receptor, G protein-coupled, *HTR3A* 5-hydroxytryptamine (serotonin) receptor 3A, ionotropic, *HTR3B* 5-hydroxytryptamine (serotonin) receptor 3B, ionotropic, *HTR3C* 5-hydroxytryptamine (serotonin) receptor, ionotropic, *HTR6* 5-hydroxytryptamine (serotonin) receptor protein-coupled, *HTRs* 5-hydroxytryptamine (serotonin) receptors, *HTT* Huntingtin, *IFNA1* Interferon, alpha 1, *IL1RN* Interleukin 1 receptor antagonist, *IL6* Interleukin 6 (interferon, beta 2), *KCNE1* Potassium voltage-gated channel, Isk-related family, member 1, *KCNE2* Potassium voltage-gated channel, Isk-related family, member 2, *KCNH2* Potassium voltage-gated channel, subfamily H (eag-related), member 2, *KCNJ11* Potassium inwardly-rectifying channel, subfamily J, member 11, *KCNQ1* Potassium voltage-gated channel, KQT-like subfamily, member 1, *LEP* Leptin, *LEPR* Leptin receptor, *LPL* Lipoprotein lipase, *MAOA* Monoamine oxidase A, *MAOB* Monoamine oxidase B, *NR3C1* Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor), *NTRK2* Neurotrophic tyrosine kinase, receptor, type 2, *PON1* Paraoxonase 1, *POR* P450 (cytochrome) oxidoreductase, *RGS2* Regulator of G-protein signaling 2, 24 kDa, *RGS4* Regulator of G-protein signaling 4, *RGS7* Regulator of G-protein signaling 7, *RIN2B* Glutamate receptor, ionotropic, N-methyl D-aspartate 2B, *SIGMAR1* Sigma non-opioid intracellular receptor 1, *SCN5A* Sodium channel, voltage-gated, type V, alpha subunit, *SLC6A2* Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2, *SLC6A3* Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3, *SLC6A4* Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4, *SOD2* Superoxide dismutase 2, mitochondrial, *SPG7* Spastic paraplegia 7 (pure and complicated autosomal recessive), *STAT3* Signal transducer and activator of transcription 3 (acute-phase response factor), *TBX21* T-box 21, *TNF* Tumor necrosis factor, *TPH1* Tryptophan hydroxylase 1, *TPH2* Tryptophan hydroxylase 2, *TSPO* Translocator protein (18 kDa), *UGT1A1* UDP glucuronosyltransferase 1 family, polypeptide A1, *UGT1A10* UDP glucuronosyltransferase 1 family, polypeptide A10, *UGT1A3* UDP glucuronosyltransferase 1 family, polypeptide A3, *UGT1A4* UDP glucuronosyltransferase 1 family, polypeptide A4, *UGT1A6* UDP glucuronosyltransferase 1 family, polypeptide A6, *UGT1A7* UDP glucuronosyltransferase 1 family, polypeptide A7, *UGT1A9* UDP glucuronosyltransferase 1 family, polypeptide A9, *UGT2B7* UDP glucuronosyltransferase 2 family, polypeptide B7, *UGT2B15* UDP glucuronosyltransferase 2 family, polypeptide B15, *UGTs* UDP glucuronosyltransferases

Table 13 Main characteristics of cytochrome P-450 genes [16, 17]

CYP2D6

OMIM	Size (kb)	Exons number	RNA transcripts (bp)	Locus	Representative allelic variants	Genotype	Phenotype	Frecuency (Caucasian population) (%)
124030	4.38	9	1684 1433 1190 2257	22q13.1	g.24825C > T g.25747C > T g.26385G > A g.12739delT g.26572G > A 2549delA 2613-2615delAGA g.27576T > C g.27714G > A g.27909G > A	*1/*1	EM	47,10
						*1/*3	IM	1,95
						*1/*4	IM	17,42
						*1/*5	IM	3,87
						*1/*6	IM	2,58
						*1/*7	IM	0,65
						*10/*10	IM	1,30
						*1xN/*1	UM	4,52
						*1xN/*4	UM	1,95
						*1/*10	EM	4,52
						*4/*4	PM	8,37
						*4/*10	IM	3,23
						*5/*5	PM	0,65
						*6/*10	IM	0,65
						*7/*10	IM	0,65

Table 13 (continued)

CYP2C9								
Omin	Size (kb)	Exons number	RNA transcripts (bp)	Locus	Representative allelic variants	Genotype	Phenotype	Frecuency (Caucasian population) (%)
601130	50.71	9	1860	10q24	g.15489579A > C g.15450573C > T g.15456202T > G g.47506179T > C	*1/*1	EM	60.56
						*1/*2	IM	18.78
						*1/*3	IM	13.62
						*2/*2	PM	3.76
						*2/*3	PM	3.28
						*3/*3	PM	0
CYP2C19								
Omin	Size (kb)	Exons number	RNA transcripts (bp)	Locus	Representative allelic variants	Genotype	Phenotype	Frecuency (Caucasian population) (%)
124020	90.21	9	1901 2395 1417	10q24.1-q24.3	g.47346080G > C g.15288936G > A g.15270989A > G	*1/*1	EM	68.54
						*1/*2	IM	30.05
						*2/*2	PM	1.41
CYP3A4/5								
Omin	Size (kb)	Exons number	RNA transcripts (bp)	Locus	Representative allelic variants	Genotype	Phenotype	Frecuency (Caucasian population) (%)
124010	27.2	13	2153 651 564 2318 2519	7q21.1	g.24589084T > A g.24601703C > T 392A > G	*1/*1	UM	1.37
						*1/*3	IM	15.88
						*3/*3	EM	82.75

be important in the pathogenesis of some CNS disorders and in the therapeutic response to conventional psychotropic drugs as well [16].

2.3.2 CYP2C9

This gene is mainly expressed in hepatocytes, where a protein of 55.63 kDa (490 aa) can be identified. Over 600 drugs are CYP2C9-related, 311 acting as substrates (177 are major substrates, 134 are minor substrates), 375 as inhibitors (92 weak, 181 moderate, and 102 strong inhibitors), and 41 as inducers of the CYP2C9 enzyme [17]. There are 481 *CYP2C9* SNPs. The phenotypic distribution is presented in Table 13. No *CYP2C9-*3/*3* cases have been found in the control population; however, in patients with depression, psychosis, and mental retardation the frequency of this genotype is 0.91, 1.03, and 1.37%, respectively [16]. Significant variation has been found in *CYP2C9* genotypes among diverse brain diseases. The plethora of metabolising profiles in CNS disorders suggest a potential pathogenic role of *CYP2C9* in brain pathology and a very strong role of the CYP2C9 enzyme on drugs with deleterious effects on cerebrovascular function (e.g. nonsteroidal anti-inflammatory drugs (NSAIDs) and thromboembolic phenomena and/or bleeding (e.g. warfarin, coumarinics).

2.3.3 CYP2C19

This gene is expressed in liver cells where a protein of 55.93 kDa (490 aa) is identified. Nearly 500 drugs are CYP2C19-related, 281 acting as substrates (151 are major substrates, 130 are minor substrates), 263 as inhibitors (72 weak, 127 moderate, and 64 strong inhibitors), and 23 as inducers of the CYP2C19 enzyme [17]. About 541 SNPs have been detected in the *CYP2C19* gene [16]. Minor variation has been reported in different brain disorders.

2.3.4 CYP3A4/5

These genes are expressed in intestine, liver, prostate and other tissues where 4 protein variants of 57.34 kDa (503 aa), 17.29 kDa (153 aa), 40.39 kDa (353 aa), and 47.99 kDa (420 aa) are identified. The human *CYP3A* locus contains the three *CYP3A* genes (*CYP3A4, CYP3A5* and *CYP3A7*), three pseudogenes as well as a novel *CYP3A* gene termed *CYP3A43*. The gene encodes a putative protein with between 71.5 and 75.8% identity to the other CYP3A proteins. The predominant hepatic form is CYP3A4, but CYP3A5 contributes significantly to the total liver CYP3A activity. This enzyme metabolises over 1900 drugs, 1033 acting as substrates (897 are major substrates, 136 are minor substrates), 696 as inhibitors (118 weak, 437 moderate, and 141 strong inhibitors), and 241 as inducers of the CYP3A4 enzyme [17]. About 347 SNPs have been identified in the *CYP3A4* gene (*CYP3A4*1A*: Wild-type), 25 of which are of clinical relevance.

2.3.5 *CYP* Clustering

The construction of a genetic map integrating the most prevalent *CYP2D6* + *CYP2C19* + *CYP2C9* polymorphic variants in a trigenic cluster yields 82 different haplotype-like profiles. The most frequent trigenic genotypes are **1*1-*1*1-*1*1* (25.70%), **1*1-*1*2-*1*2* (10.66%), **1*1-*1*2-*1*1* (10.45%), **1*4-*1*1-*1*1* (8.09%), **1*4-*1*2-*1*1* (4.91%), **1*4-*1*1-*1*2* (4.65%), and **1*1-*1*3-*1*3* (4.33%). These 82 trigenic genotypes represent 36 different pharmacogenetic phenotypes. According to these trigenic clusters, only 26.51% of the population show a pure 3EM phenotype, 15.29% are 2EM1IM, 2.04% are pure 3IM, 0% are pure 3PM, and 0% are 1UM2PM (the worst possible phenotype). This implies that only one-quarter of the population processes normally the drugs which are metabolised via CYP2D6, CYP2C9 and CYP2C19 (approximately 60% of the drugs of current use) [13, 16].

2.4 Genes Encoding Drug Transporters

ATP-binding cassette genes (ABC), especially *ABCB1, ABCC1, ABCG2* (White1), and other genes of this family encode proteins which are essential for drug metabolism and transport. The multidrug efflux transporters P-gp, multidrug-resistance associated protein 4 (MRP4) and breast cancer resistance protein (BCRP), located on endothelial cells lining brain vasculature, play important roles in limiting movement of substances into and enhancing their efflux from the brain. Transporters also cooperate with Phase I/Phase II metabolism enzymes by eliminating drug metabolites. Their major features are their capacity to recognise drugs belonging to unrelated pharmacological classes, and their redundancy, by which a single molecule can act as a substrate for different transporters. This ensures an efficient neuroprotection against xenobiotic invasions. The pharmacological induction of ABC gene expression is a mechanism of drug interaction, which may affect substrates of the up-regulated transporter, and overexpression of multidrug resistance (MDR) transporters confers resistance to anticancer agents and CNS drugs [64-66]. Also of importance for CNS pharmacogenomics are transporters encoded by genes of the solute carrier superfamily (SLC) and solute carrier organic (SLCO) transporter family, responsible for the transport of multiple endogenous and exogenous compounds, including folate (*SLC19A1*), urea (*SLC14A1, SLC14A2*), monoamines (*SLC29A4, SLC22A3*), aminoacids (*SLC1A5, SLC3A1, SLC7A3, SLC7A9, SLC38A1, SLC38A4, SLC38A5, SLC38A7, SLC43A2, SLC45A1*), nucleotides (*SLC29A2, SLC29A3*), fatty acids (*SLC27A1–6*), neurotransmitters (*SLC6A2* (noradrenaline transporter), *SLC6A3* (dopamine transporter), *SLC6A4* (serotonin transporter, SERT), *SLC6A5, SLC6A6, SLC6A9, SLC6A11, SLC6A12, SLC6A14, SLC6A15, SLC6A16, SLC6A17, SLC6A18, SLC6A19*), glutamate (*SLC1A6, SLC1A7*), and others [11, 18]. Some organic anion transporters (OAT), which belong to the solute carrier (SLC) 22A family, are also expressed at the blood-brain barrier (BBB), and regulate the excretion of

endogenous and exogenous organic anions and cations [67]. The transport of amino acids and di- and tripeptides is mediated by a number of different transporter families, and the bulk of oligopeptide transport is attributable to the activity of members of the *SLC15A* superfamily (Peptide Transporters 1 and 2 [*SLC15A1* (PepT1) and *SLC15A2* (PepT2), and Peptide/Histidine Transporters 1 and 2 [*SLC15A4* (PHT1) and *SLC15A3* (PHT2)]. ABC and SLC transporters expressed at the BBB may cooperate to regulate the passage of different molecules into the brain [68]. Polymorphic variants in ABC and SLC genes may also be associated with pathogenic events in CNS disorders and drug-related safety and efficacy complications [17].

3 Alzheimer's Disease

Alzheimer's disease (AD) is the most prevalent form of dementia with a prevalence of about 1 % at the age of 60–65 years and over 25 % in patients older than 85 years of age. Identified by Alois Alzheimer in 1906, AD is characterised by memory disorders, behavioural changes and progressive functional decline. Its neuropathologic phenotype includes the presence of extracellular deposits of aberrant forms of amyloid-beta (Aβ) protein in senile plaques, intracellular deposits of hyperphosphorylated protein tau in neurofibrillary tangles (NFT), synaptic and neuronal loss in selected regions of the neocortex, neuroinflammatory reactions, neurotrophic dysfunction, and overproduction of oxidative stress reactions [20, 24]. In the Alzgene database [69] there are over 600 genes potentially associated with AD, of which the top ten are (in decreasing order of importance): *APOE*, *BIN1, CLU, ABCA7* (ATP-binding cassette, sub-family A (ABC1), member 7), *CR1, PICALM, MS4A6A, CD33, MS4A4E*, and *CD2AP*. Potentially defective genes associated with AD represent about 1.73 % of the human genome, which is integrated by 20774 coding genes [70]. The highest number (> 5 %) of AD genes concentrate on chromosomes 10 (15.69 %), 1 (9.67 %), 6 (7.61 %), 19 (6.81 %), 12 (6.50 %), 11 (6.18 %), and 17 (5.07 %), with the highest proportion (related to the total number of genes mapped on a single chromosome) located on chromosome 10 and the lowest on chromosome X [11] (Table 2).

The genetic and epigenetic defects identified in AD can be classified into 4 major categories: Mendelian mutations, susceptibility SNPs, mtDNA mutations, and epigenetic changes. Mendelian mutations affect genes directly linked to AD, including 32 mutations in the amyloid beta (Aβ) (ABP) precursor protein (*APP*) gene (AD1); 165 mutations in the presenilin 1 (*PSEN1*) gene (AD3); and 12 mutations in the presenilin 2 (*PSEN2*) gene (AD4) [15, 16, 20, 71]. *PSEN1* and *PSEN2* are important determinants of γ-secretase activity responsible for proteolytic cleavage of APP and NOTCH receptor proteins. Mendelian mutations are very rare in AD (1:1000). Mutations in exons 16 and 17 of the *APP* gene appear with a frequency of 0.30 and 0.78 %, respectively, in AD patients. Likewise, *PSEN1, PSEN2*, and microtubule-associated protein Tau (*MAPT*) mutations are present in less than 2 % of the cases. Mutations in these genes confer specific phenotypic profiles to patients

with dementia: amyloidogeneic pathology associated with *APP, PSEN1* and *PSEN2* mutations; and tauopathy associated with *MAPT* mutations, representing the two major pathogenic hypotheses for AD [20, 33, 72, 73].

Multiple polymorphic risk variants (Table 2) can increase neuronal vulnerability to premature death. Among these susceptibility genes, the apolipoprotein E (*APOE*) gene (19q13.2) (AD2) is the most prevalent as a risk factor for AD, especially in those subjects harboring the *APOE-4* allele, whereas carriers of the *APOE-2* allele might be protected against dementia [15, 16, 20, 71]. *APOE*-related pathogenic mechanisms are also associated with brain ageing, several CNS disorders and the neuropathological hallmarks of AD.

APOE is a pathogenic gene in dementia and the prototypical paradigm of a pleiotropic gene with multifaceted activities in physiological and pathological conditions, including cardiovascular disease, dyslipidemia, atherosclerosis, stroke, and AD [15, 16]. ApoE is consistently associated with the amyloid plaque marker for AD. APOE-4 may influence AD pathology interacting with APP metabolism and Aβ accumulation, enhancing hyperphosphorylation of tau protein and NFT formation, reducing choline acetyltransferase activity, increasing oxidative processes, modifying inflammation-related neuroimmunotrophic activity and glial activation, altering lipid metabolism, lipid transport and membrane biosynthesis in sprouting and synaptic remodelling, and inducing neuronal apoptosis [20].

The distribution of *APOE* genotypes in the Iberian peninsula is as follows: *APOE-2/2* 0.32%, *APOE-2/3* 7.3%, *APOE-2/4* 1.27%, *APOE-3/3* 71.11%, *APOE-3/4* 18.41%, and *APOE-4/4* 1.59% [15, 16]. These frequencies are very similar in Europe and in other Western societies. There is a clear accumulation of *APOE-4* carriers among patients with AD (*APOE-3/4* 30.30%; *APOE-4/4* 6.06%) and vascular dementia (*APOE-3/4* 35.85%, *APOE-4/4* 6.57%) as compared to controls. The distribution and frequencies of *APOE* genotypes in AD also differ from those of patients with anxiety, depression, psychosis, migraine, vascular encephalopathy, and post-traumatic brain injury syndrome [15, 16] (Fig. 3). Different *APOE* genotypes confer specific phenotypic profiles to AD patients. Some of these profiles may add risk or benefit when the patients are treated with conventional drugs, and in many instances the clinical phenotype demands the administration of additional drugs which increase the complexity of therapeutic protocols. From studies designed to define *APOE*-related AD phenotypes, several conclusions can be drawn: the age-at-onset is 5–10 years earlier in approximately 80% of AD cases harboring the *APOE-4/4* genotype; the serum levels of ApoE are lowest in *APOE-4/4*, intermediate in *APOE-3/3* and *APOE-3/4*, and highest in *APOE-2/3* and *APOE-2/4*; serum cholesterol levels are higher in *APOE-4/4* than in the other genotypes; HDL-cholesterol levels tend to be lower in *APOE-3* homozygotes than in *APOE-4* allele carriers; LDL-cholesterol levels are systematically higher in *APOE-4/4* than in any other genotype; triglyceride levels are significantly lower in *APOE-4/4*; nitric oxide levels are slightly lower in *APOE-4/4*; serum and cerebrospinal fluid Aβ levels tend to differ between *APOE-4/4* and the other most frequent genotypes (*APOE-3/3, APOE-3/4*); blood histamine levels are dramatically reduced in *APOE-4/4* as com-

pared with the other genotypes; brain atrophy is markedly increased in *APOE-4/4* > *APOE-3/4* > *APOE-3/3*; (xi) brain mapping activity shows a significant increase in slow wave activity in *APOE-4/4* from early stages of the disease; (xii) brain haemodynamics, as reflected by reduced brain blood flow velocity and increased pulsatility and resistance indices, is significantly worse in *APOE-4/4* (and in *APOE-4* carriers in general, as compared with *APOE-3* carriers); brain hypoperfusion and neocortical oxygenation is also more deficient in *APOE-4* carriers; lymphocyte apoptosis is markedly enhanced in *APOE-4* carriers; cognitive deterioration is faster in *APOE-4/4* patients than in carriers of any other *APOE* genotype; in approximately 3–8% of the AD cases, the presence of some dementia-related metabolic dysfunctions accumulates more in *APOE-4* carriers than in *APOE-3* carriers; some behavioural disturbances, alterations in circadian rhythm patterns, and mood disorders are slightly more frequent in *APOE-4* carriers; aortic and systemic atherosclerosis is also more frequent in *APOE-4* carriers; liver metabolism and transaminase activity also differ in *APOE-4/4* with respect to other genotypes; hypertension and other cardiovascular risk factors also accumulate in *APOE-4*; and *APOE-4/4* carriers are the poorest responders to conventional drugs. These 20 major phenotypic features clearly illustrate the biological disadvantage of *APOE-4* homozygotes and the potential consequences that these patients may experience when they receive pharmacological treatment for AD and/or concomitant pathologies [12, 13, 15, 16, 20-24, 71].

When *APOE* and *CYP2D6* genotypes are integrated in bigenic clusters and the *APOE* + *CYP2D6*-related therapeutic response to a combination therapy is analysed in AD patients, it becomes clear that the presence of the *APOE-4/4* genotype is able to convert pure *CYP2D6*1/*1* extensive metabolisers into full poor responders to conventional treatments, indicating the existence of a powerful influence of the *APOE-4* homozygous genotype on the drug-metabolising capacity of pure *CYP2D6* extensive metabolisers. In addition, a clear accumulation of *APOE-4/4* genotypes is observed among *CYP2D6* poor and ultra-rapid metabolisers [21, 24].

Conventional treatments in AD include the cholinesterase inhibitors tacrine (suspended due to hepatotoxicity), donepezil, rivastigmine, and galantamine, and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine. Donepezil (ACHE and BCHE inhibitor) is a major substrate of CYP2D6, CYP3A4 and UGTs, and is transported by ABCB1. Galantamine (ACHE and BCHE inhibitor) is a major substrate of CYP2D6, CYP3A4, and UGT1A1. Memantine is a strong inhibitor of CYP2B6 and CYP2D6, and a weak inhibitor of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4 [17] (Table 7).

4 Pharmacogenomics of Other NDDs

The pharmacogenomics of current drugs in use for prevalent NDDs are summarized in the following tables: Parkinson's disease (Table 8), amyotrophic lateral sclerosis (Table 9), multiple sclerosis (Table 10), and Huntington's disease (Table 11). At the

present time, the pharmacogenomics of most drugs for the treatment of NDDs is poorly understood; however, recent data collected from the World Guide for Drug Use and Pharmacogenomics [17] allowed us to identify the most significant genes potentially involved in the pharmacogenomic output associated with these treatments.

4.1 Parkinson's Disease

Parkinson's disease (PD) is the most prevalent form of movement disorder (affects 1 % of people > 60 years), characterised by tremor, bradykinesia, and rigidity due to neurodegeneration of dopaminergic neurons of the nigrostriatal pathway. PD is a paradigm of genome-environment interactions in which genetic defects may increase susceptibility to the deleterious effects of toxic agents, cerebrovascular microlesions, and other neurodegeneration-induced phenomena in selective neurons. Most mutations (*SNCA, PINK1, PARK2, PARK7), PLA2G6, FBXO7*, and *ATP13A2*) represent rare causes of PD, and one *LRRK2* mutation is relatively common in certain populations [74-77] (Table 3). Mutations in 7 genes are robustly associated with autosomal dominant (*SNCA, LRRK2, EIF4G1, VPS35*) or recessive (*parkin*/*PARK2, PINK1, DJ1/PARK7*) PD or parkinsonism. Different SNPs in other genes have been suggested as causes for parkinsonism or PD, including genes for hereditary ataxias (*ATXN2, ATXN3, FMR1*), frontotemporal dementia (*C9ORF72, GRN, MAPT, TARDBP),* Dopa-responsive dystonia *(DYT5, GCH1, TH*, *SPR*), and others (*ATP13A2, CSF1R, DNAJC6, FBXO, GIGYF2, HTRA2, PLA2G6, POLG, SPG11, UCHL1*) [78].

In PD, several categories of drugs are currently used: (a) dopamine precursors (carbidopa, levodopa), (b) dopamine receptor agonists (amantadine, apomorphine, bromocriptine, cabergoline, lisuride, pergolide, pramipexole, ropinirole, rotigotine), (c) anticholinergic agents (benztropine, biperiden, diphenhydramine, ethopropazine, procyclidine, trihexyphenidyl), (d) monoamine oxidase (MAO) inhibitors (rasagiline, selegiline), and (e) catechol-O-methyltransferase (COMT) inhibitors (entacapone, tolcapone) [17]. Levodopa still remains the gold standard for the treatment of motor symptoms of PD. Dopamine agonists, COMT inhibitors and MAO-B inhibitors have been developed to provide oral delivery of dopaminergic stimulation in order to improve motor outcomes and decrease the risk of levodopa-induced motor complications [79]. Levodopa crosses the blood-brain barrier to be converted into dopamine by striatal enzymes. The formation of dopamine from levodopa at functional nigrostiatal dopaminergic sites may correct akinesia via DRD1, DRD2, DRD3, DRD4, and DRD5, and may also inhibit COMT. Benserazide inhibits peripheral decarboxylation of levodopa without affecting its metabolism in brain. Benserazide exerts is effects via DRD2, COMT and MAOB. Carbidopa inhibits

the plasma breakdown of levodopa by inhibiting its decarboxylation, and thereby increases available intracerebral levodopa. In addition to COMT and DRD2, some pleiotropic gene-related products, such as ACE, BDNF, ACHE, and OPRM1, are influenced by the co-administration of carbidopa and levodopa [17].

The amine oxidase inhibitors (MAOIs), of current use in neuropsychiatry, may prove useful for treating some NDDs. Some MAOIs are multifaceted compounds in which their neuroprotective properties seem to be independent of their MAO inhibition [46]. L-Deprenyl (l-N-propargyl, N-methylamphetamine, selegiline), a selective irreversible MAO-B inhibitor (and MAO-A inhibitor at higher doses) has been demonstrated to have neuroprotective or neurorescue properties in vivo and in vitro. Rasagiline, a structurally related drug, has an advantage over L-deprenyl of not being metabolised to L-amphetamine and L-methamphetamine. These two metabolites of L-deprenyl are potentially neurotoxic, whereas N-proparglyamphetamine, another metabolite, might be neuroprotective. In addition, L-amphetamine might interfere with the neuroprotective action of L-deprenyl, whereas the major metabolite of rasagiline, aminoindan, might be neuroprotective [80, 81]. L-Deprenyl and rasagiline downregulate proapoptotic proteins such as BC-associated death promoter (BAD) and BCL-associated protein X (BAX) and prevent the activation and nuclear localisation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in response to neurotoxins and reactive oxygen species [81]. Increased MAO activity and expression of tranyclypromine, an irreversible, nonselective MAOI, may cause an increase in BDNF mRNA and AMP response element binding protein (CREB) in the hippocampus. Phenelzine (2-phenylethylhydrazine, PLZ) is an irreversible, nonselective MAO inhibitor (antidepressant) that also inhibits gamma-aminobutyric acid transaminase (GABA-T), increases brain GABA, and exerts a neuroprotective effect in cerebral ischaemia models [46]. Moclobemide, an MAO-A inhibitor, displays anti-Parkinsonian effects and neuroprotective effects which might be independent of MAO-A inhibition [81]. Clorgyline, an irreversible MAO-A inhibitor, is also neuroprotective under different experimental conditions.

4.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) (Lou Gehrig's disease) is a neurodegenerative disease, described by Charcot in 1874, characterised by progressive muscular atrophy and weakness resulting from loss of both upper and lower motor neurons. Its incidence ranges from 1.5 to 2.5 per 100,000 per year, with a lifetime risk of 1:400, a mean age of onset of 60 years, with a male predominance of 1.3:1 [51, 82]. Familial and sporadic forms of ALS are clinically indistinguishable. Mutations in different genes have been associated with ALS (Table 4). The most common ALS genes, *SOD1, TDP-43*, and *FUS* mutations and the C9orf72 hexanucleotide repeat, might account for 65% of familial ALS cases in the United States, with great variability depending on the geographical region [83, 84]. Major pathogenic theories for ALS include glutamate toxicity, oxidative stress, autoinmune reactions, and protein

aggregation. In terms of therapeutic intervention, mesenchymal stem cells (bone marrow-derived) and neural progenitor cells (spinal cord-derived) are the two cell types with the most evidence for use in ALS [85]; however, riluzole, an antiglutamate drug, is the most prevalent pharmacological treatment with poor results. Many mechanistic genes might be involved in riluzole-related effects (Table 9). Riluzole is a major substrate of CYP1A2 and ABCB1, a major inhibitor of CYP1A2, and is transported by ABCB1 [17]. Other antiglutamate drugs under investigation for the treatment of ALS include: talampanel (LY300164), a benzodiazepine, noncompetitive AMPA receptor antagonist with antiglutamate properties; ceftriaxone, a third-generation cephalosporin; and glutamate carboxypeptidase II (NAALADase) [85]. Recent studies search for the identification of novel pharmacological targets through genomic analysis of deregulated genomic pathways [86].

4.3 Multiple Sclerosis

Multiple sclerosis (MS) is the commonest demyelinating disorder (prevalence < 5/100,000 in Africa, South America and Asia; > 100/100 000 in Scotland, Scandinavia and Canada), potentially caused by the interaction of multiple genetic and environmental factors [87] (Table 5). The presence of oligoclonal bands (OCB) in cerebrospinal fluid is a typical finding in MS. SNPs from the HLA complex (rs3129871and rs3817963, correlating with the *HLA-DRB1*15* and the *HLA-DRB1*04* alleles, respectively) and 6 other loci were associated to OCB status in Scandinavian patients [88].

Different categories of drugs are currently used for the treatment of MS including: (a) hormones (prednisone), (b) immunosuppressive and inmunomodulatory agents (cyclophosphamide, fingolimod, teriflunomide), (c) potassium channel blockers (dalfampridine/4-aminopyridine), (d) sphingosine 1-phosphate receptor modulators (fingolimod), (e) biologic response modifiers (glatiramer acetate, interferon beta-1a, interferon beta-1b, natalizumab), (f) antineoplastic agents (cyclophosphamide, mitoxantrone), (g) skeletal muscle relaxants (dantrolene), (h) monoclonal antibodies with selective adhesion-molecule inhibitor activity (natalizumab) and (i) fumarates (tecfidera) (Table 10). Most of these compounds show complex pharmacogenomic profiles depending on their pharmacological category [17]. Interferon-beta (IFNβ) was the first immunomodulatory treatment for multiple sclerosis, with limited clinical efficacy. Whole-genome association studies have revealed that clusters of brain-specific genes may act as IFNβ response modifiers [89]. Furthermore, gene expression studies have shown that the expression levels of IFN response genes in the peripheral blood of MS patients prior to treatment could serve a role as a biomarker for the differential clinical response to IFNβ [90].

4.4 Huntington's Disease

Huntington's disease (HD) is a neurodegenerative autosomal dominant disease caused by 36 or more CAG triplet repeat expansions in exon 1 of the *HTT* gene, coding for huntingtin protein. These expansions lead to a substantial loss of spiny neurons in the striatum causing the abnormal involuntary movements, cognitive decline, and psychiatric disturbance which characterise this disease. The mean age of onset is 35–44 years and the median survival time is 15–18 years after onset [91].

The Guideline Development Subcommittee of the American Academy of Neurology [35] recommended to prescribe tetrabenazine (up to 100 mg/day) (Table 11), amantadine (300–400 mg/day) (Table 8), or riluzole (200 mg/day) (Table 9) for varying degrees of expected benefit in patients with Huntington's disease (Table 6). However, most patients with this type of coreiform movement disorder usually receive different types of neuroleptics and other psychotropic drugs [19, 58]. Tetrabenazine is a central monoamine-depleting agent with a complex mechanism of action, acting as a major CYP2D6 substrate, and a VMAT2 inhibitor, transported by ABCB1, SLC18A1, SLC18A2, SLC6A3, and SLC6A4 [17, 92].

5 Future Trends

Historically, the vast majority of pharmacogenetic studies of CNS disorders have been addressed to evaluate the impact of cytochrome P450 enzymes on drug metabolism, and conventional targets for psychotropic drugs were dopamine, serotonin, noradrenaline, GABA, ion channels, acetylcholine and their respective biosynthetic and catalysing enzymes, receptors and transporters; however, in the past few years many different genes have been associated with both pathogenesis and pharmacogenomics of neuropsychiatric disorders [12, 15, 16, 19, 58]. Some of these genes and their products constitute potential targets for future treatments. New developments in genomics, including whole genome genotyping approaches and comprehensive information on genomic variation across populations, coupled with large-scale clinical trials in which DNA collection is routine, now provide the impetus for a next generation of pharmacogenetic studies and identification of novel candidate drugs.

Priority areas for pharmacogenetic research are predicting serious adverse reactions and establishing variation in efficacy [93]. Both requirements are necessary in CNS disorders to cope with efficacy and safety issues associated with both current psychotropic drugs and new drugs. Since drug response is a complex trait, genome-wide approaches may provide new insights into drug metabolism and drug response. Of paramount importance is the identification of polymorphisms affecting gene regulation and mRNA processing in genes encoding cytochrome P450s and other drug-metabolising enzymes, drug transporters, and drug targets and receptors, with broad implication in pharmacogenetics since functional polymorphisms which alter gene expression and mRNA processing appear to play a critical

role in shaping human phenotypic variability [94]. It is also most relevant, from a practical point of view, to understand the pharmacogenomics of drug transporters, especially *ABCB1* (P-glycoprotein/MDR1) variants, due to the pleiotropic activity of this gene on a large number of drugs [95]. It is necessary to have a better documentation related to the pharmacogenetic roles of the enormous number (>170) of human solute carrier transporters which transport a variety of substrates, including amino acids, lipids, inorganic ions, peptides, saccharides, metals, drugs, toxic xenobiotics, chemical compounds, and proteins [96]. RNAi pharmacogenomics will also bring new insights into the nature and therapeutic value of gene silencing in CNS disorders [1, 9, 97-99].

The optimisation of CNS therapeutics requires the establishment of new postulates regarding (i) the costs of medicines, (ii) the assessment of protocols for multifactorial treatment in chronic disorders, (iii) the implementation of novel therapeutics addressing causative factors, and (iv) the setting-up of pharmacogenomic strategies for drug development and drugs in the market [14-16].

By knowing the pharmacogenomic profiles of patients with complex disorders, it might be possible to obtain some of the following benefits related to efficacy and safety issues: to identify candidate patients with the ideal genomic profile to receive a particular drug; to adapt the dose in over 90% of the cases according to the condition of EM, IM, PM or UM (diminishing the occurrence of direct side-effects in 30–50% of the cases); to reduce drug interactions by 30–50% (avoiding the administration of inhibitors or inducers able to modify the normal enzymatic activity on a particular substrate); to enhance efficacy; and to eliminate unnecessary costs (>30% of pharmaceutical costs) derived from the consequences of inappropriate drug selection and the overmedication administered to mitigate adverse drug reactions [11, 18].

To make complex disorders a global health priority in the coming years, conceptual and procedural changes are needed on several grounds, such as political, administrative, economic, legal, ethical, industrial, regulatory and educational issues; the implantation of novel biomarkers (genomics, transcriptomics, proteomics, metabolomics) as diagnostic and therapeutic aids; the introduction of innovative therapeutics; the implementation of pharmacogenomics in the clinical practice in order to optimise therapeutics; and the promotion of selective preventive plans for the population at risk [11].

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Pharmacogenetics of Asthma

Anthony G. Fenech, Ian Sayers and Michael A. Portelli

Abstract The study of pharmacogenetics has expanded from what were initially casual drug response observations present in families, to a fully-fledged science with direct therapeutic applications, within a timespan of less than 60 years. Within the field of asthma therapeutics, heterogeneity in patient response to therapeutic agents has been reported from various studies, and several genotype associations with specific therapeutic-response phenotypes have been established. There is however much to be accomplished. The future of asthma pharmacogenetics lies in consolidating and validating clinically important pharmacogenes which are relevant to currently available therapy, and to integrate itself into day to day drug management and the drug development process, in order to streamline the discovery of the potential pharmacogenetic relevance of new drugs. This chapter reviews the pharmacogenetics of current asthma therapies, and discusses challenges which need to be addressed in order to enable the optimisation and personalisation of patient management in a genotype-dependent manner.

Keywords Pharmacogenetics · Asthma · Drug development · Genotype-guided prescribing · Predictive therapeutics

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1 Introduction

The internationally established Global Initiative for Asthma (GINA), defines asthma as a heterogeneous disease, characterised by chronic airway inflammation and variable airflow limitation, and described by a history of respiratory symptoms which may include wheeze, shortness of breath, chest tightness and cough that are variable over time and in intensity [1]. The joint European Respiratory Society/American Thoracic Society guidelines on the definition of asthma add that "Asthma, and severe asthma in particular, are increasingly recognised as heterogeneous processes, not all of which may respond similarly to current therapies or have the same clinical course" [2].

Addressing inter-patient variability in response to treatment is the fulcrum of the gradual but expanding practice of personalised medicine. Research in pharmacogenetics is the driving force behind the evolution of the "one size fits all" paradigm to a scenario where specific patient parameters, including genetic factors, form part of a treatment strategy which is optimised on an individual basis.

Asthma, a condition which is multifactorial and polygenic in nature, is managed by a therapeutic repertoire of drugs which target multiple pathways, and are themselves subject to pharmacogenetic influence by multiple genes. The complex molecular interactions occurring within the disease pathology, its environmental and genetic determinants, the drugs administered as well as pharmacogenetic and epigenetic contributions, make the development of genotype guided prescribing protocols for asthma, a formidable activity. The identification of genotype-drug response associations and assessment of their clinical relevance, are critical steps towards the development of such protocols.

The genotype nomenclature, adopted for different alleles throughout this chapter, refers to that adopted by the respective cited authors.

2 Pharmacological Management of Asthma

Current asthma management guidelines, including those developed by the GINA as well as several other professional organisations such as the European Respiratory Society (www.ersnet.org), the American Thoracic Society (www.thoracic.org), the British Thoracic Society (www.brit-thoracic.org.uk), the Scottish Intercollegiate Guidelines Network (www.sign.ac.uk) and the National Institute for Health and Care Excellence (www.nice.org.uk) advocate for a stepwise management approach. This approach is based on clinical severity assessments, with regular re-evaluations of treatment resulting in a step up, when asthma is not adequately managed, or a step down if disease control can be maintained with less aggressive therapy. The drug classes currently in use for asthma comprise; β_2-adrenoceptor agonists, the less commonly used anti-muscarinic bronchodilators, glucocorticoids, anti-leukotriene drugs, theophylline, and the monoclonal anti IgE antibody omalizumab which is normally reserved for severe, atopic asthma patients with elevated serum IgE titres,

who are refractory to other treatments. This chapter will focus on the current knowledge regarding the pharmacogenetics of medicines used clinically in the treatment of asthma and also review the emerging importance of both pharmacogenetics and stratified approaches to newer asthma drugs currently in Phase II development.

3 β_2-adrenoreceptor Agonists

β_2-adrenoceptor agonists act by binding to a 413-amino-acid G-protein coupled receptor (GPCR) encoded for by an intronless gene (*ADRB2*) located on chromosome 5q31.32. Binding of the ligand to the β_2-adrenoceptor results in stimulation of Gs proteins, activating protein kinase A, resulting in the activation of adenyl cyclase. This activation pathway enables the phosphorylation of several target proteins, followed by reduction in intracellular calcium which in turn causes the targeted β_2 agonist effect of airway smooth muscle relaxation.

ADRB2 is highly polymorphic containing 49 known and validated single nucleotide polymorphisms (SNPs) and two insertion/deletion variants [3]. There have been extensive studies of the pharmacogenetic effects of *ADRB2* on the efficacy of β_2-adrenoceptor agonist therapy. The vast majority of these studies have focussed on the role of four non-synonymous coding region polymorphisms, namely Arg-16Gly, Gln27Glu, Val34Met and Thr164Ile [4]. The frequency of the position 16 and 27 polymorphism was found to be Arg16Glu 59 %, Gln27Glu 29 % in the Caucasian population [4]. The Val34Met and Thr164Ile polymorphisms are rare, with approximate frequencies of <0.001 % and 0.05 %, respectively. Although the Arg16 variant has been associated with an enhanced acute response to β_2-adrenoceptor agonists, a decline of asthma control following prolonged use and a subsensitivity of response for bronchoprotection, several studies have failed to reproduce these effects. Therefore a common consensus on the contribution of Arg16 has yet to be reached. At least twelve different haplotypes (combinations of polymorphisms) have been described in the *ADRB2* gene [5], suggesting a varied population-specific genotype for *ADRB2*. This may explain the lack of consensus when examining genotype effects in isolation [6].

Functional effects of these β_2-adrenoceptor coding region polymorphisms obtained from *in vivo* studies have shown the following: (a) the rare Thr164Ile variant receptor produces impairment in agonist binding and a reduction in adenyl cyclase activity, (b) an enhanced agonist-mediated receptor down-regulation occurs in the Gly16 receptor variants, and (c) a resistance to down-regulation is associated with the Glu27 variant [6–9]

Although cell studies have not identified a significant effect of the common Arg-16Gly and Gln27Glu *ADRB2* coding region polymorphisms on the responses to a range of β_2-adrenoceptor agonists including the long-acting beta-adrenoceptor agonist indacaterol, they did however confirm that the Thr164Ile variant receptor produced an impaired agonist mediated cAMP production in recombinant cell lines [10].

A recent clinical study by Lee et al. reported associations between the Gln/Glu residue 27 status, and lung function in asthmatic children on nebulised terbutaline. More specifically, the peak expiratory flow rate (PEFR) improved by 23.4 ± 14.4 % in Glu/Glu homozygotes and by 8.2 ± 0.4 % in Gln/Gln individuals ($p < 0.05$), while Arg16Gly heterozygotes showed no bronchodilator effect [11]. The main limitation of this study was the small number of participating patients ($n = 27$). One of the largest studies to date ($n = 1182$), carried out by Basu et al. in asthmatic patients having an age range of 3–22 years, reported an increased risk of exacerbations per copy of Arg16 (odds ratio [OR], 1.30; CI 1.09–1.55 $p = 0.003$). Two patient groups were studied; one was receiving regular inhaled corticosteroid plus salbutamol *prn*, while the other was being managed with a regular inhaled corticosteroid/salmeterol combination, plus salbutamol *prn*. Interestingly, this pharmacogenetic effect was driven by those patients who used daily doses of at least one of the bronchodilators [12]. The study is however limited by the absence of control patients who were not on β_2-adrenoceptor agonist treatment. This missing control group may have provided a clearer interpretation for the detrimental effects of β_2-adrenoceptor agonists in Arg16 subjects. This is especially relevant in view of other studies where clinically relevant effects of these *ADRB2* variants could not be identified. For example, a large study conducted earlier by Bleecker et al., failed to identify any clinically relevant outcomes of the Gly16Arg genotype. The group studied 2250 asthma patients, who were assigned to three management groups for a 6 month period as follows: (a) budesonide/formoterol combination maintenance therapy plus reliever therapy *prn*, (b) fixed dose budesonide plus formoterol, and (c) fixed dose fluticasone plus salmeterol [13].

These contrasting outcomes may be rationaliszed by the different study designs employed. Furthermore, deeper data analyses (e.g. looking at data stratified by daily *prn* reliever use) was lacking. A more recent study conducted by the same group, explored the potential pharmaocogenetic effects of 11 *ADRB2* SNPs in patients administered a fluticasone/salmeterol combination ($n = 268$) or salmeterol alone ($n = 266$), Interestingly, this study found no Arg16Gly pharmacogenetic effects on clinical outcomes, including morning PEFR and number of symptom free days, while other *ADRB2* SNPs localised to regulatory regions of the gene appeared to influence clinical outcomes of bronchodilator treatment. It is however unlikely that these outcomes would survive correction for multiple testing [14]. A similar lack of clinical influence of the Arg16 genotype, on fluticasone/salmeterol clinical outcomes, has also been observed by other research workers. For example, a double blind, placebo controlled trial carried out by Wechsler et al., using Arg/Arg and Gly/Gly adult asthmatic subjects with matched morning PEF (peak expiratory flow), FEV_1 (forced expiratory volume in one second) and symptom scores (an approach undertaken in order to maximise the likelihood of identifying genotype-specific effects) failed to identify Arg or Gly related outcomes after an 18-week study period [15].

While the majority of studies have focussed on the Arg16Gly, it is important to emphasise that as in the majority of genes, *ADRB2* is highly polymorphic with multiple potential polymorphisms occurring in the gene's regulatory regions. Single SNP approaches on their own may therefore be ineffective at elucidating

genotype-phenotype relationships. Haplotype analysis, or *in vitro* "whole gene transfection" may be a more appropriate approach, and one that approximates more the *in vivo* situation. Such an approach has been recently reported by Panebra et al. who studied eight common haplotypes based on 26 SNPs, and identified differential effects on receptor expression and down-regulation that are haplotype driven [16]. This identification that four common haplotypes are related to elevated receptor expression and two haplotypes are related to enhanced receptor down-regulation, suggests that haplotypes rather than single SNPs may be the driving force between these differential effects [6].

The lack of agreement between large prospective clinical studies which have identified no pharmacogenetic effects of the common Arg16Gly and Gln27Glu *ADRB2* polymorphisms, and *in vitro* molecular research which commonly identified receptor signalling effects, questions the validity of the direct translation of *in vitro* functional work to what happens in a clinical setting. Current evidence points to haplotype rather than individual SNP-driven effects. Future clinical research in this area should perhaps focus on carefully designed studies, which are suitably powered to enable identification of both haplotype-driven and SNP-driven pharmacogenetic effects where these actually exist.

4 Novel Regulators of β_2-adrenoceptor Agonist Responses

As the mechanism of action of β_2-adrenoceptor agonists is both complex and multifactorial, further genes are expected to influence β_2-adrenoceptor agonist efficacy. Indeed the estimated heritability for β_2-adrenoceptor agonist response is thought to be 28.5%, a value too high to be driven by a single gene [17]. Using a novel algorithm implemented in a family-based association test (FBAT), Litonjua et al. screened the association of 844 genotyped SNPs in 111 candidate genes (42 involved in β_2-adrenoceptor signalling/regulation, 28 genes involved in glucocorticoid regulation, 41 genes from prior asthma association studies) in 209 children and their parents participating in the Childhood Asthma Management Program (CAMP) for their association with acute response to inhaled β_2-adrenoceptor agonist. This study identified the Arginase 1 (*ARG1*) SNP rs2781659 as being significantly associated with bronchodilator response (BDR) ($p=0.047$) [18]. Data from guinea pig models of allergic airway disease suggests that Arginase enzymes deplete stores of L-arginine in the airways, a nitric oxide synthase substrate leading to decreased nitric oxide which normally acts to relax smooth muscle resulting in airway hyper-responsiveness [19, 20]. The relationship between *ARG1* and β_2-adrenoceptor agonist response was also identified in a recent candidate gene study involving 221 asthma subjects [21]. The *ARG1* polymorphisms identified in both studies were in linkage disequilibrium (inherited together) suggesting a common causative mechanism involving potential transcriptional regulation as these polymorphisms were predominantly 5' to the gene. This alteration in transcription has now been confirmed in promoter-reporter studies with the key *ARG1* haplotype associated with improved

BDR driving the highest level of *ARG1* promoter activity [22]. Interestingly, in the study by Vonk et al., *ARG2* SNPs were also associated with patient responses to Salbutamol [21].

The S-nitrosoglutathione reductase (*GSNOR*) gene has recently been associated with β_2-adrenoceptor agonist (salbutamol) responses. Specifically, the *GSNOR* promoter SNP rs1154400, was associated with a decreased response to salbutamol in 107 African American children [23]. *GSNOR* is an alcohol dehydrogenase that breaks down GSNO, an endogenous bronchodilator [24]. In addition, GSNO regulates nitrosylation of proteins leading to alterations in function, including G protein–coupled receptor kinase 2 (GRK2) which phosphorylates and desensitises the β_2-adrenoceptor [25]. Within the same study, a post-hoc multi-locus analysis identified that a combination of rs1154400 with the *ADRB2* SNPs Arg16Gly and Gly27Glu and rs2230739, a SNP associated with the carbamoyl phosphate synthetase-1 (*CPS1*) gene, give a 70% predictive value for therapeutic unresponsiveness [23]. This implies that pharmacogenetic regulation of β_2-adrenoceptor agonist therapy may depend on several loci acting together via gene-gene interactions. In confirmation 4/5 SNPs tested within *GSNOR* were associated with asthma patient responses to salbutamol in 168 Puerto Rican asthma patients [26]. These SNPs were also associated with asthma susceptibility and the key risk haplotype was associated with increased transcriptional activity based on promoter-reporter studies [26]. The identification of *GSNOR* in two racially distinct populations underlines the importance of cross population studies to identify common regulators of β_2-adrenoceptor agonist activity, where different haplotypes of a gene may have a population dependant role on drug function, efficacy and side-effects.

A recent study by Himes et al. using RNA-seq, a cutting edge technology able to detect differential gene expression at the transcript level in a cell population, has identified the up-regulation of the cysteine-rich secretory protein LCCL domain containing 2 gene (*CRISPLD2*) on stimulation of a bronchial epithelial cell line with a long acting β_2-adrenoceptor agonist (formoterol) [27]. Although this association has not been replicated *in vivo*, we highlight this study as an example of potential novel genetic associations that remain to be discovered through developing laboratory based approaches. Although our understanding of the pharmacogenetics of β_2-adrenoceptor agonist response currently remains fragmented, a combination of laboratory based applications and larger better stratified population studies will help us to better understand this relationship.

5 Leukotriene Modifier Drugs

Leukotrienes were first recognised to be important modulators of airway constriction, by Kellaway and Trethewie, who in 1940 reported the release of a “slow-reacting smooth muscle-stimulating substance” from perfused guinea pig lung, following sensitisation with egg albumin [28]. This was later termed SRS-A (slow reacting substance of anaphylaxis) by Brocklehurst in 1960 and was eventually recognised

to consist of a combination of the cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4 [29]. Leukotrienes are generated via the 5-lipoxygenase arm of the arachidonic acid metabolism pathway, and they exert their main pro-inflammatory and bronchoconstrictive actions through their interaction with the G-protein coupled receptors $CysLTR_1$, and $CysLTR_2$. The dihydroxy-containing leukotriene LTB_4 acts via the $LTB4R_1$ and $LTB4R_2$ receptors, though there is evidence to suggest that these receptors may also exhibit some binding affinity for the cysteinyl-containing ligands. In the human lung, leukotriene receptors are primarily expressed on airway smooth muscle but *CYSLTR1* and *CYSLTR2* gene transcripts have also been identified in circulating immune cells including eosinophils, monocytes, T-cells and B-cells, where cytokines such as IL-4 and IFN-γ have been shown to upregulate their expression [30].

There are two classes of FDA approved therapeutic agents which target the leukotriene pathway; the leukotriene receptor antagonists (LTRA) zafirlukast, montelukast, pranlukast and cinalukast, which have primary activity against $CysLTR_1$, and the leukotriene synthesis inhibitors (LTSI), the main member being the 5-lipoxygenase inhibitor, zileuton. A third class, the 5-lipoxygenase activating protein (FLAP) inhibitors have offered promising benefits, but none of these agents are yet FDA-approved. More recent data has suggested that drug-induced inhibition of the leukotriene A_4 hydrolase (LTA_4H) enzyme, which is responsible for the synthesis of LTB_4 from LTA_4, may offer therapeutic benefit against the inflammatory and bronchoconstrictory effects of LTB_4-mediated pathways [17]. Indeed, earlier studies have shown some *LTA4H* gene polymorphisms to be associated with susceptibility to allergy, asthma and Chronic Obstructive Pulmonary Disease (COPD), as well as to contribute to the baseline lung function of smokers and the serum IgE levels of asthmatic patients [31–33]. In addition, specific *LTA4H* polymorphisms have been associated with LTRA treatment outcomes (Table 1). However information on whether *LTA4H* gene polymorphisms may also modify the therapeutic outcome of LTA4 hydrolase inhibitors, remains to be determined.

Functional leukotriene-related pharmacogenetic effects, may be contributed by polymorphic variation in genes regulating various steps within the pathway involved in leukotriene production or receptors that transduce cellular effects. Table 1 lists some examples of functional leukotriene-related genetic variants.

A well-studied Sp1-motif repeat polymorphism in the 5-lipoxygenase (*ALOX5)* gene promoter, is reported to cause a significant reduction in the expression of 5-lipoxygenase, in carriers of variants other than the wild-type penta-repeat. This results in a lower leukotriene-contributed inflammatory component in asthmatic patients carrying non-wild type Sp1-repeats. Consequently, drugs targeting this pathway show a reduced response in patients who are homozygous or heterozygous for Sp1-repeat variants. This was initially demonstrated with the LTSI ABT-761 [34], a 5-lipoxygenase inhibitor which halted development after Phase III clinical trials [45], and later also demonstrated with the LTRA montelukast [36]. Indeed, *ALOX5* promoter Sp1 repeats other than the wild type $(Sp1)_5$, associate with overall worse asthma control [46]. An earlier study contrastingly reported non-wild type Sp1 repeat polymorphisms to be *beneficial*, in terms of an increased reduction in the number of asthma exacerbations in montelukast-treated patients [35], while a

Table 1 A list of some pharmacogenetically relevant genes which influence treatment outcomes of drugs targeting the 5-lipoxygenase pathway

Gene	Locus	Allele	Function	Reference
ALOX5	10q112	Sp1 binding motif repeats other than $[Sp1]_5$	Loss of effect of LTSI, ABT-761	[34]
		Sp1 binding motif repeats other than $[Sp1]_5$	Reduction in asthma exacerbations in patients treated with montelukast for 6 months	[35]
		$[Sp1]_5$	Improved montelukast response in asthmatic patients with at least one $[Sp1]_5$ allele	[36]
		rs2115819	'G' allele associated with improved FEV_1 in patients treated with montelukast for 6 months	[35]
		rs4987105	Improved PEF in asthmatic patients treated with montelukast for 12 weeks	[37]
		rs4986832	Improved PEF in asthmatic patients treated with montelukast for 12 weeks	[37]
		rs2115819	Reduced FEV_1 response to zileuton and montelukast in asthmatic patients	[38]
LTC4S	5q35	−444 A>C rs730012	Reduced risk of asthma exacerbations in patients treated with montelukast for 6 months	[35]
		−444 A>C rs730012	Improved FEV_1 following 1 month treatment with pranlukast	[39]
LTA4H	12q22	rs2660845	'G' allele increases risk of asthma exacerbations in patients treated with montelukast for 6 months	[35]
		rs2540491 (Intron 3, G/A)	'A' allele associated with augmented FEV_1 response to salbutamol in patients who were also treated with one of montelukast, zafirlukast or zileuton	[40]
CYSLTR1	Xq13-q21	−642 A>G	'G' alleles causes lower CysLTR1 expression in transfected THP1 cells and may therefore potentially reduce efficacy of LTRAs	[30]
		−634 C>T	Higher allelic frequency corresponds to higher montelukast dose requirements in aspirin intolerant asthmatics	[41]

Table 1 (continued)

Gene	Locus	Allele	Function	Reference
CYSLTR2	13q14.2	rs912277	Improved PEF in asthmatic patients treated with montelukast for 12 weeks	[37]
		rs912278	Improved PEF in asthmatic patients treated with montelukast for 12 weeks	[37]
		M201V	Loss of LTD_4 binding affinity and LTD_4-mediated signalling *in vitro*	[42]
Haplotypes				
LTC4S	5q35	−1072G/A −444A/C	G-A, G-C and A-C haplotypes (but not A-A) increase dexamethasone-induced LT4CS transcription *in vitro*	[43]
Genes not directly involved in the lipoxygenase pathway				
SLCO2B1	11q13	rs12422149 (Arg312Gln)	Heterozygotes show lower plasma montelukast concentrations than similarly dosed homozygotes	[44]
ABCC1 (MRP1)	16p13.1	rs119774	'T' allele associated with improved FEV_1 in patients treated with montelukast for 6 months	[38]

recent study by Nwokoro and co-workers, reported less unscheduled medical attendences in montelukast-treated children carrying the wild type Sp1-pentarepeat than children carrying other Sp1 repeat variants [47].

ALOX5 SNPs may also carry pharmacogenetic relevance. For example, Tantisira and co-workers associated the *ALOX5* intronic SNP rs2115819 with a reduction in response to both zileuton and montelukast treatment in asthmatic patients, using FEV_1 as a clinical endpoint [38], while *ALOX5* rs4987105 and rs4986832 SNPs have both been associated with improved montelukast treatment outcomes [37]. However, the mechanisms underlying these associations remain to be determined.

The tetra-locus haplotype [−1708G-21C-270G-1728A] in the *ALOX5* gene is associated with aspirin sensitivity to asthma [48]. A potential explanation for aspirin-induced asthma exacerbations, argues for an already potentiated leukotriene production pathway, which, in the presence of aspirin, or another non-steroidal anti-inflammatory drug (NSAID) is loaded with excess arachidonic acid, which would otherwise be shunted down the cyclooxygenase pathway in an NSAID-free environment. The arachidonic acid loading generates a higher production of bronchoconstricting leukotrienes, often resulting in exacerbation of asthma symptoms. The association of the aspirin-sensitive phenotype with genetic variation has valuable clinical applications, as it could potentially discriminate patients in whom NSAID contraindications are warranted from those in whom NSAID use is permissible.

The CysLTR1 and CysLTR2 receptors are the targets through which cysteinyl leukotrienes act with CysLTR1 providing the major binding affinity for LTRAs. The genes for these receptors may therefore be considered to be strong candidates for functional pharmacogenetic variability. However it has been difficult to identify clear associations between clinical therapeutic responses and genetic polymorphisms in these genes. Two particular *CYSLTR1* variants, G300S and I206S, have been reported to show increased LTD_4 potency, and may therefore potentially also exhibit altered potency for other ligands, including LTRAs [49]. In addition, Duroudier et al. (2009) found the upstream *CYSLTR1* −642A>G SNP to be associated with reduced gene expression in transiently transfected THP1 cells, therefore potentially contributing to reduced LTRA response *in vivo* due to reduced expression of the drug target [30]. Kim et al. (2007) similarly found high allelic frequencies of another upstream *CYSLTR1* SNP −634C>T, to correspond to higher montelukast dose requirements in aspirin intolerant asthmatic patients [41]. Some *CYSLTR2* coding sequence SNPs have been associated with the presence of aspirin intolerance in asthma, and haplotype analysis further consolidated this observation [50]. The presence of rs912277 or rs912278 has been reported to result in improved response to montelukast [37], suggesting a potentially modified interaction between the CysLTR2 receptor and LTRAs in patients carrying these genotypes. The CYSLTR2 M201V receptor variant, has been shown to exhibit minimal affinity for LTD_4 in HEK293 stable transfectants, while functional analysis of LTD_4-stimulated CYSLTR2 receptor activation, showed downstream signalling endpoints to be extensively reduced for the expressed variant receptor in this cell line. LTC_4 showed an approximately 50% reduction in binding affinity to the M201V variant, and LTC_4-mediated downstream signalling was reduced at low concentrations, but appeared to be restored at higher LTC_4 levels [42]. Interestingly, this variant had also been associated with atopy [51] and asthma [52] in earlier studies.

Genes not directly involved in cysteinyl leukotriene synthesis or actions may also exert a pharmacogenetic influence (Table 1). For example, the rs2660845 SNP in *LTA4H* which codes for leukotriene A_4 hydrolase, has been reported to exert a negative influence on LTRA treatment outcomes. LTA_4 hydrolase is responsible for the synthesis of LTB_4, a dihydroxy leukotriene, with no affinity for the cysteinyl leukotriene receptors [35]. In addition, genes may also influence LTRA or LTSI treatment outcomes, by directly interfering with pharmacokinetic processes. For example the rs119774 SNP in the multidrug resistance associated protein 1 (*ABCC1*) gene has been associated with an improved FEV_1-based clinical response to both zileuton and montelukast in asthmatic patients [38]. *ABCC1* is involved in the transport of LTC_4 from the cell. In contrast, the membrane transporter gene *SLCO2B1* rs12422149 SNP (Arg312Gln) has been associated with reduced morning plasma montelukast concentrations in asthmatic patients and improved symptom assessment changes [44]. However, not all studies have been able to replicate these findings.

6 Glucocorticoids

Inhaled glucocorticoids (GC) are commonly used in the management of asthma. The molecular actions of glucocorticoids are multifaceted and complex, involving the simultaneous activation and/or repression of many genes. This has often made it difficult to establish clear genotype-phenotype associations. Moreover, although the human genome only contains one known glucocorticoid receptor (GR) gene (*NR3C1* located at cytogenetic band 5q31.3), alternative splicing and alternative translational initiation events give rise to separate receptor isoforms having different functional profiles [53]. There is also recent evidence for a membrane-bound GR isoform, possible expressed by the same gene, and which mediates rapid-onset non-genomic actions of glucocorticoids [54]. Furthermore, glucocorticoid receptor (GR) isoforms may also be subject to a variety of posttranslational modifications which result in altered receptor function.

The major pharmacogenetic issues associated with glucocorticoid therapy relate to patients who fail to respond beneficially to their therapeutic potential. These are often referred to as steroid-resistant individuals. Two forms of glucocorticoid resistance have been described. Type I is an induced type, and usually the result of cytokine-induced modification of glucocorticoid activity on T-lymphocytes, such as IL-2 and IL-4 in T-cells [55], IL-13 in monocytes [56], TNFα + IFNγ in airway smooth muscle [57] and IL-17 in airway epithelial cells [58–60]. This type of resistance may be reversed through cytokine inhibition approaches. Type II, the pharmacogenetic form of glucocorticoid resistance, is irreversible and may be due to genetic variation in the glucocorticoid receptor gene itself, or other genes which contribute to the functional pathways that relate to glucocorticoid receptor activation and function [61, 62].

The *NR3C1* gene is highly functionally conserved, and functional polymorphisms identified to date are rare, with minor allelic frequencies in various populations commonly falling below 3% [63]. The functional outcomes of these polymorphisms are strongly dependent on the specific receptor isoform in which they are expressed, and the pharmacogenetic phenotype may be the result of the combined effects of the same SNP on different isoforms. Expression of these isoforms is driven by the selective use of at least 5 recognised *NR3C1* promoters [64], and polymorphic variation in these promoters may determine the respective transcript expression level. For example the −22C>A polymorphism located upstream of the *NR3C1* gene significantly decreases the promoter transcriptional activity compared to the wild type C allele as determined by promoter luciferase reporter assays in HepG2 and HEK293 cells, and is likely to be related to lower GRα expression in the clinical setting [65]. Similarly, the G allele of the *Bcl*I GR promoter polymorphism is significantly associated with GC resistance and with the development of severe asthma [66]. Moreover, a second layer of complexity in selection of *NR3C1* isoform expression arises from the epigenetic-driven methylation of the different promoters, occurring in a tissue-dependent fashion [67]. This may partially explain the difficulty of corroborating *in vitro* functional effects of *NR3C1* promoter polymorphisms with *in vivo* clinical outcomes.

The two major GR isoforms which contribute to GC treatment outcomes are the α and β gene products. GRα, is a 777 amino acid protein which is the functional receptor responsible for the majority of glucocorticoid actions. The second isoform, GRβ, is an alternatively spliced product which carries the first identical 727 amino acids as GRα. It has a C-terminal sequence which is both shorter and non-homologous to the C-terminal sequence of GRα, and this renders it unable to bind ligand or be transcriptionally active. Both isoforms are ubiquitously expressed in human cells, with GRβ normally exhibiting lower expression levels [68–70]. Table 2 highlights some polymorphisms which are related to glucocorticoid response.

6.1 Polymorphisms Influencing GRα Function

The dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) lists more than 18000 human *NR3C1* SNPs, located throughout the 157 kilobases of the whole gene. However, most of these are localised to intronic regions, and exert no known influence on the expressed receptor protein or on its production. About 39 *NR3C1* coding region missense SNPs have been recognised to date. Though several associations with disease severity phenotypes have been reported, the *NR3C1* polymorphisms which are *pharmacogenetically relevant*, only represent a small fraction of the total SNP pool. Moreover, clinically relevant *NR3C1* variants which influence therapeutic outcomes, tend to occur at low allelic frequencies in all populations, though functional *in vitro* work occasionally suggests that more common variants may also contribute to glucocorticoid responses. Niu and co-workers identified 108 polymorphisms within exonic, intronic and untranslated regions of the *NR3C1* gene, in a sample of 240 individuals. *In vitro* functional analysis identified Phe(65)Val and Asp(687)Glu to be associated with higher hGRα protein expression. SNP 746T>C and haplotype 237delC / 238C>T/240G>C were both associated with reduced transcript expression, and Ala(229)Thr and Ile(292)Val was found to be associated with decreased ligand binding. These functional polymorphisms, present in allelic frequencies ranging from 5.8% to 18.3% in different ethnic groups, are potential contributors to reduced glucocorticoid sensitivity in a clinical setting [73].

The presence of variants AG or GG of codon 363 in exon 2 of the *NR3C1* gene has been reported to correlate with better *in vivo* anti-inflammatory effects of glucocorticoid therapy and reduced risk of development of uncontrolled asthma in a study carried out using a sample from the Polish population. The G allele causes a change of asparagine to serine, and it has been suggested that this may modify interactions of the receptor with the transcriptional factors, AP-1 and NF-κB [76]. However, earlier work by Huizenga's workgroup could not identify any alteration in the repressive capacity of the *NR3C1* N363S variant on target genes via negative glucocorticoid-responsive elements or via AP-1 and NF-κB [87]. Though this polymorphism is present at allelic frequencies of 3–7% in Caucasian and African populations, studies in the Swedish population had earlier failed to identify these genotype-phenotype associations [88]. Furthermore, several studies have reported this N363S polymorphism to be either absent, or otherwise occur at very low allelic frequencies (<0.5%) in subjects of various Asian origins [63, 89–91].

Table 2 Pharmacogenetically relevant genes which may be related to glucocorticoid actions

Gene	Locus	Allele	Function	Reference
NR3C1	5q31-q32	Val>Asp641	Threefold lower receptor binding affinity for dexamethasone	[71]
		Val>Ile729	Fourfold decrease in dexamethasone response	[72]
		Phe>Val65	Increased hGRα protein expression *in vitro*	[73]
		Asp>Glu687	Increased hGRα protein expression *in vitro*	[73]
		746T>C	Reduced hGRα transcript expression *in vitro*	[73]
		237delC 238 C>T 240G>C [haplotype]	Reduced hGRα transcript expression *in vitro*	[73]
		Ala>Thr229	Decreased ligand binding affinity	[73]
		Ile>Val292	Decreased ligand binding affinity	[73]
		*Bcl*I RFLP	Higher cortisol levels in GC than CC genotypes, both pre- and post-ICS treatment	[74]
		*Bcl*I RFLP	Increased *in vitro* sensitivity to methylprednisolone	[75]
		*Bcl*I RFLP	'G' allele associated with *in vivo* glucocorticoid resistance and severe asthma	[66]
		Asn>Ser363	Increased sensitivity to GCs	[76]
		−22 C>A	Decreased *in vitro* promoter transcriptional activity	[65]
		G-A-T haplotype (consisting of BclI SNP G, intron B 33389A, intron B 33388T)	Low post-dexamethasone cortisol levels	[77]
CRHR1	17q12-q22	rs1876828, rs242941	Delayed cortisol response, following inhaled glucocorticoid treatment	[74]
TBX21	17q21.2	His>Glu33	Larger improvements in methacholine-induced PC_{20} following treatment with inhaled glucocorticoids	[78]
		rs9910408	Larger improvements in methacholine-induced PD_{20} in asthmatics carrying the AA genotype, following treatment with inhaled glucocorticoids	[79]
ABCB1	7q21.12	G2677T	Reduced glucocorticoid response	[80]
		G2995A	Reduced glucocorticoid response	[80]
		C3435T	Increased glucocorticoid response	[81]

Table 2 (continued)

Gene	Locus	Allele	Function	Reference
TACR2 (*NK2R*)	10q22.1	Gly231Glu	Gly allele associated with improved glucocorticoid response	[82]
STIP1	11q13.1	rs4980524 (G/T) (intron 1)	Altered lung function responses to glucocorticoids	[83]
		rs6591838 (A/G), (intron 1)	Altered lung function responses to glucocorticoids	[83]
		rs2236647 (C/T), (intron 5)	Altered lung function responses to glucocorticoids	[83]
DUSP1	5q35.1	rs881152 (A/G), (5' region)	Associated with bronchodilator response in asthma	[84]
FCER2	19p13.2	rs28364072 (T/C), (intronic)	'C' allele associated with lower FcεRII expression, higher serum IgE and higher risk of asthma exacerbations during budesonide therapy	[85]
GLCCI1	7p21.3	rs37972 (C/T), (5' region)	'C' allele associated with improved glucocorticoid response	[86]

6.2 Polymorphisms Influencing GRβ Function

High expression levels of GRβ are strongly associated with glucocorticoid resistance. The early evidence to support this comes both from clinical observations [92] as well as from *in vitro* overexpression experiments in cell line models [93, 94], but the mechanisms for this action are still widely debated today. In contrast to GRα, the β-isoform interacts poorly with heat shock proteins, does not bind ligands, and is transcriptionally inactive. GRβ-induced glucocorticoid resistance is likely to occur via competition with GRα for binding to glucocorticoid responsive elements (GREs) on gene promoters, via direct GRα inactivation through heterodimerization, and by the inhibition of co-activating proteins which are necessary for GRα activity. Recent evidence suggests that GRβ competes with GRα for binding to glucocorticoid receptor-interacting protein 1 (also called nuclear receptor coactivator 2 and coded for by the *NCOA2* gene), and generates an ineffective co-activator complex. Although individuals having a high GRβ/GRα expression ratio appear to carry this as a lifelong phenotypic trait, the identification of specific gene variants which strongly associate with this aberrant ratio has been elusive. Current evidence suggests that high GRβ/GRα seems to be more related to disease-specific factors, in particular specific cytokine profiles, which may occur in diseases such as rheumatoid arthritis, asthma, and cancer and autoimmune diseases [95, 96]. It has been suggested that high GRβ may be a consequence of a patient-specific disease profile, rather than a contributor to the cause. The associated glucocorticoid insensitivity further complicates the situation, by hindering our therapeutic ability to reverse the causative inflammatory processes. For example recent evidence shows that cytokines IL-17 and IL-23 released by infiltrating T-cells in asthma, contribute to GRβ upregulation and consequently to a degree of glucocorticoid insensitivity [97].

6.3 *Non-NR3C1 Polymorphisms Which Influence Glucocorticoid Response*

The multiplicity of GR-mediated pathways, lend its actions to be potentially influenced by variability in several other genes. However, the functional contribution of such variability is often difficult to predict. For example, allelic variations in *CNTNAP2*, *LEPR*, *CRHR1*, *NTAN1*, *SLC12A3*, *ALPL*, *BGLAP*, and *APOB* have all been associated with prednisolone-induced hypertension in specific patients [98]. Most of these genes are involved in the hypothalamic-pituitary axis pathway. Polymorphic variations in genes that code for lung esterases are interesting candidates for the inhaled prodrug glucocorticoids beclomethasone and ciclesonide, since both are activated in the airways by esterase action. Beclomethasone diproprionate is metabolised to the more active beclomethasone-17-monopropionate, while ciclesonide, an inactive prodrug, is metabolised in airway epithelial cells to the pharmacologically active desisobutyryl-ciclesonide [99]. However, although genetic variability in various esterase genes has been described [100, 101], pharmacogenetic evidence for their clinical contribution to bioactivation-associated pharmacology is lacking. Some pharmacogenetically relevant polymorphism are described below.

6.4 *ATP-Binding Cassette, Sub-Family B Member 1* (ABCB1) *Gene*

The membrane transporter PgP is coded for by *ABCB1* (previously known as MDR1), a 28-exon gene located on 7q21.1. It is a cellular efflux transporter which operates on a large range of substrates including prednisolone, dexamethasone and beclomethasone monopropionate (the active metabolite of the pulmonary administered beclomethasone dipropionate). *ABCB1* overexpression is of concern, since increased Pgp efflux activity contributes to reduced therapeutic outcome of the affected drugs. Examples of high-expression-inducing *ABCB1* variants include G2677T and G2995A [80]. Conversely, low expression variants such as C3435T [81] may contribute to increased glucocorticoid response due to intracellular accumulation of the drug. There is also evidence to show that glucocorticoids upregulate Pgp expression, and may therefore amplify the effects of *ABCB1* allele-specific high activity variants [102].

6.5 *Corticotropin Releasing Hormone Receptor Type 1* (CRHR1) *Gene*

The CRHR type 1 receptor is the major corticotropin receptor, and therefore has a key regulatory role in endogenous corticosteroid synthesis. This gene, located on 17q12–22, exhibits SNPs which have been shown to be associated with cortisol

response in humans. For example, Tsartsali's workgroup carried out Synacthen testing on male asthmatic children, treated with inhaled budesonide (400 μg/day) or fluticasone (200 μg/day) for 3 months, and genotyped the patients for *CRHR1* variants. Homozygotes for the *CRHR1* variant rs242941 (TT) demonstrated a delayed cortisol response after treatment when compared to heterozygotes (GT) ($p=0.033$) and wild-type homozygotes (GG) ($p=0.018$). Homozygotes (AA) for a second *CRHR1* SNP, rs1876828, manifested lower baseline cortisol levels before treatment ($p=0.009$) compared to the GG genotype. These patients also showed marginal statistical significance for delayed cortisol response after treatment compared to the GA heterozygotes ($p=0.05$) [74].

6.6 *T-Box Expressed in T Cells* (TBX21) *Gene* and Other Genes

TBX21 encodes the T-bet transcription factor and contributes to an enhanced Th1 lymphocyte population. *TBX21*-knockout mice have been shown to develop airway eosinophilia, bronchial hyperresponsiveness and enhanced airway remodelling, thus suggesting that this gene to have a protective role in asthma [103]. An SNP causing a His>Glu33 substitution has been identified to activate Th1 cytokine synthesis in cell culture models, and this is postulated to protect against allergy and asthma by shifting lymphocyte proliferation away from the Th_2 development. Indeed, both heterozygous and homozygous Glu33 patients have been reported to show a greater reduction in PC_{20}-measured airway hyperresponsiveness, than His33 homozygotes. Unexpectedly, it has been shown that glucocorticoids inhibit T-bet induction, possibly contributing to a shift *towards* a Th_2-based response rather than against [78]. The precise mechanistic contribution of His>Glu33 in this scenario remains to be explained. A second *TBX21* polymorphism, rs9910408, has been associated with increased response to inhaled glucocorticoids in adult asthmatic patients. Work carried out by Lopert's group showed that patients carrying the AA genotype, exhibit better reductions in bronchial hyperresponsiveness, higher improvements in FEV_1, and better quality of life scores, than similarly ICS-treated GG or AG patients [79].

Polymorphisms in the neurokinin-2 receptor gene (*TACR2*–previously known as *NK2R*) influence glucocorticoid-mediated therapeutic outcome, possibly by modifying neurokinin-induced inflammation and bronchoconstriction [82]; intronic genetic variants of the stress-induced phosphoprotein-1 gene (*STIP1*) influence lung function responses to glucocorticoids by mechanisms which are unclear, and which may involve interactions with Hsp70 chaperones, thus influencing GR function [83]. *DUSP1*, a gene that codes for a p38 MAPK (mitogen-activated protein kinase) inhibitor, the expression of which is thought to be influenced by glucocorticoid-mediated regulation, influences glucocorticoid response possibly by polymorphism-dependent alterations in its ability to target the p38 MAPK signalling pathway [84]. Interestingly, polymorphic variation in the low-affinity IgE receptor (FcεRII) has been shown to increase the risk of asthma

exacerbations in children on budesonide therapy, possibly due to an associated increase in serum IgE concentrations. These findings have survived replication studies in separate asthma cohorts [85]. The *GLCCI1* (glucocorticoid-induced transcript 1) rs37972 SNP ('C' allele) was found to be associated with improved glucocorticoid response in asthmatic patients, through a genome-wide association study (GWAS) carried out by Tantisira's workgroup [86]. Since rs37972 is localised to the *GLCCI1* promoter, it may be postulated that the mechanism involves polymorphism-induced variations in the transcriptional regulation of the *GLCCI1* gene. However, the precise mechanism by which this influences glucocorticoid response is unclear.

The elucidation of clear glucocorticoid pharmacogenetic genotype-phenotype associations remains a challenge. The complex molecular network which is involved in the outcomes of glucocorticoid receptor activation, suggests that the search for pharmacogenetic variants would benefit from the use of hypothesis-free genome wide approaches. The study of multiple polymorphisms spread over a gene network may be more relevant than attempts to identify single responsible variants. Moreover, age may also influence pharmacogenetically-induced phenotypic traits. Park and co-workers have recently found rs10044254, an *FBXL7* (F-box and leucine-rich repeat protein 7) gene A>G intronic SNP, to be associated with improved response to inhaled glucocorticoids in the paediatric asthmatic population, but not in adult patients [104]. *FBXL7* codes for an F-box protein which plays a role in the phosphorylation-dependent ubiquitination of proteins.

7 Anti-Muscarinic Agents

Anti-muscarinic drugs are commonly used in the management of COPD. However they may also be used in severe asthma as second line bronchodilators, in combination with an adrenergic β_2-agonist, in those patients who do not adequately respond to β_2-agonist-induced bronchodilation alone, or who demonstrate a reduced degree of airway reversibility.

Two main anti-muscarinic agents are used to manage airway disease; ipratropium and tiotropium. Both drugs are administered by inhalation, and are pharmacologically non-selective antagonists of airway muscarinic receptors M1, M2 and M3; however receptor-bound tiotropium exhibits a much slower rate of dissociation from M3 receptors, than it does from M1 and M2, giving it long acting and kinetically M3 selective properties [105].

The coding regions of these receptor genes tend to be highly conserved with only low allelic frequency synonymous SNPs being reported (Table 3). However, a common polymorphism (1696T>A) reported by Fenech and co-workers [106] in the 3'UTR of the M2 gene, was later found by Szczepankiewicz's group to be associated with poor bronchodilator response to ipratropium bromide in paediatric asthmatic patients homozygous for the T allele [107].

The regulation of expression of M2 and M3 receptors is highly promoter-dependent, and polymorphisms have been demonstrated in the promoters of both genes [108, 109]. Indeed, 7 dinucleotide $(CA)_n$ repeat polymorphisms [(insertion $(CA)_1$, wild type $(CA)_{14}$, $\Delta(CA)_1$, $\Delta(CA)_2$, $\Delta(CA)_3$, $\Delta(CA)_4$, $\Delta(CA)_8$] have been identified in a high activity region of the *CHRM2* promoter (Table 3), and *in vitro* luciferase reporter assays carried out in human airway smooth muscle (HASM) and airway epithelial (BEAS-2B) cell transfectants have reported repeat-dependent transcriptional activities. These repeats may therefore potentially alter muscarinic M2 receptor expression *in vivo*. Since airway muscarinic M2 receptors are primarily neurologically expressed and exhibit autoreceptor function, a decrease in expression may contribute to increased airway parasympathetic activity, and thus reduce the response to antimuscarinic drugs.

Maeda reported two *CHRM1* promoter polymorphisms (−9697C>T and −4953A>G) to be associated with asthma susceptibility, with the T-A haplotype showing a significantly lower promoter activity in luciferase reporter assays carried out using IMR32 neuronal transfectants ($p=0.019$) [110]. Such SNPs could be expected to have some degree of pharmacogenetic importance *in vivo,* since the major receptors accessible to anti-muscarinic agents administered by the inhaled route, tend to be M2 and M3 rather than M1.

8 Pharmacogenetic Aspects of Disease Management: *ADRB2* Arg16 and Second Line Therapy in Asthma

While the study of genetic contributions to drug responses have been predominantly in the trial setting and focused to acute responses to medication e.g. change in lung function, a more recent study set out to look in a real life situation where genetic information could influence management of disease over a prolonged period [112]. First line treatment for asthma is a short acting β_2-adrenoceptor agonist e.g. salbutamol as required (step 1); if symptoms persist the addition of an inhaled glucocorticoid e.g. beclomethasone is considered (step 2), and for additional control, the addition of a long acting β_2-adrenoceptor agonist e.g. salmeterol or a leukotriene receptor antagonist (LTRA) e.g. montelukast (step 3) may be used. In this context, Lipworth and colleagues investigated whether genotype information at the *ADRB2* Arg16 locus is useful in order to inform the choice of prescribing add on therapy, i.e. salmeterol or montelukast in a community setting [112]. Children with persistent asthma and homozygous for the Arg16 genotype ($n=62$) were randomised to receive salmeterol (50 μg bd) or montelukast (5 or 10 mg once daily) as an add-on to inhaled fluticasone propionate for 1 year. The aim was to test the hypothesis that carriers of the Arg16 genotype may be more prone to adverse effects e.g. exacerbation associated with prolonged β_2-adrenoceptor agonist as observed in several large scale studies. Using this rationale the montelukast group should have superior control for this preselected population. The study outcomes were school absences (primary outcome), exacerbation score, reliever (salbutamol) use, morning dyspnoea

Table 3 Polymorphisms of potential pharmacogenetic importance, identified in muscarinic receptor genes. Although several polymorphisms have been identified in these genes, most clinical associations have been with disease related rather than with pharmacological response outcomes. The values in brackets provide the reported allelic frequency, where available

Gene	Locus	Allele	Function	Reference
CHRM1	11q12–q13	−9697 C>T	Associated with asthma	[110]
		−4953A>G	Associated with asthma	[110]
CHRM2	7q35–q36	1197T>C *Coding region, synonymous (ACT>ACC, Thr>Thr) (0.5%)*	No known pharmacogenetic relevance	[106]
		1696T>A 3' *UTR* (63–68%)	Paediatric TT homozygote asthmatic patients exhibit poor response to ipratropium bromide	[106, 107]
		976 A>C *Coding region, synonymous (AGA>CGA, Arg>Arg) (0.5%)*	No known pharmacogenetic relevance	[106]
		$[(CA)_{15}]$ (0.6%) $[(CA)_{14}]$ (22.3%) $\Delta(CA)_1$ (44.0%) $\Delta(CA)_2$ (0.6%) $\Delta(CA)_3$ (14.9%) $\Delta(CA)_4$ (0.6%) $\Delta(CA)_8$ (17.1%) *$(CA)_n$ located ~96 bp downstream of the most 5' transcriptional start site for CHRM2*	$\Delta(CA)_8$ results in significantly lower transcriptional activity of a reporter gene in BEAS-2B and HASM cells. This allele may potentially influence CHRM2 gene expression *in vivo*	[108]
		1050 A>G *Coding region, synonymous (TCA>TCG, Ser>Ser) (14–16%)*	No known pharmacogenetic relevance	[111]
CHRM3	1q43	261 C>T *Coding region, synonymous (ATC>ATT, Ile>Ile) (0.5%)*	No known pharmacogenetic relevance	[111]
		−708 A/G *Promoter*	No known pharmacogenetic relevance	[109]
		−627G/C *Promoter*	No known pharmacogenetic relevance	[109]
		−513 C/A *Promoter*	No known pharmacogenetic relevance	[109]
		−492 C/T *Promoter*	No known pharmacogenetic relevance	[109]
		$(CTTT)_{12–20}$ *Promoter*	No known pharmacogenetic relevance	[109]
		$(GT)_{6–19}$ *Promoter*	No known pharmacogenetic relevance	[109]

and quality of life scores which are in keeping with the more real life approach to the study. Montelukast provided superior benefit for all measures with clinically relevant differences being obtained within 3 months. No significant difference in lung function, FEV_1 (% predicted) were observed, adding to the accumulating data that there is a lack of correlation between lung function and symptom based scores [112]. These results generated in a community setting involving disease management decisions are encouraging, and suggest that larger and longer prospective studies are warranted to provide more definitive data regarding the clinical utility of Arg16 stratification. The addition of a Gly16 arm to the study and the potential use of additional markers to define the population, would potentially provide clearer interpretation of the study outcomes.

9 Asthma Pharmacogenetics and Ethnicity

Allelic frequencies are known to often differ substantially across different populations and ethnic groups who do not share a common ancestry. Since pharmacogenetic associations may be haplotype-driven rather than SNP-driven, and inter-ethnic differences may also be observed in gene penetrance, the overall pharmacogenetic genotype-phenotype relationship may vary substantially across ethnic groups. Moreover, even where the genotype-phenotype relationships are unaffected, the mere differences in allelic frequencies across these populations may influence the general effectiveness of a therapeutic agent within a specific ethnic group. Table 5 provides some examples of inter-ethnic group differences in allelic frequencies of known pharmacogenetically relevant loci. This may have implications when applying similar pharmacological treatment guidelines, because while the majority of one ancestry population may adequately respond to a specific drug, the same may not necessarily be said of a second population which happens to harbour higher gene frequencies of a particular drug response-inhibiting allele. Similarly, it may be difficult to replicate clinical pharmacogenetic genotype-phenotype association studies in separate ethnic groups, because the results of such studies may be influenced by substantially dissimilar allelic frequencies of related genes, especially in haplotype-driven phenotypes (Table 4).

Disease phenotypes may also show inter-ethnic differences, and these may in turn influence the management patterns. For example, Puerto Rican and Afro-American asthmatic patients show a higher morbidity and mortality than non-Hispanic white and Mexican American subjects [114]. Though the different health care systems may contribute to this disparity, the underlying cause is more likely to be due to different allelic profiles arising from different ancestral origins [113].

The lack of agreement between studies on the functional role of the two most commonly studied *ADRB2* coding region polymorphisms, Gly16Arg and Gln27Glu (described earlier in this chapter), may be partially attributed to the mixture of patient ethnicities recruited for the different studies, essentially generating outcomes which were difficult to replicate due to patient stratification. This may be further

Table 4 Examples of pharmacogenetic SNPs and their allelic frequencies in different ancestry groups[a] (CEU: Utah residents with ancestry from northern and western Europe; YRI: subjects from Yoruba in Ibadan, Nigeria; ASW: African American subjects from the southwestern United States; MEX: Mexican American subjects from Los Angeles, California; CHB: Han Chinese from Beijing, China; JPT: Japanese from Tokyo, Japan)

Gene	SNP	Major/minor allele	Minor allelic frequency					
			CEU	YRI	ASW	MEX	CHB	JPT
ADRB2	rs1042713 (Gly16Arg)	G/A	0.36	0.53	0.57	0.47	0.58	0.44
ALOX5	rs21158197	A/G	0.37	0.21	0.28	0.53	0.73	0.82
LTC4S	rs730012	A/C	0.32	0.007	0.06	0.22	0.14	0.18
LTA4H	rs266845	T/G	0.17	0.007	0.06	0.22	0.44	0.36
STIP1	rs2236647	C/T	0.43	0.76	0.71	0.6	0.43	0.35
GLCCI1	rs37972	C/T	0.45	0.14	0.29	0.46	0.43	0.38

[a] adapted from Ortega and Meyers (2014) [113]

appreciated, in the light of 49 *ADRB2* polymorphisms identified in a multi-ethnic population, some alleles of which may potentially operate in concert with the Gly16Arg and Gln27Glu variants under study. This generates various haplotypes with frequencies which may be specifically associated with particular groups. For example, the National Heart, Lung, and Blood Institute (NHLBI) BARGE (beta agonist response by genotype) trial, in line with earlier studies, reported that *ADRB2* Gly16 homozygotes demonstrate improved lung function and symptom control improvements during salbutamol administration that were not observable in Arg16 homozygous asthma patients [115]. In constrast, outcomes of the NHLBI's LARGE (long-acting beta agonist response by genotype) trial, showed that Arg16 and Gly16 homozygous patients both experienced similar PEFR improvements following therapy; however Gly16 homozygotes experienced a decrease in methacholine bronchial reactivity, while this was not observable in asthmatic Arg16 homozygotes participating in this study [15]. Deeper analysis of the LARGE study data, however, shows that within the subgroup of African American subjects, Gly16 homozygotes experienced significantly greater improvements in morning and evening peak flow rates with salmeterol than Arg16 homozygotes, although the numbers of subjects in these analyses were low [113].

The inclusion of ethnic groups as part of a wider sample population in clinical pharmacogenetic studies, without statistically addressing genetic diversities, may confound the study outcomes. Ortega and Meyers (2014) argue that in order for prospective pharmacogenetic studies to be carried out in ethnic minorities, the following issues should be addressed; (a) ethnic groups tend to represent subject minorities, thus making the design of suitable statistically powered studies difficult; (b) besides genetic diversity, ethnic grops may also differ in factors related to cultural or habitual issues, such as access to health care, compliance with medications, and different environmental exposures, such as cigarette smoke, (c) inter-ethnic altered allele frequencies may also contribute to ethnic-group specific

gene-gene interactions, and genotype-phenotype relationships, and (d) the accuracy of self-reported ethnicity, especially in admixed populations, may have to be considered [113]. For example, a gene-gene interaction has been reported between rs11547772 in the *GSNOR* gene and Gly16Arg in *ADRB2*, which influences salbutamol bronchodilator response in Puerto Rican asthmatic families, while this interaction could not be observed in Mexican American patients [26]. In addition, there is often an increased frequency of rare gene variants in populations with African ancestry compared to non-Hispanic white populations [116].

Addressing pharmacogenetic issues in ethnic groups remains a challenge, especially within the context of large genome wide association studies (GWAS). The sample size requirements needed to achieve the necessary statistical power, imposes restrictions on study feasibility due to difficulties in being able to enrol sufficient patients from one genetically conserved group. On the other hand, incorporating these groups into larger sample cohorts may confound outcomes for reasons previously described. Ortega and Meyers (2014) suggest that one approach would be the use of admixture mapping techniques rather than direct GWAS, since the former method uses estimates of ancestry at each SNP to test for associations with a phenotype. In contrast, GWA studies directly compare allele frequencies with phenotype. In addition, admixture mapping requires a smaller number of genetic markers and therefore offers greater statistical power, while maintaining coverage for genomic regions that contain rare variants. Though this approach offers less resolving power than standard GWAS, the data generated is a more accurate depiction of the underlying pharmacogenetic associations within these groups [117, 118].

10 The Role of Patient Stratification Based on Clinical Data and Genetics in New Drug Development for Asthma

Drug development has been slow to generate new classes of compounds for the management of asthma. The major of recent advances in patient care have come from new indications for existing drug classes, e.g. improved duration of action for LABAs. Much effort has been placed on the inhibition of cytokines in asthma including anti-IL-5, IL-13, IL-4 and TNFα, however initial results have been disappointing. One explanation for this limited progress is the design and recruitment to Phase II trials for the evaluation of new compounds. For example, initial trials of anti-IL5 (mepolizumab) in asthma demonstrated disappointing effects on lung function and asthma symptoms when patient recruitment was not stratified. Only after careful selection of patients, based on refractory asthma and sputum eosinophilia (>3%), were clinically relevant improvements in asthma observed, e.g. reduced exacerbation rates [119]. Similarly, a recent study examining anti-IL-13 antibody (lebrikizumab) efficacy in asthma used preselected individuals with an IL-13/Th_2 dominated disease (defined by elevated total serum IgE, blood eosinophilia and serum periostin levels) [120]. Periostin is an extracellular matrix protein induced by IL-13 in airway epithelial cells and lung fibroblasts, and an important biomarker of

Th_2-associated airway inflammation [121]. The preselected asthma patients showed greatest improvements in the primary outcome, being a mean change in baseline FEV_1 (% predicted) of 8.2% compared to the low periostin group which only showed a mean improvement of 1.6%, following 12 weeks treatment [120]. These Phase II studies demonstrate that careful select asthma subjects is critical in asthma drug development and evaluation. It is important to note that serum eosinophilia has a recognised genetic component involving multiple polymorphism within *IL5, IL33, lL1RL1, WDR36* and *MYB* genes [122] and that polymorphisms spanning the *IL13* locus show robust association with asthma [123]. It is therefore highly likely that these drug responsive sub-phenotypes are genetically determined.

Therefore overall, recent approaches to treat asthma include; (a) cytokine inhibition *e.g.* anti IL-5 (mepolizumab), anti-TNFα (etanercept, soluble TNFR), anti-IL-13 (lebrikizumab, tralokinumab), (b) chemokine receptor antagonists *e.g.* chemokine (CXC motif) receptor 2 (CXCR2) (SCH527123) and (c) signalling pathway inhibitors with anti-inflammatory effects *e.g.* targeting p38 MAPK (SD-282), PDE4 (roflumilast), PI3K inhibitors (IPI-145) [124–126].

The specific targeting of single mediators is unlikely to provide a therapeutic option for asthma using the broad definition of the term. However accumulating evidence suggests that targeting of specific subpopulations leads to clinical efficacy *e.g.* anti-IL-5 and anti-IL-13 (Table 5). Very recently, a study investigating a human IL-13-neutralising monoclonal antibody (tralokinumab) used a carefully balanced population of atopic and non-atopic asthmatics with exclusions for additional respiratory pathology, cigarette smoking≥10 pack-years, recent infection or treat-

Table 5 Selected asthma therapies in Phase II development and potential for patient stratification approaches

Anti-cytokine therapies	Evidence for stratification potential	Reference
IL-4Rα antagonist (pitrikinra)	Yes, based on *IL4RA* genotype	[14, 129]
IL-4Rα antagonist (dupliumab)	Yes, based on a blood eosinophil count of at least 300 cells per microliter or a sputum eosinophil level of at least 3% and using inhaled glucocorticoids plus long-acting β-adrenoceptor agonists	[128]
Anti-IL-5 (mepolizumab)	Yes, severe asthma and elevated serum and sputum eosinophils	[119]
Anti-IL-9 (MEDI-528)	Unknown	[130]
Anti-IL-13 (tralokinumab)	Yes, based on exclusions for additional respiratory pathology, cigarette smoking≥10 pack-years, recent infection or treatment with immunosuppressive medication	[127]
Anti-IL-13 (lebrikizumab)	Yes, based on elevated total IgE, blood eosinophilia and serum periostin levels	[120]
Soluble TNFα receptor (etanercept)	Unknown	[131]

ment with immunosuppressive medication in a randomised, double-blind study [127]. Here, tralokinumab was associated with improving lung function based on an increase in FEV_1 and a decrease in daily β_2-adrenoceptor agonist use [127]. Similarly, a monoclonal antibody that blocks IL-4Rα function (dupilumab) has provided some encouraging results, such as a reduction in asthma exacerbations in carefully selected patients that have a blood eosinophil count of at least 300 cells per microliter or a sputum eosinophil level of at least 3 % and who are administered inhaled glucocorticoids plus long-acting beta-agonists [128]. As pointed out, features such as eosinophilia have a genetic basis. It is thus anticipated that the phenotype may be genetically driven, although definitive pharmacogenetic studies remain to be completed for these biologics.

As novel asthma genes are identified, it is important for the evaluation of these potential drug targets, particularly in the context of Phase II trials, to take into account genetic factors. Preliminary data supports genetic testing in Phase II trials of newer compounds, as shown in a recent report of the IL-4/IL-13 dual antagonist pitrikinra. Polymorphic variation in the target receptor for this antagonist i.e. IL-4Rα significantly influenced outcomes in allergic asthma subjects [14]. This antagonist is a recombinant form of IL-4, differing at two amino acid residues, i.e. a mutein, R121D/Y124D. This has been shown to reduce late phase antigen responses (LAR) to inhaled antigen as defined by changes in lung function (FEV_1) over a period of 4–10 h post antigen exposure. The subjects under study had been following a four week period of twice daily active treatment of nebulised pitrikinra or placebo, with an increased LAR ratio correlating with a reduced FEV_1 [129]. Stratification of subjects (pitrikinra $n = 15$, placebo $n = 14$) into genotype groups for the non-synonomous IL-4Rα SNP rs1801275 (Gln576Arg) identified that Arg/Arg carriers had an attenuated LAR compared to Gln/Gln or Gln/Arg genotypes. Similarly, stratification based on rs1805011 (Glu400Ala) showed an attenuated LAR in the Glu/Ala group but not in the Glu/Glu subjects [14]. The Arg576 variant (when in combination with Val75) has been shown to be a risk factor for allergic asthma and lead to enhanced IL-4Rα signalling post IL-4 stimulation, potentially providing a putative mechanism of action [132].

Therefore, although preliminary, these data suggest that selecting a subgroup of patients based on genotype, where it is anticipated that the receptor/pathway may have a more dominant role in that individual's asthma, may be critical to interpreting Phase II clinical trials.

11 Conclusion

The field of pharmacogenetics has made great strides forward since the original coining of the word by Frederick Vogel more than half a century ago [133]. In recent years, there has been a development of high throughput technologies, both in genetic discovery and drug development, as well as the establishment of various pharmacogenetic working groups operating under both the scientific and regulatory arms of international agencies.

The future of therapeutic management lies in the individual patient tailoring of "real life" data emerging on the usefulness of genotype-driven asthma management. Pharmacogenetics is currently moving through the translational phase from bench to bedside. The establishment of curated knowledgebases such as PharmGKB (https://www.pharmgkb.org), and the setting up of international professional committees such as the Clinical Pharmacogenetics Implementation Consortium (CPIC), established in 2009, contributes to the creation of platforms which facilitate translational pharmacogenetics. The advisory and regulatory roles of major agencies such as the Food and Drug Administration, and the European Medicines Agency, in the field of pharmacogenetics, provide a link between academia and industry, for the optimisation of the discovery process.

The incorporation of pharmacogenetic studies within Phase II clinical trials of new compounds is important for the identification of genetically defined cohorts who may respond differently to the drug. Subject stratification at this stage, provides information which paves the way for more informed decision taking, relating to drug development, and optimisation of the compound's targeted use. Compounds that may fail to show adequate benefit/risk ratios in a genetically undefined study population, may be found to be ideal for genetically defined subsets. Alternatively, subject stratification during early clinical trials, may identify compounds that show unwarranted toxicities in a general population, to be detrimental only to subjects with a particular genetic profile. This information may essentially influence the availability of new drugs on the market.

These initiatives are especially relevant in diseases such as asthma for which therapies directed at new drug targets are under development. In the future, therapeutics will see an increase in the use of biologics as well as targeted therapies, and respiratory disease is no exception. The consolidation of data, together with the assessment of robustness and clinical relevance of pharmacogenotypes, are critical components for the development and establishment of personalised genotype-guided prescribing protocols in bronchial asthma. Rapidly advancing technologies, especially in the areas of GWAS and whole genome sequencing, coupled with improvements in high-throughput techniques and refined bioinformatics analyses, all contribute to accelerating the attainment of these aims.

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Pharmacogenetics and Antineoplastic Therapies

Jai N. Patel, Christine M. Walko and Federico Innocenti

Abstract The genome of cancer cells differs from that of the host cell from which it arises. These changes in molecular pathways drive cellular proliferation and ultimately tumour growth and progression. As a result, there has been a shift from categorising tumours solely based on their tissue of origin and histology to consideration of their molecular profiles. This transformation has led to the current breadth of treatments available, including largely targeted therapies. When individualising cancer therapy, it is essential to evaluate the expected individual drug exposure, risk for toxicity, and expected drug efficacy; however, a large heterogeneity in response to antineoplastic therapies exists across the human population. Dose-limiting toxicities often lead to dose reductions and delays in therapy, even in a potentially curative setting. It is therefore imperative that clinicians be able to identify the patients most likely to benefit from treatment and those at an increased risk of toxicity. This chapter will focus on the pharmacogenetic associations of antineoplastic therapies, and the prospective identification of clinically validated and/or utilised germ-line and somatic biomarkers, maximising clinical efficacy and minimising toxicity.

Keywords Pharmacogenetics · Germ-line · Somatic · Predictive · Biomarker · Antineoplastics · Cancer

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1 Introduction

Administration of an equivalent dose of an antineoplastic drug to a randomly selected population of cancer patients has been shown to result in a range of toxicities, from mild to lethal events [1]. Dose-limiting toxicities often lead to dose reductions and delays in therapy, even in the potentially curative setting. Additionally, poor prediction of driver tumour abnormalities, compensatory cellular pathways leading to resistance, and inter-individual variability of exposure to active drug may result in marginal efficacy of current regimens in the adjuvant and metastatic setting [2]. Drug exposure above a therapeutic threshold often results in severe toxicity without additional clinical benefit, while insufficient drug concentration at the site of action may result in a sub-therapeutic response. Secondary to the inherent narrow therapeutic nature of antineoplastics, the identification of optimal methods for individualising cancer therapy is in itself imperative [3]. Pharmacogenomics, or the study of how genetic inheritance influences one's response to medications, has the potential to change the way medicine is practiced. This shift in cancer treatment can potentially be achieved through optimising drug and dose selection to minimise toxicity and maximise clinical benefit [4].

Since the completion of the Human Genome Project in 2003 and the unveiling of the architecture of somatic alterations in many tumour types through The Cancer Genome Atlas Network, genomics has become a mainstay of cancer research [5]. As a result, there has been a shift from categorising tumours solely based on their tissue of origin and histology to consideration of their molecular profiles. Ultimately, this shift has introduced a transition from the drug development of broadly acting cytotoxic agents to specific targeted therapies, allowing for the current breadth of treatments available. By utilising both germ-line and somatic DNA, pharmacogenomics provides a practical method to optimise systemic drug exposures, to decrease adverse drug reactions, and to maximise clinical efficacy, thereby improving the survival and quality of life of patients. This chapter will highlight the major advances made in pharmacogenetics of antineoplastics, including many clinically validated and/or utilised genetic predictors of drug efficacy and/or toxicity.

2 Approaches to Genomic Cancer Medicine: Germ-Line versus Somatic DNA, Prognostic versus Predictive Biomarkers

A pharmacogenetic or pharmacogenomic marker is defined as any genetic or genomic marker (or set of markers) that is associated with drug response. Genomic cancer research provides a unique opportunity to analyse two sets of DNA: the patient and the tumour, also referred to as germ-line DNA and somatic DNA, respectively. These two sets of DNA differ in many ways, including, but not limited to, location, accessibility, and clinical utility. While germ-line DNA mutations are found within germ

cells (sperm or egg), and are therefore inherited and transmitted to the offspring, somatic DNA mutations occur within any other cell (i.e. tumour cell) after conception and subsequently are not passed on to progeny (see review [6, 7]).

Clinically relevant germ-line mutations are often useful for determining both pharmacokinetic and pharmacodynamic responses, including variations in the enzymes thiopurine-S-methyltransferase (TPMT), cytochrome P450 2D6 (CYP2D6), uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1), and dihydropyrimidine dehydrogenase (DPD). Determining the activity of these enzymes can ultimately help to predict drug or active metabolite exposure and the resulting implication on clinical response and toxicity. Because of their acquired nature, somatic mutations are useful in evaluating pharmacodynamic effects, and ultimately tumour response [6]. A few well known examples include presence of BCR-ABL in chronic myeloid leukaemia (CML), *KRAS* mutations in metastatic colorectal cancer, the epidermal growth factor receptor (*EGFR*) mutations in lung cancer, the overexpression of human EGFR-2 (HER2) in breast and gastric cancer, BRAF in metastatic melanoma, and anaplastic lymphoma kinase (ALK) in lung cancer. These mutated proteins drive affected cells toward transformation and addict cells to their abnormal function, where the continued presence of the abnormal function becomes required for the tumour cell to survive. This forms the basis of targeted therapies, where blockage of these mutations ultimately deprives the cancer cell from stimulation, growth, and survival (see review [8]).

Biomarkers in general can be broadly categorised into two main types: prognostic and predictive. A prognostic biomarker is a single measurable trait, or combination of measurable traits, that has been shown to be associated with clinical outcome, regardless of treatment (see review [9]). Notable examples include the 70-gene profile, MammaPrint™, for oestrogen/progesterone receptor (ER/PR) positive, lymph node-negative breast cancer [10] and microsatellite instability (MSI) in colorectal cancer patients [11]. Gene expression arrays such as MammaPrint™ help to determine the risk of recurrence in women with early stage breast cancer and help to guide treatment recommendations based on recurrence risk [10]. High-frequency MSI (MSI-H), often found in patients with Lynch Syndrome, may indicate a favourable prognosis compared to microsatellite stable/low-frequency MSI (MSS/MSI-L), independently of chemotherapy, in local and advanced colorectal cancer [11, 12]. Additionally, biomarkers such as *BRAF* mutations have been found to have stronger prognostic significance than predictive in certain tumours such as colorectal, as opposed to being a highly predictive biomarker in tumours such as melanoma [13, 14].

A predictive biomarker is a single measurable trait, or combination of measurable traits, that predicts response to specific therapies and can be used to prospectively identify patients who are most likely to benefit from treatment and/or those predisposed to toxicity (see review [15]). Notable examples of predictive biomarkers are listed in Table 1 and are discussed in further detail in the remainder of the chapter. Additionally, some biomarkers are classified as both prognostic and predictive, such as overexpression of HER2 and presence of ER/PR.

Table 1 Summary of biomarker classifications, antineoplastic drugs, and FDA package insert information

Biomarker	Germ-line (G) or Somatic (S)	Predictive or prognostic or both	Antineoplastic drug(s)	Excerpt from FDA package insert regarding the biomarker[a]
CYP2D6	G	Predictive	Tamoxifen	None
DPD	G	Predictive	Capecitabine, 5-fluorouracil	"Capecitabine is contraindicated in patients with known DPD deficiency" "Rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia and neurotoxicity) associated with 5-fluorouracil has been attributed to a deficiency of DPD activity. A link between decreased levels of DPD and increased, potentially fatal toxic effects of 5-fluorouracil therefore cannot be excluded"
TPMT	G	Predictive	6MP; azathioprine	"Homozygous-deficient patients, if given usual doses of 6-MP, accumulate excessive cellular concentrations of active thioguanine nucleotides predisposing them to 6MP toxicity" "Patients with deficient or null TPMT activity are at increased risk for severe 6MP toxicity from conventional doses of 6MP and generally require substantial dose reduction"
UGT1A1	G	Predictive	Irinotecan; nilotinib	"Individuals who are homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of irinotecan treatment" "…a reduction in the starting dose by at least one level of irinotecan should be considered for patients known to be homozygous for the UGT1A1*28 allele"
ALK	S	Predictive	Crizotinib	"…is indicated for the treatment of patients with locally advanced or metastatic NSCLC that is anaplastic lymphoma kinase (ALK)-positive as detected by an FDA- approved test" "Detection of ALK-positive NSCLC is necessary for selection of patients for treatment with crizotinib"

Table 1 (continued)

Biomarker	Germ-line (G) or Somatic (S)	Predictive or prognostic or both	Antineoplastic drug(s)	Excerpt from FDA package insert regarding the biomarker[a]
BCR-ABL	S	Both	Imatinib, dasatinib, nilotinib, bosutinib, ponatinib	"…indicated for the treatment of newly diagnosed adult and pediatric patients with Ph+CML and adult patients with relapsed or refractory Ph+ALL." (Imatinib, dasatinib) "…indicated for the treatment of Ph+chronic CML in adult patients resistant to or intolerant to prior therapy that included imatinib." (Nilotinib) "…indicated for the treatment of adult patients with chronic, accelerated, or blast phase Ph+chronic myelogenous leukemia (CML) with resistance or intolerance to prior therapy." (Bosutinib) "…for the treatment of adult patients with Ph+ALL that is resistant or intolerant to prior tyrosine kinase inhibitor therapy." (Ponatinib)
BRAF	S	Prognostic (colorectal) Both (melanoma)	Vemurafenib	"Before taking vemurafenib, patients must have BRAF V600 mutation –positive tumor status confirmed…" "The efficacy and safety of vemurafenib in patients with tumors expressing BRAF V600 non-E mutations have not been convincingly established"
C-KIT	S	Both	Imatinib	"…indicated for the treatment with KIT (CD117) positive unresectable, resectable, and/or metastatic malignant GIST"
EGFR	S	Predictive	Erlotinib	"Erlotinib inhibits the intracellular phosphorylation of tyrosine kinase associated with the epidermal growth factor receptor (EGFR)" "…indicated for first-line use in patients with metastasized NSCLC that tests positive for EGFR mutations"

Table 1 (continued)

Biomarker	Germ-line (G) or Somatic (S)	Predictive or prognostic or both	Antineoplastic drug(s)	Excerpt from FDA package insert regarding the biomarker[a]
ER/PR	S	Both	Tamoxifen, exemestane, letrozole, anastrozole, fulvestrant	"Available evidence indicates that patients whose tumors are estrogen receptor positive are more likely to benefit from tamoxifen therapy" "The estrogen and progesterone receptor values may help to predict whether adjuvant tamoxifen therapy is likely to be beneficial" "Exemestane is indicated for adjuvant treatment of postmenopausal women with estrogen-receptor positive early breast cancer…interferes with estrogen-induced stimulation or maintenance of growth of hormonally responsive (ER/PR +) breast cancers." (Same for all anti-hormonal therapies)
HER2	S	Both	Trastuzumab, pertuzumab, lapatinib, T-DM1	"…should only be used in patients whose tumors have HER2 protein over-expression." (Same for all therapies targeting HER2)
KRAS	S	Both (NSCLC) Predictive (colorectal)	Cetuximab, panitumumab	"Retrospective subset analyses of trials in patients with colorectal cancers having *KRAS* mutations noted a lack of benefit associated with these monoclonal antibodies" "…indicated for first-line treatment in combination with chemotherapy for patients with metastatic colorectal cancer who have EGFR-expressing, and KRAS wild-type tumors"
PML-RAR alpha	S	Both	Arsenic trioxide ATRA	"…indicated for those where APL is characterized by the presence of the t(15;17) translocation or PML/RAR-alpha gene expression"
CD20 antigen	S	Predictive	Rituximab, ofatumumab, tositumumab, ibritumomab	"Rituximab is indicated for the treatment of patients with relapsed or refractory, low-grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma." (Same for tositumumab and ibritumomab; however, for patients refractory to rituximab) "Ofatumumab is a CD20-directed cytolytic monoclonal antibody indicated for the treatment of patients with CLL refractory to fludarabine and alemtuzumab"

Table 1 (continued)

Biomarker	Germ-line (G) or Somatic (S)	Predictive or prognostic or both	Antineoplastic drug(s)	Excerpt from FDA package insert regarding the biomarker[a]
CD30 antigen	S	Predictive	Brentuximab	"Brentuximab is a CD30-directed antibody-drug conjugate indicated for the treatment of patients with Hodgkin lymphoma…"
CD52 antigen	S	Predictive	Alemtuzumab	"Alemtuzumab is a recombinant DNA-derived humanized monoclonal antibody that is directed against CD52"

CYP2D6 cytochrome P450 2D6, *DPD* dihydropyrimidine dehydrogenase, *TPMT* thiopurine-S-methyltransferase, *6MP 6* mercaptopurine, *UGT1A1* uridine-diphosphateglucuronosyltransferase, *ALK* anaplastic lymphoma kinase, *Ph* Philadelphia chromosome, *EGFR* epidermal growth factor receptor, *ER/PR* estrogen receptor/progesterone receptor, HER2 human epidermal growth factor receptor 2, *T-DM1* trastuzumab emtansine, *ATRA* all trans retinoic acid, *NSCLC* non-small-cell lung cancer; *CD* cluster of differentiation

[a] FDA website for table of drugs with pharmacogenomic biomarkers on drug label: http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm

3 Germ-Line Predictive Markers of Anticancer Efficacy and/or Toxicity

3.1 Thiopurine S-Methyltransferase (TPMT)

Of the germ-line predictive markers identified, *TPMT* pharmacogenetics is one of the original hallmark examples of how a germ-line genetic mutation can impact clinical outcomes and drug toxicity, particularly in children with acute lymphoid leukemia (ALL). 6-mercaptopurine (6-MP) is one of the backbone agents used to treat ALL. It inhibits the formation of purine ribonucleotides through the formation of thioguanine nucleotide analogues, ultimately leading to cell death. TPMT is a cytosolic enzyme ubiquitously expressed in the human body and catalyses the S-methylation of thiopurines into inactive compounds. Approximately 6–11 % of the population is heterozygous for the defective variants of TPMT and 0.2–0.6 % is homozygous resulting in null enzyme activity (see review [16]). TPMT *2 and *3 account for 95 % of defective TPMT activity. The risk of myelosuppression is significantly elevated in patients who, secondary to a TPMT deficiency, have increased thioguanine nucleotides [16, 17].

In a study of 180 children with ALL treated with a 6-MP-based regimen, patients were genotyped and had TPMT enzyme activity measured. A significant inverse relationship was observed between concentration of thioguanine nucleotides and TPMT enzyme activity. The percentage of wild-type, heterozygous, and homozygous-deficient patients who were able to tolerate the full dose throughout treatment was 84, 65, and 7 %, respectively ($P < 0.001$) [18]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends an initial 90 % dose reduction in homozygous variant patients and a 30–70 % dose reduction in heterozygous patients [19]. The Food and Drug Administration (FDA) recommends, but does not require, genetic testing prior to treatment with 6-MP (Table 1) [20]. As a result, a number of institutions, including St. Jude Children's Hospital, routinely test all patients receiving 6-MP for *TPMT* genetic variants. As noted in Table 2, several key regulatory bodies support genetic testing of *TPMT* to individualise 6-MP dosing.

3.2 UDP Glucuronosyltransferase 1 Family, Polypeptide A1, UGT1A1

Irinotecan is effective in a variety of cancers including those of the gastrointestinal tract and lung. It is metabolised by carboxylesterases to the active metabolite, SN-38. UGT1A1 conjugates SN-38 to an inactive glucuronide metabolite, SN-38G, which can undergo enterohepatic recirculation, and is eventually excreted in the bile and urine (see reviews [21, 22]). Polymorphic *UGT1A1* variants may lead to a reduction in enzyme activity or expression, resulting in an increase in SN-38, and ultimately an increased risk of severe neutropenia. The wild type allele is referred

Table 2 Summary of biomarker implication on treatment, clinical assay availability, and incorporation into guidelines and practice

Biomarker	Clinical assay(s) available[a]	Clinical implication	Federal/Regulatory/ National Guidelines supporting association/use	Year of discovery[b, c]	Year of implementation of first targeted therapy or genetic test
CYP2D6	AmpliChip Cytochrome P450 Genotyping test[a], xTAG CYP2D6, P450-Glo	Altered tamoxifen metabolism (potential for increased rates of breast cancer recurrence) [30, 106]	DPWG	2005 [30, 106]	Genetic test not yet frequently utilised
DPD	DPD Enzyme Assay	Increased risk of 5-fluorouracil or capecitabine induced toxicity [39]	CAP, CPIC, DPWG, FDA, NICE	1998 [39]	Genetic test not yet frequently utilised
TPMT	TPMT Enzyme diagnostic test, Prometheus TPMT genetic test	Increased risk of 6-mercaptopurine induced neutropenia [18]	ASCO, CAP, CPIC, DPWG, EMA, ESMO, FDA, NCCN, NICE	1999 [18]	2010 [25]
UGT1A1	Invader UGT1A1 Molecular Assay[a], INFINITI UGT1A1 Assay	Increased risk of irinotecan or nilotinib induced toxicity [23]	CAP, DPWG, FDA, NCCN	2004 [23]	Genetic test not yet frequently utilised
ALK	Vysis ALK Break Apart FISH probe kit[a]	Sensitivity to crizotinib [43]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	2007 [107]	2010 [43]
BCR-ABL (Ph chromosome)	BCR-ABL Mutation test	Sensitivity to imatinib, dasatinib, nilotinib, bosutinib, ponatinib [108]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	1973 [109]	2001 [110]
BRAF	Cobas 4800 V600E[a]	Sensitivity to vemurafenib [13]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	2001 [111]	2011 [13]
C-KIT	c-Kit pharmDx[a]	Sensitivity to imatinib [59]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	2001 [112]	2001 [112]

Table 2 (continued)

Biomarker	Clinical assay(s) available[a]	Clinical implication	Federal/Regulatory/ National Guidelines supporting association/use	Year of discovery[b, c]	Year of implementation of first targeted therapy or genetic test
EGFR	EGFR pharmDx[a], EGFR Mutation Assay[a] Cobas EGFR Mutation Test[a]	Sensitivity to erlotinib [65]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	2004 [64]	2003 [113]
ER/PR	ER/PR pharmDx[a], TargetPrint	Sensitivity to hormonal therapy (tamoxifen, exemestane, letrozole, anastrozole) [71]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	1896 [114]	1896[d] [114]
HER2	HercepTest[a], Pathway[a], Insite[a], PathVysion[a], SPOT-Light[a], HER2 CISH[a]	Sensitivity to trastuzumab, pertuzumab, lapatinib, T-DM1 [115–117]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	1985 [118]	2001 [119]
KRAS	Therascreen KRAS RGQ PCR Kit[a], DxS KRAS Mutation Test Kit, Genzyme's KRAS Mutation Analysis	Resistance to cetuximab or panitumumab [14]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	1987 [120]	2006 [121]
PML-RAR alpha	PML/RARA t(15;17) Quantitative Real-time PCR; FISH AML M3,	Sensitivity to all-trans-retinoic acid and arsenic trioxide [55]	ASCO, CAP, FDA, EMA, ESMO, NCCN, NICE	1990 [122]	1987 [123]

NSCLC non-small-cell lung cancer, *ASCO* American Society of Clinical Oncology, *FDA* Food and Drug Administration, *EMA* European Medicines Agency, *ESMO* European Society for Medical Oncology, *NCCN* National Comprehensive Cancer Network, *CPIC* Clinical Pharmacogenetics Implementation Consortium, *DPWG* Dutch Pharmacogenetics Working Group, *NICE* National Institute for Health and Care Excellence, *CAP* College of American Pathologist

[a] FDA-cleared (and CLIA-approved); Clinical assay(s) available adapted from Patel et al. [124]

[b, c] For germ-line variants (CYP2D6, DPD, TPMT, UGT1A1), the year of first discovery of the association with clinical phenotype was used. For somatic variants (all others), the year of the discovery of the actual aberration was used

[d] 1896—oophorectomy; date of approval of first modern day anti-hormonal therapy: 1998 (letrozole)

to as *UGT1A1**1, while the most common and studied variants include *28, *93, *60, and *6; *UGT1A1**28 leads to a 70% reduction in expression of the gene. The frequency of the *28 allele is 39% in patients of European, 16% of Asian, and 43% of African origins [22].

The *UGT1A1* genotype and total bilirubin levels were found to be strongly associated with severe neutropenia in 66 patients with advanced cancers receiving irinotecan 350 mg/m^2 every 3 weeks. The relative risk of grade 4 neutropenia was 9.3 (95% CI, 2.4–36.4) for patients with the *28/*28 genotype versus wild-type and heterozygous patients [23]. One meta-analysis demonstrated a significant increase in toxicity for *28/*28 patients at medium (150–250 mg/m^2) and high doses (>250 mg/m^2) (odds ratio [OR] 3.2 and 27.8, respectively) [24]. The FDA label recommends an initial dose reduction be considered for patients who are homozygous for the *UGT1A1* *28 allele [20]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) and Dutch Pharmacogenetics Working Group (DPWG) guidelines suggest an initial dose reduction by 30% in *28/*28 patients if the starting recommended dose is >250 mg/m^2 [2, 25]. Additionally, new studies define individualised dosing of irinotecan. A genotype-guided phase I dose escalation study defined the irinotecan maximum tolerated dose (MTD) to be 370 and 310 mg/m^2 for *1/*1 and *1/*28 patients, respectively, suggesting higher tolerable doses in wild-type and heterozygous patients [26]. The observed toxicities were mainly gastrointestinal and neutropenia. A subsequent and similar genotype-guided phase I study determined the MTD in *1/*1, *1/*28, and *28/*28 patients to be 390, 340, and 150 mg/m^2, respectively [27].

3.3 Cytochrome P450, Family 2, Subfamily D, Polypeptide 6 (CYP2D6)

The selective Estrogen Receptor (ER) modulator, tamoxifen, is widely used in locally advanced and metastatic ER positive breast cancer patients. Tamoxifen metabolism via CYP2D6 produces a more potent metabolite, endoxifen. *CYP2D6**1 is the wild-type allele (resulting in normal enzyme activity), while *3, *4, and *5 result in null enzyme activity, and *9, *10, and *17 result in decreased enzyme activity. Patients with two *CYP2D6* alleles with normal enzyme activity are considered to be 'extensive metabolisers (EM).' Patients with one or more *CYP2D6* alleles with reduced enzyme activity or heterozygous for an inactive allele are considered to be 'intermediate metabolisers (IM),' while patients with two inactive alleles are considered to be 'poor metabolisers (PM)' [28]. The frequency of the most common variant allele, *4, is approximately 18% in patients of European origin, while rarely (<1%) found in patients of Asian origin and moderately (7–9%) in African origin [29]. Additionally, the frequency of the *10 allele is approximately 40% in patients of Japanese origin, while the *17 allele frequency is about 25% in patients of African origin [30, 29].

A retrospective review in 225 breast cancer patients who received adjuvant tamoxifen identified a significantly shorter time to recurrence (hazard ratio [HR] 3.20; $P=0.007$) and worse 2-year relapse-free survival (RFS) (HR 2.69; $P=0.009$) in PMs (*4/*4) as compared to EM patients [31]. A subsequent retrospective study demonstrated higher recurrence rates for PMs and IMs when compared to EMs (29 and 20.9% vs. 14.9%, respectively); compared with EMs, there was a significantly increased risk of recurrence for IMs (HR 1.40; $p<0.05$) and PMs (HR 1.90; $p<0.05$) [32]. An ongoing debate exists on the clinical utility of *CYP2D6* testing as two large studies published in 2011 demonstrated no association between genotype and recurrence rates [33, 34]; however, major flaws in the genomic study design, such as use of tumour DNA as opposed to germ-line DNA, may help explain these opposing results (see commentary [35]). Advocates of the uptake of *CYP2D6* genetic testing indicate that until prospective, adjuvant trial data is available, the current evidence is sufficient to accept the *CYP2D6*-tamoxifen pharmacogenetic relationship in postmenopausal women [36]; however, the appropriate dose adjustment and ultimate impact on breast cancer recurrence appear to be unclear. The DPWG recommends consideration of aromatase inhibitor (AI) therapy in postmenopausal women who are PMs and IMs (along with avoidance of concomitant CYP2D6 inhibitors) [37]. Regardless of expert recommendations, very few regulatory bodies support the use of *CYP2D6* genetic testing (Table 2). Of the validated germ-line biomarkers, *CYP2D6* is the only one not included on the FDA label (Table 1).

Dosing of tamoxifen may be dependent upon the *CYP2D6* genotype. An interventional genotype-guided study in patients of largely Caucasian origin demonstrated a significant increase in endoxifen concentrations in PM and IM patients when doubling the dose from 20 to 40 mg daily [28]. A subsequent study in patients of Japanese origin identified a significant 1.4 and 1.7-fold higher concentration of plasma endoxifen in *CYP2D6* *1/*10 and *10/*10 patients, when dosed at 30 and 40 mg/day, respectively, compared to concentrations prior to dosage increase (all patients received 20 mg/day of tamoxifen before the intervention) [38]. Additionally, these plasma concentrations of endoxifen achieved similar levels of those in *CYP2D6* *1/*1 patients receiving 20 mg/day of tamoxifen [38].

3.4 Dihydropyrimidine Dehydrogenase, DPD

Fluorouracil has been the backbone therapy of several cancers, including but not limited to colon, rectal, and gastric. DPD is the rate-limiting enzyme involved in the metabolism of fluorouracil to the inactive 5,6-dihydrofluorouracil (see review [39]). Genetic variations in the gene coding for DPD, *DPYD*, resulting in deficient DPD activity is estimated to occur in approximately 1% of patients, which has the potential to prolong the half-life of fluorouracil by up to 100-fold, ultimately increasing the risk of fluorouracil-induced toxicity, namely neutropenia and/or diarrhea (see review [40]). To date, among the many proposed defective variants of *DPYD*, IV14+1G>A is the one that has shown the most predictive value. This

variant leads to splicing of mature DPD mRNA. Only 1.8% of the overall population carries at least one variant allele [41].

In one study, sixty patients who experienced severe fluorouracil-induced toxicity were genotyped for the splice site mutation [41]. Results indicated that 28% of all patients were heterozygous or homozygous for the mutation compared to 3% who were wild-type ($P<0.001$). An overall decreased DPD activity was noted in roughly 60% of all cases, independently of genotype [41]. In a multicenter trial of 683 patients with various cancers treated with fluorouracil monotherapy, patients were genotyped for *DPYD*. Investigators noted a positive predictive value of 0.46 for overall toxicity in patients with the splice site mutation; however, of interest was a gene/sex-interaction resulting in an OR for toxicity of 41.8 ($P<0.0001$) for male patients but only 1.33 ($P>0.05$) in female patients [42]. Establishing DPD deficiency measuring catalytic DPD activity in peripheral blood mononuclear cells might be challenging, as standardised cut-offs for deficient DPD activity are not available and different laboratories use different thresholds. The FDA label includes a warning for increased risk of toxicity in patients with deficient DPD activity; however, the definition of what constitutes "deficient" is not defined, nor does the FDA mandate genetic testing for any SNPs in *DPYD* (Table 1) [20].

4 Somatic Predictive Markers of Anticancer Efficacy

4.1 ALK

EML4-ALK, a fusion gene found in several types of cancers including anaplastic large-cell lymphoma, neuroblastoma, inflammatory myofibroblastic tumours, and non-small-cell lung cancer (NSCLC), results in constitutive kinase activity allowing activation of the RAS-MEK-ERK, JAK3-STAT3, and PI3K-AKT pathways [43]. This translocation is relatively uncommon occurring in approximately 2–7% of all NSCLC cases [43]. Crizotinib, an oral ATP-competitive selective inhibitor of ALK and MET tyrosine kinases, was granted accelerated approval in 2011 by the FDA, along with a companion diagnostic test for the *ALK* genetic abberation. The FDA label indicates genetic testing is necessary to confirm an *ALK* positive tumour prior to a patient receiving crizotinib (Table 1). Additionally, several key regulatory bodies support genetic testing for *ALK*, along with a number of other somatic biomarkers included in this chapter (Table 2).

In an open-label, multicenter, two-step phase I trial, 82 NSCLC patients harboring the fusion *ALK* gene rearrangements were treated with crizotinib. An overall response rate of 57% was observed along with a disease control rate of 87% at 8 weeks. Historical data indicates a 15–20% response rate with standard chemotherapy [43]. The probability of progression free survival (PFS) at 6 months was estimated at 72% [43]. The impressive activity and clinical benefit from phase I and II clinical trials led to the rapid FDA approval of crizotinib for treatment in

ALK positive NSCLC, prior to validation in phase III clinical trials (see review [44]). As with many targeted therapies, the initial therapeutic benefit is short-lived secondary to acquired resistance, which may present in the form of novel *EGFR*, *KIT*, or *ALK* mutations not previously identified [45].

4.2 BCR-ABL

The development of targeted treatment was made promising by landmark discoveries that identified the chromosomal abnormality responsible for CML: the Philadelphia chromosome (Ph) formed by a translocation between chromosomes 9 and 22, resulting in a fusion between the breakpoint cluster region (*BCR*) and the *c-ABL* oncogene [46]. This chimeric BCR-ABL tyrosine kinase (TK) occurs in >95% of all CML cases and causes constitutive activation, triggering numerous signal transduction pathways associated with cell survival, proliferation, and resistance to apoptosis [47]. The original tyrosine kinase inhibitor (TKI), imatinib, was discovered in a high-throughput screening assay and was subsequently approved in 2001. As indicated in Table 1, the FDA label recommends therapy with TKIs in hematological malignancies which are Ph positive.

At a 5-year follow up, imatinib demonstrated significantly higher rates of complete hematologic response compared to interferon-alfa plus Ara-C (97 vs. 56%, $P<0.001$), and significantly higher major and complete cytogenetic response (85 and 74% vs. 22 and 8%, respectively, $P<0.001$) [48]. The substantial superiority of imatinib over standard chemotherapy resulted in the study results being disclosed early and most patients being crossed over to the imatinib arm [48]. Secondary to emerging resistance, subsequent TKIs with slightly different mechanisms of action were developed, including nilotinib, dasatinib, bosutinib, and ponatinib. Approximately 10% of patients will present initially with resistance to TKIs and close to 20% will develop resistance over time (1–5 years) [49]. The primary mechanisms of resistance are point mutations occurring in the kinase domain resulting in steric hindrance; however, newer agents such as ponatinib feature a carbon-carbon triple bond, which allows the molecule to take up a position with no steric hindrance, ultimately inhibiting a variety of TKs and overcoming the initial resistance (see review [50]).

4.3 PML-RAR Alpha

Similarly to BCR-ABL in CML, the routine detection of the *PML-RAR alpha* translocation has provided a significant and widely incorporated predictive biomarker for diagnosis and treatment of acute promyelocytic leukaemia (APL). Nearly all APL cases, which account for approximately 10% of all acute myeloid leukaemia cases, express a translocation between chromosomes 15 and 17 (see review [51]). The resulting fusion between the PML gene on chromosome 15 and the retinoic acid

receptor alpha gene on chromosome 17, t(15;17), allows increased differentiation of leukaemic cells [51]. This disease has traditionally been the most malignant form of acute leukemia with a fatality within weeks, without treatment. Chemotherapy (daunorubicin, idarubicin, and cytosine arabinoside) became the initial front-line therapy resulting in a complete remission (CR) rate of 75%; however, nearly all patients rapidly progressed within 11 to 25 months [52].

Treatment with all-trans-retinoic acid (ATRA) directly down-regulates PML-RAR alpha, subsequently inducing disease remission in 90–95% of newly diagnosed patients with a 5-year disease free survival (DFS) of 75% (see review [52]). In a phase III study comparing ATRA to daunorubicin, the rates of overall survival (OS) at one, two, and 3 years after entry into the study were 75, 57, and 50%, respectively, among patients assigned to chemotherapy, and 82, 72, and 67% among those assigned to ATRA ($P=0.003$) [53]. Unfortunately, relapse frequently ensues within months following treatment, which is routinely followed by combination chemotherapy [54]. Another agent, arsenic trioxide, triggers the rapid degradation of PML-RAR alpha through the targeting of the promyelocytic leukemic moieties of the fusion protein, demonstrating a high CR rate (close to 60%) in relapsed APL (see review [55]). As a result, current therapeutic approaches for APL in patients with PML-RAR alpha often require a combination approach involving ATRA plus arsenic trioxide plus or minus chemotherapy (anthracycline or gemtuzumab ozogamicin). As noted in Table 1, treatment of APL with these agents requires confirmatory genetic testing to identify PML-RAR alpha gene expression.

4.4 BRAF

Approximately 40–60% of cutaneous melanomas on non-sun damaged skin carry mutations in *BRAF* (frequency is approximately 15% on sun damaged skin), which led to constitutive activation of downstream signalling through the MAPK pathway. Roughly 90% of these mutations result in the substitution of glutamic acid for valine at codon 600 (V600E). Vemurafenib, a potent inhibitor of *BRAF* V600E, has marked antitumour activity against metastatic melanoma tumours harboring the *BRAF* mutation and was subsequently approved in 2011 [13]. As noted in Table 1, verification of positivity for *BRAF* V600E is required prior to therapy with vemurafenib.

In a large randomised phase III trial, 675 untreated metastatic melanoma patients expressing the *BRAF* mutation were randomly assigned to receive standard of care (dacarbazine) or vemurafenib. Results demonstrated a HR for death in the vemurafenib arm of 0.37 when compared to dacarbazine ($P<0.001$). At 6 months, OS was 84 and 64% in the vemurafenib and dacarbazine arm, respectively (further follow up is ongoing). The HR for tumour progression in the vemurafenib group was 0.26 ($P<0.001$) [13]. Despite dramatic initial responses, resistance eventually occurs in all patients with a mean time to diagnosis at first lesion at roughly 10 weeks.

Likely resistance mechanisms include the paradoxical activation of the MAPK pathway through *RAS* mutations and signalling of downstream pathway proteins such as MAPK kinase (MEK). Combining a BRAF inhibitor with a MEK inhibitor

may thus help overcome this resistance mechanism. In fact, a randomised study demonstrated significantly improved PFS (4.8 vs. 1.5 months; HR 0.47; $P<0.0001$) in patients receiving a MEK inhibitor, trametinib, in combination with a selective BRAF inhibitor, dabrafenib [56]. Both of these drugs ultimately received FDA approved indications in 2013 for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E or V600K mutation who have not already received a BRAF inhibitor.

4.5 C-KIT

The *C-KIT* proto-oncogene (also known as CD117) encodes a receptor TK, for which the stem cell factor is the ligand. This interaction results in the development of melanocytes, erythrocytes, germ cells, and mast cells, owing to dimerization, autophosphorylation and continuous signal transduction [57, 58]. Roughly 85% of gastrointestinal stromal tumours (GIST) express gain-of-function C-KIT mutations in exon 9 or 11 [59]. These tumours are often larger in size and more frequently invade adjacent tissues compared to mutation-negative tumours [60]. Before the identification and targeting of C-KIT with imatinib, tumours with gain-of-function *C-KIT* mutations were more aggressive and resulted in a poorer prognosis. However, the addition of imatinib resulted in a partial response rate of 83.5% for GISTs harboring the exon 11 *C-KIT* mutations. This was compared to patients with tumours containing an exon 9 *C-KIT* mutation or no detectable mutation, having partial response rates of 47.8% ($P=0.0006$) and 0% ($P<.0001$), respectively [59]. While imatinib primarily induces tumour shrinkage in GIST via C-KIT inhibition, further inhibition of other TKs, such as platelet derived growth factor receptor-A (PDGFR-A), may play an additional role in the observed antitumour activity of imatinib [61]. As a result, imatinib is only FDA indicated once a diagnosis of C-KIT (CD117) positive GIST has been made (identified by qualitative immunohistochemistry) (Table 1). Although molecular genetic analysis of *C-KIT* may also help identify patients with *C-KIT* positive GIST, the FDA only recommends genetic testing to identify genes associated with imatinib resistance, not for initial diagnosis.

4.6 EGFR

Mutations resulting in the activation of EGFR are located in the TK domain and allow constitutive signalling through activation of the PI3K-AKT and RAS-MEK-ERK pathways (see review [62]). Deletions in exon 19 and a missense mutation at exon 21, resulting in an arginine to leucine substitution (L858R), account for 90% of all *EGFR* activating mutations found in patients with NSCLC. Subgroup analyses from initial clinical trials revealed that patients with certain clinical and histologic characteristics, including female patients of East Asian descent, those with adenocarcinomas, and never-smokers, are more likely to harbor *EGFR* mutations

[63]. Erlotinib, an oral small molecule TKI, binds to the ATP binding site of EGFR, inhibiting the downstream cascade of events, resulting in greater efficacy over traditional chemotherapy. Approximately 20% of NSCLC patients have activating *EGFR* mutations and are candidates for first-line therapy with erlotinib [64]. Erlotinib, an oral small molecule TKI, binds to the ATP binding site of EGFR, inhibiting the downstream cascade of events, resulting in greater efficacy over traditional chemotherapy. Approximately 20% of NSCLC patients have activating *EGFR* mutations and are candidates for first-line therapy with erlotinib (Table 1).

In a randomised phase III trial, investigators evaluated erlotinib versus standard chemotherapy (gemcitabine + carboplatin) as first-line treatment in 165 advanced NSCLC patients harboring activating *EGFR* mutations. The median PFS was significantly longer in the erlotinib arm compared to the chemotherapy arm (13.1 vs. 4.6 months; HR 0.16; $P<0.0001$). The objective response rate (ORR) was 83 and 36% for erlotinib and chemotherapy, respectively ($P<0.0001$). Interestingly, there was a significant association between reduced PFS and the presence of the L858R mutation as compared with a deletion at exon 19 in patients receiving erlotinib (HR 1.92; $P=0.01$) [65]. Two mechanisms of resistance to EGFR inhibitors include a secondary point mutation in *EGFR* (T790M), which blocks the ability for erlotinib to bind to the ATP binding pocket due to steric hindrance, and the amplification of MET, which activates similar downstream signalling pathways [43]. However, newer agents such as afatinib, an irreversible ErbB-family blocker, has shown activity in patients with the L858R activating mutation, exon 19 deletion, and mutations that confer resistance to erlotinib, including the T790M mutation [66].

4.7 ER/PR

As mentioned, ER/PR status can be used to measure disease prognosis (both distant and locoregional recurrence) utilising the 21-gene recurrence score, Oncotype Dx®, in lymph-node negative breast cancer [67], and also can be used as a predictive marker to identify patients most likely to respond to hormonal therapies. Close to 75% of all breast cancers are ER positive and grow in response to oestrogen while close to 65% of these are also PR positive. Studies indicate that ER/PR positive breast tumours are 60% likely to respond to hormonal therapy, whereas the response to hormonal therapy in ER/PR negative tumours is decreased to 5–10% [68]. Frequently administered hormonal therapies include tamoxifen, a selective oestrogen receptor modulator, and AIs (anastrozole, exemestane, and letrozole). As indicated in Table 1, only patients whose tumours are ER/PR positive are likely to benefit from these therapies.

A meta-analysis of women with early stage ER/PR positive breast cancer demonstrated that AIs produced significantly lower rates of recurrence compared with tamoxifen in postmenopausal women in the adjuvant setting; however the contribution of *CYP2D6* genetic variants on tamoxifen treatment was unknown in this study and may have contributed to the observed difference [69]. AIs are not an option for

premenopausal women, as these drugs may stimulate the ovaries to produce oestrogen through a negative feedback loop, making tamoxifen the preferred therapy for premenopausal women (see review [70]). Additionally, one study in women with early stage ER/PR positive breast cancer demonstrated that continuing adjuvant tamoxifen to 10 years rather than stopping at 5 years (which has been traditionally done in clinical practice) produces a further reduction in recurrence and mortality, particularly after year 10 (recurrence rate ratio 0.90 [95% confidence interval (CI) 0.79–1.02] during years 5–9 and 0.75 [95% CI 0.62–0.90] after year 10). In fact, these data speculate that 10 years of tamoxifen treatment can roughly halve breast cancer mortality during the second decade after diagnosis [71].

4.8 HER2

HER2 is a transmembrane TK receptor that helps control cellular signalling and proliferation at normal expression levels; however, in approximately 20–25% of breast cancers, the *HER2* gene is amplified resulting in HER2 protein overexpression, allowing the breast cancer cell to proliferate uncontrollably, resulting in aggressive tumour growth and progression [72, 73]. As mentioned previously, HER2 represents both a prognostic and predictive biomarker (Table 1), as overexpression is associated with a higher rate of recurrence without therapy (see review [74]) but is also predictive of a beneficial therapeutic response and enhanced survival from several HER2-targeted therapies including trastuzumab, lapatinib, pertuzumab, and T-DM1, over traditional cytotoxic chemotherapy. As indicated in the FDA label, these targeted agents should only be used in tumours that have HER2 protein overexpression and/or gene amplification (Table 1). Standard practice involves determining HER2 protein over-expression by immunohistochemistry (0 or +1 being negative, +2 being borderline, and +3 being positive), as this method is less costly. If the test result is IHC +2, fluorescence in-situ hybridisation (FISH) must be used to confirm gene amplification.

In one of the original hallmark trials evaluating the clinical utility of trastuzumab, 1694 HER2 positive locally advanced breast cancer patients were randomly assigned to 2 years of treatment with trastuzumab, 1694 randomly assigned to 1 year of trastuzumab, and 1693 assigned to observation [75]. The unadjusted HR for an event (recurrence of breast cancer, contralateral breast cancer, second non-breast malignant disease, or death) in the trastuzumab group, as compared with the observation group, was 0.54 ($P<0.0001$) [75]. Subsequently in a large randomised phase III trial, investigators randomised 3222 women with HER2-positive early-stage breast cancer to receive doxorubicin and cyclophosphamide followed by docetaxel (AC-T), the same regimen plus 52 weeks of trastuzumab, or docetaxel and carboplatin plus trastuzumab (TCH). The estimated DFS at 5 years were 75% among patients receiving AC-T, 84% among those receiving AC-T plus trastuzumab (HR 0.64 compared to AC-T; $P<0.001$), and 81% among those receiving TCH (HR 0.75 compared to AC-T; $P=0.04$) [76]. Lapatinib, a small molecule TKI targeting the intracellular domain of HER2, led to significant improvements in time to progres-

sion in advanced or metastatic HER2 positive breast cancer in combination with capecitabine in patients who have progressed on prior therapy (including an anthracycline, a taxane, and trastuzumab) compared to capecitabine alone (HR 0.49; $P < 0.001$) [77]. Pertuzumab, an anti-HER2 humanised monoclonal antibody that inhibits receptor dimerisation, has been shown to prolong PFS in combination with trastuzumab and docetaxel compared to placebo, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer patients (HR 0.62; $P < 0.001$) [78]. T-DM1, an antibody-drug conjugate incorporating the targeted therapy of trastuzumab with the cytotoxic properties of emtansine (DM1), significantly prolonged PFS and OS with less toxicity than lapatinib plus capecitabine in patients with HER2 positive advanced breast cancer (previously treated with trastuzumab and a taxane) (HR for PFS and OS 0.65 and 0.68, respectively; $P < 0.001$ for both) [79].

4.9 KRAS

KRAS, a member of the Ras family of small G proteins, functions as a mediator between the extracellular ligand binding and intracellular signal transduction from the EGFR to the nucleus where transcription occurs (see reviews [80, 81]). The autophosphorylation of the intracellular TK domains at codons 12 and 13 confers constitutive activity of downstream signalling pathways, including RAS-RAF-MAPK and PI3K-AKT pathways (see review [82]). Activating mutations of the *KRAS* oncogene (codon 12 G12V, G12S, G12R, G12D, G12C, G12A, and codon 13 G13D) have emerged as a negative predictive biomarker to identify patients with metastatic colorectal cancer who will not benefit from EGFR inhibitor therapies, panitumumab and cetuximab. Approximately 35–40 % of colorectal cancer patients will harbor *KRAS* mutations [82]. The FDA (Table 1), along with several other regulatory bodies (Table 2), support and recommend testing for *KRAS* status prior to initiating therapy with EGFR inhibitors.

Investigators prospectively evaluated FOLFIRI (fluorouracil, leucovorin, and irinotecan) alone versus FOLFIRI plus cetuximab in patients with evaluable *KRAS* status [14]. The addition of cetuximab to FOLFIRI in patients with *KRAS* wild-type disease resulted in significant improvements in OS (23.5 vs. 20.0 months; HR 0.796) and PFS (57.3 % vs. 39.7 %; OR 2.069) compared with FOLFIRI alone. In patients whose tumours carried mutations in *KRAS*, there was no evidence of a benefit associated with the addition of cetuximab [14]. In a similar trial, investigators prospectively analysed FOLFOX alone versus FOLFOX plus panitumumab by tumour KRAS status in metastatic colorectal cancer patients. In the wild-type *KRAS* patients, the addition of panitumumab significantly improved PFS (HR 0.80; $P = 0.02$), while in mutant *KRAS* patients, PFS was significantly reduced in the panitumumab arm (HR 1.29; $P = 0.02$) [83].

Additionally, *KRAS* mutations have been identified as a poor prognostic indicator of NSCLC disease and a predictor of resistance to EGFR-TKIs in NSCLC [84,85]. *KRAS* is mutated in approximately 20 % of NSCLC and is associated with

adenocarcinoma histology and a positive smoking history [86]. NSCLC patients with *KRAS* mutations treated with erlotinib experienced a shorter median time to progression than patients without *KRAS* mutations (HR 2.14; $P=0.01$); however, *KRAS* mutation status did not appear to affect OS [87]. Although *EGFR* and *KRAS* mutations appear to be mutually exclusive, *KRAS* mutations may represent an important biomarker of non-response to EGFR-TKIs in NSCLC.

5 Chemoimmunotherapy

Chemoimmunotherapy, or chemotherapy combined with immunotherapy, utilises a cytotoxic or cytostatic compound plus immunotherapy which stimulates the body's immune response against the cancer cells (see review [88]). Monoclonal antibodies exert their cytotoxicity on malignant cells via three major mechanisms: antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), or directly induced apoptosis [89, 90]. Flow cytometry and cytogenetics can be used to identify antigen markers present in hematologic malignancies, which can then be targeted with various monoclonal antibodies to increase response rates and survival. For example, chemoimmunotherapy involving rituximab targeting the CD20 antigen on B-cell malignancies, such as Non-Hodgkin's Lymphoma (NHL) and chronic lymphoid leukaemia (CLL), has proved superior to chemotherapy alone in terms of response rates, PFS, and OS [91, 92]. In a large phase III trial, 817 patients with CD20 positive CLL were randomly assigned to standard fludarabine plus cyclophosphamide versus fludarabine, cyclophosphamide, and rituximab. At 3 years after randomisation, 65% of patients in the rituximab arm were free of progression compared to 45% in the other arm (HR 0.56; $P<0.0001$) [91]. In another study, rituximab administered as induction and/or maintenance therapy with CHOP chemotherapy significantly prolonged failure-free survival (FFS) in diffuse large B-cell lymphoma patients compared to CHOP alone (2-year FFS was 76% vs. 61%; $P=0.009$) [93]. Other malignancies rituximab is commonly used in include lymphoma, ALL, and other B-cell CD20 expressing tumours and autoimmune diseases, such as rheumatoid arthritis.

Although rituximab has proven to increase survival in combination with chemotherapy in CLL patients, it has minimal activity as a single agent for induction purposes. This prompted the development of novel anti-CD20 monoclonal antibodies that may be more effective than rituximab, particularly as monotherapy [89]. Ofatumumab, for example, is a novel fully humanised monoclonal antibody that binds to the CD20 molecule [94]. A phase II study demonstrated encouraging ORR, PFS, and OS in patients who were refractory to fludarabine+/− alemtuzumab (an anti-CD52 monoclonal antibody) [95], and also in refractory patients irrespective of previous rituximab treatment (5.6 and 5.3 months PFS in rituximab-naïve patients and those who had received prior rituximab, respectively [$P=0.04$]; however, no difference in ORR or OS) [96]. The results of another phase II trial demonstrated even higher ORRs (77%) than the previous study in refractory patients (43–53%)

when previously untreated patients were treated with a combination of fludarabine, cyclophosphamide, and ofatumumab [97]. Other anti-CD20 immunoconjugates include the older tositumumab and ibritumomab, which take advantage of radiolabeled compounds. The use of radiolabeled anti-CD20 antibodies is associated with substantial delayed hematologic toxicity related to nonspecific targeting of the bone marrow [98]. Consequently, these antibodies should be used as single agents or in sequence with conventional chemotherapy. Phase II and III trials of these agents have demonstrated high CR rates (>70%) and ORRs (>90%) [99, 100].

A novel approach to chemoimmunotherapy are antibody-drug conjugates, such as T-DM1 (discussed in the HER2 section) and brentuximab. Brentuximab was granted FDA approval in 2011 for the treatment of relapsed Hodgkin's lymphoma and anaplastic large cell lymphoma. This agent is a chimeric antibody-drug conjugate directed towards the CD30 antigen. Its composition involves a highly stable valine-citrulline linker and a potent chemotherapeutic agent, monomethyl auristatin E, which inhibits microtubule polymerisation [101]. The stable linker conjugating a highly potent compound with a highly specific targeted antibody allows for effective delivery of the cytotoxic agent to the tumour cells of interest. A study of 45 CD30-positive Hodgkin's lymphoma patients treated with brentuximab as a single agent (heavily pre-treated) demonstrated an ORR of 60% and a CR rate of 22%. The median duration of response was 8 months [102]. Another phase II study assessed the efficacy of brentuximab in relapsed or refractory CD30-positive Hodgkin's lymphoma. Results demonstrated an ORR of 75% with CR rate of 34% [103]. Brentuximab efficacy has also been evaluated in 58 patients with relapsed anaplastic large cell lymphoma with an overall response rate of 86% with a median duration of response of 12.6 months [104].

As indicated in Table 1, the FDA recommends routine flow cytometry or cytogenetics to confirm the CD positivity of various haematological malignancies prior to receiving any targeted chemoimmunotherapy.

6 Conclusion

Over 100 drugs currently approved by the FDA require pharmacogenetic information on the drug label, resulting in approximately 25% of all outpatients who receive one or more drugs which are vulnerable to pharmacogenetic variation [105]. Secondary to their unique mechanisms of action and narrow therapeutic indices, antineoplastic therapies are one class of medications greatly impacted by pharmacogenetics. Cancer biomarkers can be used for the early detection of cancers during the diagnostic phase, and subsequently used to determine disease prognosis. Predictive biomarkers are particularly useful in determining a patient's response to therapy, including efficacy and/or toxicity. Elucidating the molecular profile of tumours will help to identify driver mutations, provide a greater number of treatment options and allow for better patient selection in biomarker-driven clinical trials. Because tumour samples also contain both acquired and inherited alterations, cancer

sequencing efforts will also capture germline information, which may inform drug selection and/or dose optimisation, as well as genetic susceptibility to disease. These efforts will generate a tremendous amount of data and clinicians must be prepared to interpret and utilise this information to optimise cancer therapeutics.

7 Expert Recommendation

A concerted effort must be made by researchers, clinicians, and cancer centres to adopt genomic-based cancer medicine as the current standard of care. As cost and turnaround time of genomic testing decreases, more and more patients will have their tumours sequenced, allowing for more effective assignment of targeted therapies for each patient. There is likely to be a shift from single-gene testing to multiplex genomic sequencing. It can be argued that the threshold required to warrant single-gene tests differs greatly from the threshold to consider when sequencing information is readily available. Arguable, it is unethical to ignore this information given the validated phenotypes that exist to predict efficacy and/or toxicity. Generation of actionable clinical recommendations, however, is often burdened by the level of evidence needed to warrant implementation.

Although prospective, randomised controlled clinical trials are the gold standard for an intervention to be accepted into standard of care and enforce clinical implementation, this may not be the most effective method for pharmacogenetic studies. The inherent excessive costs, prolonged time, and large sample sizes associated with these trials may deprive many patients of safer and more effective treatments and dosing. The future of genomic-based cancer medicine should focus on adequate sampling of both tumour and germ-line DNA from large phase III clinical trials with prospectively collected data. This information will be critical in both discovery and validation of drug-gene pairs. Well-validated genes that have passed replication should be assessed for clinical implementation through adaptive, biomarker-driven clinical trials. A shift towards large retrospective validation and replication, randomised phase II clinical trials and adaptive-biomarker designs may allow for a more efficient means of translation.

As our knowledge of cancer at the molecular level continues to expand, clinicians must understand these intricate pathways, the therapeutic implication of mutations within these pathways, and the availability and clinical application of such genetic tests in clinical practice.

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Pharmacogenetics of Coumarin Anticoagulant Therapy

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Abstract Coumarins are effective drugs for treatment and prevention of thromboembolic events. However, their use requires a balancing act between the chance of underdosing which increases the risk of thromboembolic events and the chance of overdosing which increases the risk of haemorrhages. It has been shown that polymorphisms in *VKORC1* and *CYP2C9* explain 35–50 % of the dose variability, although patient characteristics and environmental factors also play a role. In this book chapter we discuss the pharmacogenetics of coumarin derivatives, clinical trials investigating the effectiveness of pre-treatment genotyping and the cost-effectiveness of pharmacogenetic-guided dosing.

Keywords Adverse drug reaction · Pharmacogenetics · Predictive genotyping · Translation · Abacavir · Hypersensitivity · Malignant

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1 Introduction

Coumarin derivatives, such as warfarin, phenprocoumon and acenocoumarol, are very effective in the prevention and treatment of thromboembolic diseases, for example in patients with atrial fibrillation or venous thromboembolism [1–5]. Patients with atrial fibrillation have an annual stroke risk of 4.5 %, which decreases to 1.4 % during treatment with warfarin [1]. Warfarin is the most prescribed coumarin in the world while phenprocoumon and acenocoumarol are the coumarins of first choice in continental Europe [6–8]. Although these drugs have already been on the market for decades, finding the right dose for each patient is still challenging. Coumarins have a narrow therapeutic index, often resulting in an unacceptably low anticoagulant effect with an increased risk of thromboembolism or unacceptably high anticoagulant effect with an increased risk of haemorrhages [9–13]. Furthermore, they are subject to inter- and intra-individual variability in dose requirements [14, 15]. Also, the use of coumarins frequently results in drug-related hospitalisation [16–19]. It has been established that anticoagulation response is affected by environmental, clinical, and genetic factors such as age, height, weight, concurrent drug therapy, morbidities, dietary vitamin K intake, and genetic variation in Cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1) [20–25]. This chapter elaborates on the inter- and intra-patient variability in the response to coumarin derivatives, mainly focusing on the pharmacogenetics of these drugs.

2 Mechanism of Action

Inactive coagulation factors II, VII, IX and X require γ-carboxylation of the glutamic acid (Glu) residues into γ-carboxyglutamic (Gla) residues for their coagulation activity (see Fig. 1) [26–28]. In this process, the γ-carboxylase cofactor vitamin K-hydroquinone is oxidised to vitamin K-epoxide. Vitamin K- epoxide is recycled for the carboxylation of new coagulation factors in a 2-step reduction to vitamin K-hydroquinone [27, 28]. Vitamin K epoxide reductase (VKOR) is the catalyser of the first step in the reduction of vitamin K-epoxide into vitamin K-quinone and also contributes to the second reduction step, in which vitamin K-quinone is further reduced to vitamin K-hydroquinone [27, 28]. Cytochrome P450 4F2 (CYP4F2) is a vitamin K-oxidase and metabolises vitamin K-quinone to hydroxyvitamin K [29]. Coumarins, also called vitamin K antagonists, inhibit the reduction of oxidised vitamin K by binding to a small trans membrane protein in the endoplasmic reticulum called vitamin K epoxide reductase complex 1 (VKORC1), which is part of the VKOR complex [30, 31]. As a result, vitamin K-hydroquinone will not become available for the γ-carboxylation of coagulation factors. Coumarins thus act indirectly on the coagulation factors. The half-lives of the coagulation factors range from approximately 6 h for factor VII to 2.5 days for

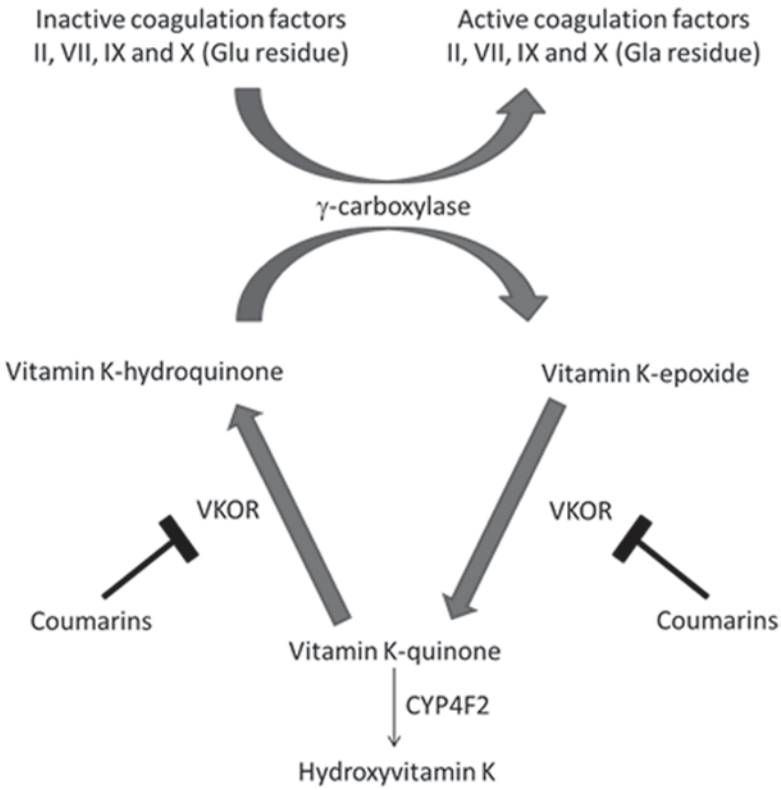

Fig. 1 The mechanism of action

factor II (prothrombin) [32]. This means that the effect of the coumarins in inducing an anticoagulant effect starts 15 h after administration [33] and ends 36–72 h after start of coumarin use [34, 35].

3 Pharmacokinetics

All three coumarin derivatives have a similar chemical structure and belong to the group of 4-hydroxycoumarins. Each coumarin has a single, chiral centre with a R-enantiomeric form or a S enantiomer, which is approximately 2- to 5-fold more potent [36]. Even though the mechanism of action is identical for the three coumarins, there are clear differences in their pharmacokinetic properties and therefore we discuss the pharmacokinetics of the coumarins separately. After administration, all coumarins (except S-acenocoumarol) are absorbed from the gastrointestinal tract with almost complete bioavailability [36].

3.1 Warfarin

Warfarin is metabolised to five different monohydroxylated metabolites (i.e. 4'-, 6-, 7-, 8- and 10-hydroxywarfarin), cis- and trans-dehydro-warfarin, and two diastereomeric alcohols [36, 37]. Metabolism to hydroxylated and dehydro- metabolites is dependent on Cytochrome P450 (CYP) enzymes and occurs in the microsomal fraction of hepatocytes [38], while reduction to alcohols is dependent on NADPH and takes place in the endoplasmic reticulum and cytosol [39, 40]. Different monohydroxylated warfarin metabolites are formed, which suggests involvement of different CYP-isoenzymes. The largest proportion of hydroxylation is catalysed by CYP2C9, resulting in the formation of 7-hydroxywarfarin, the most abundant metabolite. To a much smaller extent, CYP2C8, CYP2C19, CYP1A2 and CYP3A4 are involved [36]. The half-life of warfarin is 24–33 h for S-warfarin and 35–58 h for R-warfarin [36, 41].

3.2 Acenocoumarol

Acenocoumarol is metabolised to 6-, 7-, and 8-hydroxy-acenocoumarol, amino and acetamido acenocoumarol and two diastereometic alcohols [42, 43]. Enzymes involved in the formation of amino and acetamido metabolites and alcohols have not yet been identified. Hydroxylation is dependent on CYP-enzymes [44]. Hydroxylation is catalysed by CYP2C9, the main metabolite being 7-hydroxyacenocoumarol. As for warfarin, CYP2C9 regioselectivity for the 6- and 7- position and stereoselectivity for the S-enantiomer seem to play a role [36]. In contrast, the role of CYP2C19 and CYP1A2 is much smaller [36]. The half-life of acenocoumarol is 1.8 h for S-acenocoumarol—the most potent form—and 6.6 h for R-acenocoumarol [43].

3.3 Phenprocoumon

The metabolites of phenprocoumon are 4'-, 6-, 7- and 8-hydroxy-phenprocoumon and in contrast to warfarin and acenocoumarol all metabolites are hydroxyl-metabolites [36]. The hydroxyl-metabolites are all formed by CYP-enzymes [45, 46]. The 6- and 7-hydroxy phenprocoumon are the most abundant metabolites, 45 and 52%, respectively [36]. The main metabolising enzymes involved are CYP2C9 for approximately 60–65% and CYP3A4 for approximately 35–40% of 6- and 7-hydroxy-phenprocoumon. These CYP-enzymes and CYP2C8 are also involved in the formation of the other metabolites [36]. The half-life of phenprocoumon is much longer compared with the two other coumarins: 110–130 h for S-phenprocoumon (the most potent form) and 110–125 h for R-phenprocoumon [47]. The contribution of CYP2C9 to the metabolism of the different enantiomers of the three coumarins varies [36] and is shown in Fig. 2.

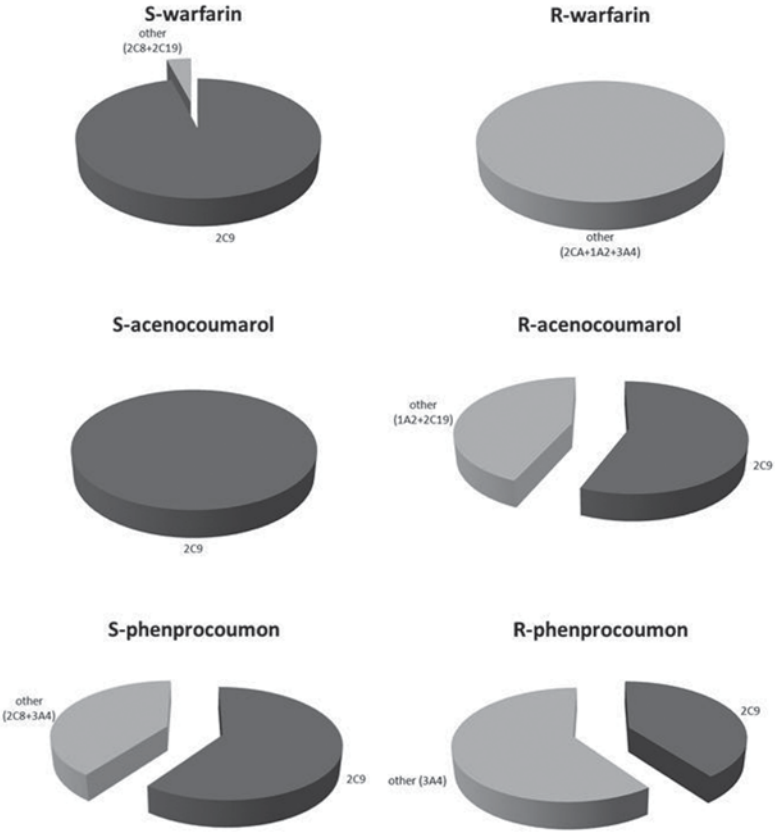

Fig. 2 The contribution of CYP2C9 and other CYP enzymes to the metabolism of the different enantiomers

4 Anticoagulant Therapy

In order to find the most effective and safe balance between underanticoagulation (with a risk of thromboembolic events) and overanticoagulation (with a risk of haemorrhage), a recommendation was made during the first American College of Chest Physicians (ACCP) conference in 1986 that therapy with coumarins should be monitored using the International Normalised Ratio (INR) established by the World Health Organisation [48, 49]. A dose that prolongs the INR to two to three times control (i.e. INR of 2.0–3.0) was recommended for indications such as prophylaxis and treatment of venous thromboembolism, and atrial fibrillation [49]. Higher ranges (i.e. INR of 3.0–4.5) were recommended for other indications including, for example, recurrent venous thrombosis despite adequate anticoagulation [49]. These recommendations are widely accepted and have increased the safety of coumarins [48]. The treatment is often managed by the general practitioner (GP) or a physician

in the hospital. In contrast to most other countries, there are specialised anticoagulation clinics in The Netherlands that follow dosing strategies to maintain the INR between the 2.0 and 3.5 for the low intensity range (e.g. atrial fibrillation, venous thromboembolism) or 2.5 and 4.0 for the high intensity range (e.g. artificial heart valves, recurrent venous thrombosis despite adequate anticoagulation) [28, 50, 51]. Dutch patients regularly visit the anticoagulation clinic for INR measurements and subsequent dose adjustments. Anticoagulation clinics improve the quality of the anticoagulant therapy and are cost saving because haemorrhages and thromboembolic events are prevented more adequately compared to usual clinical care (monitoring by GPs or in the hospital) [52, 53]. In 2010, the Dutch anticoagulation clinics achieved a median percentage time spend in target INR range of 77.9% for patients in the low intensity range and 73.2% for patients in the high intensity range [50]. This is a very high percentage time in range compared with what has been reported in other countries (for example, 63% in the UK, 56% in Germany, and 66% in Austria) and comparable to Sweden (76%) [54], but it still means that over 20% of the time, INRs are above or below the target range. This can be explained by intra-individual dose variability over time, which will be discussed, together with inter-individual variability, in the next paragraph.

5 Inter- and Intra-Individual Dose Variability

The coumarin dose that is optimal for one patient may cause haemorrhages in another patient and thromboembolic events in a third patient. Patients need very different dosages which can differ by up to 10 fold [14]. For example, the maintenance dose of warfarin ranges from 1.5 to 12 mg/day, acenocoumarol from 1 to 9 mg/day and phenprocoumon from 0.75 to 9 mg/day [36]. In addition, the required dose may also change over time in an individual patient. There are several factors that cause inter- and intra-individual variability.

5.1 Patient Characteristics and Environmental Factors

Effects of patient characteristics and environmental factors can roughly be divided into 3 categories: effects on the coumarin dose, effects on the stability of the anticoagulant therapy, and effects on clinical outcomes.

5.1.1 Effects on Coumarin Dose

Coumarin dose requirements decrease with increasing age, but increase with increasing weight and height [25, 55]. Many diseases affect the coumarin dosages as well. Patients with hepatic disorders need lower dosages because the synthesis of

coagulation factors is reduced in these patients because of Vitamin K deficiency, decreased metabolism due to reduction in hepatocyte mass or hypo-albuminaemia [56, 57]. Hyperthyroidism leads to decreased coumarin dosages compared to euthyroidism, while hypothyroidism is associated with a decreased catabolism of vitamin K-dependent coagulation factors, attenuating the response to oral anticoagulant therapy and resulting in increased dose requirements [56]. Heart failure may cause hepatic congestion, resulting in a decreased synthesis of coagulation factors and therefore lower coumarin maintenance dose requirements [56, 58]. Malignancies might affect the coumarin dose by metastatic liver disease, malnutrition, or use of chemotherapy [56]. Fever decreases coumarin dose requirements, probably by increasing degradation of coagulation factors [9]. Dehydration might affect the INR and therefore the coumarin dose by changing the volume of distribution of the coumarins [57]. Hypo-albuminaemia affects the concentration of unbound coumarins and therefore the coumarin dose requirements [57]. Kidney disorders might also affect the albumin concentration and therefore coumarin dose requirements [57]. Comedication use is also of importance and there are many drugs that can increase or decrease the anticoagulation effect and thereby influence the coumarin dose requirements [22, 23, 25, 59–62]. In the Netherlands, clinically relevant drug interactions with coumarins have been described and regulated in the guidelines for anticoagulation clinics [63, 64]. There are two main categories of drug interactions: first, the pharmacokinetic interactions affecting the absorption, distribution or elimination and second, the pharmacodynamic interactions affecting production or metabolism of coagulation factors, or directly affecting coagulation [57]. Besides affecting the coumarin maintenance dose, comedication might also increase the risk of haemorrhages.

5.1.2 Effects on Stability of the Anticoagulant Therapy

Dietary vitamin K intake interferes with the stability of the oral anticoagulant therapy [65]. Daily supplementation of vitamin K intake possibly contributes to a more stable anticoagulant therapy [66–68]. Other nutrition factors can also be of influence [57]. Because vitamin K is a fat-soluble vitamin, the absorption of vitamin K through the intestines is influenced by fat intake and absorption disorders which might result in instability of the anticoagulant therapy. Gavage feeding might cause fluctuating INRs [57, 69]. This could be due to different concentrations of vitamin K in the gavage in comparison to normal diet. Also, vitamin K might bind to proteins in the gavage feeding, or vitamin K might get lost in the preparation of the gavage or due to adsorption to the tube wall. Disorders of the gastrointestinal tract (e.g. vomiting, diarrhea, malabsorption of fat, or antibiotic use which may affect bacteria in the intestines that produce vitamin K) might affect the stability of anticoagulant therapy [57]. Increased levels of stress are thought to be associated with increased INRs and varying amounts of physical exercise may cause a fluctuation in INR as well [57]. Travelling (and any resulting changes in diet or alcohol consumption) and poor compliance might cause instability as well [57, 70].

5.1.3 Effects on Clinical Outcomes

Hematological disorders, such as thrombocytopenia, might affect the anticoagulant therapy by increasing the risk of haemorrhage. In addition, local disorders such as polyps increase the risk of haemorrhage. Malignancies may increase the risk of both venous thromboembolism and haemorrhages [57].

5.2 Pharmacogenetics

In 1992, Rettie et al. reported that CYP2C9 is the main metabolising enzyme of warfarin [71]. CYP1A2 and CYP3A4 were also found to contribute to the metabolism of the drug [71]. Furuya et al. hypothesised that polymorphisms in *CYP2C9* (resulting in proteins with different catalytic activities) might have a major effect on the clearance of the most potent enantiomer (S-warfarin) and therefore might affect the warfarin maintenance dose [72]. They recruited almost 100 patients who attended the anticoagulation clinic for routine INR monitoring. Information on body weight, height, age, sex, drug history, INRs history, indication for coumarin use, and comorbidities was collected. A blood sample was used to determine the *CYP2C9*2* genotype. Of the 94 included patients, 58 (62%) were wild type (*CYP2C9*1/*1*) and 36 (38%) heterozygous for *CYP2C9*2*. There were no patients homozygous for *CYP2C9*2*. Patients carrying the variant allele required significantly lower warfarin dosages than wild type patients (Mann-Whitney U-test, $p = 0.02$). In addition, they found an association between age and warfarin dose requirements. The results suggesting an effect of *CYP2C9* genotypes on the coumarin maintenance dose have since been replicated by many research groups [25, 73–78]. Not only *CYP2C9*2* but also *CYP2C9*3* is a common variant allele in Caucasians that reduces the coumarin maintenance dose significantly [25, 73–78]. The CYP2C9*2 allele frequencies vary from 8 to 19% and the CYP2C9*3 alleles from 3 to 16% in Caucasians [79]. East Asian and African or Afro-American populations show an absence of CYP2C9*2 and a reduced frequency of CYP2C9*3 (79). The *CYP2C9* genotype explains approximately 4.5–17.5% of the coumarin (warfarin, acenocoumarol and phenprocoumon) dose variation [25, 76, 80–85].

Rost et al. and Li et al. identified VKORC1 as a target of the coumarins in 2004 [30, 31]. This introduced a new possibility for explaining the coumarin dose variability. Indeed, many researchers showed decreased coumarin dose requirements if patients carried one or two variant alleles in the *VKORC1* gene [73–75, 82, 86, 87]. Two SNPs in *VKORC1*, the −1639G>A and the 1173 C>T, were found to be associated with decreased warfarin dose requirements [28]. It was demonstrated that promotor SNP −1639G>A causes the variability in VKORC1 activity by suppressing the gene expression, but a role for 1173 C>T could not be excluded because of the complete linkage disequilibrium between the two SNPs [88]. Patients carrying one or two variant alleles have decreased levels of *VKORC1* mRNA in the liver and therefore need lower coumarin dosages compared to wild type patients

[88]. Because the two SNPs are in complete linkage disequilibrium [88, 89], studying either of the two SNPs will give the same results. Allele frequencies for the *VKORC1* variant allele are 37–41 % in Caucasians, 10–12 % in African Americans, and 88–92 % in East-Asians [28].

There are many other genes that could potentially affect the coumarin maintenance dose. The association with the coumarin dose might for example be based on other pharmacokinetic or pharmacodynamic mechanisms, for example by affecting the transport of coumarins or vitamin K or by affecting the vitamin K cycle. In the metabolism of phenprocoumon, other metabolising enzymes, especially CYP3A4, also play an important role [36, 90] and therefore SNPs in the genes encoding for these metabolising enzymes are hypothesised to affect the phenprocoumon dose requirements. However, Teichert et al. did not find an association between *CYP3A4*1B* and the phenprocoumon dose [91]. Another gene that has been associated with coumarin response is *CYP4F2* [91–97], which is a vitamin K oxidase. Patients carrying one or two V433M variant alleles in *CYP4F2* have a reduced capacity to metabolise Vitamin K, resulting in increased vitamin K levels and therefore also resulting in higher coumarin dose requirements when compared to non-carriers [29]. SNPs in *CYP4F2* have a nominal effect on the coumarin maintenance dose; it explains an additional 1–2 % of the coumarin dose requirements [92, 94]. Polymorphisms in the gene encoding γ-glutamylcarboxylase (*GGCX*), which is involved in the carboxylation of coagulation factors, have also been shown to have a minor effect on the coumarin dose [74, 98] however other research groups did not find an association between the coumarin dose and polymorphisms in *GGCX* [99, 100]. Other minor influences on the coumarin maintenance dose might be caused by polymorphisms in the genes encoding for the coagulation factors VII and X [101], epoxide hydrolase (*EPHX1*) [100, 102] which encodes a protein subunit of VKOR, apolipoprotein E (*APOE*) [103–107] which encodes for the protein responsible for the vitamin K uptake, and in protein C (*PROC*) [103] which encodes for protein C, responsible for the inactivation of coagulation factors Va and VIIIa. All these polymorphisms show low or no clinical relevance.

Until now, only *VKORC1*, *CYP2C9* and *CYP4F2* genotypes were found to be associated with the coumarin maintenance dose in genome wide association studies (GWAS) [91, 93, 94, 97]. Ross and co-workers studied the allele frequencies of these genes in different populations and found that there are significant differences between populations worldwide [108]. The allele frequencies of the common and variant alleles of *VKORC1, CYP2C9*2, CYP2C9*3* and *CYP4F2* are shown in Fig. 3. One study also found an association between CYP2C18 and the acenocoumarol dose [97]. Another study of 1496 Swedish patients starting warfarin treatment investigated possible associations between183 polymorphisms in 29 candidate genes and warfarin dose and only found an association for *CYP2C9* and *VKORC1* [83].

CYP2C9 and *VKORC1* genotypes together explain approximately 35–50 % of the coumarin dose requirements [83, 87, 109]. To date, a number of studies have reported the development of pharmacogenetics-guided algorithms for coumarins in order to predict the personalised coumarin dose before start of the anticoagulant

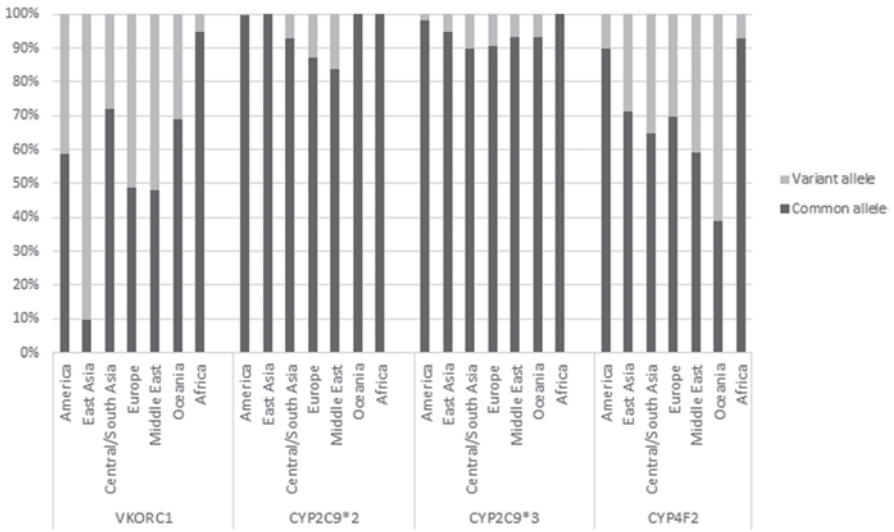

Fig. 3 Allele frequencies of genes associated with coumarin dose requirement among different populations

therapy [25, 76, 80–85]. The predictive value of these algorithms varied from 47 to 60%. Because of ethnic differences in allele frequencies, it can be expected that pharmacogenetic algorithms have a different predictive value in different populations. Several authors have included race as a parameter in their pharmacogenetic-guided algorithm [76, 80, 81, 83]. The International Warfarin Pharmacogenetics Consortium showed that a model that was adjusted for race performed better than specific models for each ethnicity. However, racial differences were not significantly associated with the required dose when genetic information was added to the model [76].

5.2.1 Clinical Trials

In 2005, the first (pilot) randomised trial on pharmacogenetic-guided warfarin dosing in 38 patients was published [110]. These authors reported no differences in percentage time in INR range or the risk of supratherapeutic INR values. In another randomised trial with 191 patients, the time to stable dose was decreased and the time spent in therapeutic range was increased by pharmacogenetic-guided dosing [111]. In both these studies, only *CYP2C9* genotype was assessed and not *VKORC1* genotype. Anderson et al. [112] investigated the impact of genotyping for both *CYP2C9* and *VKORC1* genotypes in 220 patients. No effect on the number of out-of-range INR values could be demonstrated when looking at all patients, but in wild-type

patients and patients carrying multiple variant alleles, genotyping decreased the risk of out-of-range INRs by 10%. In two small randomised trials in Chinese patients, a stable dose was reached faster in patients receiving a pharmacogenetic-guided dose than in patients receiving a standard dose [113, 114]. Burmester et al., compared dosing using a pharmacogenetic algorithm to a clinical algorithm instead of standard dosing and found no differences in percentage time in therapeutic range between the two arms [115]. The Applying Pharmacogenetic Algorithms to Individualise Dosing of Warfarin (Coumagen-II) trial (NCT00927862) showed that pharmacogenetic dosing was superior to standard dosing for percentage time in and out of therapeutic range [116]. During the first month of the treatment, 31% of the INR measurements were below or above the therapeutic range in the intervention group vs. 42% in the control group. The reduction in out-of-range INRs was mainly due to a reduction in INRs below the therapeutic range. The percentage time within the therapeutic range was 69% in the intervention group and 58% in the control group. Also, less serious adverse events (including haemorrhagic and thromboembolic events) occurred in the genotype-guided group (4.5 vs. 9.4%, $p=0.001$). The limitation of this study was the lack of randomised comparison.

The European Pharmacogenetics of Anticoagulant Therapy EU-PACT trial (unique ClinicalTrials.gov Identifiers: NCT01119274, NCT01119261, and NCT01119300) compares a dose algorithm with patient characteristics (or in the case of warfarin standard clinical care) to a dose algorithm with patient characteristics and *VKORC1* and *CYP2C9* genotype [117, 118]. The primary outcome is the time within target INR range. It is the only RCT that investigates all three coumarins (warfarin, phenprocoumon and acenocoumarol). The EU-PACT warfarin arm showed a positive effect of the genotype-guided dosing taking percent time in therapeutic INR range as an outcome. The patients that were genotyped spent 7% more time in range in the first 12 weeks of warfarin therapy compared with the patients in the standard care arm. In the EU-PACT phenprocoumon/acenocoumarol arm there was no statistically significant difference in time in therapeutic range in the first 12 weeks, however there was a statistically significant effect in the first 4 weeks of treatment. Patients in the genotyped arm spend 5% more time within therapeutic range in these first 4 weeks [117]. On the other hand, the Clarification of Optimal Anticoagulation Through Genetics (COAG) (NCT00839657) trial results in no significant difference in the time spent within the therapeutic range in the first 4 weeks of warfarin treatment [119]. These conflicting results are compared in Table 1. One of the reasons for these observed differences might be the comparator, since for warfarin dosage, the genotype guided dose was compared to standard care in the EU-PACT trial, whereas the comparator in the EU-PACT phenprocoumon/acenocoumarol arm and in the COAG trial was a clinical algorithm. Furthermore in the COAG trial it was shown that for African Americans the time in therapeutic range was less in the genotyped arm compared with the clinical algorithm arm. This implies that different algorithms are necessary for different race groups.

Table 1 Overview of randomised clinical trials

	EU-PACT [117]	COAG [119]
Coumarin derivative	Phenprocoumon, acenocoumarol, warfarin	Warfarin
Population	Patients with atrial fibrillation or venous thromboembolism	Patients requiring warfarin therapy with a target INR range of 2–3
Genotypes included	VKORC1, CYP2C9	VKORC1, CYP2C9
Comparator	Clinical algorithm (acenocoumarol, phenprocoumon) Standard care (warfarin)	Clinical algorithm
Number of patients	911	1015
Primary outcome	Percentage time within target INR range	Percentage time within target INR range
Result	Genotype-guided Warfarin Algorithm is superior	No difference

6 Cost-Effectiveness

Clinical trials can provide valuable information about the safety and effectiveness of genotyping before starting coumarin therapy. This information is not only valuable for clinicians but also for policymakers who need to make a decision about whether or not to implement genotype-guided dosing. However, this decision will not only depend on the effectiveness of genotyping, but also on the cost-effectiveness since an important factor for implementation will be reimbursement of the genetic tests. This is the primary reason for performing cost-effectiveness analyses. Some of the cost-effectiveness analyses of genotyping performed in the past have estimated the costs to avoid an adverse event. But for a health insurance company, this way of describing cost-effectiveness makes it difficult to compare with the cost-effectiveness of other drugs for other diseases. Reimbursement authorities therefore often require a so-called *cost-utility analysis* in which the extra costs to gain one quality-adjusted life-year (QALY) are estimated. Since the QALY represents a generic measure of overall health that can be improved by increasing life expectancy and/or quality of life, the cost per QALY gained can therefore be applied for any health technology for any disease area.

One of the first estimates of the cost-effectiveness of genotyping warfarin users was published in 2003. These authors estimated that the cost to avoid one bleeding event were US$5940 [120] if patients were given a dose based on their *CYP2C9* genotype, compared with standard care. Very similar results were obtained by You et al., who calculated a cost-effectiveness ratio of US$5778 per bleeding event avoided [121]. Schalekamp et al. reported that the cost-effectiveness of genotyping acenocoumarol users for their *CYP2C9* genotype was US$5151 per bleeding event avoided [122]. This study focused on the Netherlands, while the other two studies focused on the

US. After the relevance of the *VKORC1* genotype was demonstrated, it was assumed that genotyping the patient for both *CYP2C9* and *VKORC1* genotypes would lead to better dose prediction and therefore a larger effect of pharmacogenetic-guided dosing than genotyping for *CYP2C9* alone. More recent cost-effectiveness analyses therefore also included *VKORC1* genotyping in their assessment. Several authors estimated the cost-utility ratio of genotyping for these two genes compared with standard care in the US [123–127] and reported results that vary from US$60,750 to US$347,000 per QALY gained. Eckman and co-workers performed a meta-analysis of the three trials that were available in 2008 [110–112] and found that pharmacogenetic-guided dosing could reduce the risk of bleeding by 32 % [124]. When they used this data in their economic model, they found that genotyping would cost US$170,000 per QALY gained, a value much higher than the willingness-to-pay thresholds of US$50,000–US$100,000 that are often applied in the US to conclude whether or not an intervention is cost-effective [128]. Sensitivity analyses by Eckman et al. showed that the costs per QALY gained would be less than US$50,000 only if the test would be restricted to patients with a high bleeding risk or if all of the following criteria were met: more bleeding events could be avoided, the test would cost less than US$200 and the results would be available within 1 day. Patrick and co-workers also found that genotyping only patients with a high bleeding risk would increase its chance of being cost-effective [126]. Meckley and co-workers used data from the CoumaGen trial [112] and found a cost-effectiveness ratio of US$60,740 per QALY gained [127]. You et al. reported a much higher cost per QALY gained than previous studies (US$347,000) as well as high costs per life saved (US$1,106,000 per life saved) and high cost per adverse event averted (US$170,000), which combined bleeding events with thromboembolic events [123]. The chance that genotyping would cost less than the US$50,000 threshold was low (38 %) and increased with lower genotyping costs, greater reduction in out-of-range INRs and in specific settings where poor INR control was seen. Using data from the CoumagenII trial [116], in which the time in therapeutic range in the first month was increased by 11 % in the first month, Verhoef et al. reported that pharmacogenetic-guided phenprocoumon dosing would be cost-effective [129] given a cost per QALY gained of 2700 euro.

Recently, novel oral anticoagulant drugs such as dabigatran, rivaroxaban and apixaban have been developed, which appear to be good alternatives to coumarin anticoagulants [130]. You et al. studied the cost-effectiveness of dabigatran and genotype-guided warfarin treatment and showed that dabigatran seems to be a cost-effective treatment [131]. However, they reported that pharmacogenetic-guided warfarin dosing had a higher chance of being cost-effective if it was able to increase the percentage time in target INR range to > 77 %.

The main limitation of the cost-effectiveness studies published up to now has been the lack of robust data from appropriately powered clinical trials [132]. Also, the costs of genotyping *VKORC1* and *CYP2C9* polymorphisms are not clear yet. Previous studies have used costs that vary from US$175 to US$575 when the genotype is determined in the lab and US$50 for a point-of-care test [127, 132, 133]. These costs are expected to decrease over time and with increased usage, which will influence the cost-effectiveness as well. In the analysis by Verhoef and

co-workers, the use of a point-of-care test was assumed, which provides the results within 2 h and costs less than US$50 [133]. In sum, most of the studies found that, pharmacogenetic-guided dosing did not seem to be cost-effective and their results underline the large influence of effectiveness of genotyping and the costs of the test. Genotype-guided dosing will only be cost-effective if the costs of the test can be kept low or if it has a large effect on INR control and related incidences of adverse events. The results also show that genotyping could be cost-effective if it would be used only with specific patients (with a high bleeding risk) or in specific settings (with a low quality of INR control).

A more reliable estimate of the cost-effectiveness or cost-utility of pharmacogenetic-guided coumarin dosing can be calculated after the results of the large RCTs become available. Because of many differences between countries in costs and organisation of anticoagulation services, the cost-effectiveness of genotyping coumarin users probably varies between countries [54]. Therefore it will also be necessary to carry out country-specific analyses in the future.

7 Conclusion

Coumarins are effective drugs for treatment and prevention of thromboembolic events. However, their use requires a delicate balancing act between the chance of underdosing (which increases the risk of thromboembolic events) and the chance of overdosing (which increases the risk of haemorrhages). It has been shown that polymorphisms in *VKORC1* and *CYP2C9* explain a large part (35–50%) of the dose variability but patient characteristics and environmental factors also play a role. Clinical trials have researched the added value and cost effectiveness of pre-treatment genotyping. The results from the trials were not convincing, and at this moment there is not enough evidence to recommend genotyping for CYP2C9 and VKORC1 in routine clinical practice. Recent cost-effectiveness studies have shown that the small improvement of time in therapeutic range does not weigh against the costs of genotyping all patients. However, the cost-effectiveness of the intervention will depend on the costs of genotyping and on the availibility of other anticoagulation therapy such as the Direct Oral Anticoagulants (DOACs) [118].

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Implementation of Genomic Medicine: Tools and Challenges

Godfrey Grech, Ron van Schaik and Joseph Borg

Abstract The challenge of personalised medicine have been the centre of constructive debate in the last years, and have activated multidisciplinary approaches to solve the issues for translation to the clinic. In the preface, the editors summarise the challenges to translate genetic-based knowledge to the clinical setting. In this chapter, we will be reviewing the pre-requisites that will lessen the struggles associated with the implementation of pharmacogenetic testing protocols. To achieve this we will focus on the needs and recommendations to promote patient molecular classification to enhance risk assessment and drug response research; stratification of well-defined therapeutic groups, using genetic analysis, into subgroups of responders to specific therapies; the development of technologies and integrative information systems to provide the healthcare system with optimised and sustainable genetic testing protocols; the need of harmonised guidelines for the proper selection of patient groups for clinical trials; and advances in research to generate evidence based knowledge that can be smoothly translated for healthcare use.

Translation from research to the clinic is mainly associated with (1) the co-evolution of biomarkers and targeted therapy and (2) genotypes associated with drug metabolising enzymes. Although significant pharmacogenetic analyses are currently in use, the main obstacles associated with the efficient implementation include the uptake by the health care systems, incorporation into clinical guidelines as well as economic, educational, legal and commercial aspects. Understanding the approach for successful translation to clinical practice and resolving, in-part, the confounding

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aspects that negatively impact efficient implementation, shall support the transition from an empirical approach to a personalised health care system.

Keywords Genomic medicine · Pharmacogenetics implementation · Biomarker-driven prescribing · Genomic medicine in health care · Evidence-based medicine · Predictive genetics · Ethnic variations · Genetic-guided therapy · Pharmacogenetic technologies · Next generation sequencing

1 Current Knowledge and Expectations

Understanding the cause of disease at a molecular level permitted the development of drugs, following the discovery of potential therapeutic targets. This biomarker-driven prescribing deals mainly with somatic mutation that are causative of the disease. Somatic DNA, derived from the tissue (biopsies or resections), provides information on mutations that represent the variations associated with the tumour initialisation, progression of disease or secondary mutations due to the tumour itself [1]. The presence of the fusion protein BCR/ABL in chronic myeloid leukaemia (CML), overexpression of human Epidermal Growth Factor Receptor 2 (Her2) in breast cancer (Fig. 1), Epidermal Growth Factor Receptor (*EGFR*) mutants in lung cancer, are genetic markers currently used in the clinic to select therapy. The translation of these genetic tests into the clinic was smooth, mainly due to the clear patient benefits, offering patients access to improved healthcare management, avoiding unnecessary treatment and reducing adverse drug reactions. In addition, the simple algorithm used to apply the results in the health care system, provides the health care provider assurance for correct clinical decisions. A major challenge in targeted therapy is resistance due to acquired mutations of the drug target protein, exemplified by the development of resistance to imatinib, due to acquired mutation in BCR/ABL fusion protein in CML patients or cKit mutants in gastrointestinal stromal tumours (GIST) [2, 3]. Second-generation drugs were developed to treat secondary resistance to imatinib. Hence, genetic analysis to assess development of disease is required for patient management. In addition, to patient-specific adjusted therapy and dosing, classification of the tumour type in individual patients is important to derive information to predict re-occurrence of the disease. This is achieved by using advanced technologies (discussed below) to measure panels of gene expression utilising well established gene signatures associated with disease re-occurrence, such as the FDA approved tests, Mammaprint (Agendia BV) and Oncotype DX (Genomic Health) designed to predict event free survival in breast cancer patients.

After identification/classification of the disease, drug treatment specific for this disease can be started. But also for the same disease, patients may benefit from altered dosing based on their inherited capacity to metabolise these drugs in the liver, as detailed in Chap. 5. The clinical therapeutic approach is evolving from the

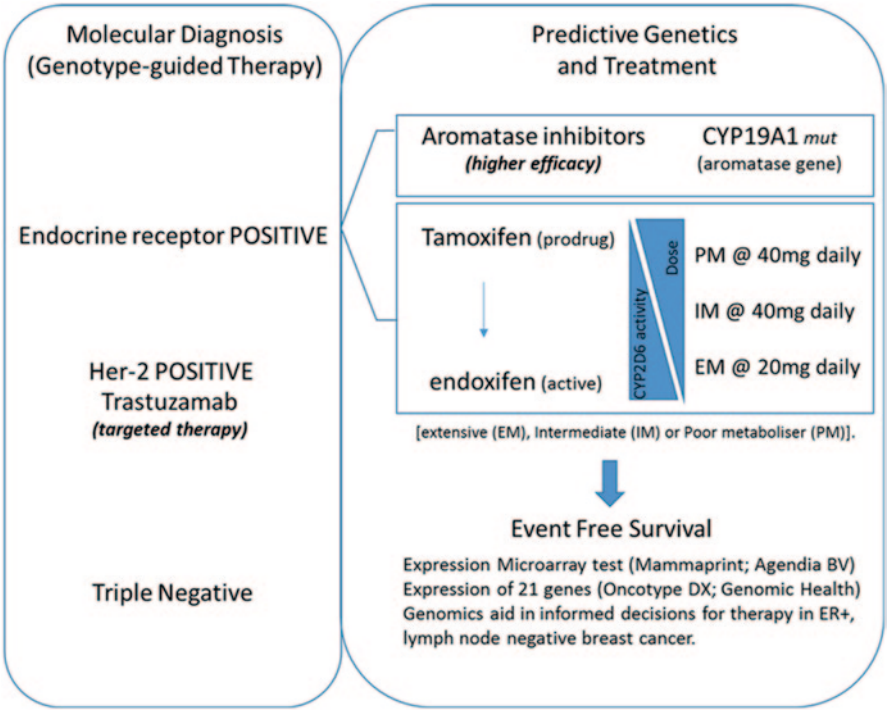

Fig. 1 Genotype-Phenotype evidence. Molecular diagnostics provides genotype-guided therapy by classifying patients into therapeutic groups (*left* panel). Taking endocrine receptor positive cases, the prediction of therapy outcome is summarised in the *right* panel. The efficacy of aromatase inhibitors depends on mutations in the aromatase gene, and the dosage of tamoxifen is guided by *CYP2D6* genotypes associated with the enzyme activity

knowledge of the effect of a drug in the average patient, towards the understanding of the response of drugs in individual patients. Today, it is recognised that personalised medicine is the future. Anticipation of a drug response, adverse drug reaction and selection of a specific therapy contributes significantly to a better quality of life due to evidence-based prescribing and personalised management of a therapy. The potential use of genotype-drug response, well established for *HLA-B*5701* screening for Abacavir therapy, and currently debated for tamoxifen dosing used for estrogen receptor positive breast cancer patients [4–6], warfarin dosing [7], *DPYD* screening for colon cancer treatment with 5-FU or capecitabine [8] and *CYP19A1* SNPs associated with enhanced efficacy of aromatase inhibitors [9], may increase patient safety and therapy efficacy (Fig. 1). Genotype-based prescribing is gaining importance in the clinic, and interrogation of genomic data is expected to overcome the complexity of multifactorial drug-genotype associations. The algorithms used to improve therapy based on genotype alone may not always be successful, depending on the non-genetic factors contribution to drug efficacy and toxicity.

2 Genetic Variance Within and Across Populations

It is recognised that there is inter-patient genetic variability and this accounts significantly to the prediction of therapy outcome. The association of drug response with patient variability highly depends on the patient group under study. Of interest, the polymorphic nature of drug metabolising enzymes is even more pronounced across populations [10]. In addition, the differences in the DNA variants and their frequencies, in different ethnic groups is another confounding factor. This is important to appreciate since a pharmacogenetic test which is developed using data derived from a Caucasian population, might not reflect the requirements of an Asian population. The ethnic differences present drastic constraints on the worldwide commercialisation of pharmacogenetic tests and the standardisation of clinical protocols. Of course one has to mention that the constant inter-ethnic genetic mix in today's society offers an additional hurdle.

Pharmaceutical and diagnostic companies are working together to formulate SNP typing for particular drugs to allow predictive information (select the most appropriate drug) and drug dosing, prior to prescribing of a drug to a patient. The goal of SNP typing is to achieve optimal therapeutic response and minimise toxicity, including adverse drug reactions. The polymorphic nature and the differences between ethnic groups makes this very challenging, since genotyping developed using data from a particular population, does not necessary mean that it can be utilised worldwide. The main challenge for successful genotype-phenotype studies is the selection of patients, design of the study and a thorough and uniform registration of phenotypes. There are various variables that needs to be harmonised to allow population studies and meta-analysis. First, phenotype definition for proper selection of patients should follow specific guidelines. Questions on how are the efficacy and toxicity measured, and which thresholds are being used, if standardised therapy protocols are used, and at which dose is being administered, should be addressed before initiating any study. Secondly, outcome measures used for genetic associations, should be defined properly [11]. There are various outcome measures including (a) genotypic eg viral load; standardised qPCR kits, (b) biochemical assays (c) pharmacokinetics with also various measurable variables such as maintenance dose; half-life; (d) pharmacodynamics and drug response, and (e) clinical outcome. In addition, clinical assessment of a phenotype can be reported as a presence/absence value; grading and scoring of toxicity grade levels. In addition, there are various methodologies used to correlate genotypic differences with drug responses. Randomised controlled trials are commonly used to establish genetic determinants of drug efficacy and toxicity, although also this approach may not always give the clear outcome expected, as was recently demonstrated with two randomised controlled trials on pharmacogenetics for warfarin, where two randomised controlled trials reached opposing conclusions [7, 12], due to slightly different set up of these studies. One approach is to compare outcomes of genetic-guided therapy with clinical-guided care [7, 13]. This approach provides the required evidence, but the method will delay treatment. To overcome this limitation, the use of case-control studies is preferred. This study design depends on defining cases with adverse reaction or

reduced drug efficacy. Differential allele frequencies between cases and controls are measured. Case-control studies provide genotype-phenotype association, but require prospective studies to validate the effect of the genotype on the measured outcome. This variability in patient selection, outcome measures and study design, hampers the analysis of data shared from different studies, and results in failure to replicate pharmacogenetic findings [14, 15]. Hence, the lack of reproducibility of results slows down the implementation of genetic testing in the clinic, as was demonstrated with the *CYP2D6*/tamoxifen controversy [16, 17]. Although knowledge is increasing, the clinical response to drugs is still unexpectedly low, and this may be improved by implementing genetic testing in the clinical setting. The challenges to implement genomic medicine are discussed in the following section.

3 Uptake of Genomic Medicine in the Health Care System

The intended use of targeted drugs are clearly indicated on the drug labels. The health care provider is provided with clinically validated companion diagnostic tests to ensure compliance with the recommendations. To date the FDA labelled more than 120 drugs with label notifications that address the use of genetic tests to predict efficacy and toxicity of specific drugs, prior to prescription (http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm). The challenge is to encourage the adoption of companion diagnostics in the day to day clinical practice.

The expression and activity of various drug metabolising enzymes, determine the pharmacokinetic properties of the drugs, as detailed in Chap. 5. In addition, accumulation of active metabolites is associated with the occurrence of serious adverse drug reactions (ADRs), which is of major concern in therapeutics as explained in Chap. 6. A group of enzymes, the CYPs are the major contributors in drug metabolism and activation. Genetic variations in drug metabolising enzymes, such as thiopurine-S-methyltransferase (TPMT), cytochrome P450 2D6 (CYP2D6) and uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1) are germ-line variants that are useful to determine both pharmacokinetic and pharmacodynamics responses, as detailed in Chap. 10. Algorithms using information on genetic variants are used to determine the dose of specific pharmaceuticals. This genotype driven drug dosing has been extensively used in dosing of warfarin, which is described in Chap. 11.

3.1 Education of the Healthcare Professionals

Clinical implementation of new technologies does not necessary depend on the in-depth understanding of the technological process by the healthcare professionals,

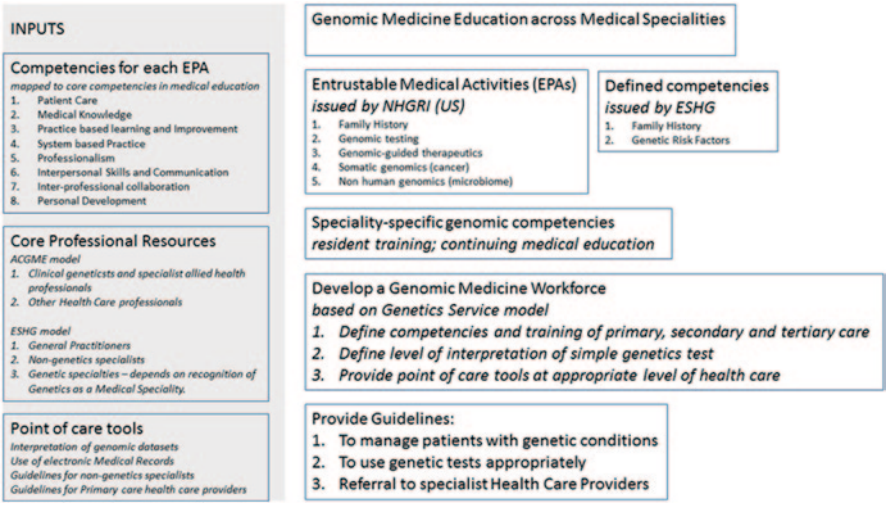

Fig. 2 Genomic Medicine Education across Medical Specialities. Composition of the proposed core professional resources, issued by the National Human Genome Research Institute (NHGRI) as a model for the American Council of Graduate Medical Education (ACGME) and competencies defined by the European Society of Human Genetics (ESHG). The *grey* box lists the competencies, target professional resources and tools required (inputs) and the *right* panel summarises the framework of the models including the deliverables of the proposed education programme

but is highly dependent on the presentation of results in a clear and user friendly manner, specifically addressing the clinical aspect that raised the need of the test. Hence, education of healthcare professionals, should include basic understanding of the technology used, but focus on knowledge to gain competency in the clinical utility of genomics.

The first challenge in providing the proper outcome-based education is to stratify the health care providers into core professional resources and define the competencies to be achieved for each group of providers (Fig. 2; [18]). Of interest the composition of the core professional resources, differ in the models proposed by the American Council of Graduate Medical Education (ACGME) and the European Society of Human Genetics (ESHG) [19]. The competencies are defined based on the desired outcomes [20], using an educational programme designed to establish competence in genetics throughout medical education, residency and speciality training [21]. Speciality-specific genomic competencies are defined to ensure proper residency training and continuous medical education. The proposed Genomic Medicine workforce includes training of primary, secondary and tertiary health care professionals [19, 22]. The educational programmes are supported by guidelines, providing details to use genetic tests appropriately, treatment and management of patients with genetic conditions, tools for clinical decisions, including proper referrals to specialist healthcare providers, use of electronic medical records and guidelines to support primary care health care providers [22]. Various models are current used to implement genetic services in the healthcare system, and different sectors, such as rare and common disorders require different means to deliver proper ser-

vices. Whereas, specialist genetic clinics drive the service for rare genetic diseases, inter-professional collaboration bringing together geneticists and other specialists is required for common diseases, such as cancer care [23]. The coordination of activities between different health care professionals in primary, secondary and tertiary care require well defined competencies, implementation of training and educational programmes, sharing of data through a regional hub with well-defined outreach procedures, and harmonised guidelines. An overview of the models used to implement genetic services are described in detail in Chap. 2. In addition to screening and diagnosis, the implementation of genetics services within the healthcare system includes the molecular classification of patients into therapeutic groups and predictive genetics/genomics with the ultimate aim to predict therapy response, resistance and relapse. Implementation of pharmacogenetics is purely exercised by specialists in the field and the educational component is mainly covered by continuous professional education and inter-professional communication.

3.2 Evidence Generation

The Clinical Pharmacogenetics Implementation Consortium (CPIC) under the governance of the Pharmacogenomics Research Network (http://www.pgrn.org), and the Pharmacogenomics Knowledge Base (PharmGKB, http://www.pharmgk.org; 24] provide open access, peer-reviewed, updated, evidence-based pharmacogenetic clinical practice guidelines [25]. Justification of pharmacogenetic test implementation require an understanding of the clinical utility and a level of evidence defined by the CPIC framework. Evidence generation is measured against a set of considerations including (a) a significant association between genomic variation/s and the phenotype, (b) the possibility to alter dosages or give alternative drugs for patients with high-risk genotypes, (c) the cohort (size and age group) used to generate the data. Genetic information is periodically updated online on the PharmGKB website. The Pharmacogenetic Clinical Practice Guidelines should be discussed in the various clinical disciplines to promote uptake in the appropriate clinical guidelines. The guidelines can take the form of a drug data sheet or indicate testing of a particular gene for various drug administration. These guidelines are based on studies in specific populations and due to global genetic variation, the guidelines shall be revised to conform to this variance. In addition, the relevance in terms of incidence and phenotype-association, of a particular genetic variant needs more investigation at a global perspective.

4 Current Technologies

With the advent of new technologies in the post-genomic era, a new field called pharmacogenomics has flourished. The aims of pharmacogenomics are to correlate genomic variations and gene expression with drug efficacy, response and/or

toxicity [26]. There are several genes, also referred to as pharmacogenes, which are associated with absorption, distribution, metabolism, excretion and toxicity of several drugs [ADMET; www.pharmgkb.org; Denus et al. 2013], that are actively studied using current technologies for gene specific or genome-wide typing. There are a number of genotyping methods, namely Polymerase Chain Reaction- or even microarray-based assays to perform genetic screening of known pharmacogenomic markers in well-documented pharmacogenes.

The most commonly used microarray-based platforms are the AmpliChip P450 platform (Roche Diagnostics, Basel, Switzerland), a CE-IVD certified assay that analyses 33 known *CYP2D6* gene variants, including 7 gene duplications and deletion, as well as 2 frequent *CYP2C19* gene variants (http://molecular.roche.com/assays/Pages/AmplichipCYP450Test.aspx). The Roche AmpliChip Cytochrome P450 Genotyping test is used in connection with the Affymetrix GeneChip Microarray Instrumentation System. This particular platform aims to assist doctors and medical specialists to prescribe personalised treatment options for their patients. Medical professionals can then use their patient's DNA profile to assist them further and better to prescribe appropriate medicine and at correct doses. In turn this will help reduce the unwanted harmful drug reactions and able to prevent patients from being improperly treated with sub-optimal and incorrect doses. The test uses human DNA extracted from either patient's blood or saliva to detect common genetic variants that alter the body's ability to break down (metabolise) specific types of drugs. The enzyme produced from the gene that is tested, called cytochrome P4502D6 (CYP4502D6), is active in metabolising many types of drugs including antidepressants, antipsychotics, β-blockers, and some chemotherapy drugs. Variations in this gene can cause a patient to metabolise these drugs abnormally fast, abnormally slow, or not at all as described in detail in Chaps. 5 and 6.

Also, the DMET™ Plus assay (Affymetrix, Santa Clara, CA, USA), allows simultaneous analysis of 1936 pharmacogenomic biomarkers in 231 pharmacogenes [27]. The DMET™ Plus Solution consists of a Molecular Inversion Probe (MIP) panel that amplifies the precise target DNA of interest, and the allele-specific oligonucleotide array provides a single color readout on the GeneChip® Scanner 3000 or GeneChip® Scanner 3000Dx v.2. The MIP method employed by the DMET™ Plus assay is an efficient technology for large-scale Single Nucleotide Polymorphisms (SNPs) analysis and hence well suited for SNP discovery and genotyping. This technique produces "inverted" probes in which SNPs are introduced into tag sequences that could be analysed using a universal sequence tag [28].

In addition, a cost-effective genotyping array using *TaqMan* chemistry is also available [29]. This method enjoys the concept of a customised array. The array is developed by utilising the Life Technologies QuantStudio 12 K Flex system with OpenArray technology. This array platform was designed with important clinical diagnostic elements in mind that are deemed important for clinical application of the genotyping information. Even more so, the turnaround time from DNA to genotype is only 5 h. The QuantStudio system requires minimal technical support time. Each SNP genotype is run in a separate reaction; therefore new SNPs can be substituted into the array based on new data, hence providing substantial flexibility.

Chips on the open array can be designed in any combination of 3072 SNPs, and heavily depend on the curated pharmacogenetics data and clinical annotations in PharmGKB database.

The above platforms, like every other genetic screening approach, have the inherent danger of missing novel unique or rare variants in ADMET-related genes, which may either affect ADMET gene expression or enzyme structure and may hence lead to variable response or increased toxicity in commonly prescribed drugs. The advent of next-generation sequencing technologies have created unprecedented opportunities to analyse whole genomes, which, unlike conventional medium or even high throughput genetic screening approaches, allows obtaining a full picture with respect to people's variomes. To date, whole exome and/or whole genome sequencing can be easily performed using several commercially available or proprietary platforms, to comprehensively analyse genome variation with a high degree of accuracy and with reasonable costs, compared to the not so distant past. Despite the fact that whole-exome sequencing is currently more cost-effective than whole genome sequencing, it has a number of limitations [30–32]: (a) our knowledge of all truly protein-coding exons in the genome is still incomplete, so current capture probes can only target known exons, (b) there is a degree of variability between the various commercial target enrichment kits, (c) regulatory and untranslated regions are not sequenced, (d) there is a significant bias in the target enrichment step, since the efficiency of capture probes varies considerably and some sequences fail to be targeted by capture probe design altogether. As such, not all templates are sequenced with equal efficiency, not all sequences can be aligned to the reference genome so as to allow base calling and as a result a significant proportion of variants may go undetected.

4.1 Next Generation Sequencing

The way we think about scientific approaches in clinical and applied research was changed by the introduction of Next Generation Sequencing (NGS) technologies and their ability to produce large volumes of data at a relatively cheaper cost than it used to be [33]. Different NGS platforms such as Roche/454, Illumina/Solexa, SOLiD/Life/APG, Helicos BioSciences and Polantor Instrument are commercially available and these technologies differ from the traditional Sanger sequencing mainly in three ways that include; (1) DNA sequencing libraries are clonally amplified in vitro; (2) DNA is sequenced by synthesis and (3) the amplified DNA templates are sequenced simultaneously.

4.1.1 Sample Preparation for NGS

One similarity between all current NGS technologies is the immobilisation of the DNA sample template to a solid support. This facilitates the sequencing of bil-

lions of reactions to be performed in massive parallel runs. Two different methods are employed by the different NGS platforms for DNA sample preparation. These are clonally amplified templates that originate from single DNA molecules [33]. For clonally amplified DNA templates, solid-phase amplification [34] or emulsion Polymerase Chain Reaction (emPCR) are utilised and they are the two most common methods used [35]. The solid-phase amplification employed by the Illumina/Solexa involves two consecutive steps. The primary step involves the initial priming and extending of the single-stranded DNA (ssDNA) followed by bridge amplification of the immobilised template with immediately adjacent primers resulting in the formation of clusters. This type of amplification can give rise between 100 to 200 million spatially separated clusters. A hallmark of this method is the fact that these clusters contain free ends to which universal sequencing primers can be added and hybridised therefore enabling the NGS reaction to commence [33].

In emPCR, the sequencing DNA templates are prepared in a cell-free system, resulting in the formation of fragment targets. The universal priming sites adaptors are ligated to the target ends. This allows relatively complex genomes to be amplified and extended using common PCR primers. The next step involves separation of dsDNA to ssDNA and these are then captured onto beads under conditions that preferentially bind to one DNA molecule per bead. The emPCR is used by different platforms. After successful amplification and enrichment of emPCR beads, in the case of Polantor platform, millions of beads are immobilised in a polyacrylamide gel on a standard microscope slide [36]. In the case of SOLiD/Life/APG platform, the beads are chemically cross-linked to an amino-coated glass surface [37] while in the case of Roche/454 the beads are placed into individual Pico Titre Plate [38].

Single-molecule template preparation requires much less starting DNA template than clonally amplified templates and it is perhaps easier to perform. Between 3 and 20 μg of starting material is required in clonally amplified templates whilst in single-molecule templates only 1 μg or less of starting material is required. This method involves the immobilisation of single molecule templates on a solid support that can be carried out by three different approaches before NGS reaction is performed [39]. The Helicos BioSciences platform uses two different approaches. The first approach involves attachment of spatially distributed individual primer molecules to the solid support. The template, which is then hybridised to the immobilised primer, is prepared by randomly fragmenting the starting DNA template into smaller sizes and common adaptors are added to the fragment ends. The second approach also involves the attachment of spatially distributed single-molecule templates to the solid support. These are covalently attached by priming and extending single-stranded, single- molecule templates from immobilised primers. This is followed by hybridisation of a common primer to the template. In both approaches, DNA polymerase binds to the immobilised primer template configuration to initiate the NGS reaction [39]. Pacific Biosciences platforms uses the third approach in which spatially distributed single polymerase molecules are attached to the solid support that contains a bound primed template molecule. In contrast to the first two approaches, the third approach can be used with larger DNA molecules and it can also be used with real-time methods, which give rise to potentially longer read lengths [40].

4.1.2 Sequencing and Imaging

When coming to sequencing, there are fundamental differences between sequencing clonally amplified templates and sequencing single molecule templates. When carrying our clonally amplification it gives rise to a population of identical templates each of which has undergone the sequencing reaction. When imaging is carried out, the observed signal is in harmony with the probes or nucleotides, which were added to the identical templates for a given cycle. Incomplete extension of the template ensemble can result in lagging-strand dephasing while leading-strand dephasing can occur when there is the addition of multiple nucleotides and probes. Signal dephasing give rise to an increase in fluorescence noise resulting in basecalling errors and shorter reads [41]. With single molecules, dephasing is not a problem but these can give rise to multiple nucleotides or probe addition. When this occurs, it gives rise to deletion errors that in turn result in quenching effects between adjacent dye molecules. In the case where there is the incorporation of a nucleotide or probe that does not contain a fluorescent label, no signal will be detected. For sequencing and imaging the different platforms uses four different strategies which are; (a) cyclic reversible termination (CRT), (b) sequencing by ligation (SBL), (c) single nucleotide addition—pyrosequencing, (d) real-time sequencing [33] and (e) DNA nanoball sequencing.

CRT technique uses reversible terminator nucleotides in a cyclic method that encompasses incorporation of nucleotide, fluorescence imaging and cleavage [42]. The first step involves the addition of one fluorescently labeled nucleotide by the bounding of the DNA polymerase to the primed DNA template and simultaneously any unincorporated nucleotides are washed away. The terminating group and the fluorescent dye are removed by a cleavage step and before the next incorporation step, an additional washing step is carried out. Reversible termination can be either of two types; 3’ blocked or 3’ unblocked [33]. The Illumina/Solexa Genome analyser uses a four-colour CRT cycle. All four nucleotides together with DNA polymerase are added simultaneously to the flow cell channels. These result in the incorporation of the nucleotides together with DNA polymerase into the oligo-primered cluster fragments. The nucleotides carrying a base-unique fluorescent label and the 3-OH group are chemically blocked and therefore each incorporation is a unique event. After each nucleotide incorporation, an imaging step follows. Once the imaging step terminates, the 3’ blocking group is chemically removed and this prepares each strand for the next incorporation by DNA polymerase [43]. Total internal reflection fluorescence (TIRF) imaging is used and it detects the four colours. The Helicos BioScience platform uses a similar methodology, however it employs a non-colour cycle.

Sequencing by ligation (SBL) is another cyclic method, however uses DNA ligase instead of DNA polymerase as in the CRT. In SBL one-base-encoded probes or two-base encoded probes are used. It involves the hybridisation of a fluorescently labeled probe to its complementary sequence adjacent to the primed template. Addition of DNA ligase follows and this joins the dye-labelled probe to the primer. Washing is carried out to remove any unligated probes. The identity of the ligated

probe is determined by fluorescence imaging [44]. SBL is used by SOLiD/Life/APG platform and sequencing of the *Escherichia coli* MG 1655 genome was carried out by the SBL method [36].

Pyrosequencing, which is a non-electrophoretic, bioluminescence technique, measures the release of inorganic pyrophosphate. This is carried out by proportionally converting the release of inorganic pyrophosphate into invisible light by using a series of enzymatic reactions [45, 46]. Instead of using modified nucleotides to terminate DNA synthesis, this method uses single addition of dNTP in limiting amounts to manipulate DNA polymerase. DNA polymerase extends the primer and then pauses upon the incorporation of the complementary dNTP. Upon addition of the next complementary dNTP in the dispensing cycle, DNA synthesis is reinitiated. The underlying DNA sequence is revealed by flow grams, which are recorded by the order and intensity of the light peaks [33]. Pyrosequencing is used by Roche/454 instrument.

Real-Time sequencing, used by Pacific Biosciences, is different from other sequencing methods, it does not halt the process of DNA synthesis instead during the process of DNA synthesis it involves imaging of the continuous incorporation of dye-labelled nucleotides [42]. The single molecule real time sequencer used in the Pacific Biosciences platform, segregates DNA templates and single polymerase molecules onto a plate containing thousands of nanometre-sized wells. Polymerase molecules are bound to the bottom of the wells and the fluorescence emitted from the bottom of the well is measured by a finely tuned optical system. Fluorescently labelled nucleotides are then incorporated into the wells, and once incorporation takes place, the optical system detects it and a fluorescent signal is given off [40].

DNA nanoball sequencing, is a high throughput next generations sequencing technology that is used to determine the entire genomic sequencing of an organism. The method uses rolling circle replication to amplify small fragments of genomic DNA into *DNA nanoballs*. Fluorescent probes bind to complementary DNA and the probes are then ligated to anchor sequences bound to known sequences on the DNA template. The base order is determined *via* the fluorescence of the ligated and bound probes [47]. This platform has been chiefly pioneered by Complete Genomics, that has recently merged with BGI Technologies and offering clinical and basic research at the whole genome level.

4.1.3 Data Analysis

NGS sequencing data can be analysed by a large variety of software tools, whose functions can fit into several categories such (a) alignment of sequence reads to a reference, (b) basecalling and/or DNA variant detection, (c) *de novo* assembly and (d) genome variation browsing and annotation [48]. Once NGS reads have been generated they are either aligned to a known reference sequence or assembled *de novo* [49, 50]. Intended biological application, cost, effort and time considerations are the basis on which one decides to use one strategy or the other [33]. Alignment to a reference genome in terms of computational methods is the simplest to perform

but relatively challenging to decide which reference genome to choose, and whether its ethnically correct for your samples of interest since this may obstruct the detection of structural variations and single nucleotide polymorphisms (SNPs) in the patients specimen. Alignment of short-read sequence data to a reference genome is performed by a variety of computational methods. The most common strategies used are either the performance of a Burrows-Wheeler transform to construct a matrix of all possible rotations of a given sequence or converting the sequence data into a servers of unique integer values (Hash tables) [51]. When performing de novo genomic assembly one must create long stretches of DNA sequence from shorter read length data. Algorithms have been incorporated into software programmes to perform successful *de novo* whole genome assembly [52].

4.1.4 Applications of NGS

The production of large numbers of low-cost reads makes the NGS platforms useful for many applications [33], including pharmacogenetic testing as described above. It has helped research laboratories to investigate disease-causing genes, and mechanisms by identifying novel DNA variants, that can lead to perturbation to transcriptional regulation and hence RNA expression. One of the biggest challenges associated with complex diseases is the identification of specific genetic loci underlying the disease. Until now, one approach to this challenge is to catalogue SNPs across the genome and then using genome-wide association studies to associate the variants with a particular phenotype [53]. In large-scale projects such as the International HapMap Consortium, the predominant methodology for SNP genotyping to date was the high-density SNP arrays but this approach has achieved its limited scope due to the density of the array [54, 55]. Single nucleotide resolution can now be achieved by the new NGS platforms and therefore rare variants can now be detected and categorised. Recently unknown causative mutations have been identified in a family with a recessive form of Charcot-Marie-Tooth disease using SOLiD platform while another family with primary ciliary dyskinesia and Miller syndrome [56, 57]. NGS can improve the detection of rare sequence variations in the discovery to novel somatic mutations in cancer such as acute myeloid leukaemia and myeloma [43]. With NGS the predictive power of GWA studies in the comprehensive SNP identification will be improved and this can lead to more understanding of complex disease trait loci and pharmacogenomics.

4.2 Haplotype Analysis

The determination whether two sequence variants are present *in cis* (on the same copy of a chromosome) or *in trans* (on opposite chromosome) is referred to as haplotype analysis. By using the Sanger sequencing protocols it is difficult to assess the *cis/trans* distinction but the new NGS offers a clever solution without the need to

carry out bacterial cloning through an *in vitro* clonal amplification step. The 454, SOLiD platform is able to carry out haplotype analysis [58]. Complete Genomics has also optimised accurate whole genome sequencing coupled with haplotyping [59] over the whole genome using a technique called Long Fragment Reads (LFR). LFR is similar to sequencing long single DNA molecules without cloning or separation of metaphase chromosomes. Other applications of NGS involve copy number variation, epigenetics, transcriptome anlaysis and metagenomics and minimal residual disease detection.

5 New Challenges and Recommendations

The main challenge in genomic medicine is to create the proper vehicle to communicate genome information within the healthcare system. Health care systems are heterogeneous and it is imperative to engage institutional leaders to promote electronic health records including clinical data, disease progression and therapy outcome. The Global Alliance for Genomic Health (GA4GH) aims to standardise data creation, access and retrieval in an effort to realise the full potential of genetic and clinical data in research. To achieve this objective the alliance established an international framework for data sharing promoting effective and ethically responsible approaches. Similarly, the International Collaboration for Clinical Genomics (ICCG) was set to acquire and annotate genomic variation with clinical utility information and submit the genotype/phenotype data into a database for public access. The Clinical Variation (ClinVar) Public Database [60] is an example of these databases. Various databases are being annotated and a concerted action to allow proper interface for simultaneous acquisition of data is required [61]. Hence creation of databases shall comply with an international set of guidelines that define the process of data submission, data formatting and sharing and ensure proper quality checks and curation. In addition, proper annotation depends on the clinical data captured and hence the clinical data elements must be clearly defined. In addition to clinical data, the collection of cost-related data elements are useful for pharmacoeconomic studies.

To the same extent, the presentation of the genetic data to the clinic shall ensure a simple and clear understanding of the use of genetic data to allow health care providers to perform genetic-based informed decisions. Adoption of electronic health records (EHRs) to store and interpret genetic results, based on clinically validated algorithms, provides a tool that will allow genetic based informed decisions [62].

Provision of clinical data between countries is challenging and might be one of the major hurdles. The issue of data-sharing at the level of clinical data is not only challenging, but creates concern in protecting patient identity and dignity. Hence, although data sharing at the level of genomic information is of great benefit, the use of clinical data should be restricted within a regional/national database. Algorithms generated following genetic data sharing, can be easily interrogated for clinical utility using annotated data sets within national databases.

In conclusion, a well defined interface between genomic data sets, clinical data, therapeutic options and dosing algorithms is required to create a vehicle to communicate genome data with the healthcare system. Provision of this interface needs to be accompanied with proper education and selection of actionable genetic tests with robust evidence to support the genotypic association with the specific phenotype and subsequently clinical action.

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Ethical Considerations in the Genomic Era

Bridget Ellul

Abstract Pharmacogenomics is a powerful molecular tool in biomedical research aimed at providing personalised medicine in everyday clinical practice, best described as the provision of 'the right drug for the right patient at the right dose', that is safe, effective therapy, with minimal adverse reactions. The patient, is the main beneficiary but is also the indispensable key player, providing biological material for research.

This chapter focuses primarily on ethical issues as they affect the patient undergoing pharmacogenetic tests for personalised treatment, the subject enrolled in a clinical trial or participating in genomic research or the healthy person donating biological material for biobanking and research. Issues affecting the other stakeholders will also be pointed out, but again mainly from the perspective of the consumer.

Discussion centres on the right to beneficience, explored through benefit to risk ratio and the right to autonomy, exercised through informed consent with safeguards to ensure privacy and confidentiality in the handling of biological samples and data. Elements of justice will be introduced in relation to the target of equitable access to healthcare.

The basic ethical principles must be upheld through regulatory frameworks. States have embraced various instruments, from local and international guidelines to national legislation, but as genomic research increasingly moves into the global non interventional arena, the vision is of facilitation of international cooperation through harmonised regulations.

Keywords Informed consent · Ethical approval · Pharmacogenetic test uptake · Data protection · Clinical trials · Biobank

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1 Introduction

The concept that genes play a part in controlling response to drugs was recognised in the late 1950s [1]. The speciality of pharmacogenetics [2–4] led to the discovery of specific DNA polymorphisms, notably in drug metabolising enzymes. As molecular techniques developed, in the late 1990's, pharmacogenomics emerged as a new discipline [5, 6] with an important role in the field of drug development. The identification of person to person 'variations of DNA and RNA characteristics as related to drug response' [7] can be applied to the quest for new drug targets and for safe drugs, balancing efficacy with minimal adverse reactions.

Though pharmacogenetics and pharmacogenomics are terms that are often used interchangeably, pharmacogenomics is centred on information from the entire genome. In fact it was the advent of new technologies, such as whole-genome sequencing, that drove research swiftly. Next generation sequencing, whole-exome sequencing and the development of bioinformatics, make the possibility of carrying out large population studies and data analysis, more feasible and will prove effective tools to identify biomarkers related to an individual's likely reaction to a particular medicinal product. This shall support the development of drugs and the required predictive genetic tests and companion diagnostic tests. Once such clinical application becomes an everyday reality, the promise of personalised medicine, the ideal healthcare programme, will finally be realised.

Such a goal requires solid interaction between the scientific and medical communities and the public. The index patient, or the healthy person seeking a predictive test, may well be seen as the ultimate beneficiary of personalised medicine but he is also the key player in the quest for the 'right drug for the right patient at the right dose' [8] since he is the one to contribute the biological material. This complex relationship must be fostered and nurtured on a sound foundation of ethical principles that enhance trust between all stakeholders.

Ethical behaviour in medical science, and specifically in genomic research, offers the foundation for the protection of the basic human rights of an individual and of society, but is also relevant to the other parties involved in research practices, the healthcare providers, the scientific investigators, the pharmaceutical and diagnostic companies, the funders and the policy makers. The perspectives of each group will vary but as all have a vested interest in a successful outcome, and since they depend on each another, there is a willingness to harmonise effective practices to move forward. However to guarantee that ethical issues are respected, this is not enough and there is a requirement for good governance, with a variety of regulatory instruments applicable at various stages of research.

This paper will focus primarily on ethical issues as they affect an individual, as a patient undergoing pharmacogenetic tests for personalised treatment, or as a subject enrolled in a clinical trial or participating in basic scientific research or as a healthy person donating biological material for research. Issues affecting the other stakeholders will also be pointed out, but again mainly from the perspective of the donor.

Ethical aspects will centre on the right to beneficience, explored through benefit to risk ratio, the right to autonomy, exercised through informed consent with safeguards of privacy and confidentiality, and on elements of justice, in relation to the target of equitable access to healthcare.

2 Personalised Therapy

2.1 Benefits v Risks

The pharmacogenomics target is to change healthcare management, in particular drug therapy, from general to personalised prescribing of evidence based effective and safe medicines. The benefits of taking the right medicine at the right dose, with minimal side effects are obvious. There can be little doubt that this is the ideal situation for the patient but it also embodies a prime objective in the provision of healthcare. Moreover providing optimum treatment is also a lack of maleficience, one of the main tenets of ethical medical practice. However the benefits must be weighed against the risks.

Personalised medicine is most practised in oncology [9]. For some tumours, genetic testing has become essential, and sometimes mandatory for Good Clinical Practice, before therapy is started. A success story is the clinical application of mandatory pharmacogenetic testing for gefitinib by the European Medicines Agency, EMA. Gefitinib, a tyrosine kinase inhibitor, is an epidermal growth factor receptor, EGFR, antagonist, and when used in patients with advanced non small cell lung cancer with EGFR activating mutations, it provides a significant increase in median survival [10].

However the best known example of pharmacogenomics testing is for variants of the enzyme CYP2D6 [11, 12] in the treatment of breast cancer with tamoxifen [13], where response to tamoxifen is reduced. Ethnic differences in genetic polymorphisms are very variable, with decreased activity, more commonly present in non Caucasians, making them less likely to benefit from tamoxifen [14]. Yet so far there is no mandatory regulation for pharmacogenetic testing prior to starting therapy. In fact recent review and meta-analysis concluded that there is ‘insufficient evidence to recommend CYP2D6 genotyping to guide tamoxifen treatment’ [15]. This exemplifies the problem of translation of genomic research to clinical use, some issues giving rise to risks for the individual, as will be discussed below. It also highlights the need for assessing the benefit to risk ratio for a particular individual.

The prime risks of a medication are the adverse drug events. Side effects exist for all drugs. In fact ‘any drug involves some kind of risk-taking on the part of the patient’ [16], but particularly burdensome are the serious adverse drug reactions requiring hospitalisation. However they are difficult to quantify, both as to prevalence and severity. Research has focused on the resulting hospital admissions, but often in individual hospitals rather than national [17] studies. The often quoted

meta-analysis study by Lazarou [18] way back in 1998 had estimated they were the 4th to 6th commonest cause of mortality in the states, when hospital admissions due to serious events were 6.7%. In a more recent overview of 95 published studies, related to hospitalisation following adverse drug events, with admissions ranging from 0.1 to 54%, there was such great variation in methodology that it was concluded that 'extrapolation based on a meta-analysis of unselected studies may be biased' [19]. Some have also attempted to quantify the economic impact from the burden on healthcare management [20, 21].

Marketing of drugs is based on a balance between efficacy and safety but for the patient, the decision to take a medicine depends on the benefit to risk ratio, which 'must always be compared with existing alternatives' [16], that is one must ensure that the proposed new therapy is better than the current treatment and management available [22] for each specific patient. However patients with disease causing serious morbidity may be prepared to take more risks, such as use a medication with higher adverse reactions than normally accepted by less ill individuals. Also one might be prepared to try a drug with serious adverse reactions if there is no other alternative available.

Availability and access to a specific therapy and its accompanying diagnostic test may be related to area of residence or may be a question of cost of treatment, not necessarily whether a medicine is actually on the market. This of course raises issues of justice and will be discussed later on in this paper.

3 Pharmacogenomic Tests

To benefit from personalised therapy, the patient needs to know whether his genotype puts him at risk for serious side effects or if a drug will be inefficacious or if an adjustment in dose is required. He has therefore to submit to a pharmacogenomic test developed for the relevant predictive biomarkers.

To put this into perspective, at present, pharmacogenomic information in drug labelling, by the Food and Drug Administration, FDA, is only available for about 150 drugs [23], with 16 having information about more than one gene. However mandatory pharmacogenomics testing prior to starting therapy is only required for 31 and recommended for 6 drugs by the FDA and for 17 by EMA [24]. Ideally regulation of diagnostic tests and drugs should occur together [24].

3.1 Informed Consent

The personal choice to consent to take a pharmacogenomics test respects the right of the patient to be personally involved in his own healthcare management. For valid consent, an adult must be competent to understand and evaluate options and so come to a decision. However for autonomy to be entirely respected, consent must

be genuinely informed and not be reduced to the legal requirement of validity and signing a form. The patient must be given the tools to reach a decision, specifically sufficient information, in a language they can understand, to effectively be engaged in evaluating the benefits versus the risks.

Consent by vulnerable patients such as the elderly or the very young may prove problematic as the person may not be fit to fully comprehend information regarding the state of health, let alone the relevance of a genomic test or of treatment options. These groups of people are well protected by regulatory mechanisms that insist on a guardian or legal representative to give consent.

Information should be provided about the specific indication for the pharmacogenetic test, the genetic abnormalities being detected and the interpretation of the result in relation to treatment options available. However the patient must also be made aware of issues regarding handling and secure storage of the sample and the data generated, including the long term dispositions and particularly if there are plans for use in future research. This allows the patient to assess the level of privacy and confidentiality afforded.

The patient must also be given enough time to consider all options and time to ask questions and clarify any confusing issues. The patient has the right to be informed as to benefits and risks, common and unusual. It stands to reason that choice must be free of any coercion. Healthcare professionals are ethically bound to offer only tests that are relevant to the medical problem and tests that are clinically valid, in keeping with good medical practice guidelines.

However there is also a fundamental right not to know [25] and such refusal of consent must be accepted, provided it is a genuine autonomous decision based on evaluation of adequate information.

3.2 Benefits: Uptake of Tests

For therapeutic purposes, when there is a definite recommended medicinal available, with the promise of beneficial impact on choice of treatment as well as a better patient outcome, most physicians value the tests [26]. Such tests are generally well accepted also by patients [27, 28] since the patient expects to benefit greatly from knowing the genetic variations which will predict the efficacy of the recommended treatment and/or whether there is any significant toxicity or if the dose needs to be adjusted. A 2009 study among a diverse US population revealed that 77% were ‘very likely’ or ‘somewhat likely’ to take a pharmacogenetic test [29]. A US patient survey, with just a response rate of 42%, showed that 73, 85, 91 and 92%, respectively were in favour of obtaining a test to identify if they were likely to have mild side effects, suffer serious side effects, to have the appropriate dose prescribed or to choose a specific drug [30].

Moreover it has been argued that a patient whose genetic test identifies an expected good response or at least an absence of serious side effects, is much more likely to comply with medication [31]. Taking a test may also be beneficial as it reduces anxiety [32].

3.3 Risks: Limitations to Uptake of Tests

3.3.1 Understanding the Value of Genomic Tests

For an individual the decision to take a test may actually prove to be a very difficult and painful decision. The primary reason may be that few patients can really comprehend genetic and genomic tests [33, 34] and appreciate the real benefits or risks. Healthcare providers use the term 'genetic tests' loosely for a multitude of different procedures that explore the function of genes and their products. So it is not surprising that there is a 'confused public perception of genetic testing' [35]. Just the mention of a genetic test to a patient can be a highly emotive experience, with the spectre of genetic exceptionalism in the background, leading one to immediately equate all tests with the possibility of establishing identity. There is also the immediate association that any genetic test must necessarily indicate inheritable disease of the monogenic type. The patient must be educated as to the possible value of a pharmacogenomic test, one that only provides estimates of risks for a particular variant, not definitive results [31].

Uptake of these genomic tests by society may depend on the perception of individuals as to the uncertainty of results of predictive testing. Patients require assurance as to the value of tests, that development is in line with the US ACCE framework model [36], which applies to analytic validity, clinical validity, clinical utility and associated ethical, legal and social implications that ensure adequate safeguards to the scientific measures.

Analytical validity is the ability to measure the relevant biomarker, with reproducible accuracy and reliability, which is the first step before any test can be developed further for the market. Estimate of errors in identifying a gene variant in whole genome sequencing is given as less than 0.5% [37] but this does assume great importance when dealing with rare variants of disease. Different results in genome sequences of the same sample have been quoted as between 4–14% [38]. Such variances may give rise to imperfect or erroneous deductions in the interpretation of a predictive test.

Many cannot appreciate that the clinical validity lies in the ability of the test to identify the phenotype from the genotype [39]. Clinical validity is a function of the complex relationship between penetrance of the genomic variant, gene heterogeneity and the test sensitivity and specificity. Genomic tests, though less invasive than phenotypic tests, often have lower sensitivity and specificity [40]. Variations of the latter two test characteristics will give rise to false positives and false negatives.

A low predictive value may be one of the reasons why a test is underused [39]. However it may be even harder to explain that clinical validity may be much lower than expected because response to drugs is not limited only to genetic factors. There is interrelationship with the environment, lifestyle, age, race, comorbidities and other drug treatments [6, 39]. Moreover gene variants are sometimes pleiotropic [41], and are associated with more than one disease or drug response. The contribution of the genotype to a particular drug response is reported as very variable, anything between

20–95 % of all variability [42]. For warfarin, over 40 % of variability in dose requirement cannot be attributed to any known genetic or non genetic factor [43].

Moreover genuine laboratory errors may also occur, for example analysis of the wrong sample or a problem with techniques or equipment. Laboratories providing genetic tests should be accredited and there are established practice guidelines and standards for laboratories conducting pharmacogenetic tests [44] and for ensuring quality assurance of genetic tests [45].

To be useful in clinical practice, a predictive test requires to show clinical utility [39]. It must reflect the expected health benefit attributed to the result of the test, which may include adherence to the drug, for which the patient was tested [46]. Realising there is a specific treatment might actually improve compliance to drug taking [31].

A new concept is that of personal utility [47] that is how a test will prove of benefit to the patient in terms of disease outcomes. Obviously for pharmacogenomics tests, this relates to how useful is the drug therapy available or how important it is to avoid a particular drug with serious adverse reactions. This may be a very individual assessment, depending on the seriousness of the disease being managed. Clinical utility may however also spur a healthy person to take a test in the asymptomatic stage, just for relief of uncertainty. This alone may provide psychological benefit. On the other hand lack of sufficient clinical utility may still push a patient with a serious disease to ask for the therapy even though the test result may not be promising [48].

The test result will always have a psychological effect with the risk of anxiety and depression from false positive tests to misplaced relief or a euphoric state from false negative tests. However a negative test, or one that suggests a reduction in drug dose, may not only cause anxiety but may make the patient unduly worried of the inability to take the recommended therapy at the usual dose, and so the patient may not adhere to what is regarded as less than best therapy [31]. The latter scenario may prove most difficult since it gives a false hope, which is doomed to total shattering effects if the disease actually manifests itself, let alone opening the spectre of litigation for the clinicians. An unexpected lack of response or increased risk may thus occur leading to possible litigation [31, 49]. This alone should encourage the doctor to think twice about which information to give the patient and the reliability of the test is one piece of information which should always be imparted.

Understanding pharmacogenetic tests is even more difficult when the patient has a lower educational level [50]. In a survey of oncology services providers, many cancer patients are thought to be unable to ‘adequately comprehend the purposes and complexities of pharmacogenomic testing’ [9], in particular in appreciating differences between somatic and germline testing and that only the latter have potential for inheritance and an effect on family members.

It is also probable that uptake of tests depends on the effort made by the healthcare provider in obtaining consent. The onus of promoting a test through enhancing the patient’s understanding lies with the medical provider, who is morally and professionally expected to aim at maximising benefit to patients. Yet studies indicate that there is little expertise in genomics among clinicians in oncology [9]. This leads

to problems in provision of services [26] and in giving the right advice to patients and/or in interpreting genetic results [51].

Clinicians also worry that they do not have adequate guidelines how to use tests [52], although now there are many such guidelines [52, 53] to consult. Canadian cardiologists, oncologists and family physicians identified various difficulties that prevented use of pharmacogenetic tests, mainly lack of clinical guidelines (60%), lack of personal knowledge (57%), no evidence based clinical information (53%) and expense (48%). 37% also recognised that they did not have the time and resources to educate patients [26]. US primary care physicians also reported being uncomfortable with the level of knowledge expected to interpret the genomic tests [54]. There is little formal training of healthcarers, with as many as 92% reporting no formal undergraduate training [26, 55] and there is a good argument for introduction or increase in the teaching of pharmacogenomics in medical curricula [56–58].

Such physician surveys highlight the need for adequate explanation and counselling by well trained individuals. Counselling requires ensuring that the patient understands the implications of testing, whichever result is obtained. This requires commitment by the healthcare providers to explain in lay man's terms and to ensure there is adequate understanding. Such consultation is time consuming. With regard to counselling for hereditary disease, there are recommendations for counselling pre and post test when the disease is severe, with the counsellor giving the patient sufficient time to weigh up the odds [59].

3.3.2 Discrimination

Patients do refuse to take tests because of fear of the test results finding their way into the wrong hands, such as an employer, or an insurer, which exposes them to discrimination. Those with higher levels of education express fewer concerns about possible misuse of genetic information [34]. Despite anti discriminatory laws being enacted in all democratic countries, patients are still worried [9]. In a Canadian study, 40% of clinicians admitted that their patients had suffered from the fear of discrimination in relation to genetic testing [26].

Even when there is legislation against discriminatory practices, it may not offer comprehensive protection. The US Genetic Information Nondiscrimination Act, GINA, prohibits employers from discriminating against their employees on the basis of genetic information and prohibits health insurers from refusing to provide insurance, or asking for higher premiums, on the basis solely of genetic tests, but it does not offer protection for life insurance or disability insurance.

Legislation usually takes a firm stand against discrimination in terms of employment because the right to work is a fundamental human right but as to insurance, this is often a personal voluntary choice of the consumer to buy certain products. There may be instances where the consumer feels coerced into making a choice, for example in requiring insurance related to certain transactions, like obtaining a bank loan or buying a house.

3.3.3 Ethnicity

Certain gene variants will be predominant in particular ethnic groups. So clinical validity is higher in such groups. In a multicultural society, due to heterogeneity and cross culture, gene variations within a racial group, may differ more than the variations between different races and so there seems to be no need to have gross racial sub classifications [60]. A focus on race might not take into account the environmental factors. However one cannot negate the fact that certain drugs are certainly contraindicated in certain ethnic groups, e.g. ACE inhibitors in African Americans, so there certainly remains scope for more research into racial genetic differences. African Americans are more likely than other groups to believe that genetic test results will be misused [34], that genetic test results lead to racial discrimination [61] or for the racial/ethnic group to be labelled as inferior [33, 62].

3.3.4 Privacy and Confidentiality

Patients also feel threatened by the risk of breach of privacy and confidentiality. In a telephone survey of US adults, 78% stated that they were unlikely to have a pharmacogenomic test if there was a risk that their DNA sample or test result could be shared without their permission [30].

The family doctor offering companion diagnostic tests may also be put in a dilemma as to whether they should inform the family, of any positive results, especially when they are also the doctor's patients. This disclosure is always to be considered as unethical professional behaviour, without the consent of the index patient. In fact a person may refuse to be tested just because of fear that they may be asked to make their test results available to relatives. Although one can argue, from an altruistic point of view, that such disclosure should occur, most patients are reluctant to show others their disease status. Also, patients may be prepared to tell their family doctor the result of a pharmacogenetic test but they may have some reservations at sharing the results with other healthcarers involved in their health care management [63].

If tests become widely available, a healthy patient might decide to take a test years before he is likely to develop a disease. So the result will end in his medical file [64]. Protection of data from access by third parties must therefore be ensured through regulatory instruments. Patients should be reassured as to storage facilities for samples and data from results, both paper and electronic formats.

What does the physician do if a patient refuses to take a pharmacogenomic test? Should the medication still be provided, even if there may be adverse reactions? The answer lies in ensuring real informed consent has been obtained because an individual retains the fundamental right of refusing treatment. However physicians are concerned as to possible litigation in the future from the patient or the family.

The American College of Medical Genetics recommends that incidental findings obtained in a clinical (not research) setting should be disclosed to the patient and the clinicians [65]. However not everyone agrees with these guidelines [66–68] and

it is best to have an agreed policy at the time of the initial consent, with the added safeguard of utilising counselling if there are unexpected results.

3.4 Direct to Consumer Tests

It is increasingly possible now to obtaining genetic information without direct consultation of a physician by purchasing personal genome tests, PGTs, also called direct to consumer tests, bought directly through the company developing and marketing the test, or via retailers. Commercially available tests, based on whole genome sequencing are now widely available for multifactorial diseases and some also for pharmacogenomic tests. The companies may offer a bundle, with tests providing results about several diseases or groups of diseases, or about therapy options, which vary in nature and therefore also have differing ethical implications. Some pharmacogenomic tests for antidepressants are also being combined with susceptibility tests [69] in psychiatric disease and this raises concern in relation to clinical validity of such tests [70].

Common to all there is the central issue of consent and how to ensure that it is really informed. Article 7 of the Additional Protocol of the Council of Europe on Genetic Testing for Health Purposes [71] states that 'a genetic test for health purposes may only be performed under individualised medical supervision' with a view to offering protection to the person tested and also the possibility of informed consent and counselling.

Bunnik et al state that 'because of the complexity and the quantity of the information offered in PGT, informed consent cannot be fully specific' [72]. They propose a model of consent, with three layers, tiered, layered and staged, which can also be intertwined. Tiered consent is based on giving a choice to the individual as to which type of disease the consumer is interested in. The layered consent relates to the amount of information made available at different times, starting with a minimum basic amount of knowledge, labelled as the first layer; so there are options to know more, with the choice left freely to the consumer. Staged consent refers to provision of information over a specific timeframe, when the consumer has to give consent at various stages of the process, in relation to a certain process, for example pre purchase of the test, prior to being sent the results and prior to receiving updates.

The other ethical dilemma is disclosure of information. The companies provide different levels of assurance as to confidentiality and disclosure of information for their clients. Consumers have expressed a preference for tiered consent schemes that allow individuals to specify the level of data sharing permitted with respect to their genome [73]. Of course not everyone is prepared to share their results, not even with their doctor [74].

There has not been any strong evidence of harm to consumers from availability of direct to consumer tests. In fact a study revealed that the type of information received did not result in any psychological harm [75]. Possibly this reflects the personality of the person willing to obtain such tests.

Other concerns relate to criminal abuse, such as the possibility of submitting biological samples of third parties who have not actually consented, as well as lack of transparency as to what type of research is carried out on the samples submitted [76].

4 Research

4.1 Clinical Trials

4.1.1 Informed Consent

The ethical issues related to research in general and to clinical trials in particular are well established and safeguards are faithful to the principles in the Declaration of Helsinki, with its latest amendment in 2013 [77]. From a participant's point of view, informed consent, privacy and confidentiality are guaranteed through scrutiny by Research Ethics Committees, RECs, based in universities and in health departments and institutions, sometimes covered by state legislation. For clinical trials there is specific legislation in most countries reflecting the higher stakes. In Phase I research there may be healthy volunteers while in Phase II or III clinical trials, the patient may get the placebo or the least effective drug. So the information prior to obtaining consent has to be comprehensive and transparent and well explained.

In the EU, states have transposed the EU Directive [78] into national legislation. The Directive lays down detailed guidelines as to what the RECs should assess. Again there are guidelines available through the Oviedo Convention [25] and the Additional Protocol [79], which clearly distinguish subjects capable of giving consent from those who for some reason (age, mental infirmity, and emergency situations) are unable to consent. Research on such vulnerable people, including persons with mental impairment and minors, carries the same problems in relation to consent, as that for diagnostic purposes but there are some specific issues. The subject should only participate if the trial is personal of benefit, or to others suffering from the same type of disease, or if there is no other way of obtaining the same information. Although not able to understand all information, vulnerable individuals may be able to decide and consent to simple procedures and to take part in the decision making process. With respect to children, a child should be involved in evaluation of the benefits and risks of participating in research, and in coming to a decision, in accordance with maturity but consent from the parent or legal guardian is also required, though the minor is allowed to object [80, 79]. Moreover if a competent child refuses trial participation, they must not be coerced to participate just because the parents agree to participation. Ethics mandates that their wishes should be respected, even though legislation only requires consideration of their views [80]. Yet again the main problem here is ensuring adequate information and time to enable the potential trial participants to make up their minds.

Obtaining consent in multicentre clinical trials may be hampered by lack of harmonisation of law. Even in the EU states this has this been possible, for example there is no harmonisation of standards for ethical committees.

4.1.2 Consent for Genetic Studies

Consent for genetic tests, either on blood or tissues, in a clinical trial is usually obtained completely separately from the consent for the rest of the protocol. However the content of the legally binding form to be signed and the information supplied, vary from one trial to another.

The main problem is obtaining consent for future studies. The Oviedo Convention allows additional use of biological materials if 'done in conformity with appropriate information and consent procedures' [25]. The pharmaceutical companies claim that often it is impossible at the first instance to outline exactly the future research. So researchers have sought different models of consent, focusing mainly on broad [81, 82] or open consent for any future research, whether of a genetic nature or not, that is, there would be no need to get back to the trial participant to ask for consent for future studies, or for studies not contemplated in the original design of the trial or because new technologies become available. However broad consent does not mean 'vague' [83] but broad in relation to the original idea of consent to a specific protocol. It usually implies consent related to future research, either on the same disease or some new biomarker, but not tied to a specific project. Effectively this is 'consent to governance' [81] by some authority or person to take the decision in future as to whether to use the material or data for research. However sometimes such distinctions are not even mentioned and this actually amounts more to 'blanket' rather than a broad type of consent. The only safeguard is the requirement to have the future project reviewed by a REC, which is always a prerequisite for substantial amendments to a trial.

Other researchers may opt to give the participant the option to choose whether to be recalled in the future for further consent. Yet this is very cumbersome, not to mention that of course it limits anonymisation of material collected, as the participants have to be traced to be recalled, thus compromising privacy.

There is some disagreement as to whether broad consent can ever be equated with genuine informed consent [85, 86] although most ethicists are in favour of its use and agree that this is a decision that fulfils the original intention behind the introduction of informed consent, that of ensuring the participant's autonomy is protected [82]. Similarly the trial participant may affirm that they do not want to be re-contacted in future and are prepared to give authorisation for the researchers to use their material anyway. From an ethical perspective such a position would also be a voluntary decision and thus guarantees the principle of autonomy [87].

Legally, broad or open consent or waiver of consent cannot by its very nature be considered as informed consent as covered by the EU Directive or by the Council of Europe. However the Nuffield Council on Bioethics did approve broad consent for the 'use of samples that are anonymous or anonymised' [88] while it recommended collection of a separate broad consent if the samples where identifiable.

The possibility to opt out of the trial, must be present for the length of the trial, but also for the stored samples and data. It may be possible at the outset to refuse to be part of the genetic research arm, or to refuse to all future research, although often these comprise exclusion criteria from the trial. When opting out occurs after destruction of the biological sample, the data already collected is generally retained for the research - this must be highlighted in the original consent.

Finally, the issue of coercion arises when a patient agrees to being a trial subject if they obtain access to a new medicine, which they hope will work [88]. Is this a valid consent? One can argue that this decision is conditioning a better outcome in the participant, akin to a placebo effect.

4.1.3 Privacy and Confidentiality

Participants may be worried about privacy and confidentiality of stored biological samples, and sharing of data and results, which in the EU are offered protection through the EU Directive [88, 89]. However Data Protection laws are not comparable in non EU states.

The EU Directive on Clinical Trials does emphasise the 'rights of the subject to physical and mental integrity, to privacy' in accordance with the Data Protection Directive. In general privacy issues related to fear of discrimination are not an issue, as occurs in the diagnostic field. However subjects need to know that insurance companies do not get access to data. For this reason, they may be more likely to agree to participate in a trial if there is anonymisation of samples, though they may be satisfied by coding which allows them potential access, particularly if they trust the pharmaceutical company or researcher.

However the problem of confidentiality is paramount for uncommon orphan diseases, particularly in a small community. With rare diseases, it seems pointless to have anonymity when it might be beneficial to contact the participants to impart individual results. Should there be disclosure of results to such participants, and/or to other family members, particularly if the information is beneficial to them or their family? Potential participants should be encouraged to speak with their families regarding genetic trials, so that the subject can share information with family members [90–92].

4.1.4 Data

There is a duty on the scientists to impart sufficient information at the time of recruiting participants to a clinical trial to enable informed consent. The potential trial subjects should be told about the benefits and risks of the research but there is a need to balance the knowledge divulged with what a reasonable person would expect to be told and to express consent forms in a straightforward unambiguous language. The investigator should find out what is important to a specific group of persons or ethnic group or study group in the inclusion criteria.

Subjects must be informed as to the length of time and site of storage of biological samples and data, the security provided and who has access to the data, whether

only researchers or local authorities or third countries, in conformity with Data Protection legislation.

In the process of data sharing, data should be anonymised and some countries have regulatory mechanisms in place, particularly in the case of clinical trials, e.g. EMA guidelines. Data sharing is of course enhanced and expedited through publication in open access journals but this needs to be funded. A survey among trialists reported willingness to share data among respondents (albeit there was only a 46% response) but they were concerned about appropriate interpretation of data, protecting their own interests with respect to publication or academic recognition, as well as some concern about patient confidentiality being maintained [93]. In the interests of the public, negative results should be reported, so trials can be repeated.

There has been a campaign to increase the transparency of clinical trials and to make results available to the public. It is not clear how companies decide which information to make available to the public or third parties. RECs should actually make sure that both trial registration and the publication of results are mandatory prior to ethics approval [94]. EMA's policy of providing clinical trial data to third parties was weakened due to legal action from pharmaceutical companies. Similar incentives are happening in many countries. Once the EU Directive regulating clinical trials is repealed in 2016 and replaced by the Clinical Trials Regulation [95] registration of all trials in the EU will become mandatory as will the publication of trial results. A full study report must be published in line with guidelines by ICH [7] and again this would become available in the public domain.

Trial subjects may worry about the commercial interests of the company overriding their basic rights. They have to be informed as to commercial interests of the company as well as to the fact that intellectual property rights are vested with the sponsors or the pharmaceutical company.

Drugs that are new on the market need to be followed up for a considerable length of time, in fact ideally throughout the drug's lifetime, the so called 'life-cycle approach to risk management' [96]. This will ensure that rare side effects are identified [16, 96]. EMA guidelines [97] aim to strengthen evidence about the effect of genomic labelling and the use of genomic biomarkers in the post marketing stage, to use it in clinical practice. Post marketing surveillance is when certain rare adverse reactions are identified. These may be due to complex interactions of genetic variations with environment and lifestyle. The pharmaceutical companies may proceed to utilise these results to develop personalised medicines.

4.2 Genomics Research and Biobanks

4.2.1 Informed Consent

Genomics research may lead to development of new drugs, which can then be assessed through clinical trials. By its very nature such research requires a large database so as to enable examination of genomes from a large number of individuals, to obtain meaningful results that identify either susceptibility genes for specific diseases or for variability in drug responses.

This has led to the establishment of biobanks, repositories of biological materials and / or data derived from such samples, from specified populations, sometimes healthy individuals, invited to participate in being donors for research purposes. Some material is in private collections but most is in the public domain. Many banks contain more information, in the form of personal data, which may be also linked to medical and lifestyle data. This data is necessary because of the interrelationship between genes and the environment but it is often linked to other information held on a national level, for example social security or tax information.

Such biological collections are now often managed by multinational groups or consortia. This means we have entered the global era of research where international collaboration is the only way to obtain sufficient research material that can be shared globally for meaningful results for practical clinical application.

Recruitment is increasingly coming indirectly through the use of the biological material already stored in national archives or biobanks. Consent is obtained at the time of donation of samples, often long before the research project is fashioned. The commonest type of consent used currently is broad consent [98]. Such research is non interventional and offers a low risk to participants [99], seeing that most results do not apply directly to the individual who has submitted the material; this renders broad consent more acceptable. To donors, 'practical utility' may be more important than knowing the details of each project [84].

As for clinical trials purposes, broad consent is acceptable provided certain safeguards are in place; these would include measures to maintain confidentiality by ensuring all personal information, whether in the form of biological material or data, is stored securely in a suitably coded fashion. Anonymisation is not usual as it would preclude clinical monitoring or adding new data to the bank. Maintaining privacy is important because of the links to other data. Consent should include information related to who has access to data and with whom data is shared. The principal investigator should not be involved in obtaining consent but a contact person needs to be identified.

The autonomy of consent can also be preserved if, within the constraints of the biobank set up, donors are allowed the possibility to have samples and data withdrawn [100]. A crucial safety measure is to ensure adequate governance regulations of the biobank facility and to have RECs approval for any new research project that uses material from the bank.

Because most of the research is carried out by international collaboration, of different bodies, difficulties arise in obtaining consent from the individual national RECs. In an effort to simplify such authorisation and to ensure it is timely, there is a need for setting up a framework based on cooperation between the multiple research centres, be they health institutions or industry based companies, with consideration of the local policies, regulatory mechanisms and legislation.

A recent recommendation is to set up a Safe Harbor Framework for International Ethics Equivalency [101], creating an International Ethics Review body that harmonises procedures based on the same principles and satisfying the varied national legislation [102]. Management will rely on electronic means to expedite matters.

One way to promote donation to biobanks is to induce patients to donate residual biological material after diagnostic procedures, material that may otherwise be discarded after biopsy [103] or even to obtain material during recruitment in clinical trials. This may be quite acceptable for donors as it benefits them by promoting a sense of altruism in helping society. Some ethicists argue that there is no need for consent [104], particularly if the material stored can be considered as having already been discarded by the patient. On the other hand there are advantages to the patient giving consent. This would enhance trust between patients and researchers, auguring well for future participation in research, since it would show respect for patients' views. They may have strong objections to being involved in any genomic research, especially if it has commercial potential but may be prepared to consent to use of their biological material if the research allows clinically relevant results to be passed on to the patient or their family [105]. Current opinion is that national banks should be able to inform clients of any positive results if it is going to be to their benefit.

In fact in a study [106] of the UK public in 2012, 55% of those surveyed believed that it was 'extremely important' and 25% that it was important to be asked to provide consent for residual samples, and the majority agreed to have an opt in type of consent, to speak directly to a healthcare professional and not just fill a form. 27% of those surveyed and 57% of those in focus groups did prefer an opt out type of consent. However this again would be broad consent at the time of collection.

Much biological material is already present as archived biological material, initially retrieved with another purpose in mind, in particular human tissues in pathology departments in all hospitals, previously collected with consent for diagnostic purposes. The question is whether such material should now be available for genomic research and then how to obtain consent to use such material. Recall of patients is very unlikely to be feasible. In some countries, legislation provides for use without consent if there is appropriate protection, such as ethics approval by RECs, for each new project. If samples are securely coded and stored and access to data is limited, and if the donor has not specifically refused consent, then most agree that specific consent is not necessary [107]. The Council of Europe provides for use without consent if reasonable efforts to contact the owner are not possible, provided the research is of important scientific interest and could not be addressed by using other biological material for which consent was available [108].

There should be a policy as to what to do with samples from minors. Should these be initially excluded from inclusion in a biobank until the minor turns 18?; should they then be contacted for consent?

Other models of consent may be used, such as the authorisation model [85], which allows the donor to decide for which type of research they are willing to contribute and it is up to the donor to lay down particular conditions as to the level of involvement they want in the future, especially regarding recall for future consent or not. Staged or stepwise [109] informed consent would allow potential donors to understand what is happening. Dynamic consent [110] takes involvement a step further as the donors are kept informed by the researchers as to what is happening and are asked to re-consent, with various interactions occurring between the donor and

the biobank, regarding samples and data use. It is claimed that this would increase recruitment, trust and transparency. However Segerdahl [111] has been critical of such consent, claiming that this places a lifelong burden on the donor while the researchers have the liberty to move on.

Another problem particular to biobanks is what would happen to banked material after death of the donor. The OECD [112] leaves the options to the biobank but the relevant policy must be made known to the participant from the outset. The sample and / or data could be withdrawn or the option given to relatives to decide or they can be anonymised.

Biobanks have variable policies [113] regarding return of unexpected findings to research participants, whether these are incidental findings unrelated to the aims of the research or whether they are 'individual research results' that are part of the study variables, with less likelihood of the latter being passed on to the subjects. Such policies must be clear before consent is obtained.

4.2.2 Data Privacy

Now that technology has really advanced, the limiting factor in genomic research is actually the data analysis. Electronic records facilitate the extraction of results but electronic data is never really completely safe. With today's bioinformatics tools it is possible to analyse large public data bases and use data linkage disequilibrium to identify individuals, even if data was anonymised.

Electronic medical records may be less secure than envisaged but even researchers have claimed that 'efficiency and utility of securing accurate personal genomic information through genomic testing and electronic medical records may outweigh patient privacy concerns if cancer treatment outcomes can be improved' [9]. The US has an electronic Medical Records and Genomics network, eMERGE, with biobanks linked to electronic medical records, specifically aimed at finding genomic markers and genotype-phenotype associations.

The Personal Genome Project [37] hinges on publicly sharing genome date from self referrals, such genomic data being combined with public health data. The participants are 'explicitly not promised anonymity' because the project leaders argue that although protection of data is possible in such research, privacy cannot be always ensured when there is public release of data for sharing among researchers.

Therefore it is crucial to ensure confidentiality. This is possible in EU states where it is covered by the Data Protection Directive [89]. However the collection, storage and sharing of samples is not harmonised. Data may be transferred across the EU in line with the Data Protection Directive but transfer to third parties is allowed if they are deemed to have data protection laws similar to the EU; this excludes the United States of America. In an attempt to solve this impediment to sharing information, in 2000, the European Commission and the US Department of Commerce agreed on a Safe Harbor Framework, agreeable to both sides [114] for ensuring privacy.

It is a much bigger problem if such material is unfairly disclosed to authorities for educational decisions, employment and legal decisions on culpability [87]. This

will erode donors' trust in the system but there is no specific legislation as yet, at least on an EU level, to give biobanks the right to protect their data at all costs. It is envisaged that the data may be useful for allocation of health resources. Accessibility to biobanks must therefore be controlled.

5 Responsibilities of Other Stakeholders

Commercial companies in the pharmaceutical industry have a duty to society to invest in research and development of new drugs to provide efficacious and safe drugs through properly conducted clinical trials. But Phase I development of new drugs is expensive and traditionally drugs have been aimed at 'one size fits all'.

It is now accepted that it is unethical to ignore the needs of non responders, when such reaction is the result of recognised pharmacogenomic differences that can be targeted in drug development for the production of personalised drugs for these people.

However if the number of potential patients is low, it will not be economically feasible for a pharmaceutical company to invest in drug development specific to such patient groups since the drug will be too expensive to manufacture, even if altruistically this will be of long term benefit to such individuals, if not a life saver.

The company may need to look into allele distributions for specific populations before deciding to invest in developing drugs for such a group of people. Of course this seems unjust to a minority of people with orphan diseases.

Some have argued that companies have to be given incentives to pursue drug development, such as allowing them exclusive research for rare diseases for which they aim to provide therapy. This may be public funding [115], as happened for orphan diseases, for which research has been adopted by international networks. The US Orphan Drug Act of 1983 allowed tax refunds and 7 year exclusivity for drugs for orphan diseases.

Knowing which individuals are likely to benefit from a new drug based on their genetic variations can lead to clinical trials only in patients with the particular genotype, thus leading to a new personalised product at a quicker rate. This minimises costs.

Maybe this is why the major investment has been in personalised therapy in oncology. The increasing number of elderly people has brought about an increase in the prevalence of cancer and an easily available source of biological material to work on. However, one can argue that either someone looked far ahead and invested in oncology work, or it was fortuitous and initial success led to development of more drugs in oncology [9].

A company may also consider to rescue drugs that have failed to pass a clinical trial because the majority suffered toxic effects. Yet the drug may be developed for the relatively few responders. Such a scenario is more likely for specific ethnic groups [116], as happened with BiDil (isosorbide and hydralazine) [117]. Repurposing of drugs, the use of approved drugs for another indication, may also prove possible.

The Nuffield Council on Bioethics [88] had proposed that licences for marketing of medicines could be tied to a pharmacogenetic test to ensure that it is carried out and not bypassed in a measure to avoid costs.

5.1 Justice

Healthcare providers may be willing to allocate resources for pharmacogenetic tests or personalised drugs if there is evidence of the benefit in terms of savings in health expenditure, in the long term, for treating the rare conditions.

More commonly the problem is that the tests are still expensive [49] and for pharmacogenetically designed drugs they are likely to be more expensive tests, leading to inequalities in access [118]. In fact not all genomic tests are covered by insurance [119] and this deters patients from obtaining the test and moreover the availability of insurance cover is not uniform, for example in the US, this varies from state to state [120].

Access to genetic diagnostic testing may be limited for various reasons, including residence, distance from healthcare facility, socio-economic status and insurance coverage. It may not be available at all in certain countries or at least it may only be available in tertiary centres, rendering access difficult and entailing expenses to contact the providers. If the test is positive, there is the problem that the relevant therapy, again likely to be more expensive than for common diseases, may not be available or may not be free to all. Treatment for rare conditions is much more expensive than expected. Sometimes the impetus must come from lobbying by patient groups to put pressure on insurance companies to provide cover and on healthcare providers to make expensive tests available [3, 121].

Pharmacogenomics will benefit society by improving the benefit to risk ratio for a particular drug in a particular population but it can never guarantee improvement for an individual [49]. Putting the onus on a person to take decisions about their health can be interpreted as empowerment but it may also be placing a burden of responsibility on their shoulders, instead of healthcarers. Patients might have to conform to social expectations [122].

6 Outcome and Recommendations

It is the hope of all stakeholders, but primarily of the main beneficiary, the potential patient, that the promise of pharmacogenomics to provide ‘the right drug for the right patient at the right dose’ is fulfilled. Advances in molecular technology have enabled the application of genomics to the development of specific drugs for personalised therapy. At present the number of drugs with pharmacogenomic labels is limited and the challenge is to identify genetic variability of drug targets and develop biomarker diagnostic tests for common diseases. This requires investment

in large scale research and robust clinical trials on an international basis, with multidisciplinary international collaboration. However the starting point for such projects is the pool of biological material from the patient or potential patient.

This chapter has focused on the ethical issues that guarantee standards and promote cooperation by society. The primary emphasis is on informed consent, which is central to the involvement of the patient. It is a crucial step for the uptake of a pharmacogenetic test, for enrolment in a clinical trial or genomic research and for the donation of biological material for a biobank. Continuing cooperation of the patient is enhanced through safeguards to ensure privacy and confidentiality in the handling of biological samples and data. Consent is also promoted through dissemination of the knowledge that there is equitable access to personalised medicine and not solely a reliance on the cost effectiveness of the tests and the therapy.

So society must be kept fully informed about pharmacogenomic progress to encourage open interaction with scientific and medical researchers. There is a definite educational role for healthcare professionals, provided they are themselves also well informed and trained to explain the benefits of personalised medicine, to choose and interpret relevant genomic tests and to encourage research participation. Genomic training must be included in medical and health science curricula at all levels, with emphasis on continuing professional development to keep abreast of advances.

Participation in multidisciplinary international research may be enhanced by opting for broad consent for genetic studies in clinical trials and for collection of biobank samples. The move to increased transparency in the conduct of clinical trials will provide more information to individuals as trial results are made public. Post marketing surveillance of drugs needs to be pursued on a regular basis to identify the effects of genomic labelling, pick up rare side effects and identify potential new niches for specific drugs.

Ethical principles are now embedded in various regulatory frameworks and legal instruments, but states have embraced different norms. As genomic research increasingly moves into the global arena, the vision is to work towards establishing harmonised regulations to facilitate international collaboration and so achieve the goal of making personalised medicine a reality in everyday medical practice.

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