CHAPTER 1

Pharmacogenetics: A Historical Perspective

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1.1 INTRODUCTION

It has been known for thousands of years that some individuals show toxic responses following consumption of fava beans, especially in countries bordering the Mediterranean. This is probably the earliest pharmacogenetic observation, although the biological basis for this has been established only quite recently (see Section 1.2). The foundation for much of modern pharmacogenetics came from experiments on chemical metabolism during the 19th century. These studies included the establishment that benzoic acid undergoes conjugation with glycine *in vivo* in both humans and animals, that benzene is oxidized to phenol in both dogs and humans and that some compounds can undergo conjugation with acetate (for a review, see Ref. 1).

1.2 EARLY PHARMACOGENETICS STUDIES (FROM 1900 TO 1970)

The development of genetics and Mendelian inheritance together with observations by Archibald Garrod on the possibility of variation in chemical metabolism in the early 20th century has been well reviewed elsewhere see [2]. Probably the first direct pharmacogenetic study was reported in 1932 when Synder's study on the ability to taste phenylthiocarbamide within families showed that this trait was genetically determined [3]. The gene responsible for this variation and common genetic polymorphisms have only recently been identified (for a perspective, see Ref. 4).

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Although not a prescribed drug, phenylthiocarbamide shows homology to drugs such as propylthiouracil.

The initial drug-specific pharmacogenetics observations appeared in the literature during the 1950s. These were concerned with three widely used drugs at that time, that are all still used today: isoniazid, primaquine, and succinylcholine. The earliest observation concerned primaquine, which was found by Alf Alving to be associated with acute hemolysis in a small number of individuals [5]. Subsequent work by Alving and colleagues found that this toxicity was due to absence of the enzyme glucose-6-phosphate dehydrogenase in red blood cells of affected individuals [6]. The molecular genetic basis of this deficiency was later established by Ernest Beutler and colleagues in 1988 [7].

Isoniazid was first used against tuberculosis in the early 1950s, although it had been developed originally a number of years previously as an antidepressant. As reviewed recently, its use in tuberculosis patients represented an important advance in treatment of this disease [8]. Variation between individuals in urinary excretion profiles was described by Hettie Hughes [9], who soon afterwards also found an association between the metabolic profile and the incidence of a common adverse reaction, peripheral neuritis, with those showing slow conversion of the parent drug to acetylisoniazid more susceptible [10]. Further studies by several different workers, particularly Mitchell and Bell [11], Harris [12], and David Price Evans [13], led to the conclusion that isoniazid acetylation was subject to a genetic polymorphism and that some individuals ($\sim$10% of East Asians but 50% of Europeans) were slow acetylators. Slow acetylation was shown to be a recessive trait. As summarized in Section 1.4, the biochemical and genetic basis of slow acetylation is now well understood.

Also during the 1950s, a rare adverse response to the muscle relaxant succinylcholine was found to be due to an inherited deficiency in the enzyme cholinesterase [14]. Succinylcholine is used as a muscle relaxant during surgery, and those with the deficiency show prolonged paralysis (succinylcholine apnea). This observation was then further developed by Werner Kalow, who showed that the deficiency is inherited as an autosomal recessive trait and devised a biochemical test to screen for the deficiency, as he described in a description of his early work [15]. The gene encoding this enzyme, which is now usually referred to as *butyrylcholinesterase*, has been well studied, and a number of different mutations responsible for the deficiency have been identified. However, the original biochemical test is still the preferred method for identifying those affected by succinylcholine apnea due to the rarity of both the problem and the number of different mutations.

While these initial studies showing the clear role for genetics in determining adverse responses to primaquine, isoniazid, and succinylcholine were in progress, the general importance of the area was increasingly recognized. Arno Motulsky published a key review on the relationship between biochemical genetics and drug reactions that highlighted the adverse reactions to primaquine and succinylcholine in 1957 [16]. The term *pharmacogenetics* was first used in 1959 by Friedrich Vogel in an article on human genetics written in German [17] and was soon adopted by others working in the field.

1.3 PHARMACOGENETICS OF DRUG OXIDATION

As described in Section 1.1, studies in the 19th century had demonstrated oxidation of benzene to phenol in vivo [1]. Pioneering studies on drug metabolism, especially those in the laboratories of the Millers and of Brodie and Gillette during the 1950s, showed that many drugs undergo oxidative metabolism in the presence of NADPH and molecular oxygen in liver microsomes [18,19]. In 1962, Omura and Sato described cytochrome P450 from a rat liver microsome preparation as a hemoprotein that showed a peak at 450 nm in the presence of carbon monoxide and dithionite [20]. Shortly afterwards Ron Estabrook, David Cooper, and Otto Rosenthal showed that cytochrome P450 had steroid hydroxylase activity [21], and further studies confirmed its role in the metabolism of drugs such as codeine, aminopyrene, and acetanilide [22]. At this time, it was still assumed that cytochrome P450 was a single enzyme, but evidence for multiple forms emerged in the late 1960s [23,24] with purification of a range of rat and rabbit enzymes achieved during the 1970s [25,26].

Independent metabolism studies on two newly developed drugs sparteine and debrisoquine in Germany by Michel Eichelbaum and in the United Kingdom by Robert Smith in the mid 1970s resulted in findings indicating that some individuals were unable to oxidize these drugs, although the majority of individuals showed normal metabolism [27,28]. These studies estimated that 10% of Europeans showed absence of activity, and the term *poor metabolizer* was first used. At this time, the enzymes responsible for this absence of activity were not known, but further studies confirmed that the deficiency in metabolism of both drugs cosegregated [29] and that the trait was inherited recessively [30]. It became clear that a number of different drugs, including tricyclic antidepressants, were also metabolized by this enzyme [31]. Studies on human liver microsomes confirmed that the enzyme responsible was a cytochrome P450 [32,33], and this enzyme was then purified to homogeneity [34]. The availability of antibodies to the purified protein facilitated the cloning of the relevant cDNA by Frank Gonzalez and colleagues, who initially termed the gene CYPIID1 [35]. On the basis of emerging data for cytochrome P450 genes in humans and other animal species, it was decided subsequently that the gene encoding the debrisoquine/sparteine hydroxylase should be termed CYP2D6. Studies on human genomic DNA led to the identification of several polymorphisms in CYP2D6 associated with the poor metabolizer phenotype, including the most common splice site variant, a large deletion, and a small deletion [36–40]. A major additional contribution to the field was made in 1993 by Johansson, Ingelman-Sundberg, and colleagues, who described the phenomenon of ultrarapid metabolizers with one or more additional copies of CYP2D6 present [41]. These ultrarapid metabolizers had been previously identified on the basis of poor response to tricyclic antidepressants, and this was one of the first accounts of copy number variation in the human genome. Agreement regarding the current nomenclature for variant alleles in CYP2D6 and other cytochromes P450 was reached in 1996 [42].

In an approach similar to that used in the discovery of the CYP2D6 polymorphism, Kupfer and Preisig found that some individuals showed absence of metabolism of the anticonvulsant *S*-mephenytoin [43]. It was demonstrated that *S*-mephenytoin

metabolism did not cosegregate with that of debrisoquine and sparteine, as this polymorphism was due to a separate gene defect. Identification of the gene responsible for *S*-mephenytoin hydroxylase proved difficult initially, probably because the relevant enzyme was expressed at a low level in the liver. The gene, now termed CYP2C19, was cloned by Goldstein and Meyer and colleagues in 1994, and the two most common polymorphisms associated with absence of *S*-mephenytoin hydroxylase activity were identified [44,45].

A number of other cytochrome P450 genes are now known to be subject to functionally significant polymorphisms. In the case of one of these, CYP2C9, which metabolizes a range of drugs, including warfarin, tolbutamide, and nonsteroidal antiinflammatory drugs, some evidence for the existence of a polymorphism appeared in 1979 when a trimodal distribution in the metabolism of tolbutamide was reported [46]. Subsequently, it was shown that tolbutamide metabolism was distinct from debrisoquine metabolism [47]. The enzyme involved was purified and cloned and later named CYP2C9 [48,49]. Analysis of CYP2C9 cDNA sequences provided evidence for the presence of coding region polymorphisms resulting in amino acid substitutions, and expression studies suggested these were functionally significant [48,50,51]. Genotyping of patients undergoing warfarin treatment confirmed the functional importance of the two most common coding region CYP2C9 polymorphisms [52–54].

Using a similar approach involving comparison of cloned cDNA sequences, evidence for a nonsynonymous polymorphism in CYP2A6 was obtained [55]. Following expression studies and population screening, it was demonstrated that this polymorphism was associated with a rare absence of CYP2A6 activity, but additional polymorphisms (including a large deletion) in CYP2A6 that also lead to loss of activity have been reported [56].

Biochemical studies on human liver demonstrated that some individuals express an additional cytochrome P450 with homology but not identity to the major drug metabolizing P450 CYP3A4 [57–59]. Expression of this isoform, now termed CYP3A5, is also determined by a common genetic polymorphism affecting splicing that was first identified by Erin Schutz and colleagues in 2001 [60].

From the early studies in the 1970s, it is now clear that at least four CYPs, namely, CYP2D6, CYP2C19, CYP2A6, and CYP3A5, are subject to polymorphisms leading to absence of enzyme activity in significant numbers of individuals and that CYP2C9 activity is very low (although not completely absent) in some individuals. There are also a large number of polymorphisms leading to smaller changes in cytochrome P450 activities (see Chapter 3 for more details). Current knowledge of phenotype–genotype relationships within the cytochrome P450 family is now more comprehensive than for the majority of human genes, although a better understanding of some aspects such as regulation of gene expression is still needed.

1.4 PHARMACOGENETICS OF DRUG CONJUGATION

As discussed in Section 1.2, a polymorphism affecting conjugation of drugs such as isoniazid with acetyl CoA had been known to exist since the 1950s. Other

conjugation polymorphisms were subsequently described from phenotyping studies. In particular, Richard Weinshilboum identified several polymorphisms affecting methylation of xenobiotics and endogenous compounds by measurement of enzymatic activities in blood cells. He described the most pharmacologically important of these polymorphisms, in thiopurine methyltransferase (TPMT), in 1980 [61]. Approximately 1 in every 300 Europeans lacks this enzyme with lower activity observed in heterozygotes. Other conjugation polymorphisms identified by phenotypic approaches included a deficiency in the glutathione *S*-transferase M1 (GSTM1), which affects 50% of Europeans and was originally detected by measurement of *trans*-stilbene oxide conjugation in lymphocytes [62]. The classic paper by Motulsky on genetic variability in metabolism [16] mentioned the mild hyperbilirubinemia described previously by Gilbert in 1901 and usually referred to as *Gilbert's syndrome* [63]. This was later shown to relate to impaired activity in glucuronidation by a form of the enzyme UDP-glucuronosyltransferase, and there were suggestions that glucuronidation of prescribed drugs might also be affected in this syndrome [64].

With the development of molecular cloning techniques, the basis of the various conjugation polymorphisms known previously became clear during the late 1980s and early 1990s, and evidence also emerged for additional functionally significant polymorphisms by sequence comparisons. The molecular basis of the GSTM1 deficiency was established quite early in 1988, probably because it is due to a large gene deletion that was readily detectable by a number of different approaches [65]. Cloning of the NAT2 cDNA, encoding the enzyme responsible for isoniazid metabolism, was achieved in 1991 by Blum and Meyer with two common variant alleles with several base substitutions in their coding regions found to be associated with absence of activity [66]. Other inactive variants were identified elsewhere [67,68], and, as in the case of the cytochrome P450 alleles, a standardized nomenclature system was developed [69]. In the case of TPMT, gene cloning and identification of two common alleles associated with absence of activity was achieved in 1996 [70,71]. The most common variant allele giving rise to Gilbert's syndrome was identified in the same year and found to be a TA insertion in the promoter region of the UGT1A1 gene, which encodes the major UDP-glucuronosyltransferase responsible for bilirubin conjugation [72].

Genotyping for the TPMT polymorphisms in patients being prescribed 6-mercaptopurine or azathioprine and the UGT1A1 variant associated with Gilbert's disease in patients receiving irinotecan are now recommended but not mandated by the US Food and Drug Administration (FDA). Knowledge of genotype can enable either dose adjustment or an alternative drug to be prescribed.

1.5 PHARMACOGENETIC STUDIES ON RECEPTORS AND TRANSPORTERS

Progress on pharmacogenetics of drug receptors and other targets has been slower mainly because phenotypic evidence for the existence of functionally significant polymorphisms was generally not available. However, as discussed in

Chapter 5, data from the human genome sequencing project have provided new insights into this area. Studies on polymorphisms in both the adrenergic receptor and dopamine receptor genes appeared in the early 1990s with Stephen Liggett leading in the area of adrenergic receptors [73]. As discussed in Chapter 6, polymorphisms in the various adrenergic receptors have been demonstated to be of considerable relevance to drug response, especially for the β2-receptor, but the overall pharmacological importance of polymorphisms in dopamine receptors is still less well established.

Among other drug targets, vitamin K epoxide reductase, the target for coumarin anticoagulants, which is also discussed in detail in Chapter 6, is another example of a gene with well-established pharmacogenetics. In particular, limited phenotypic data from the 1970s suggested that the target for warfarin was subject to interindividual variation in some individuals with resistance to the drug occurring in some families [74]. The gene encoding vitamin K epoxide reductase in humans was finally identified only in 2004 [75,76], but this advance quickly led to identification of isolated mutations associated with warfarin resistance and also to common genetic polymorphisms affecting response to anticoagulants [77–79].

1.6 PHARMACOGENOMICS, GENOMEWIDE STUDIES, AND PERSONALIZED MEDICINE

As reviewed by Meyer [2], the term *pharmacogenomics* first appeared in the literature in 1997. One of the first articles using this term [80] described its relevance to personalized medicine. Pharmacogenomics is often described as the whole-*genome* application of *pharmacogenetics*. There is clearly a large overlap between the two disciplines, but pharmacogenomics is broader and may involve the development of new drugs to target specific genes as well as more effective use of existing medicines. Prior to the 1990s, pharmacogenetic studies were concerned with the effects of single genes, but in the era of pharmacogenomics, the combined effects of a number of genes on a particular phenotype is typically investigated.

Probably the best example of an area in which there has been some implementation of pharmacogenomics is in cancer chemotherapy. Although pharmacogenetic polymorphisms such as TPMT (see Section 1.4) are important in determining the metabolism of selected drugs used in chemotherapy and their possible toxicity, it was realised that tumor genotype and phenotype in addition to host genotype will be predictors of response. The licensing of trastuzumab (Herceptin) as a targeted therapy for breast tumors in 1998 is the earliest example of a drug used as a personalized medicine on the basis of tumor phenotype (for review, see Ref. 81). A test to determine estrogen receptor status is needed before the drug is prescribed as only tumors that are estrogen receptor–positive respond. Other similar drugs followed, most notably imatinib (Glivec) in 2001. Imatinib is a tyrosine kinase inhibitor effective only in tumors with a particular chromosomal translocation [81]. In a separate development, it is now possible to classify tumors by signature for expression of a number of different genes and to use this signature to predict the

most appropriate cancer chemotherapy regimen. As discussed by Bonnefoi and colleagues [82], clinical trials are now in progress in breast cancer patients to confirm previous retrospective trials showing that determining mRNA expression levels for a set of either 21 or 70 genes in tumor tissue was of value in predicting whether patients with early-stage breast cancer should undergo chemotherapy or be treated only by hormone therapy.

Another area of pharmacogenomics that is currently developing is the use of genomewide association studies to identify genotypes associated with either drug response or drug toxicity. Such studies have been widely reported for complex polygenic diseases with some interesting novel genes affecting disease susceptibility already identified [83]. A number of genomewide association studies on drug response or adverse reactions have appeared since 2007 [84–86], but these have generally pointed to only one or two genes having a major effect rather than the larger number of genes each with a small effect typically seen in the complex polygenic disease studies. Further similar studies, especially on serious adverse drug reactions, are already in progress.

1.7 CONCLUSION

During 1957–1997, pharmacogenetics evolved to pharmacogenomics. There has been considerable further progress in the subsequent 12 years. Our understanding of single gene effects, especially in relation to drug metabolism, is now comprehensive, but our understanding of effects by multiple genes is still limited. In addition, we still need to translate the range of well-validated and clinically relevant pharmacogenetic discoveries that have been made over the years into more widespread use in patient care. Despite the predictions that we are entering an era of personalized medicine [80], except for the few examples discussed in Sections 1.5 and 1.6 in relation to cancer treatment, this has not yet occurred to any great extent.

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