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Pharmacogenomics

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Summary

Pharmacogenomics, a revolutionary chapter in the history of pharmacology, has received new impetus from the development and accessibility of molecular biotechnologies, notably DNA chips. The longstanding notion of responders/non-responders has given way to a more organic approach, where idiosyncrasy becomes an obsolete concept. This is a major step towards predictive, individualized medicine. In this review, several applications of pharmacogenomics are considered. Genetic polymorphisms of metabolism reactions, mainly with cytochrome P450, explain most of the cases described today. More fundamental and innovative studies have tried to link the structure of receptors or transporters and drug response. A leading topic in neuropsychopharmacology is the relation between the polymorphism of dopaminergic receptors and the efficacy of, or adverse reaction to, neuroleptics. In asthma, the structure of the β_2 -adrenergic receptor has been associated with response to treatment. Intrinsic genetic predisposition also plays an important role in cardiovascular diseases, and the role of ion channel mutations will be discussed. Research in oncological molecular epidemiology has explored the connection between the predisposition to certain cancers and specific enzymatic equipment hindering the detoxification of potentially carcinogenic exogenous compounds, or, on the contrary, promoting metabolic activation implicated in the formation of reactive compounds. The search for determinants of addictive behavior is another vast field of pharmacogenomics. Finally, we consider the impact of pharmacogenomics on the methodology of drug development in preclinical and clinical trials. Progress in methods of phenotyping/genotyping should promote diagnosis, guide the choice of drug for an individual (benefit/risk ratio), and determine dosage and regimen.

key words: pharmacogenomics • genetic polymorphism • cytochromes • genotyping/phenotyping

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BACKGROUND

In the early 1950s, two interesting findings – prolonged muscle relaxation after curarization with suxamethonium in patients with congenital cholinesterase deficiency, and acute hemolysis induced by anti-malaria drugs in patients with low glucose-6-phosphate-dehydrogenase (G6PD) activity – set the stage for new developments. By the end of the 80s, the causal genes coding for debrisoquine hydroxylase, or CYP2D6, had been cloned and characterized, inaugurating what we now know as pharmacogenomics. Pharmacogenomics focuses on the link between structural polymorphism in genes and variable response to drugs, or more generally, the consequences of exposure to xenobiotics. Polymorphism explains a significant amount of the interindividual variability in treatment effects [1]. Thus a small number of individuals in a given population exposed to a given drug will have a high risk of non-responding or of developing an adverse effect, a risk that can be identified because it is biologically determined [2]. A gene is considered functionally polymorphic when stable variants altering protein activity exist in a population, with a given level of frequency. These pharmacological concepts became common knowledge after the identification of two types of drug accidents: antihistamine H₁ and *torsades de pointes* when associated with an inhibitor of CYP3A4, and secondly the withdrawal from the market of mibefradil, an antihypertensive compound, inhibitor of calcium channels; this drug, when associated with simvastatine, induced rhabdomyolysis complicated by anuria due to accumulation of the hypocholesterolemic [3]. Today, most of the cases described are directly linked with metabolism modifications (mainly P450 cytochromes); however, more and more studies are observing the potential association between a genotype and the target of a given drug. The development and accessibility of molecular biotechnologies, notably DNA chips or microarrays, have given new impetus to pharmacogenomics. The knowledge thus acquired should promote accurate diagnosis, guide the choice of a given drug for an individual (benefit/risk ratio), and determine dosage and regimen [4]. This approach is a step towards predictive, individualized medicine.

INVESTIGATIVE TOOLS

Genotyping

The tools currently used for genotyping studies were developed for molecular biology [5,6]. Of particularly importance are PCR (Polymerase Chain Reaction) associated with RFLP (Restriction Fragment Length Polymorphism), as well as SSCP (Single-Stranded Conformation Polymorphism) and TGGE/DGGE (Temperature or Denaturing Gradient Gel Electrophoresis). Gene sequencing is also making a highly significant contribution.

DNA chips are a major technological innovation. DNA chips are derived from Southern blots and will undoubtedly provide the technical support needed for the development of pharmacogenomics. The basic principle is to hybridize complementary sequences of

labeled target nucleic acids. Labeling is achieved in the liquid phase using probes carried on a solid support. These chips enable large-scale investigations for the detection of allelic variants and recognition of differences even as small as one nucleotide (SNP, single-nucleotide polymorphism), as well as deletions or duplications. They can also be used to study changes in gene expression. An entire supplementary issue of *Nature Genetics* has been devoted to DNA chips [7].

Two principal techniques have been described [8–12]:

1. Spots of several hundred DNA bases can be inscribed on a support with an arrayer or an ink-jet printer. A glass support measuring 3.6 cm² can accommodate 30,000 spots. The DNA to analyze is generally obtained by inverse transcription of mRNA extracted from tissues (complementary DNA, cDNA), but genomic DNA can also be used. Prior to DNA amplification, a fluorescent agent labels the nucleotides. Genetic materials carried on a single chip coming from reference cells and cells to be analyzed are compared by labeling with different fluorophores. After hybridization and washing, the chips are read with a laser-argon scanner which excites fluorescence. A confocal microscope is used to identify the position of the hybridized DNA probe. The mass and complexity of the collected signals requires computer-assisted data processing. Chip systems labeled with radioelements are also available. These systems are read by measuring the radioactivity.

2. Oligonucleotides can be synthesized *in situ* using photolithography techniques (GeneChip[®], Affymetrix), 1.6 cm² is sufficient for 65-400,000 oligonucleotides. A sequence is tested four times by four oligonucleotides composed of 20 to 25 nucleotides that differ only in their central base. Chips can be dedicated, grouping a collection of adapted probes designed for a specific purpose (oncology, metabolism), or custom-made. For instance, chips can be specifically designed for the diagnosis and therapeutic management of acute lymphoid leukemia. These dedicated chips can simultaneously genotype lymphoblastic tumor markers (BCR/ABL, etc.), other markers (p53, etc.), enzymes implicated in the metabolism of anticancer drugs (thiopurine and TPMP, etc.) or drugs used for complementary treatments (CYP, etc.), endogenous substances implicated in anti-infection defense mechanisms (TNF, IL2, etc.), and specific vulnerability factors (receptor polymorphism, ion channels, etc.) [13].

Although the high cost of these sophisticated chips and the requirement for highly specialized equipment for their implementation puts a limit on widespread use, several systems have already been marketed.

The limitation of this approach is the extreme variability of the human genome (6 to 30 million SNP). Theoretically, directly linking changes in genetic information to phenotypic and clinical expression would require a complete catalogue of human gene sequence variations. This catalogue, an extension of the Human Genome Project, remains to be written.

Phenotyping

The phenotype (trait) is defined as the visible expression of the genotype. Phenotypes determined by more than one gene are termed polygenic or multifactorial. Phenotyping can provide qualitative or quantitative information [14,15]. In pharmacogenomics, phenotyping is used as a functional test to evaluate, for example, the level of activity of a given enzyme implicated in the metabolism of a group of drugs. In order to implement this technique it is necessary to administer an exogenous substance, then determine the circulating or excreted concentrations of the drug or its principal metabolite. These concentrations are used to calculate the index of metabolism. Different protocols have been developed using the appropriate probes of the metabolic pathway to be tested [16,17].

The limitation of this approach is that it provides a 'snapshot' of the phenotypic expression, a process that might be altered by another variable, for example by transient exposure to an inducer.

The example of CYP2D6 polymorphism illustrates very well the potential of these techniques. CYP2D6 intervenes in the hydroxylation of some forty different drug compounds. Different tests have been designed to explore its activity, for instance the sparteine test or the dextromethorphan test, but the most widely used is the debrisoquine test. A test dose of this anti-hypertensive agent is administered orally to the subjects to be phenotyped. Urine is collected over a predetermined period. The ratio of the urinary concentration of the principal metabolite of debrisoquine, 4-hydroxydebrisoquine, is used to establish the metabolic index that defines the enzyme phenotype. This ratio is quite variable, but ranges from 0.2 to 12.6 for intermediate persons considered normal. A lower ratio corresponds to ultra-rapid metabolism and is observed in patients who are recognized as non-responders to treatment with compounds metabolized by this pathway. Higher ratios correspond to poor metabolizers who are consequently exposed to overdosing and adverse effects. The distribution of the different phenotypes is illustrated in Figure 1.

This same enzyme can be genotyped using RFLP-PCR. More than 70 alleles have been characterized. The principal mutations determining the poor metabolizer phenotype are shown in Figure 2. The most frequent mutation carried on CYP2D6*4 is an adenosine replacement of guanine in position 1934 at the limit between intron 3 and exon 4. This mutation induces synthesis of a truncated inactive protein containing 181 amino acids instead of the 457 amino acids in the wild type. Ultra-rapid metabolizers have on the contrary a duplication of CYP2D6*2. The large number of identified allelic variants easily explains the wide variability of the metabolic index.

Genotyping can thus be used to determine the molecular basis of an observed phenotype.

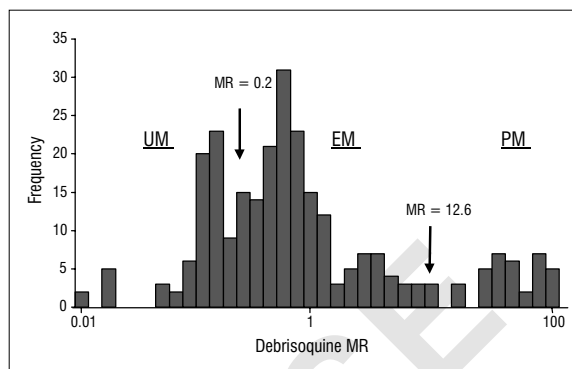


Figure 1. CYP2D6 metabolic index: distribution in a Swedish population (from [101]). MR – metabolic ratio; UM – ultra-rapid metabolizers; EM – extensive metabolizers (= normal); PM – Poor metabolizers.

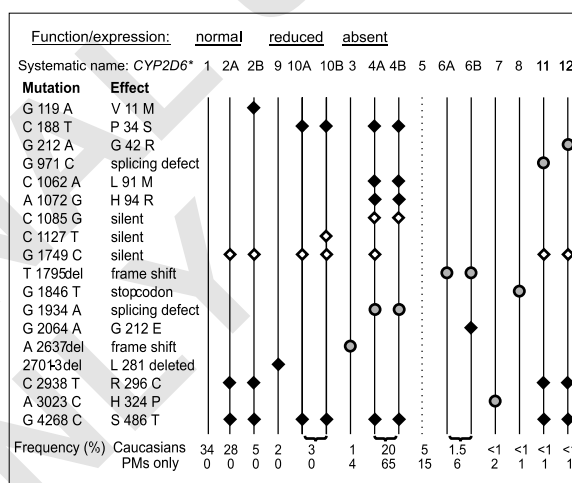


Figure 2. Distribution and consequences of the most frequent mutations of the CYP2D6 gene (from [102]). Empty diamonds: silent mutation; full diamonds: mutation modifying one amino acid; full circles: mutations causing loss of function. Dotted line (CYP2D6*5) indicate a deletion.

GENETIC POLYMORPHISM OF METABOLIZATION REACTIONS

The enzymes implicated in drug metabolism are classed into categories according to their implication in phase I reactions (changes of functional moieties) or phase II reactions (conjugation with an endogenous compound) [18]. The principal enzymes that are involved in xenobiotic metabolism and express polymorphism with clinical implications are given in Figure 3.

P450 cytochromes

P450 cytochromes [19,20] play a key role in the polymorphism mechanisms modifying the pharmacokinetic properties of a given compound. P450 cytochromes are enzymatic hemoproteins associated with the flavoproteins that intervene in endogenous biosynthesis pathways and in oxidative drug metabolism. They are also involved in the activation of procarcinogens. These mono-oxygenases are mostly located in liver tissue, but

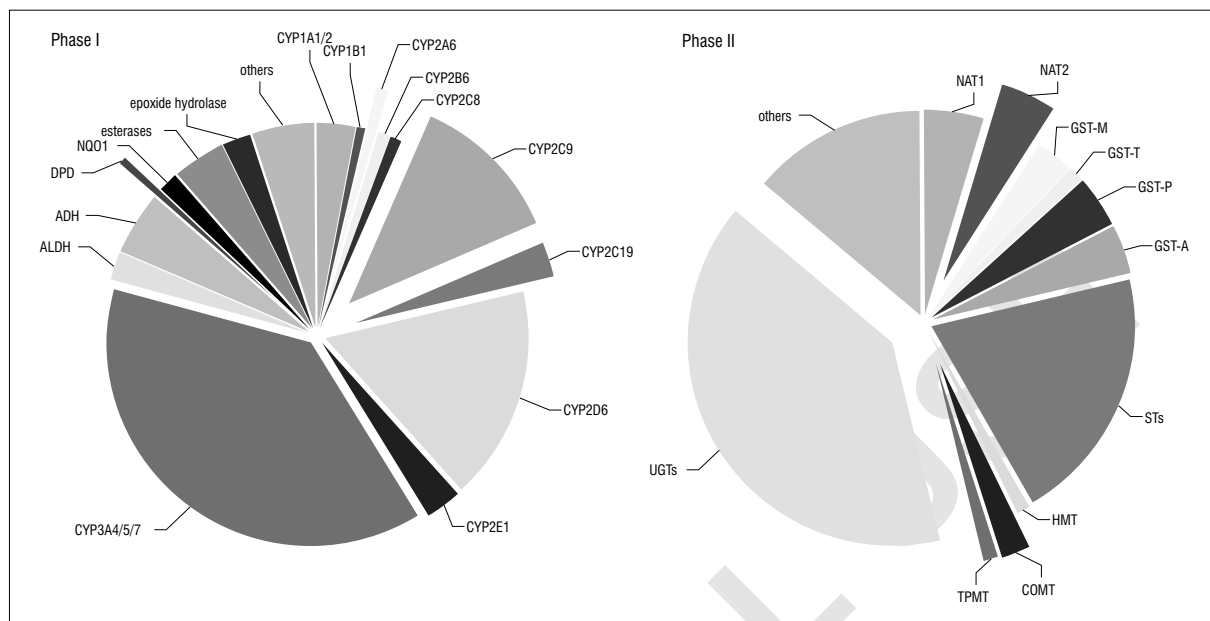


Figure 3. Principal enzymes exhibiting a polymorphism implicated in drug metabolism (from [13]). Phase I – modifications of functional moieties, Phase II – conjugation with endogenous compounds, AHD – alcohol dehydrogenase; ALDH – aldehyde dehydrogenase; CYP – cytochrome P450; DPD – dihydropyrimidine dehydrogenase; NQO1 – NADPH-quinone oxidoreductase; COMT – catechol-O-methyl transferase; GST – glutathion S-transferase; HMT – histamine methyl-transferase; NAT – N-acetyl transferase; STs – sulfotransferases; TPMT – thiopurine methyltransferase; UGTs – uridine 5'-triphosphate glucuronosyl transferases

Table 1. Distribution of the principal alleles of four human cytochromes (from [28]).

Enzyme	Predominant allelic variant	Mutation	Consequences for enzyme function	Allelic Frequency (%)			
				Caucasians	Asians	Africans	Ethiopians and Saudis
CYP2A6	CYP2A6*2	Leu160His	Inactive enzyme	1–3	0	ND	ND
	CYP2A6del	Deletion	No enzyme	1	15	ND	ND
CYP2C9	CYP2C9*2	Arg144Cys	Reduced affinity for P450	8–13	0	ND	ND
	CYP2C9*3	Ile359Leu	Specificity for the substrate altered	6–9	2–3	ND	ND
CYP2C19	CYP2C19*2	Aberrant splicing site	Inactive enzyme	13	23–32	13	14–15
	CYP2C19*3	Premature stop codon	Inactive enzyme	0	6–10	ND	0–2
CYP2D6	CYP2D6*2xN	Gene duplication or multiduplication	Increased enzyme activity	1–5	0–2	2	10–16
	CYP2D6*4	Defective splicing	Inactive enzyme	12–21			
	CYP2D6*5	Deletion	No enzyme	2–7	1	2	1–4
	CYP2D6*10	Pro34Ser, Ser486Thr	Unstable enzyme	1–2	6	4	1–3
	CYP2D6*17	Thr107Ile, Arg296Cys	Reduced affinity for substrates	0	51	6	3–9
		Ser486Thr			ND	34	3–9

ND – not determined

can also be found in the intestine. At the subcellular level, P450 cytochromes are anchored to the endoplasmic reticulum. The accepted nomenclature identifies these cytochromes with the letters CYP followed by an Arabic numeral designating the family (1 to 4), a letter, (subfamily A to F) and another Arabic numeral designating the isoenzyme (1 to 20). The number after the asterisk is the number of allelic variant. Listed by order of decreasing importance in drug metabolism, the P450 cytochromes include CYP3A4 [21,22], CYP2D6 [23], CYP2C9 [24], CYP2C19 [25] and CYP2A6. Their prin-

cipal allelic variants and their frequencies are given in Table 1 (except for CYP3A4, for which these data are not known). Table 2 lists three cytochromes of the principal molecules whose clearance is decreased in poor metabolizers. All these cytochromes, except for CYP2D6, are inducible. Induction is dose-dependent and reversible. Several substrates competing for the same binding site produce an inhibiting effect. Lists of the various substrates, inhibitors, or inducers of these cytochromes, as well as their known mutations, can be found in several publications and internet sites.

Table 2. Therapeutic consequences of poor metabolizer phenotype for three cytochromes (from [28]).

Enzyme	Reduced clearance	Adverse effects	Reduced transformation of the prodrug
CYP2C9	S-Warfarin	Bleeding	Losartan
	Phenytoine	Ataxia	
	Losartan		
	Tolbutamide	Hypoglycemia	
	NSAID	Digestive tract bleeding (?)	
CYP2C19	Omeprazole	Sedation	Proguanil
	Diazepam		
CYP2D6	Tricyclic antidepressants	Cardiotoxicity	Tramadol
	Haloperidol	Parkinsonism (?)	
	Antiarrhythmic drugs	Arrhythmia	
	Perphenazine		
	Perhexiline	Neuropathies	
	Serotonin reuptake inhibitors	Nausea	
	Zuclopenthixol		
	S-Mianserine		
	Tolterodine		

NSAID – non-steroidal antiinflammatory drugs

Thiopurine methyltransferase (TPMT)

Azathioprine is used in combination with other drugs for the prevention of acute allograft rejection and for the treatment of certain autoimmune disorders, including Crohn's disease. The active metabolite of azathioprine, 6-mercaptopurine (6-MP), is formed in the liver. This drug is used directly for the treatment of acute lymphoblastic leukemia in children.

There are three metabolic pathways for 6-MP. Hypoxanthine guanine phosphoribosyl transferase (HGPRT) transforms 6-MP into 6-TGN (6-thioguanine nucleotides), which is responsible for the drug's activity and toxicity. Xanthine oxidase inactivates 6-MP into thiouric acid. TPMT transforms active 6-MP into inactive 6-MMP (6-methylmercaptopurine) by methylation of the thiol moiety. TPMT is an enzyme mainly found in the liver, but it also occurs in erythrocytes. TPMT has a genetic polymorphism. The gene is situated on chromosome 6.

The phenotypic distribution of TPMT follows a trimodal pattern, with at least eight identified allelic variants of the TPMT gene locus that are associated with weak enzymatic activity. The most common alleles are TPMT*2, TPMT*3A and TPMT*3C [26]. The TPMT*3A variant is the most frequent in Caucasians, and has intermediate activity (heterozygous persons). TPMT is very weak or undetectable in 0.3% of the population (homozygous persons). There is a good correlation between the presence of TPMT*2, TPMT*3A and TPMT*3C alleles and phenotype. The phenotype-genotype correlation is 98%.

Persons deficient in TPMT accumulate nucleotide derivatives (6-TGN) in quantities inversely proportional to TPMT activity [27]. Doses must be carefully adapted for patients with deficient TPMT activity, due to the risk of fatal hematological toxicity reactions. Thus therapy can be successful in selected children with acute lymphoblastic leukemia at doses as low as 10 to 15 % of the conventional dose for 6-MP [28]. On the contrary, patients who have the wild alleles may require high doses to achieve therapeutic efficacy.

Children with deficient TPMT activity have a high risk of secondary myelodysplasia or acute myeloid leukemia, probably associated with the DNA damage resulting from 6-TGN accumulation. One hundred percent of all homozygous persons, 54% of heterozygous persons, and 23% of the persons with the wild phenotype develop toxic reactions to treatment [29]. Nevertheless, dose reduction to limit toxicity is counterbalanced by the fact that high-dose 6-MP is an important factor predictive of survival in children with leukemia.

The genetic polymorphism of TPMT activity is a significant clinical example of the use of pharmacogenomics to determine individual treatment with increased activity and reduced toxicity. Phenotypic tests are designed to determine the presence of the enzyme in red cells; TPMT activity can be measured. The red cells are incubated with 6-MP and 6-MMP is assayed with HPLC to determine TPMT activity [30]. Blood transfusion, frequent in these persons, affects the results.

ROLE OF PHARMACOGENOMICS IN SELECTED MAJOR SYSTEMS

Pharmacogenomics and the central nervous system

Metabolic polymorphism

Based on eight clinical cases, Balant-Gorgia et al. [31] clearly demonstrated the therapeutic impact of metabolic polymorphism in psychiatric patients in terms of therapeutic failure or adverse effects. Most of the patients (90%) required nortriptyline for major depression at a dosage of 75–150 mg/d to reach the target plasma concentration of 200–600 nmol/l. Poor metabolizers require a 10-fold smaller dose to reach the same plasma level. Without proper screening, these poor metabolizers would be exposed to a highly increased risk of adverse drug effects. Inversely, rapid metabolizers appear, wrongly, to have drug-resistant depression, but respond to other compounds metabolized via a different pathway [32].

More fundamental and innovative research has been conducted to search for a link between the structure of a receptor or transporter and the response to drugs. This approach is similar to that adopted in psychiatric genetics, searching for an association between a particular vulnerability and a given phenotypic or genotypic profile [33]. The search for an association is done by comparing the frequency of a given candidate allele or gene in persons with a key symptom of the disease. The different steps in this type of pharmacogenomic

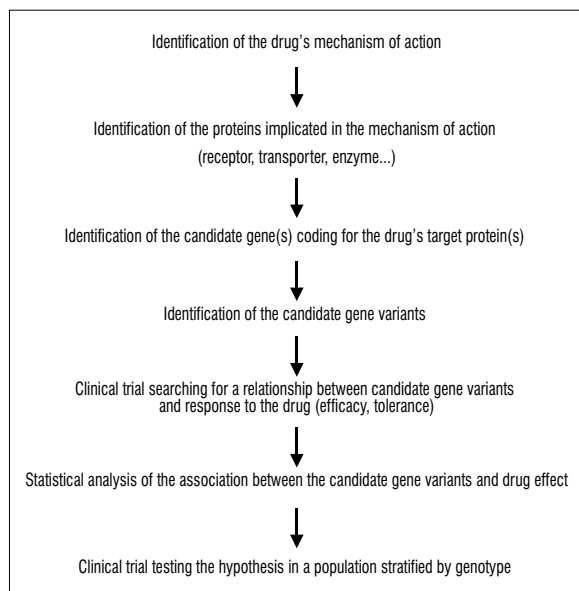


Figure 4. Steps involved in searching for a pharmacogenomic association (from [103]).

research are outlined in Figure 4. Notwithstanding the body of work on drug metabolism in the central nervous system, these studies provide a better understanding of the variability of response to treatment in different pathological conditions observed in patients with neurological disorders (such as migraine [34], epilepsy [35], or neurodegenerative disorders [36], especially Alzheimer's disease) or psychiatric illnesses (schizophrenia, depression, hyperactivity-attention deficit). We will detail two examples.

Dopaminergic receptors and neuroleptics

Neuroleptics include a variety of compounds prescribed for patients with psychotic disorders, particularly schizophrenia. Their least common denominator is their antagonist effect on receptor D_2 . Two categories of neuroleptics can be described: conventional and atypical. Clinically, atypical neuroleptics are characterized by their dual action on both the positive and negative symptoms of schizophrenia and by the absence of undesirable extrapyramidal effects. Clozapine, the leading compound in this class, has many interesting features and is a good candidate for association research. Clozapine can produce a very serious adverse effect, agranulocytosis. Pharmacologically its affinity for D_4 receptors is greater than its affinity for D_2 receptors. Clozapine is also a 5-HT_{2A} receptor antagonist. The gene of the D_4 receptor exhibits strong polymorphism, with a hyper-variable region in the third intracytoplasmic loop, which can carry repetitions of a series of 48 base pairs. These variants are designated by D_{4x} , where x is the number of repetitions [37]. Among six published studies searching for an association between these variants and therapeutic response to clozapine, only one [38] demonstrated a link between patients homozygous for four types of alleles and response to clozapine. On the other hand, a meta-analysis by Arranz et al. [39] showed an association

between the 5-HT_{2A} receptor polymorphism (T102C and His452Tyr) and response to clozapine. A combination of six polymorphisms involving several receptors demonstrated a strong association with the clinical efficacy of clozapine, exhibiting 77% predictability [40]. This approach is more focused and appears to produce better insight into the complexity of the disease [41]. The agranulocytosis induced by clozapine, a risk limiting its use, would be associated with a dominant gene of the major histocompatibility complex [42]. Late onset dyskinesia, a severe adverse effect that is observed in about 50% of patients under long-term treatment with conventional antipsychotic drugs, is associated with Ser9Gly polymorphism of the gene coding for receptor D_3 [43].

Serotonin transporter and antidepressants

Drug therapy for curable mood disorders has been an undeniable success of modern psychiatry, but in the field of drug-resistant depression, the clinical impact has been much less satisfactory. Serotonin transporter (5-HTT) is a protein capable of recapturing serotonin released at nerve ends. It constitutes the principal mechanism eliminating this neurotransmitter from the synaptic cleft. This transporter is the target of several antidepressants belonging to the class of specific serotonin reuptake inhibitors (SSRI). As early as 1966, Heils et al. [44] identified two alleles, one long (16 copies of a 20-23 base pair repeat unit) and one short (14 copies), with different transcriptional properties. The long allele leads to the synthesis of twice as much mRNA. Preliminary results suggest that the presence of at least one copy of the long allele is associated with a favorable response to SSRIs [45-47]. According to Mössner et al. [48], Parkinsonian patients with a short 5-HTT allele would have significantly higher scores on the Hamilton scale used to evaluate depression. Currently, at least 14 allelic variants have been described and further studies are expected.

Pharmacogenomics and the respiratory system

Asthma is a multifactorial disorder occurring in genetically predisposed persons exposed to environmental factors. Different mechanisms can trigger bronchoconstriction. Several treatments known to modify the 5-lipoxygenase pathway are only beneficial for certain patients whose leucotrienes contribute to their disease susceptibility. Drazen et al. [49] demonstrated a polymorphism of the promoter region of gene *ALOX5*, linked to reduced transduction of this gene and a weaker clinical response to ABT-761, a selective inhibitor of lipoxygenase. This study focused attention on examining not only the candidate gene sequences, but also their regulatory regions.

β_2 -adrenergic receptors play an important role in the control of airway smooth muscles and are one of the principal therapeutic strategies in bronchial obstruction. β_2 -adrenergic receptor agonists constitute one of the principal treatments for acute asthma [50]. Nine mutations of the β_2 receptor have been identified (five are

silent); the frequency of certain alleles has been associated with particular phenotypic expressions, such as nocturnal asthma or IgE level [51]. Arg16→Gly and Gln 27→Glu substitutions regulate the internalization of this receptor, and persons homozygous for Gly16 respond less well to salbutamol [52].

Pharmacogenomics and the cardiovascular system

Pharmacogenomics has great potential in the field of cardiovascular diseases, the leading cause of death in developed countries. The economic factor here is enormous. Cardiovascular diseases include atherosclerosis, hypertension, myocardial infarction, heart failure, and rhythm disorders, among others. In addition to extrinsic factors related to lifestyle and infectious diseases, intrinsic genetic predisposition plays an important role. Currently, the most important therapeutic consequences, which we will discuss briefly, result predominantly from the polymorphism of enzymes involved in xenobiotic metabolism: transferases, cytochromes and other oxidoreductases. As in other diseases, the pharmacokinetic and pharmacodynamic impact of the genetic polymorphism of membrane transporters is not well known in the field of cardiovascular diseases. Finally, the genetic polymorphism of proteins targeted for therapy, e.g. receptors and signal transduction enzymes, certainly contributes considerably to individual variability in the efficacy and toxicity of cardiovascular treatments. It should also have important consequences in the development of individualized treatment schemes. The real impact of this genetic polymorphism remains poorly investigated.

Metabolic polymorphism

In 1990, Lee et al. [53] hypothesized that the greater β -blocker effect of propafenone at low dose observed in poor metabolizers could be explained by genetically determined diminution in the transformation of this anti-arrhythmic agent and its less active 5-hydroxy metabolite. We now know that the enzyme that metabolizes propafenone is CYP2D6, a cytochrome with a major polymorphism as mentioned above.

The therapeutic window for drug treatment in cardiovascular diseases is rather narrow, providing an excellent example of the impact of genetic polymorphism. The *2 and *3 allelic variants of CYP2C9 induce major variability in xenobiotic metabolism. When a patient has one of these variants, a frequent situation in the Caucasian population, the enzymatic metabolism of several drugs, e.g. the S (-) enantiomere of warfarin, losartan, tolbutamine, and phenytoine, is reduced. Persons heterozygous for the Ile359Leu mutation, and even more so homozygous persons, require lower warfarin doses to achieve anticoagulation. The corollary is a higher risk of hemorrhage [54–56]. In addition, metabolic enzymes are not modulated solely by genetic polymorphism. Other factors are also involved, such as co-administration of other medications with induction or inhibition effects. The importance of individual titration is obvious. In the delicate field of antihypertensive, anti-

ischemic and anti-arrhythmic agents, CYP2C9 is inhibited by co-administration of amiodarone.

In certain cases, knowledge of the phenotype can be helpful not only in limiting the risk of toxicity, but also in increasing treatment efficacy. The recognized beneficial effect of carvedilol on mortality in heart failure results, at least in part, from the α_1 -blocker sympatholytic properties of the racemic mixture of the two enantiomers; the S (-) enantiomere also induces β -blocker activities [57]. In poor metabolizers, the slower clearance (3-fold reduction) is limited to the R (+) enantiomere and is related to the CYP2D6 genotype [58]. Treatment could be optimized by knowledge of the phenotype or genotype, depending on the desired β - or α_1 -blocker effect [59].

Transporter polymorphism

There are only a few known examples of this phenomenon. P-glycoprotein is a transmembrane ATP-dependent pump that protects the cell from the toxic effect of accumulated products by transporting them or their metabolites outside the cell. The rate of expression and function of intestinal P-glycoprotein is correlated with a mutation of exon 26 (C345T) of the multiple-drug resistance gene *MDR1C*. This polymorphism could be of major importance for adjusting the individual dosage of certain drugs in persons with this mutation. Persons homozygous for the variant (24% of the Germanic population) have serum concentrations of digoxin four-fold higher than the mean after a single oral dose. The plasma peak of digoxin is also higher after long-term administration [60]. P-glycoprotein is also a transporter of verapamil and several other therapeutic agents.

Polymorphism of target proteins

Drugs exert their final effect by the interaction of their active metabolite with an endogenous protein. This protein can be:

- a receptor. When the agonist (or antagonist) binds to the receptor, a specific biochemical reaction is stimulated (or inhibited). The effect is mediated by a specific effector, such as:
 1. an ion channel or
 2. another type of transmembrane protein or
- a transformation enzyme that catalyzes a specific biochemical reaction.

Genes coding for expression of these proteins often present a polymorphism that can contribute to the pathogenesis of genetic diseases, but can also alter response to drugs.

re 1. In the growing list of hereditary heart diseases, the most well known example concerns the different mutations of the cardiac sodium and potassium channels leading to the long QT syndrome (LQTS), characterized by abnormally long ventricular repolarization with a high risk of severe ventricular tachyarrhythmia. Care-

ful study of these mutations greatly contributed to the development of anti-arrhythmic strategies.

The Romano-Ward syndrome is the most common type. This is a purely cardiac autosomal dominant syndrome. The variable clinical presentation results from the heterogeneous nature of the mutations, often involving inversions, and occurring at different positions within the same gene. The Romano-Ward syndrome results from mutations of five genes, each coding for a subunit of a protein channel or for an entire channel. Mutations LQT 1 to 5 are recognized (the gene carrying the LQT4 mutation has not yet been identified on chromosome 4). LQT1 is the most common, and is targeted on gene *kVLQT1* on chromosome 11, which codes for subunit α of the potassium channel I_{Kr} . Mutation LQT2 occurs in gene *HERG* on chromosome 7, which codes for the potassium channel I_{Kr} . Mutation LQT3 is in gene *SCN5A* on chromosome 3, which codes for the sodium channel I_{Na} . Mutation LQT5 occurs in gene *KCNE1* on chromosome 21, which codes for an ancillary subunit *minK* of the potassium channel complex I_{Kr} . Other genes are probably involved in the disease; the phenotypic expression is quite heterogeneous for a given allelic variant, suggesting that other 'modifier' genes, yet to be identified, participate in the clinical expression.

Expression of the mutations *KvLQT1*, *HERG*, or *KCNE1* alone or with the wild allele lead to loss of function, *i.e.* a dominant negative mutation. Potassium flow is decreased by more than 50%. Inversely, *SCN5A* mutations lead to a gain of function. Prolonged activity or defective inactivation of late sodium flow delays ventricular repolarization.

It is generally difficult to develop specific treatments for loss of function. Inversely, an abnormal gain in function subsequent to *SCN5A* mutation can respond to treatments blocking the excessive sodium flow (mexiletine and lidocaine are especially effective).

LQTS and *torsades de pointes* can be induced by various drugs. The risk would be particularly high in a relatively large number of persons carrying a silent mutation of the LQTS genes that do not in themselves provoke long QT at rest [61]. These drug-induced rhythm disorders would result from the combination of the drug's effect on potassium flow and the person's modest degree of potassium flow inhibition. This is how cisapride [62] can trigger potassium channel mediated LQTS. Another mechanism would be the secondary effect of certain xenobiotics, antibiotics for example, specifically macrolides [63].

re 2. Coronary artery constriction subsequent to the activation of α_1 and α_2 adrenergic receptors is mediated by two different G proteins: the G protein associated with α_1 is not sensitive to *Bordetella pertussis* toxin (PTX), while the G protein associated with α_2 is. The gene *GNB3*, which codes for subunit β_3 of the G proteins, presents an important polymorphism (C825T). Persons with allele 825T exhibit increased response of the PTX-sensi-

tive G protein. This was demonstrated by measuring coronary flow [64]: coronary vasoconstriction resulting from the action of BHT 933, a specific agonist of α_2 receptors, is more marked in carriers of the 825T mutation than in persons homozygous for the allele C825, and more marked than the vasoconstriction obtained by the action of methoxamine, a specific agonist of the α_1 receptors. This result is important both for clinical understanding and for treatment, since in case of atherosclerosis, the vasoconstriction resulting from α_1 stimulation is greater than that resulting from α_2 stimulation more specific for healthy arteries. It has also been demonstrated that the allele 825T is significantly associated with diverse cardiovascular diseases, including essential hypertension [65].

re 3. Angiotensin converting enzyme (ACE) inhibitors have an antihypertensive effect with an effective benefit in terms of morbidity and mortality from heart failure. The human gene for ACE is situated on chromosome 17 and presents an insertion/deletion (I/D) polymorphism [66]. Hypertension, especially in patients with a risk of ventricular hypertrophy and congestive heart disease, is also linked to the genetic polymorphism of the ACE gene. To date, however, it has not been possible to clearly relate the polymorphism of the ACE gene to the highly variable individual response to the anti-hypertensive effect of various ACE inhibitors [67].

PHARMACOGENOMICS IN ONCOLOGY

Pharmacogenomics has made a wide range of contributions in the field of oncology. The advantage of personalized therapeutic protocols, recognized for patients with benign disease, is patently obvious for patients with malignant disease, the more so, that many of the anti-cancer drugs are administered as prodrugs requiring enzymatic transformation (with the underlying potential of polymorphism) for activation. In addition, many of these anti-cancer drugs have a very narrow therapeutic index [68]. Many examples can be given, including the TPMT polymorphism described above. We may also mention irinotecan, a camptothecin derivative indicated in the treatment of colorectal cancer, and *UGT1A1* polymorphism. Irinotecan is a prodrug transformed by carboxylesterases forming SN-38, the active compound inhibiting topoisomerase I. Patients with a low rate of glucuronidation accumulate SN-38 and develop toxic reactions manifested by severe diarrhea. The reduced capacity for gluconidation is related to (A [TA]nTAA) repetitions in the promoter region of gene *UBT1A1*; transcription activity falls with increasing n [69].

The search for susceptibility genes in breast cancer, such as the BRCA1 or A2 system, is not directly related to pharmacogenomics; here the focus is placed on searching for gene-disease linkage, irrespective of the effect of exogenous compounds. Researchers in molecular epidemiology have, however, searched for links between the predisposition to certain cancers and specific enzymatic equipment hindering detoxification of potentially carcinogenic exogenous compounds, or, on the contrary, promoting metabolic activation implicated

in the formation of reactive compounds. A further possibility is to search for other susceptibility factors, such as enzyme polymorphism implicated in the repair of DNA damaged by xenobiotics, radiation, or free radicals [70]. Smith et al. [71] demonstrated that a low activity level of NAD(P)H quinone oxido-reductase (NQO1), linked to the C609T polymorphism, is associated in adults with a higher risk of developing acute leukemia. Conclusions can be found in the meta-analysis published by Errico et al. [72]. This meta-analysis grouped together over 100 epidemiological studies published between 1978 and 1995. The common feature was the search for an association between lung, bladder, breast, colon, or stomach cancer and the polymorphism of enzymatic systems implicated in the metabolism of specific carcinogens: CYP2D6, CYP1A1, GSTM1 (glutathion-S-transferase) and N-acetyltransferase. The two cytochromes are phase I enzymes responsible for the activation of procarcinogens, the other two are phase II enzymes implicated in detoxification reactions.

PHARMACOGENOMICS AND ADDICTION

Another vast field of pharmacogenomics is the search for genetic determinants of addictive behavior. Much work has been devoted to this notion of 'biological fate'. Addiction can be defined as a compulsive search for and intake of substances (alcohol, tobacco, and drugs), despite recognized undesirable and serious consequences related to their consumption. Two opposing theories have been developed to explain the origin of drug addiction. The first is that drug intake induces biological and functional changes in the brain, leading to a state of dependency. The second theory considers drug addiction to be a pathological state resulting from an interaction between a vulnerable phenotype and the toxic substance, which implicates pathological intake. For the first theory, the drug is the determining agent, for the second it is the individual's traits [73]. On a neurobiological level, the reward process and the subjective sensation of pleasure is mediated by the dopaminergic neurons of the mesencephalus, particularly their projections to the nucleus accumbens [74]. Dopaminergic hyperactivity appears to be a common pathway of response to drug intake [75,76].

The metabolism of alcohol requires two enzymatic steps catalyzed by 1) five classes of alcohol dehydrogenases (ADH) that produce acetaldehyde, and 2) aldehyde dehydrogenases that transform acetaldehyde into acetate. These two enzymes exhibit polymorphism [77,78]. The accumulation of acetaldehyde induces what is called an anti-abuse reaction manifested by unpleasant or dangerous symptoms (congestion, headache, nausea, hypotension, rhythm disorders). This reaction can be triggered by the ingestion of substances blocking enzyme activity (disulfiram, metronidazole, etc.). The presence of the *ADH2* His 47 allele increases the rate of acetaldehyde formation, while the allele *ALDH2* Lys 487 limits its clearance, leading to accumulation in both cases. These two alleles are frequent in Asian populations. It has been established that persons with a genotype favoring abnormal concentration of acetaldehyde

(*ALDH2**2 for example) have a protector effect against the development of alcohol dependence (homozygous state) or a lower risk (heterozygous state). The implication of the dopaminergic system in the development of addiction has led to much research into the linkage between the genes coding for dopamine receptors and alcoholism. The results of the preliminary studies have been rather contradictory, but several meta-analyses have demonstrated an association of gene *DRD2* allele A1 with alcoholism, an association that is even more robust when alcoholism is more severe (see [79] for review). The presence of the allele A1 is associated with reduced activity of dopaminergic transmission, linked with a weaker density of D₂ receptors.

Various studies, particularly studies of twins, have demonstrated the importance of genetic factors in the development of tobacco addiction, although the causal genes have not been identified. The principal site of action of nicotine is the acetylcholine nicotinic receptor, but like almost all addictive compounds, nicotine also stimulates dopaminergic transmission, particularly in the nucleus accumbens shell. A large number of candidate genes possibly implicated in smoking behavior have been proposed, particularly those governing the different steps of dopamine metabolism and the structure of dopamine receptors (see [80] for review), as well as those implicated in the metabolism and activity of nicotine [81]. But here again, it is too early to draw any conclusion. Published studies are still too sparse and sometimes contradictory. For example, Batra et al. [82] did not find a link between 'heavy smoking' and *DRD2*, while Noble et al. [83] and Comings et al. [84] found a positive link.

Opiate dependency provides our final example. In the United States, it is estimated that among the 3 million persons who have experimented with heroin, half have or will become dependent, raising the question of determinism. Several family studies have shed some light on a certain inheritable characteristic of the risk of developing opiate dependency, but no specific genes have been identified. It is generally hypothesized that innate differences in phenotypic expression are related to multiple allelic combinatory possibilities yet to be elucidated [85]. All the genes coding for proteins implicated in opiate transmission (μ , κ , δ receptors, proopiomelanocortin, enkephalines, and dynorphine) exhibit polymorphism. Several SNP have been described for each (see [86] for review). Dopaminergic transmission is also involved, since opiate binding to μ receptors situated on gabaergic interneurons of the ventral tegmental region provoke an increase in dopamine concentration in the nucleus accumbens. But the majority of the eleven studies examining candidate genes implicated in the synthesis of receptors or transporters of the dopaminergic or serotonergic systems, or the gabaergic, cannabinoid or opiate receptors listed by Lichtermann et al. [86], have been unable to authenticate their implication. The A118G mutation of the μ receptor gene, identified in 10% of the population, produces a modified protein (Asn40/Asp) with a much higher affinity for β endorphines, but without a formal link between the modified

risk of addiction and opiate consumption. Li et al. [87] found an excess of gene *DRD4* LL alleles in a population of Chinese addicts. These findings were confirmed by Kotler et al. [88] in an Israeli population, but Lusher et al. [89] were unable to find any predisposition to opiate addiction related to the presence of the LL allele, which, however, compared to the SS genotype, may be involved in the severity of the dependence. The protective effect of a deletion of the *CYP2D6* gene, which leads to anomalous metabolism of oral opiates (these persons are also insensitive to the analgesic effect of codeine), has also been reported by Tyndale et al. [90], although the methodology used may be questioned.

PHARMACOGENOMICS AND DRUG DEVELOPMENT

Preclinical research

Drug development [91] is a long and costly process. Preclinical research must necessarily identify any prohibitory property of a candidate compound which would inevitably lead to the failure of the approval procedure or withdrawal, after initial authorization, due to serious adverse effects (fail early – fail cheap). Any compound strongly and specifically metabolized by a polymorphous cytochrome carries a high risk of triggering serious undesirable effects in persons with an unfavorable polymorphism. In addition, this type of compound would be implicated in drug interactions, further compromising its future. There is a long list of historical cases: mibefradil, terfenadine, perhexiline, etc. Undesirable pharmacokinetic properties lead to the interruption of clinical trials for 30–40% of all compounds tested [92]. Inversely, the development of a truly innovative molecule should not be prevented by the polymorphism of its metabolism [93]. Several methods have been developed to test *in vitro* drug metabolism: human recombinant cytochrome P450 proteins, liver microsomes, human hepatocyte cultures, liver slices [94].

Clinical trials

When designing clinical trials, genotype can be used *a priori*, as an exclusion criterion. With this methodology, the study group can be smaller and more homogeneous, though less representative. Using this method, Murphy et al. [95] determined the pattern of the *CYP2D6* allele in patients with major depression, using the 'poor metabolizer' trait as an exclusion criterion in order to test the effect of antidepressants. Phase I trials can thus be designed for representative populations of the principal metabolic patterns [96]. Alternatively, the genotype can be used *a posteriori*, as a stratification factor. Certain authors advocate the use of a 'CYP passport' for volunteers who participate regularly in clinical trials. The passport summarizes all tests performed and would optimize their exploitation [96]. It should also be recalled that ethnic origin has an effect on allelic polymorphism, and that trials conducted in Caucasians may not necessarily be extrapolated to other ethnic groups [97].

Applying pharmacogenomics to clinical trials also requires a revolution in the underlying philosophy. The selection of safe effective drugs designed for the widest possible population can and should be replaced by the search for compounds dedicated to genetically defined subpopulations with specific indications and contraindications. A compound with a truly beneficial effect for only 20% of the general population should not be rejected but prescribed for selected responders. The FDA has awarded marketing approval for monoclonal antibodies for the treatment of breast cancer that are active in only 10–40% of the population, *i.e.* patients who over-express *HER2* [98].

Finally, the impact of pharmacogenomics is not limited to drug development. Pharmacogenomics will also change the course of new drug discovery by revolutionizing drug design, based on a new approach to search-and-screen for new molecular targets.

CONCLUSIONS

Pharmacogenomics is a new and revolutionary chapter in the history of pharmacology. It will replace the long-standing notion of responders/non-responders with a more organic approach, where idiosyncrasy becomes an obsolete concept. Likewise, certain drugs will have to be marketed with a kit assay or with a validated dosage method, to follow circulating concentrations and individually titrate dosage. Other drugs will be marketed with their own genetic diagnosis kit [99]. The widespread use of pharmacogenomic tests will prompt necessary debate on a large number of ethical issues beyond the scope of this article: confidentiality, availability of information disquieting for the patient and his/her family, anonymous sampling for banks, etc. [100]. The principal limitation to the application of pharmacogenomic techniques is strictly theoretical, and results from the fact that current knowledge on phenotype-genotype correlation is based on statistical observations not totally verifiable on the individual level.

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