

## Cardiovascular pharmacogenetics in the SNP era

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**Summary.** In the past pharmacological agents have contributed to a significant reduction in age-adjusted incidence of cardiovascular events. However, not all patients treated with these agents respond favorably, and some individuals may develop side-effects. With aging of the population and the growing prevalence of cardiovascular risk factors worldwide, it is expected that the demand for cardiovascular drugs will increase in the future. Accordingly, there is a growing need to identify the 'good' responders as well as the persons at risk for developing adverse events. Evidence is accumulating to indicate that responses to drugs are at least partly under genetic control. As such, pharmacogenetics – the study of variability in drug responses attributed to hereditary factors in different populations – may significantly assist in providing answers toward meeting this challenge. Pharmacogenetics mostly relies on associations between a specific genetic marker like single nucleotide polymorphisms (SNPs), either alone or arranged in a specific linear order on a certain chromosomal region (haplotypes), and a particular response to drugs. Numerous associations have been reported between selected genotypes and specific responses to cardiovascular drugs. Recently, for instance, associations have been reported between specific alleles of the apoE gene and the lipid-lowering response to statins, or the lipid-elevating effect of isotretinoin. Thus far, these types of studies have been mostly limited to *a priori* selected candidate genes due to restricted genotyping and analytical capacities. Thanks to the large number of SNPs now available in the public domain through the SNP Consortium and the newly developed technologies (high throughput genotyping, bioinformatics software), it is now possible to interrogate more than 200 000 SNPs distributed over the entire human genome. One pharmacogenetic study using this approach has been launched by GlaxoSmithKline to identify the approximately 4% of patients who are predisposed to developing a hypersensitivity reaction to abacavir, an anti-HIV agent. Data collected thus far on the HLA locus on chromosome 6 indicate that this approach is feasible. Extended linkage disequilibrium can be detected readily, even

across several haplotype blocks, thus potentially reducing the number of SNPs for future whole-genome scans. Finally, a modest number of cases and controls appears to be sufficient to detect genetic associations. There is little doubt that this type of approach will have an impact on the way cardiovascular drugs will be developed and prescribed in the future.

**Keywords:** cardiovascular disease, genes, pharmacogenetics, single nucleotide polymorphisms.

### 'The right drug for the right patient', a new therapeutic paradigm

Age-adjusted cardiovascular mortality has markedly decreased in Western countries over the last few decades [1]. There is substantial evidence to indicate that this success is partly accounted for by the development of thrombolytic therapies and the prescription of antiplatelet and other agents in the secondary prevention of cardiovascular diseases, as well as by a wider use of more effective pharmacological interventions to treat hypertension and dyslipidemia [2]. Despite these major accomplishments, cardiovascular diseases remain the major cause of death in industrialized countries. This is due in part to the facts that cardiovascular risk factors remain highly prevalent, underdiagnosed and/or insufficiently treated [3]; not all patients respond equally well to pharmacological interventions; and the population is aging. Moreover, there is ample evidence to indicate that, with the growing epidemics of obesity [4,5] and Type 2 diabetes [6] progressively affecting Western countries, and the increasing prevalence of cardiovascular risk factors in developing countries [7,8], the incidence of cardiovascular diseases and the demand for safe and effective drugs to prevent/treat these diseases will steadily increase worldwide. Accordingly, there is a growing need for ways to better identify people who have the highest chance to benefit from pharmacological interventions, and those who have the lowest risk of developing side-effects when exposed to cardiovascular drugs.

To date, only a fraction of people treated with a particular drug fully benefit from such interventions. This is particularly apparent in the case of antihypertensive therapies. It is estimated that only 25–50% of patients who receive one type of therapy (for instance an ACE inhibitor or a calcium channel blocker) will have their blood pressure controlled by this

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treatment [9]. The remaining 50–75% will have received the treatment, may experience side-effects, and yet their blood pressure will not be normalized. This lack of consistency [10] in terms of efficacy not only affects the individual patients, but also drug-producing companies. Indeed, many programmes have been terminated because the average response to one particular drug has been insufficient, yet a substantial proportion of patients may have adequately responded to this intervention. Similarly, effective drugs have been removed from the market because a fraction of patients have developed intolerable side-effects. It is obvious that a better way to identify the people who will adequately respond to the drug, and those who are prone to develop side-effects, would have a major impact on the development and prescription of new cardiovascular drugs. Evidence is accumulating to indicate that pharmacogenetics may significantly assist in meeting the challenge of ‘the right drug for the right patient’ [11,12].

### The pharmacogenetics challenge

The way individuals respond to a particular drug – in terms of both efficacy and side-effects – depends on a variety of parameters, including compliance, bioavailability, drug–drug interactions, catabolization of the drug and its metabolites, the molecular mechanism responsible for the disease for which patients are treated, and what our ignorance prompts us to designate as ‘idiosyncratic reactions’. Because a variety of genes encoding enzymes, transporters or receptors involved in drug absorption, metabolism, excretion and mode of action are polymorphic (with some of these polymorphisms being functionally active), a genetic predisposition is likely to account for part of the interindividual variability in response to drugs [13,14]. The example of slow acetylators who carry a particular sequence variant within the N-acetyl-transferase 2 gene and thus poorly catabolize isoniazid or procainamide, illustrates how one single gene variant can contribute to the occurrence of severe side-effects, in this particular case hypersensitivity reactions [15]. Another recent example of a particular response to drugs that is mostly dependent on one single gene is provided by hyperbilirubinemia during administration of Tranilast. This side-effect preferentially develops in carriers of one particular variant of the UDG-glucuronosyltransferase 1 gene, the gene responsible for Gilbert syndrome [16].

It is anticipated, however, that atypical drug responses are rarely due to one gene only. For instance, it is generally accepted that African-Americans respond better to diuretics (and less well to ACE inhibitors [17]) than Caucasians, due to a higher susceptibility to salt retention (and thus a lesser activated renin-angiotensin system), and that this susceptibility does not seem to be dependent on one single gene. Elucidation of the genetic basis for this particular response to antihypertensive agents may not only allow us to accurately predict who will most likely benefit from one particular type of drug, it would also significantly contribute to our understanding of the hypertensive disease.

This latter example illustrates how discoveries in pharmacogenetics may impact on disease genetics, and vice versa, even if

these two disciplines have their own characteristics. Traditionally, the goal of disease genetics is to identify genetic variants associated with a particular susceptibility to developing a disease. The genetic contribution to diseases and the way such diseases are inherited can be estimated from twin and family-based studies. Moreover, because genetically determined diseases can be evaluated in multiple members within families, it is possible to perform family-based molecular genetic studies, such as linkage analyses. Linkage studies have been shown to be very powerful in identifying the molecular basis of Mendelian disorders (for instance Liddle’s syndrome, a rare form of salt-sensitive hypertension due to gain-of-function mutation within the epithelial sodium transporter in the proximal tubule of the kidneys [18]). Most common diseases, however, are thought to be polygenic and multifactorial, due to interactions of environmental factors and a particular genetic make-up. As an example, it has recently been demonstrated that smoking is very prevalent in early onset (usually considered highly genetically determined) forms of coronary artery disease [19]. This observation further illustrates the need to include the environment as a factor in genetic studies on complex conditions.

Pharmacogenetics can rarely rely on family-based studies, because usually only one family member is treated with a particular drug. Accordingly, the heritability and the mode of inheritance of such responses are hard to predict, and family-based molecular genetic studies would be difficult to perform. At this stage pharmacogenetics is an experimental science that mostly relies on association studies, i.e. studies comparing the distribution of sequence variants between cases who develop a particular response to the drug and appropriate controls who do not develop this type of response. The chance is that, in contrast to disease genetics, the environmental factor is limited (i.e. to the drug), simplifying the analysis to some degree.

A recent study illustrates how pharmacogenetics may assist in exploring the genetic basis of a complex disease, in this particular case hypertriglyceridemia. In this study, the authors postulated that hypertriglyceridemia, which occasionally accompanies the administration of isotretinoin (a vitamin A derivative used to treat acne), occurs preferentially in individuals who have a predisposition to lipid disorders, and that elucidation of the genetic basis for this side-effect may assist in our understanding of hyperlipidemia. The data showed that those individuals have a higher risk of developing metabolic syndrome (an aggregation of lipid disorders, hypertension and glucose intolerance) than individuals for whom plasma triglyceride levels remained unchanged during this therapy. As a proof of concept, it was shown that the lipid response to isotretinoin was closely associated with the apoE E2 allele (which is associated with Fredrickson Type III hyperlipoproteinemia) and apoE E4 allele (which is associated with higher lipid levels in the plasma than the wild-type apoE E3 allele) [20].

### Pharmacogenetics: the candidate–gene association studies

Thus far, restricted genotyping and analytical capacities have limited pharmacogenetics to association studies of *a priori*

**Table 1** Pharmacogenetic studies: candidate-gene vs. whole-genome SNP association studies

	Candidate-gene approach	Whole-genome SNP scan
SNP selection	<i>A priori</i>	Unprejudiced
Number of SNPs examined	3–5 per gene	>100 000 per genome
Genotyping technology	Low-tech	High-throughput
Analytical support	Limited	Sophisticated
Number of subjects	Large	Possibly modest
Costs	Low	High
Additional benefits	Backed by biology, more targeted and sensitive approach	Chances to elucidate novel pathogenic mechanisms (disease genetics)

selected candidate genes. In this approach, genes to be tested are usually selected based on a previous understanding of the way drugs are metabolized, or based on the biological pathway that is affected by the drugs.

A variety of conditions need to be met simultaneously for genetic associations to be detected, both for medical genetics and pharmacogenetics. The example of apoE-associated lipid response to isotretinoin may help illustrate this point. For such an association to be detected, genetic variants obviously need to be identified and accurately analyzed (using methods like restriction fragment length polymorphisms, allele-specific amplification or direct sequencing). These variants should by themselves be functionally active (for instance by modifying the affinity of apoE to the LDL-receptor), or should be in linkage disequilibrium (see below) with other variants located in exons, in intron-exon junctions or regulatory sequences that are functionally active. In addition, the functional impact of these variants should not be fully compensated by other mechanisms (for instance by upregulation of the LDL-receptor) that would abolish the phenotypic expression of this particular genotype. Moreover, the distribution of the variant should be sufficiently different in cases and controls, and the number of cases and controls examined should be large enough (usually several hundred individuals) for associations to be detected. Finally, appropriate environmental factors may need to be evaluated and included in the analysis to reveal genetic associations.

A large number of associations between predefined candidate-genes and specific responses to drugs have been described so far [for reviews, see, among others refs 14, 15, 21 and 22]. In particular, associations have been reported between sequence variants within genes encoding metabolizing enzymes (like CYP2D6) and increased response to warfarin (and subsequent risk of bleeding) or higher incidence of side-effects when exposed to  $\beta$ -blockers. Similarly, severe arrhythmias have been associated with sequence variants within genes encoding potassium channels and exposure to antiarrhythmics. In the same way, the lipid-lowering effect of statins has been associated with specific alleles of the apoE or the CETP genes.

### Pharmacogenetics: moving to whole-genome SNP association studies

Rapid technological improvements in high-throughput genotyping, and developments in bioinformatics are now opening the way for an unprejudiced exploration of the entire genome to

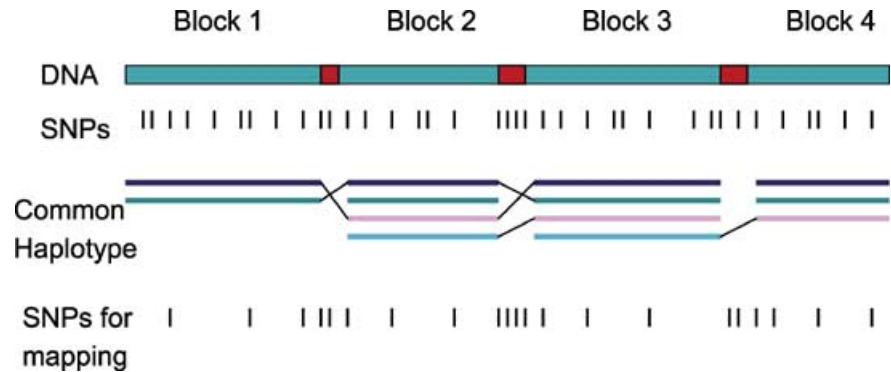
identify genes of susceptibility to a particular drug response [16] (Table 1). One particular advantage of such an approach is that, beyond finding tests to better predict the response to the drug, this unbiased approach may reveal totally unexpected genetic associations. As such, this type of pharmacogenetic approach may yield major benefits to disease genetics, as discussed above.

The concept of whole-genome-based association studies is relatively simple and takes advantage of the accumulating knowledge on SNPs. SNPs represent changes in nucleotides that are present in a substantial proportion of the population. Most SNPs are bi-allelic, making binary technologies applicable to identify and analyze them. It is estimated that there is one SNP on average for every 1000 base pairs (hence the concept that humans share 99.9% of the genome in common), and that there are approximately 3 million SNPs in the human genome (which contains  $\sim 3$  billion base pairs). Accordingly, the basic idea of a whole-genome SNP association study is to compare the frequency of these variants between cases and controls. To be successful, this type of approach relies on several factors: the availability of SNPs in sufficient numbers to cover the entire genome; very powerful and accurate genotyping capabilities to examine large collections of SNPs; appropriate analytical approaches to detect significant associations; and the availability of genomic DNA from large cohorts of well-phenotyped cases and controls. Moreover, because analysis of 3 million SNPs for each case and control is not presently feasible, one must rely on the fact that several SNPs will provide the same information as many SNPs in a particular region, due to the phenomenon of linkage disequilibrium (Fig. 1).

The SNP Consortium (<http://www.snp.cshl.org/>), a joint academia–industry initiative, was commissioned to identify and release sequences of SNPs. The success of this initiative has been tremendous, in the sense that the Consortium has recently made available in the public domain more than 2 million SNP sequences distributed over the entire human genome (for examples see <http://www.ncbi.nlm.nih.gov/>). The major questions that now arise are (i) how to genotype these SNPs at a reasonable cost, (ii) how many of these SNPs are needed to provide adequate sensitivity and specificity for pharmacogenetic studies, and (iii) what is the required sample size.

At present, the only reliable way to answer these questions is to perform the experiments and generate experimental data. GlaxoSmithKline has recently launched such an initiative. The goal of this project is to identify genetic variants that are

**Fig. 1.** Schematic representation of the linkage disequilibrium block structure of genomic DNA. Haplotype blocks and recombination hotspots are shown in green and red, respectively. Identification of recombination hotspots is based on observed recombinations in the common haplotypes, shown as gaps or crossovers in the haplotypes. Once this structure is known, SNPs that contain all the mapping information can be chosen. Using this type of strategy, the numbers of informative SNPs is expected to be reduced to 3–5 per haplotype block.



associated with hypersensitivity reaction to abacavir, an anti-HIV agent [23]. Data accumulated thus far on the HLA locus on chromosome 6 are very encouraging. They indicate that such an experiment is feasible. Moreover, large regions of linkage disequilibrium have been detected, forming haplotype blocks. These data are important, as they suggest that the number of SNPs to be examined can be reduced in other similar future initiatives (see below), and that cluster-analysis algorithms may be developed and standardized to generate individual SNP profiles (SNP Prints<sup>SM</sup>) that define genetic-susceptibility responses to drugs. Finally, the data indicate that a modest number of cases and controls may be sufficient to achieve adequate sensitivity and specificity [23,24].

### Linkage disequilibrium and haplotype blocks: potential opportunities and issues

Haplotypes are ancestral segments of chromosomes that have been inherited as a unit throughout the generations with little genetic shuffling or mutation. They can be directly observed by typing individuals within families for genetic markers and following the coinheritance of alleles from neighbouring markers through the generations. An alternative way to determine haplotypes is to perform allele-specific sequencing, a technology that is just starting to be utilized for that very purpose [25]. Alleles that occur together in this fashion are said to show allelic association, and 'linkage disequilibrium' is the extent of this co-occurrence in the population.

It was originally thought that linkage disequilibrium would mostly reflect genetic distance and would decay in a fairly linear manner over increasing distances. However, closer examination of specific chromosomal regions has revealed instead irregular linkage disequilibrium patterns that are mostly position dependent. These patterns are composed of large stretches of DNA (~5–100 kb) where recombinations are not observed and linkage disequilibrium is high (haplotype blocks), and short intervening regions (~1–5 kb) where linkage disequilibrium is low. Jeffreys *et al.* showed that regions of linkage disequilibrium breakdown in the class II region of the major histocompatibility complex correspond precisely with meiotic recombination hotspots observed by typing sperm [26], suggesting a possible explanation for the position-dependent nature of linkage disequilibrium breakdown. However, replication in other areas of

the genome is required to establish the degree of generality of these findings.

Whatever the mechanism, the observation of haplotype blocks has clear implications for linkage disequilibrium mapping, be it for whole SNP genome scans or for specific gene regions (Fig. 1). One advantage is that fewer SNPs will be required to map associations, as a relatively smaller number will be required to provide sufficient representative information within the blocks. Furthermore we will know exactly where a greater saturation of markers is required, in the regions of low linkage disequilibrium. However, the presence of large haplotype blocks will also limit the resolution of association methods to fine-map a susceptibility gene, if it falls within one of these blocks, as additional markers will not necessarily provide any more information.

Determining these haplotype blocks may therefore be pivotal in the success of whole-genome association studies, and to this end a major worldwide effort is underway to create a haplotype map of the human genome, which is expected to be completed in 2–3 years. The question then arises, how different are the patterns of linkage disequilibrium among different ethnic groups, and will it be necessary to have separate maps for each group? A recent study found that Caucasian and Asian haplotypes are very similar, but haplotypes of African origin are quite different [27]. The authors estimate that approximately half the human genome exists in blocks of 44 kb or larger in Caucasian and Asian samples, and 22 kb or larger in African and African-American individuals. Within each block 3–5 haplotypes typically capture about 90% of all chromosomes in each population. They propose that to perform a fully powered association study will require as many as 300 000 SNPs in non-Africans and 1 million SNPs in Africans. Theoretically, this would suggest that populations who have undergone population bottlenecks and hence have more extensive linkage disequilibrium would be more useful for initial localizations, and that populations with shorter range linkage disequilibrium would be more useful for fine mapping. At this stage, it is still debated whether the optimal strategy will be that simple.

A haplotype map of the human genome may therefore be critical in streamlining the process involved in association mapping and may provide much information on the structures and histories of human populations. However, assuming that the 'common variant–common disease' theory is correct, it is possible that rare variants will easily be missed with this

strategy and more than one mutation in one disease gene will split the association signal, rendering it undetectable. Thus, there is a great deal of speculation and projection at the current time, which will only be resolved by the elucidation of some common disease or drug-response genes that will allow proof of principle methods to be developed.

### Cardiovascular pharmacogenetics: hopes, hurdles and challenges

The data accumulated thus far are very encouraging, and suggest that the concept of 'the right drug for the right patient' is becoming a closer reality. There are still major hurdles to overcome. Large clinical trials need to be performed (in various ethnic groups) to consolidate the validity of this approach; such initiatives are underway in industry and academia (ex GenHat) [21], and the results of these experiments should be available within the next few years. Next, additional technological improvements are necessary to bring the cost of genotyping down to a level that allows pharmacogenetics to become economically attractive; here again, DNA chip technologies are presently being developed that should generate tests that are affordable. In addition, regulators, payers, physicians and patients should agree with such projects. In this respect, pharmacogenetics may raise ethical issues that are similar to the ones raised by disease genetics. A major effort will be required from the patients, their physicians, academia and industry to overcome these hurdles. In our opinion, the potential beneficial impact of pharmacogenetics for the patients and society in general is well worth the effort.

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