

# Pharmacogenetics: a molecular sophistication or a new clinical tool for cardiologists?

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The study of genetic risk factors for multifactorial diseases is attracting increasing interest. In particular interest has been focused on the interaction between genetic polymorphisms and environmental factors in determining the risk of disease. Among environmental factors therapeutic approaches should be considered. Therapeutic responses to a given drug, failure of drug efficacy, interindividual variability in side effects and toxicity of drugs could be at least partially accounted for by genetic polymorphisms. This paper summarizes the presently available applications of genetic concepts to some drugs commonly used in patients with cardiovascular disease. Statins and probucol fail to lower cholesterol levels in carriers of specific polymorphisms. The progression of cardiovascular disease is decreased by pravastatin only when certain polymorphisms are present. Induction problems and bleeding complications of warfarin occur in subgroups of populations carrying specific genetic variants of key enzymes in the drug metabolism. A new interpretation of the results of a thrombosis prevention trial will be given in the light of a genetic approach to pharmacology; indeed, prevention and treatment of thrombotic disease could be better focused on the basis of this knowledge. Future clinical trials and cost-effectiveness evaluation of drugs should be conducted taking these gene-drug interactions into account.

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Pharmacogenetics, polymorphisms, gene therapy, the human genome project describe a specialized subject which is developing in biomedical laboratories. Do they anticipate new clinical applications and future approaches for the health of the general population of possible interest for cardiologists<sup>1</sup>?

Revolutionary diagnostic and therapeutic expectations are emerging from the knowledge of our genetic background. They will be important for clinicians if the latter will be prepared to manage the possibilities and limits of this emerging approach.

A state of the art on the possible applications of genetic concepts to some drugs commonly used in patients with cardiovascular disease may help clarify the expected impact.

## Polymorphisms for a better understanding of diseases

Polymorphisms indicate variants of genes, which differ mostly only by one base pair from the original gene. By definition they are present in at least 1% of the gener-

al population. Sometimes up to 50% of the population is carrying a given polymorphism. Each individual is characterized by a sequence of 3 billion base pairs. One out of 300 base pairs varies from person to person, meaning that anyone of us can be distinguished from his neighbor by 10 million base pairs. Only 1% of the base pairs is functional and accounts for modifications of proteins. In this way "only" 100 000 of the 10 million base pairs is able to modify our proteins. Moreover, an indefinite number of combinations of different polymorphisms is possible.

Variability in protein levels and protein activity in blood, attributable to the presence of polymorphisms, has also been found for proteins considered as risk factors for cardiovascular diseases<sup>2</sup>. This concept allows to make direct associations between these polymorphisms and the risk of disease: a B2 allele of BclI polymorphism of the gene encoding for the  $\beta$ -chain of fibrinogen has been associated with high blood levels of fibrinogen. The risk of familial myocardial infarction is twice in carriers of this allele<sup>3</sup>. Factor VII gene 353Q polymorphism is as-

sociated with low levels of coagulation factor VII. The presence of one of these alleles reduces the risk of myocardial infarction by 50%<sup>4</sup>.

However, since polymorphisms only make a relatively small contribution to the overall risk of disease, it may be difficult to understand the impact of genotypes on the risk of disease. Epidemiological studies have not been able so far to make these differences evident<sup>5</sup>. Meta-analyses have shown that contribution of polymorphisms to the risk of disease is often limited to specific subgroups. In the case of cardiovascular disease, which is a multifactorial disease, the risk results from an interaction between several environmental and genetic factors. Rather than influencing basal levels of proteins, the presence of polymorphisms may increase or decrease the susceptibility to disease by modulating the response to environmental factors, such as diet, smoking, physical activity and exposure to infectious agents<sup>2,6</sup>. The B2 allele of the BclII polymorphism has been shown to increase the risk for cardiovascular disease related to seropositivity for *Helicobacter pylori*<sup>7</sup>. The latter increases fibrinogen levels and, consequently, the risk for myocardial infarction. The presence of the B2 polymorphism has an additive effect on both fibrinogen levels and the risk for myocardial infarction in patients seropositive for *Helicobacter pylori*. In contrast, in the absence of *Helicobacter pylori* infection, B2 polymorphism seems to have no effect on the risk of cardiovascular disease.

The presence of 353Q allele of coagulation factor VII gene modulates the risk for cardiovascular disease particularly by decreasing the detrimental effect of smoking<sup>8</sup>. Smoking remains a potent risk factor, but compared to carriers of the 353R allele, carriers of the Q allele appear in a way less exposed. On the contrary, the effect of smoking on the risk of myocardial infarction at young age is expressed more in carriers of P1<sup>A2</sup> allele of platelet glycoprotein IIIa gene<sup>9</sup>.

The consequence of these findings for clinical practice would lie in identifying subgroups of patients in whom the impact of genetic or environmental factors could be stronger. Identifying them, in turn, can help address more effective preventive and therapeutic strategies.

### Polymorphisms as the basis for different response to drugs

The therapeutic effect of drugs, indeed, should be reconsidered in this context<sup>10-12</sup>. Indeed, individual variation in response to drugs is an important problem in terms of clinical pharmacology and public health.

There are several examples that show the potential importance of genetic modulation of drug therapy in daily clinical practice. Different pathways are possible for such a modulation. First, polymorphisms can influence drug metabolism by modifying the function of drug

metabolizing enzymes. In this case, the polymorphisms shall determine the extent of the metabolism and, as a consequence, the same administered doses may reach therapeutic levels in one individual but exceed safety margins for adverse effects or toxicity in another.

Secondly, polymorphisms are able to modify the pharmacokinetics of a given drug affecting its binding capacity to target proteins as carriers or receptors. Absorption, distribution, excretion and targeting to the site of action can also be influenced and so the ultimate pharmacological response of the drug<sup>10</sup>. From a clinical point of view, such variations can determine effective pharmacological response or failure to respond to a given drug.

### Polymorphisms and the efficacy of cholesterol lowering drugs

Lipid lowering drugs, such as statins, are widely used to reduce cholesterol levels as they may prevent the development of ischemic events in patients with dyslipidemia or cardiovascular disease<sup>13,14</sup>. However, they fail to do so in a large number of patients. Patients in clinical practice who do not reach the expected reduction in cholesterol levels have been considered up to now not to comply with the therapy or to make dietary mistakes. Genetic determinants could be instead responsible for these variable responses and should be taken into consideration.

CYP2D6 gene codifies for one of the enzymes of the hepatic cytochrome P450 system that catalyzes the metabolism of many drugs, including simvastatin. Polymorphisms of the gene encoding for CYP2D6 may modify the metabolism of this drug. A study has been conducted on healthy volunteers to measure the catalytic activity of this polymorphism<sup>15</sup>. The hepatic metabolism of simvastatin is inhibited by the presence of the variant allele of the CYP2D6 polymorphism, so that homozygous carriers are called "poor metabolizers". In these people, who represent up to 7% of the white population, a given dose of simvastatin has a higher cholesterol lowering effect compared to carriers of the wild type. In contrast, carriers of the duplicated or multiduplicated wild type CYP2D6 gene, called "rapid" or "ultrarapid" metabolizers, need higher doses of simvastatin to reduce cholesterol levels to the same extent. Knowing in advance the genetic characteristics of the patients might help establishing the right doses to prescribe. Therefore, new clinical trials are needed to evaluate the effects of these drugs in genotype-based subgroups. Indeed, a comparison of "poor" metabolizers against "rapid" metabolizers might show totally different outcomes.

Besides their effect on pharmacological end-points, polymorphisms may also affect the efficacy of statins in reducing cardiovascular events. The B1 polymorphism of the cholesteryl ester transfer protein (CETP), present in 50% of the white population, is responsible for

increased LDL plasma levels. Increased levels of CETP in the blood enhance the transport of cholesterol to the liver and simultaneously decrease HDL plasma levels. A dose-dependent relationship exists between genetically determined enhanced CETP levels and progression of coronary disease. Therapy with pravastatin seems to reduce, independently of the levels of cholesterol, the progression of coronary atherosclerosis only in carriers of the B1 polymorphism. In contrast, homozygous B2B2 carriers, representing 16% of the population, do not benefit from pravastatin treatment<sup>16</sup>.

Another example, in the context of the use of cholesterol lowering drugs in hypercholesterolemia, demonstrates an interindividually different therapeutic response to probucol among patients classified in the same category of dyslipidemia according to genetic polymorphisms. Carriers of the ApoEε4 polymorphisms are characterized by higher LDL plasma levels in comparison with carriers of the ApoEε3 and ApoEε2 polymorphisms. The cholesterol lowering effect of probucol, an antioxidant substance, is differently distributed among carriers of the ApoE polymorphisms. Familial and non-familial polygenic hypercholesterolemia might both benefit from decreasing LDL with probucol but the highest decrease might be found in carriers of ApoEε4<sup>17</sup>.

On the basis of these findings, the design of clinical trials and the cost-effectiveness of drugs should be reconsidered since a subgroup analysis of apparently comparable populations could result in beneficial or adverse effects not detected so far<sup>18</sup>. This approach seems of little advantage for the pharmaceutical industry as many drugs might not be used anymore on a large scale. However, a burst of new drugs, focused on specific effects in specific conditions, should be expected. Again, cardiologists should be prepared to these new evolutions.

### Polymorphisms and the safety of oral anticoagulants

Control of side effects is one of the major challenges of modern pharmacology<sup>19</sup>. Indeed, despite the progress in obtaining more safe and specific drugs, adverse events are still unpredictable and account for a large part of the health care costs. Pharmacogenetics can help resolve the problem, as illustrated by the following example.

Warfarin is the oral anticoagulant drug most largely used in patients with venous and arterial thromboembolism. To keep the WHO International Normalized Ratio (INR) values between 2.0 and 3.0, doses of warfarin fluctuate from 1 to 20 mg or more daily. Standardized induction schemes with monitoring of INR over the first 4 days have only a 69% success rate. Such interindividual variations are partly due to the presence of polymorphisms of the CYP2C9 gene<sup>20</sup>.

CYP2C9 is a cytochrome P450 enzyme responsible for the metabolism of S-warfarin. S-warfarin, the more potent isoform of warfarin, is converted by CYP2C9

into inactive 6-hydroxy and 7-hydroxy metabolites. CYP2C9\*2 and CYP2C9\*3 polymorphisms are point mutations of the wild type gene CYP2C9. *In vitro* data show that the CYP2C9\*3 variant is less than 5% and the CYP2C9\*2 about 12% as efficient as the wild type enzyme. *In vivo* data show that the presence of the mutant polymorphism is responsible for an impaired metabolism of warfarin. Carriers of the mutant allelic forms behave as "poor" warfarin metabolizers. Up to 20% of the general population could be carrier of at least one of these variations.

To verify the impact of the CYP2C9 gene polymorphism on the warfarin dose requirements, a study has been conducted on patients requiring low daily doses of warfarin<sup>20</sup>. The frequency of the two allelic variants of CYP2C9 polymorphism in this population has been compared to that of a control group of patients with a wide range of dose requirements attending an anticoagulation clinic and to a control group of healthy volunteers. A strong association has been found between the need for a low dose of warfarin and the presence of at least one mutant allele of the CYP2C9 gene. Moreover, these subjects have difficulties at the time of induction of anticoagulation as indicated by longer hospitalization or repeated visits to the patient clinic. The decreased metabolism of warfarin also makes carriers 4 times more susceptible to bleeding complications in spite of low dose administration. The concern for this complication is justified by the high mortality risk, which is increased in the aged population. Therefore, identification of these polymorphisms before starting the therapy should help prevent complications. However, the cost of large-scale genetic screening for hyperresponsiveness to warfarin should be verified and weighted against prolonged hospitalization, emergency admission for complications and mortality risk.

### Pharmacogenetics: a new key of interpretation of thrombosis prevention trials

Analyzing the results of the Thrombosis Prevention Trial<sup>21</sup> in the light of a genetic approach to pharmacology, new interpretations for drug efficacy and new clinical trial design emerged<sup>22</sup>. This trial was performed by general practitioners in the United Kingdom and aimed at finding differences in a primary prevention approach of high risk individuals for cardiovascular disease, by treating them with low dose warfarin and/or low dose aspirin against placebo. The prospective follow-up revealed that the group receiving warfarin had the same reduction in all ischemic heart diseases as did the group receiving aspirin. This effect was greater for the group receiving the combined therapy. Bleeding being still a problem of anticoagulant and antiplatelet therapy, subjects receiving the combination showed an increased hemorrhagic risk as compared to placebo and individual therapies. Considering genetic categories related to

platelet function and coagulation factor activity could provide a model to predict drug efficacy and risk of bleeding of patients and ultimately the choice of the antithrombotic drug.

The glycoprotein IIb/IIIa complex acts as a receptor for fibrinogen and other adhesive molecules on the surface of platelets and is required for their aggregation. Polymorphism  $PI^{A2}$  is a variant of the gene encoding for glycoprotein IIIa and is present in 25% of the white population. This polymorphism has been associated with an increased risk of coronary heart disease, especially at young age and in patients undergoing coronary bypass<sup>23,24</sup>. It has recently been shown that the  $PI^{A2}$  allele is associated with an increased reactivity of platelets to aggregation and with an altered integrin-mediated function of adhesion, spreading and clot retraction<sup>25</sup>. Moreover, the presence of the  $PI^{A2}$  polymorphism might influence the antiaggregatory effect of aspirin. Indeed, to reach the same antiaggregation effect in carriers of the  $PI^{A2}$  polymorphism, a higher dose of aspirin was required<sup>26,27</sup>.

However the relevance of genetic modulated platelet reactivity should be confirmed in large studies.

Polymorphisms in coagulation factor VII and their effect in reducing factor VII levels and the risk of myocardial infarction have been mentioned above.

On the basis of these considerations, the population included in the Thrombosis Prevention Trial could be divided into four groups according to the polymorphisms  $PI^{A1/A2}$  of platelet glycoprotein IIIa and R356Q of factor VII gene (Table I)<sup>21</sup>. The first group includes subjects carrying the alleles  $PI^{A2}$  and 353Q. The second one carries the allele 353R combined with  $PI^{A1}$ . The third group includes carriers of factor VII allele 353R but combined with  $PI^{A2}$ . Finally, the fourth group consists of factor VII allele 353Q carriers combined with  $PI^{A1}$  allele of glycoprotein IIIa.

According to the associations genotype/phenotype described in the literature, these groups shall have: increased platelet aggregability and low factor VII levels (first group), decreased platelet function and high factor VII levels (second group); increased platelet function and high factor VII levels (third group), and finally de-

creased platelet function and low factor VII levels (fourth group). We could expect that the first group would benefit from aspirin alone. Indeed, reduction by warfarin of already low levels of factor VII could only expose patients to hemorrhagic side effects. In the same way, the second group should only benefit from warfarin, the third group from the combination of aspirin and warfarin, and finally the fourth group should not benefit either from aspirin or from warfarin. The latter, could, indeed, be an ideal group for testing new antithrombotic drugs.

## Conclusions

Therapeutical and preventive approaches in large populations need a personalized evaluation of their genetic background. As the risk chart for primary and secondary prevention represents a first attempt to personalize therapy on the basis of individual combinations of risk factors, efforts should be made in the future to adjust for genetic characteristics. Polymorphisms could modify the effect of environmental factors such as diet and smoking. They also modify pharmacological effects such as dose relationship, adverse effects, therapeutic doses, and finally the overall beneficial effect. Nowadays clinical trials become almost impossible to organize because of the large sample size required to show a minimal effect of a new drug added to a long list of already recommended drugs. Screening for polymorphisms could allow magnification of drug effects in subgroups of susceptible subjects. The problem of poly-medication could be partly resolved by this approach defining in advance which patients should really benefit, maximizing efficacy of treatment and reducing adverse effects. Cost-effectiveness should be recalculated in this context. Contradictory results derived from epidemiological studies are presently matter of confusion for physicians: some of them should be revised and may find new answers taking genetic backgrounds into account.

How long should we wait?

Although DNA chips, able to scan the entire genome will be available in the near future, many years will probably be necessary to really affect the daily therapeutic

**Table I.** Simulation of results of the Thrombosis Prevention Trial<sup>21</sup> if patients were categorized according to platelet glycoprotein (GP) IIIa  $PI^{A1/A2}$  and R353Q factor VII (FVII) polymorphisms.

GP IIIa $PI^{A1/A2}$ allele	Platelet reactivity	R353Q allele	FVII levels	Warfarin	Aspirin
A2	↑	Q	↓	-	+
A1	↓	R	↑	+	-
A2	↑	R	↑	+	+
A1	↓	Q	↓	-	-

↑ = increased; ↓ = decreased; + = therapeutical benefit; - = no therapeutical benefit.

decision-making of cardiologists by genetic technologies. The low penetrance of single polymorphisms and the necessity to analyze combinations of predisposing alleles, with or without environmental exposure, make the prediction of genetic risk of disease very difficult. However, testing for factor V Leiden as a risk factor for recurrent thromboembolic events has already been introduced in clinical decisions<sup>28</sup>. The measurement of the polymorphism of the enzyme thiopurine methyltransferase is used currently in clinics for successful treatment of childhood leukemia to avoid severe toxicity in some patients and to reach optimal doses in others<sup>29</sup>. These examples show how close we are to a real and important application in daily clinical decisions even if studies have to be conducted to clarify and deepen this complex and wide terrain. In the meanwhile, cardiologists should be aware of polymorphic susceptibility of complex diseases and of pharmacogenetic variability.

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### References

1. Fears R, Roberts D, Poste G. Rational or rationed medicine? The promise of genetics for improved clinical practice. *BMJ* 2000; 320: 933-5.
2. Humphries SE. The genetic contribution to the risk of thrombosis and cardiovascular disease. *Trends Cardiovasc Med* 1994; 4: 8-17.
3. Zito F, Di Castelnuovo A, Amore C, D'Orazio A, Donati MB, Iacoviello L. Bcl I polymorphism in the fibrinogen beta-chain gene is associated with the risk of familial myocardial infarction by increasing plasma fibrinogen levels. A case-control study in a sample of GISSI-2 patients. *Arterioscler Thromb Vasc Biol* 1997; 17: 3489-94.
4. Iacoviello L, Di Castelnuovo A, De Knijff P, et al. Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. *N Engl J Med* 1998; 338: 79-85.
5. Kluft C. Genetic research stimulates development of individual treatment. *Cardiologia* 1999; 44: 119-24.
6. Humphries SE, Panahloo A, Montgomery HE, Green F, Yudkin J. Gene-environment interaction in the determination of levels of haemostatic variables involved in thrombosis and fibrinolysis. *Thromb Haemost* 1997; 78: 457-61.
7. Zito F, Di Castelnuovo A, D'Orazio A, et al. Helicobacter pylori infection and the risk of myocardial infarction: role of fibrinogen and its genetic control. *Thromb Haemost* 1999; 82: 14-8.
8. Iacoviello L, Di Castelnuovo A, D'Orazio A, Donati MB. Cigarette smoking doubles the risk of myocardial infarction in carriers of a protective polymorphism in the blood coagulation factor VII gene. *Thromb Haemost* 1999; 81: 658.
9. Ardissino D, Mannucci PM, Merlini PA, et al. Prothrombotic genetic risk factors in young survivors of myocardial infarction. *Blood* 1999; 94: 46-51.
10. Sadee's W. Pharmacogenomics. *BMJ* 1999; 319: 1286.
11. Roses AD. Pharmacogenetics and future drug development and delivery. *Lancet* 2000; 355: 1358-61.
12. Wolf CR, Smith G, Smith RL. Science, medicine, and the future: pharmacogenetics. *BMJ* 2000; 320: 987-90.
13. Marchioli R, Marfisi RM, Carinci F, Tognoni G. Meta-analysis, clinical trials, and transferability of research results into practice. The case of cholesterol-lowering interventions in the secondary prevention of coronary heart disease. *Arch Intern Med* 1996; 156: 1158-72.
14. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; 344: 1383-9.
15. Nordin C, Dahl ML, Eriksson M, Sjoberg S. Is the cholesterol-lowering effect of simvastatin influenced by CYP2D6 polymorphism? *Lancet* 1997; 350: 29-30.
16. Kuivenhoven JA, Jukema JW, Zwinderman AH, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. *N Engl J Med* 1998; 338: 86-93.
17. Nestruck AC, Bouthillier D, Sing CF, Davignon J. Apolipoprotein E polymorphism and plasma cholesterol response to probucol. *Metabolism* 1987; 36: 743-7.
18. Fijal BA, Hall JM, Witte JS. Clinical trials in the genomic era: effects of protective genotypes on sample size and duration of trial 2000. *Control Clin Trials* 2000; 21: 7-20.
19. Wood AJ, Stein CM, Woosley R. Making medicines safer - the need for an independent drug safety board. *N Engl J Med* 1998; 339: 1851-4.
20. Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999; 353: 717-9.
21. The Medical Research Council's General Practice Research Framework. Thrombosis Prevention Trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischaemic heart disease in men at increased risk. *Lancet* 1998; 351: 233-41.
22. Iacoviello L, Donati MB. Interpretation of Thrombosis Prevention Trial. *Lancet* 1998; 351: 1205.
23. Weiss EJ, Bray PF, Tayback M, et al. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. *N Engl J Med* 1996; 334: 1090-4.
24. Laule M, Cascorbi I, Stangl V, et al. A1/A2 polymorphism of glycoprotein IIIa and association with excess procedural risk for coronary catheter interventions: a case-controlled study. *Lancet* 1999; 353: 708-12.
25. Vijayan KV, Goldschmidt-Clermont PJ, Roos C, Bray PF. The Pl(A2) polymorphism of integrin beta(3) enhances outside-in signaling and adhesive functions. *J Clin Invest* 2000; 105: 793-802.
26. Cooke GE, Bray PF, Hamlington JD, Pham DM, Goldschmidt-Clermont PJ. PlA2 polymorphism and efficacy of aspirin. *Lancet* 1998; 351: 1253.
27. Michelson AD, Furman MI, Goldschmidt-Clermont P, et al. Platelet GP IIIa Pl(A) polymorphisms display different sensitivities to agonists. *Circulation* 2000; 101: 1013-8.
28. Simioni P, Prandoni P, Lensing AWA, et al. The risk of recurrent venous thromboembolism in patients with an Arg<sup>506</sup> → Gln mutation in the gene for factor V (factor V Leiden). *N Engl J Med* 1997; 336: 399-403.
29. Krynetski EY, Tai HL, Yates CR, et al. Genetic polymorphism of thiopurine S-methyltransferase: clinical importance and molecular mechanisms. *Pharmacogenetics* 1996; 6: 279-90.