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**REFLECTION PAPER ON THE USE OF GENOMICS IN CARDIOVASCULAR
CLINICAL INTERVENTION TRIALS**

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Relection Paper on the use of Genomics in Cardiovascular Clinical Trials

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Summary:

The paper reviews scientific matters concerning the use of genomic data in assessing therapeutic efficacy and tolerance of drugs in cardiovascular clinical intervention trials, focusing on genetic association with clinical endpoints. Taking into consideration genetic issues in the design of such trials is perceived as potentially very important for improving their outcome. Considering however present limitation in the state of knowledge concerning the pharmacogenetic relevance of genomic polymorphisms related to cardiovascular disease, recommendation for general principles to be taken into account for future research are given. The issues of trial's power, data replication, and mechanistic insight into gene-treatment interaction effect are especially discussed

1. INTRODUCTION

Large clinical intervention trials in cardiovascular medicine have documented the beneficial effect of several classes of drugs on mortality and other major morbid events like myocardial infarction, stroke, and end stage renal failure. The effect of ACE inhibitors, beta-adrenergic receptors blockers, diuretics, statins and platelet aggregation inhibitors has been especially well documented. On the other hand, although major effects are observed at the population level (in the HOPE study, conducted in a large cohort of high cardiovascular risk patients, an ACE inhibitor reduced the incidence of major events by 22%, $P < 0.001$,¹), it is clear that all patients do not equally respond to these treatments and the residual number of morbid events remains high. Among all hypotheses that can be put forward to explain the variability in the individual response to established treatments is the role of genetic factors.

There are strong arguments, both theoretical and experimental, to suggest that the genetic background of the patients can contribute to the therapeutic efficacy, safety and tolerance of drugs. This is certainly true for the treatment of complex, multifactorial diseases, such as degenerative cardiovascular diseases: In this situation the variability in drug response will result from both the underlying genetic and environmental factors affecting disease progression, and those affecting drug response.

- The susceptibility to develop a disease, and its rate of progression, are often genetically determined, at least in part, with the consequence that disease susceptibility genes may interfere with the beneficial effect of treatment, especially in the long term.
- The drug targets and the metabolizing enzymes can display genetic variations in their structure, abundance or biological activity. These variations are often identified at the early stages of drug development and studied in relationship to pharmacokinetic and pharmacodynamic issues, while their possible influence on the therapeutic effect should be assessed in subsequent clinical trials.
- The pharmacological effect of the agent triggers physiological counter-regulations that can also be modulated by genetic factors.
- The tolerance to a drug, and the occurrence of adverse drug reaction, may be influenced by genetic traits.

It is possible to study genetic factors involved in drug effects in clinical trials. Beyond clinical considerations such as ethnicity and familial history, analysis at the genome or protein level of the molecular basis of the human genetic polymorphism provides a large amount of information that may then be related to therapeutic efficacy and tolerance of drugs. Individual variation in genomic DNA sequence, can be determined by using a blood sample and relies on well-standardized and often automated techniques. It is now feasible to perform these analyses on a large scale for a large number of genes, at a reasonable cost. These techniques have been largely developed in both academic and industrial laboratories.

Large clinical intervention trials now often include ancillary genetic studies aimed at discovering the genomic variations associated with the progression of the disease considered, and whether the genetic variations considered interfere with the therapeutic intervention being tested. This approach is being especially developed in the fields of cardiovascular medicine and diabetes. It is based on well-accepted scientific concepts, as discussed above, and robust biological parameters and would thus be expected to improve our understanding of the mechanisms that determine individual response to drugs. This could lead to refined claims for therapeutic indications in the future.

There is already abundant literature reporting on potential association between genomic polymorphisms and responses to cardiovascular medicines. However it appears that in most cases, a clear association between genetic traits and clinical outcomes of treatments cannot be established from these studies. It is perceived that the current state of knowledge of the polymorphisms relevant to cardiovascular disease does not appear to be sufficiently mature yet for being use as a major lead in clinical development of drugs, particularly because of the limited and fragmented knowledge that has been acquired with the type of clinical trials undertaken so far. Newer and more robust scientific

approaches for conducting clinical intervention trials where one of the specific objectives of the study is the analysis of the influence of genetic factors on treatment outcome are needed in cardiovascular medicine. A critical review of the experience gathered from clinical trials exploring the association of genomic polymorphisms and the response to medicines in the main cardiovascular diseases, followed by recommendations based on perceived strengths and deficiencies of available studies will provide below a useful document setting out general scientific principles. This will assist applicants, so that valuable approaches are undertaken for incorporating genomic variables in the development of new medicines, for submission for Regulatory review and approval.

2. SCOPE

The scope of this paper is:

- to review scientific matters concerning the use of genomic data in assessing therapeutic efficacy and tolerance of drugs in clinical intervention trials related to cardiovascular diseases, including diabetes vascular and renal complications, focusing on major clinical endpoints.
- to identify scientific and regulatory issues pertaining to the consideration of these data in the drug approval process.

Recommendations for general principles to be taken into account for future research are given. Regulatory issues concerning the collection and the use of DNA samples in pharmacogenetic studies, are reviewed in a separate document.²

3. MAIN POINTS

3.1 Generation and interpretation of data:

State of the art

Clinical intervention trials in cardiovascular medicine are aimed at evaluating the effect of a given drug, or combination of drugs, on the incidence of major life threatening events such as myocardial infarction, stroke, end stage renal disease, and eventually on mortality, all assessed prospectively in high-risk populations. The incidence of such events in these populations is generally of no more than few tenths per thousands patients per year and therefore the trials, in order to be adequately powered, will need to include several hundred or several thousand patients with a follow up time of several years, typically three to five. Although these trials have not been primarily designed for testing genetic association, genomic analyses have been performed on a subset of the included subjects, from whom DNA samples have been collected. These genetic association sub studies rely on the candidate gene approach. For example the Diabhycar-Diabhycargene or DCCT-EDIC studies⁴ include an ancillary genetic study.

To the best of our knowledge no information has been reported so far concerning the association of genomic variations with treatment effect in these major clinical intervention trials. The testing of candidate genes in these studies is currently in progress;

Most observations published so far have been gathered by studying relatively small cohorts of treated patients (no more than a few hundreds). Genetic association studies have also been performed by extracting information on disease status at a given time point from a clinical database of treated patients, or on disease progression assessed retrospectively, considering either major or surrogate end points. Such information has then been analyzed for association with genomic variations in candidate genes. This approach is obviously prone to bias in data interpretation related to power limitation, accuracy of clinical information and use of stratification, and has an increased risk of generating false positive or false negative results.

The diseases most commonly studied in cardiovascular pharmacogenomic trials are hypertension, congestive heart failure and diabetic nephropathy. Diabetic nephropathy is an interesting setting for conducting genomic association studies because it is clearly genetically determined, clinically easily to

diagnose, at least in type I diabetes, on the basis of elevated microalbuminuria, and associated not only with a high risk of renal insufficiency but also with an exaggerated incidence of major cardiovascular events.

The candidate genes tested for interaction with treatment may be genes coding for drug targets, or other genes previously associated with the risk of the disease and its progression. In the cardiovascular field the most studied genes so far have been those coding for the isoforms of the beta adrenergic receptors and associated G proteins, those coding for the components of the renin-angiotensin system, especially ACE (angiotensin converting enzyme). Genes coding for enzymes involved in the metabolism of warfarin and clopidogrel, and those coding for platelet components involved in the antiplatelet action of drugs have been extensively studied in relationship to pharmacokinetic issues, and anti-clotting effect.

Examples of genomic variations in drug target genes reported to be associated with disease outcome under treatment are: the beta1 and beta2 adrenergic receptor gene and beta blocker therapy in acute coronary syndrome, congestive heart failure, dilated cardiomyopathy and hypertension⁵; the ACE gene and ACE inhibitor treatment in diabetic nephropathy, congestive heart failure and hypertension.⁶

Not all studies are consistent but the association of the ACE gene with the beneficial effect of ACE inhibitor treatment in diabetic patients has been replicated in several studies, although most of these studies have power and design limitations, as discussed below. Polymorphisms in genes coding for proteins that are not the primary drug target, but may participate in its pharmacological effect, have also been tested. Thus the adducin and WNK1 kinase genes have been linked to the antihypertensive effect of diuretics.⁷

Specific points to be considered for the interpretation of data.

Data quality

Genomic polymorphisms are robust biological parameters but should not be considered differently from other biomarkers as far as data quality is concerned. Whilst the accuracy of genotyping is generally high, false results do exist and may amount to a few percent per polymorphism in large samples (Re paper on samples and data handling). This however is dependant on the gene studied and the genotyping method used. Hence data quality assessment for genotyping should be included in trial protocols. Methods for detecting mis-genotyping at the population level have been described.⁸

The reliability and interest of data rely primarily on the quality of the clinical information gathered, and on the power of the study for detecting or excluding association with the tested genes. In the cardiovascular field major events, such as myocardial infarction, stroke or death are relatively easy to diagnose. However procedures for definition and validation of all relevant clinical events should be designed. In major intervention trials these procedures include review by an independent committee. When intermediate phenotypes like clinical symptoms, or results of haemodynamic and angiographic explorations are considered, these phenotypes should be carefully validated for their relevance to the therapeutic effect, and the data should be interpreted by taking into account sensitivity, reproducibility and inter-subject variability in measured parameters. The same applies to laboratory measurements. For example, micro-albuminuria which is a robust biochemical parameter and a promising biomarker for cardiovascular morbidity in diabetic patients should be measured with repeated testing before stratifying patients according to microalbuminuria status.

Need of adequately powered studies

The study should be adequately powered for detecting the genetic association(s) tested. Power depends in part on the design of the study, the residual number of events occurring under treatment, or the magnitude of the therapeutic effect measured and the frequency of the polymorphisms tested. Almost all genetic studies appended to large clinical intervention trials, rely on marker by treatment interaction design, and stratification of genotype groups, in broad, non-genotype enriched, disease populations. Power will also depend on allele frequency and vary for each gene. For example the

common I/D polymorphism of the ACE gene, strongly associated with ACE levels and largely studied in cardiovascular and renal diseases, has an allele frequency of 0.44/0.56 in Caucasian populations and this implies relatively balanced numbers of patients in each genotype group. On the other hand informative polymorphisms in several genes, for example the beta-receptor genes, have a low frequency and this requires studying a larger number of subjects for comparing genotype groups. Furthermore statistical power may be reduced when considering haplotypes rather than SNPs, although studying multiple polymorphisms of a given gene can strengthen the study's power for detecting or excluding physiologically relevant associations. Power calculation for detecting or excluding genotype related differences in trial endpoints should be performed for each tested gene. Analyses should take into account the baseline demographic characteristics of the patient's population, like ethnic composition.

Design of studies

Although no large, prospectively conducted clinical intervention trial in an allele enriched population has been reported so far in the cardiovascular or renal fields, enrichment has been performed in post-hoc analysis of clinical databases. This approach, although quickly generating potentially valuable hypotheses, is prone to bias and should be considered at best as an exploratory design.

On the other hand a design with enrichment in specific allele(s) makes the study better powered for the considered gene, but unsuitable for studying other genes. This design would therefore be useful for confirming the association and the magnitude of the gene treatment interaction and also for evaluating genotype based therapeutic regimen adaptation.

Expected improvement

Finally it appears that a large part of the current uncertainty concerning the association of candidate genes with disease outcome relies on the low power of most studies, their design and the fragmentation of the genetic hypotheses being tested. In this context meta-analyses may not be scientifically appropriate to draw conclusions. Progress will probably come from adequately powered studies, such as those nested in large intervention trials assessing the effect of a new drug or comparing two treatment regimens. In addition new, dedicated studies should be designed for studying the interaction of genomic polymorphisms with the effect of specific treatments in large cohorts of patients. These studies may have a relatively simple design, with or without allele enrichment, but will require a logistic support level equivalent to other large clinical trials. Such a study is for example warranted for establishing beyond doubt the role, if any, of the genetic polymorphism of ACE levels in the therapeutic effect of ACE inhibitors, especially in diabetes. These dedicated studies may also facilitate exploratory work to characterize lesser known targets.

Physiological relevance of the data.

Clinical studies provide data on association, which is different from causality (see below). Furthermore association between a gene and disease outcome under treatment can reflect several different phenomena:

- The gene may be involved in drug metabolism or properties of drug target and related pathways;
- The gene may be a marker or a causal factor for disease progression and its effect is not suppressed by the treatment. In this case the association is also observed in non-treated patients;
- The gene is involved both in drug target properties and disease prognosis, a situation that may be frequently encountered, because recognized pathogenic pathways are logically targeted in drug design. This is for example the case of the ACE or adrenergic receptors genes;
- The genomic variation is only a neutral marker in linkage disequilibrium with another, causal variation concerning a different gene at the same locus.

Irrespective of the underlying mechanism behind the association observed, the unambiguous association of a genetic variation with the outcome of a disease under treatment suggests that an identifiable subgroup of patients have a poorer prognosis despite the treatment, and should be targeted

for improved therapeutic intervention, while another subgroup is recognized as an eligible set of patients for the treatment, depending also on its benefit/risk profile. Good records of phenotypes and samples of both responders and non-responders should be maintained for several years in order to explore in the future the non-responder population genetics in the light of discovery of new candidate genes.

3.2 Validation of hypotheses

Validation of the genetic hypotheses being tested relies on clinical replication of the association observed⁹, and also on strength of mechanistic insight obtained. Although useful practical options can often be designed from associations without proof or knowledge of causation, definitive medical advances will depend on establishing causation and mechanisms.

The following points can be considered for establishing the scientific validity and medical relevance of a proposed gene-treatment interaction effect:

- The scientific soundness of the hypothesis tested (choice of candidate gene, data on functionality of polymorphisms being tested). This may however not apply to genomic markers identified by whole genome scans.
- The replication of the genetic association observed in other, adequately powered trials, as discussed above. These confirmatory trials may be designed with or without allele enrichment, and include disease-related biomarkers or carefully justified intermediate phenotypes (clinical or laboratory). They may be required to grant a marketing authorisation incorporating the PG biomarker.
- The gathering of experimental evidence supporting causality and suggesting pathogenic mechanisms linking the genomic variation studied to the therapeutic effect. In particular, genetic manipulations in mice aimed at reproducing the consequences of human genetic polymorphisms on the protein phenotype (either gain or loss of function or quantitative variations) have proven a powerful approach in cardiovascular genetics for establishing causality between genes and diseases, and deciphering pathogenic mechanisms.

4. CONCLUSIONS AND RECOMMENDATIONS

Although major beneficial effects are observed at the population level in large clinical intervention trials conducted with several classes of drugs in the cardiovascular field, it is clear that all patients do not equally respond to the treatments and the residual number of morbid events remains high. Among the hypotheses that can be put forward to explain the variability in the individual response to treatments is the role of genetic factors, that could be important contributors to the variability in individual response. This avenue is worth exploring especially at the molecular level, because genomic polymorphisms are easily accessible and are robust biomarkers. Genetic association studies in disease populations are also relevant to the clinical development of new classes of drugs,.

Considering the current state of knowledge concerning the pharmacogenetic relevance of genomic polymorphisms related to cardiovascular disease, it is considered that newer scientific approaches for conducting clinical intervention trials with the objective of analyzing the influence of genetic factors on treatment outcome are needed.

Recommendations

Based on the above, the following general principles, that may be applicable to pharmacogenomic association studies for other common complex diseases, are to be taken in to account for future studies:

- The issue of a trial's power for detecting association of genomic variations with treatment efficacy is a central one, especially because the number of targeted morbid events may be low and further reduced by the treatment, and the data have to be analyzed by allelic subgroups.

Adequate power is likely to be only achieved in large trials. Genomic association studies nested in some currently ongoing large intervention trials may provide useful information. However the design of dedicated trials, based on follow-up of cohorts of treated patients and testing of selected candidate genes after appropriate power calculation, is strongly warranted.

- Replication is another important issue, especially in those cases for which data derived from mechanistic studies are not available and/or the strength of the initial association appears to be low. To confirm the validity of the association either a second trial based on similar design and endpoints, adequately powered, may have to be conducted or allele enriched designs can be considered.
- Mechanistic insight into the gene-treatment interaction effect observed should be pursued.

Footnotes

1. Yusuf S et al. *N Engl J Med*. 2000 Jan 20;342(3):145-53.
2. Reflection Paper on Pharmacogenomic Samples, Testing and Data handling
3. See Yusuf S et al . *N Engl J Med*. 2000 Jan 20;342(3):145-53; Marre M et al. *BMJ*. 2004 Feb 28;328(7438):495
4. Boright AP, et al; DCCT/EDIC Research Group. the DCCT/EDIC Genetics Study. *Diabetes*. 2005 Apr;54(4):1238-44; Hadjadj S et al *Diabet Med*. 2003 Aug;20(8):677-82.
5. See Lanfear DE et al *JAMA*. 2005 Sep 28;294(12):1526-33; Johnson JA et al *Clin Pharmacol Ther*. 2003 Jul;74(1):44-52; Liggett SB et al *Proc Natl Acad Sci U S A*. 2006 Jul 25;103(30):11288-93 and other publications on this topic
6. See Penno G et al *Diabetes*. 1998 Sep;47(9):1507-11 ; So WY et al *Kidney Int*. 2006 Apr;69(8):1438-43; McNamara et al *Circulation*. 2001 Mar 27;103(12):1644-8 and *J Am Coll Cardiol*. 2004 Nov 16;44(10):2019-26 and other publications on this topic.
7. See Sciarrone T et al *Hypertension*. 2003 Mar;41(3):398-403; Turner ST et al *Hypertension*. 2005 Oct;46(4):758-65.
8. See for example Moskva V et al *Hum Hered*. 2006;61(1):55-64; Becker T et al *Hum Hered*. 2006;61(1):55-64
9. NCI-NHGRI Working Group on Replication in Association Studies. Replicating genotype–phenotype associations *Nature* **447**, 655-660 (7 June 2007) | doi:10.1038/447655a; Published online 6 June 2007