Mini-review

Genetic tests and genomic biomarkers: regulation, qualification and validation

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Summary

The identification of new risk factors for specific diseases is an enduring theme in medical research. Advances in molecular biology, genetics, and computational biology are accelerating the pace of this work. The research seeks to increase our understanding of the causes of diseases, but there is also hope that the recognition of new risk factors will lead to improved methods for identifying persons who are in the early stages of, or at high risk for, the diseases of concern. Research has shown, however, that a genomic biomarker must have a much stronger association with the disease outcome than we ordinarily see in etiologic research if it is to provide a basis for early diagnosis or prediction in individual patients. However, even if the literature contains ~150,000 reports of disease-associated molecular markers, there are still very few validated biomarkers of proven and robust clinical utility. At present there is no established, standardized means for validating the association between a marker (or set of markers) and clinical outcomes. The Regulatory Authorities have undertaken a number of initiatives in order to enhance the use of biomarkers in drug development, to promote a more informed drug development and maximise the benefit of innovative medicines to the patients.

KEY WORDS: genetic testing, Genomic Biomarkers (GBs), validation, qualification, regulation, pharmacogenomics.

Introduction

Genetic testing is a type of medical test that identifies changes in chromosomes, genes, or proteins. Most of the time, testing is used to find changes that are associated with inherited disorders. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or passing on a genetic disorder. Several hundred genetic tests are currently in use, and more are being developed.
The rapidly expanding knowledge of disease pathogenesis at the molecular level is providing new targets for disease characterization, early diagnosis, and drug discovery and development. Several decades of intensive research have originated multiple factors or biomarkers that are likely to be helpful in the diagnosis, characterization, and therapy selection. A deep understanding of the relative relevance of each biomarker will be key to efficiently diagnose diseases, adverse drug responses and direct our patients towards the drugs more likely to be of benefit based on their particular profile. The development of new preclinical models is of paramount importance to achieve these goals. There is an enormous effort to identify, characterize, and validate meaningful biomarkers because its successful development will represent a step forward in the individualization of diagnosis, therapy and monitoring. Advances in methods and technology now enable construction of a comprehensive biomarker pipeline from six essential process components: candidate discovery, qualification, verification, research assay optimization, biomarker validation and commercialization. To accommodate for a swift regulatory appraisal of these new technologies in the processes of drug development and approval, the European Medicines Agency has put in place dedicated experts panels, new procedures.

Genetic testing and genomic biomarkers

It is important to distinguish between genetic testing for the diagnosis and prognosis of disease and genetic test performed for pharmacogenomics purposes (Table I).
In the first case, the test is carried out to identify causative gene mutations or polymorphisms of susceptibility while in the second case the genetic test will reveal DNA and RNA characteristics as related to drug responses. Historically, successful genetic markers have been linked to single effects for both the patient and the patient's family (e.g. cystic fibrosis testing, spinal muscular atrophy testing etc.) and/or to monitor particular effects on large populations (i.e. HIV mRNA, HCV mRNA etc.). In this context, genetic testing should be described as a particular assay to detect:
– a particular genetic variant (or set of variants)
– for a particular disease
– in a particular population
– for a particular purpose.

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Table I - Classification of genetic tests according to their purpose*.

Population screening	Conducted to identify asymptomatic individuals from within a particular community or a subsection of that community who have an increased chance of having a specific genetic disorder, of carrying a specific genetic predisposition to disease or of being a carrier of a recessive genetic variant. For example, women may be tested for BRCA1&2 - genes associated with breast cancer, so that preventative measures and early intervention can be considered.
Diagnostic testing	These tests are conducted to confirm or rule out a known or suspected genetic disorder in a symptomatic individual. For example, genetic testing is often used to confirm the clinical diagnosis of cystic fibrosis (CF).
Predictive testing	These tests are conducted to determine the probability of asymptomatic individuals who are suspected of having an inherited disorder developing the clinical manifestations.
Carrier testing	Conducted to determine if an individual is a "carrier" of a gene for an autosomal recessive or X-linked genetic disorder. For example, couples undergo carrier testing for disorders such as Tay-Sachs disease, to assist in their reproductive decisions.
Prenatal testing	Conducted during pregnancy to determine whether there is an increased risk of having a child with a genetic disorder. Down's Syndrome is the most common genetic disease screened by this method.
Newborn screening	These tests focus on the identification of metabolic disorders in newborns. Early detection and treatment may be crucial to reduce the progression of such diseases. One example is the newborn screening for phenylketonuria (PKU).
Pre-symptomatic testing	These tests are conducted on healthy individuals to determine whether or not they carry a genetic mutation that increases their likelihood of developing late-onset diseases and disorders. Examples include Huntington's disease and Alzheimer's disease.
Susceptibility (or predisposition) testing	These tests are conducted to determine the risk or probability that individuals with the genetic variant will develop a particular disease.
Pharmacogenetic testing	Conducted to determine individual genetic variability impact on drug efficacy and toxicity.
Forensic/Identity testing	These tests are conducted to discover genetic linkages in criminal investigations between suspects and evidence or between children and their biological parents.

* Modified from OECD (DSTI/STP/BIO 2006)17.

Rapid advances in research are leading to growing knowledge about the genetics and, thus, to an expanding array of genetic markers which may be useful not only for more accurate diagnosis but also for better therapeutic interventions. In the context of drug development, the traditional approach to genetic testing needs to be expanded because:

a) it is at odds with goals for individualized therapy;
b) does not recognize multidimensional quality of clinical response;
c) does not include possibility of multiple biomarkers providing useful information in aggregate.

Therefore, recently, regulatory agencies (CHMP/ICH/437986/2006) introduced the concept of "Genomic Biomarker" (GB) which is defined as a DNA or RNA characteristic that is an indicator of normal biologic processes, pathogenic processes, and/or response to therapeutic or other intervention. Therefore, a genomic biomarker should reflect:

- The expression of a gene
- The function of a gene
- The regulation of a gene.

DNA characteristics include, but are not limited to:

- Single nucleotide polymorphisms (SNPs)
- Variability of short sequence repeats
- DNA modification, e.g. methylation
- Insertions
- Deletions
- Copy number variation
- Cytogenetic rearrangements, e.g. translocations, duplications, deletions or inversions.

RNA characteristics include, but are not limited to:

- RNA sequence
- RNA expression levels
- RNA processing, e.g. splicing and editing
- MicroRNA levels.

Robust, reproducible accessible genomic biomarkers are of diagnostic value, and may lead to identification of causal factors. They therefore can be used clinically to screen for diagnose, to monitor the activity of diseases, and also may be useful to guide molecularly targeted therapy and personalised regimens or to assess therapeutic response.

The importance and the potential utility of GB has been recognized by substantial public and private funding, and GB discovery efforts are now commonplace in both academic and industrial settings.

For years GBs have been used to test for single gene mutations predisposing to disease. Examples of these include familial hypercholesterolemia and mutations in LDL receptors (1), high blood pressure, hyperkalemia and mutations in WNK kinase genes (2) and many other monogenic disorders (3). However, while GBs give diagnosis, causality and pathophysiology

information in monogenic disorders, they seem to have a scarce or no detectable impact on polygenic and complex disorders. Complex disorders are caused by multiple genetic and environmental factors, are characterized by high population prevalence, lack of clear Mendelian patterns of transmission, etiologic and phenotypic heterogeneity, and are involved in a continuum between disease and non disease states. Another complicating factor in developing biomarkers for complex traits is the difficulty in understanding the role these genetic factors play in the pathophysiology of disease.

The identification of "key" genes influencing complex traits such as heart disease, diabetes, and cancer will not only assist in predicting those individuals who are predisposed to disease, but may potentially have significant impact on the pharmaceutical industry as the identification of GBs for disease susceptibility may lead to *new targets, better classification of disease* and *more informed clinical development*.

A major challenge facing the development of GBs for complex diseases, is in the etiologic or phenotypic heterogeneity of the clinical conditions. Heterogeneity influences the ability both to discover a biomarker and to prove the clinical utility of the biomarker once identified. Clearly, a biomarker that is specifically associated with the phenotype of interest has more clinical utility than one associated with a range of phenotypes.

Following an understanding of the relationship between the identified biomarker and the phenotype, all additional polymorphisms in the gene or its regulatory region and their relationship to the observed functional effect should be investigated. This phase of investigation often includes studies in a variety of populations, as a specific polymorphism may have utility in one but not in another.

GBs are frequently evaluated in relation to drug development in order to accumulate information on their ability to help refining treatment effects, in terms of efficacy and/or adverse drug reactions (4-7). The most common application of genetic markers in drug development and medicine today is the genotyping of the drug metabolism enzymes. The genetic polymorphisms in these enzymes do not predict disease or response to therapy but lead to differences between individuals in metabolic activity and clearance of drugs and therefore in the overall exposure of patients to the compound. The drug metabolizing enzymes are not targets for new therapeutics but rather may guide dosing regimen alterations for therapeutics metabolized by the polymorphic enzyme. Even though these tests are not widely used outside of clinical trials, there is growing acceptance of using such GBs in determining *proper dosing regimens*.

In addition individual's clinical response to treatment may be influenced by genetic variants in the coding or regulatory regions of genes involved in the therapeutic pathways. New biomarkers that identify incipient damage that leads to preclinical and clinical toxicities will enable better decision-making during drug development and clinical management of patients. In the case of genetic predisposition to a drug adverse reaction caused by a specific drug in a small number of individuals, identification of the responsible genetic variants may prevent exposure to patients at risk of an adverse reaction while enabling the successful use of a drug to the many who would benefit. Therefore, genetic *biomarkers for response* may facilitate classification of individuals by response, improving therapeutic outcome, and personalizing prescriptions.

Some examples of GBs associated to drug response include:

- UGT1A1 in the irinotecan therapy in patients with colon cancer (8-11)
- CYP2C9/VKORC1 impact on the warfarin dosing in patients in anticoagulant treatment (12-15)
- HLA-B*5701 and abacavir hypersensitivity syndrome (16-18)
- EGFR status and Erbitux (19-22)
- Her2/neu status and Herceptin (23-26)
- Philadelphia chromosome (~ Bcr-abl) and Gleevec (27, 28).

Already there are over 50 drugs that mention the possibility to test biomarkers in their labels. The expectation is that the next few years will see a rapid increase in the number of drugs approved with GB data in their labels, and older drugs that will have GBs data added to their labels.

Despite the presence and promise of a growing body of research relating GB to treatments effects, in addition to the still unveiled complexities of genomic interactions and regulation, numerous barriers exist to a widely use of GB in the clinic of complex diseases. Lack of sufficient evidence for consistent phenotype-genotype associations, lack of evidence to support generalizability to diverse populations, significant overlap between genotypes and measurable disease, influence of polygenic factors, difficulty in defining the true clinical phenotype and lack of quality assured, cost-effective and feasible laboratory technologies have all been considerable hurdles in this process together with the lack of algorithms available to the physicians for an informed choice among the available therapeutic options to be tailored to the specific characteristics of the patients and of their condition.

Therefore, prior to widespread clinical application of a GB, multiple scientific and clinical studies must be completed to characterize the genetic variants and delineate their functional significance in the pathophysiology of a carefully defined phenotype and to develop the tools needed for their clinical use.

Biomarkers validation and qualification

Increased knowledge of genes biology is generating promising marker candidates for more accurate diagnosis, prognosis assessment, and therapeutic targeting. To apply these exciting results to maximize patient benefit, a disciplined application of well-designed clinical trials for assessing the utility of markers should be used. Clinical trial designs for evaluating the usefulness of molecular traits or markers within the context of genetic testing are fundamentals. Ideally, the population studied should be one in which knowledge of the marker would have substantial clinical relevance and where the feasibility of obtaining appropriate specimens is established. Although biomarkers are emerging as key indices for individualized patient management, oversight and regulation of their safety and validity have lagged. About 1,000 biomarkers are available as diagnostic tests, almost universally marketed as home-brew tests without formal approval from regulatory agencies. Although biomarkers have been used in decision-making, clinical practice, drug development and regulatory evaluation of new drugs for many years, there is currently an increased focus on them as a means to facilitate and expedite regulatory decision-making (29).

There are numerous potential biomarkers that could be related to different clinical events or processes, highlighting the need for consensus among investigators and regulators on the basic principles of validating and qualifying biomarkers for decision-making. In order to provide a forum for informal discussion the regulators both at EU level (European Medicines Agency – EMEA) and at US level (Food and Drug Administration – FDA) since 2003 have established special experts panel (The Pharmacogenomics Working Party – PGWP) and the Interdisciplinary Pharmacogenomic Review Group – IPRG) able to initiate the reflection on the implication of the use of genomic biomarkers in the development of medicinal products. These two expert committees were able to discuss with Industry data submitted on a voluntary basis, including genomic and non-genomic biomarkers and spanning from analytical platforms validation studies, toxicology testing, clinical development designs.

The relevance of the biomarkers in clinical development has been recently re-emphasized in the Final Report of the EMEA/CHMP think-tank on innovative drug development (Innovative drug development approaches (EMEA/127318/2007 - http://www.emea.europa.eu/pdfs/human/itf/12731807en.pdf):
"Use of biomarkers in early non-clinical and clinical development to provide a more informed scientific basis for the design of pivotal trials has already proven useful in streamlining the development of targeted therapies. The identification and validation of predictive efficacy and safety biomarkers in particular could be very important in future drug development as changes in biomarkers following treatment may reflect the pharmacodynamic/clinical response to the product".
A research plan should include assay validation and biomarker qualification. Method of validation has been the object of the FDA "Guidance for Industry on Bioanalytical Method Validation" (2001). Anyway there is a little regulatory rules on validation of biomarkers assays. Method validation should demonstrate that a particular assay is reliable for the intended application (29) and thus the rigor of method validation increases from the initial validation proposed for exploratory purposes, to the more advanced validation for a biomarker and the use of results. Different applications of biomarkers require targeted method validation. Validation of candidate biomarkers is typically achieved through the use of independent sample cohorts. This is essentially replication of results in different sample populations. Many studies today are performed on both a training cohort to identify susceptibility loci and a validation cohort to replicate and validate the results, both within the scope of the same investigation. These replication studies can be difficult because of the complexity involved in controlling environmental factors between different populations. Thus, true correlates that could contribute to disease in one population may not replicate in another population that was subject to a uniquely different environment. In contrast to validation, the biomarker qualification focuses on evidence linking a biomarker with disease biology and as appropriate with clinical end points (29).
All the above taken in to account, we think that biomarker development and use should be guided by the pragmatic principle of being linked to how they will be used in terms of context (e.g., in the context of non-clinical toxicology) and purpose (e.g., in the same context-detection of acute drug-induced injury). A biomarker research plan is a graded process. Moving from an initial exploratory approach, often retrospective, a key feature of a biomarker development plan is that it prospectively describes the studies required for its qualification.
Biomarkers that will be used as surrogate endpoints (SEPs) in clinical efficacy trials or as markers of toxicity will require substantial qualification, partly because it is not self-evident what relationship exists between the effects of the drug on the biomarker and the effects of the drug on outcomes, and partly because the consequences of unreliable SEPs or toxicity biomarkers, in terms of regulatory decision-making, are much graver. Two conditions, if simultaneously true, would be sufficient to accept an SEP (30): (a) the SEP (biomarker) must be correlated with the clinical endpoint; and (b) the marker must fully capture the net effect of the intervention on the clinical efficacy end point. However, it is clear from regulatory guidance and the history of clinical practice that SEPs and toxicity biomarkers cannot be qualified on theoretical grounds alone, but must be based upon objective data. Moreover, to ensure their robustness, the data should originate from multiple studies by different investigators. All these are practical arguments for a collaborative framework for the development of biomarkers. Indeed a collaboration permit to avoid the duplicative efforts and to enable cost-sharing, joint solutions to legal issues, intellectual property. It was for these reasons that the Pharmaceutical Research and Manufacturers of America entered discussions with FDA in 2004 with a view to creating a consortium for the qualification of biomarkers for regulatory decision-making. The Pharmaceutical Research and Manufacturers of America/FDA Biomarker Consortium is set up with the Foundation for the National Institutes of Health and launched formally in October 2006. The consortium will manage biomarker projects to ensure scientific rigor, appropriate prioritization and funding, and compliance with relevant laws.
In parallel the Regulatory Authorities at international level (the European Medicines Evaluation Agency – EMEA, the US Food and Drug Administration – FDA) have initiated new regulatory processes aiming at providing scientific advice both for the development of novel biomarkers and for their use in the drug development context as acceptable regulatory standards (biomarkers qualification). It is also worth mentioning that in December 2007, the EU Parliament and Council agreed – within the Research Framework Program 7 – FP7 – on the Innovative Medicines Initiative (IMI), which is aiming at establishing solid public-private partnerships to enhance the establishment of the collaborative framework needed to promote innovative development approaches in the pharmaceutical sector, inter alia the development of biomarkers.

Personalized medicine and biomarkers

Most patient populations show large inter-individual variability in drug response and toxicity. Individuals may have excellent responses, respond partially, or experience adverse drug reactions to standard doses (4-7). This individual variation can be due to genetic, physiological, pathophysiological, or environmental factors. A drug's absorption, distribution and metabolism, and interactions with its target can be determined by genetic differences. Genetic factors account for 15% to 30% of differences in drug metabolism and response between individuals. For some drugs or classes of drugs, genetic factors can account for up to 95% of inter-individual variability in drug disposition and effects. Potential GB may be in genes involved with the drug target, the metabolism of the drug, or in the disease pathway (4). The ability to identify GB corresponding to a therapeutic effect is the basis for the concept of the so-called personalized medicine (31). Personalized medicine can be defined as a specific and selected algorithm addressed to a particular patient to optimize treatment to maximize efficacy and minimize risk, based on the genetic make-up of the patient (31). The develop of a personalized medicine is closely linked to GB, which may serve as the basis for diagnosis, drug discovery and monitoring of diseases. New molecular technologies and new biomarker development processes have emerged over the few years towards this goal. However, a complete agreement on processes to qualify new GBs for developing novel prognostic biomarkers for a possible personalized medicine is lacking. In prostate cancer, for example, the prostate-specific antigen (PSA) changes is an accepted preclinical biomarker, but this marker is far from perfect in terms of specificity and sensitivity. Several new potential GBs with potentially better specificity and sensitivity than PSA have been discovered and published. These include specific gene mutations (e.g., HPC1, RNase-L) and combined single nucleotides polymorphisms (SNPs) located within five chromosomal regions (32). However, these new genomic biomarkers have a mechanistic importance but a scarce clinical utility since are unable to distinguish between indolent and aggressive prostate cancer. Other examples concern the colorectal cancer that is the second leading cause of cancer-related death. Current clinical practice in colorectal cancer screening (fecal occult blood test, FOBT; colonoscopy) has contributed to a reduction of mortality. However, despite these screening

programs, about 70% of carcinomas are detected at advanced tumor stages (UICC III/IV) presenting poor patient prognosis. Thus, innovative tools and methodologies for early cancer detection can directly result in improving patient survival rates. Biomedical research has advanced rapidly in recent years with the availability of technologies such as global gene and protein expression profiling. We developed a low density homemade oligoarray ("AndroChip 2") containing 190 genes, selected on the basis of their proved or potential role in prostate cancerogenesis related to androgen signalling (33, 34). This array was successfully utilized to monitor the gene expression profiles in androgen-dependent and androgen-independent cells for pharmacogenomic purposes. Multiple genes were identified exhibiting differential expression during drug treatment. Importantly, we recently demonstrated that all genes fixed on the "Androchip 2" show a detectable expression levels in peripheral blood cells (PBMC). Therefore, "Androchip 2" may be helpful in developing novel prognostic biomarkers and therapy for androgen-sensitive and androgen-refractory prostate cancer. PBMC represents an attractive, clinically accessible tissue for the identification of novel biomarkers. In fact, circulating blood cells come into contact with every cell in the human body and provide an active defence against insult and injury. This peculiarity, combined with the fast turnover rate of blood cells, gives rise to the possibility that subtle changes occurring in association with injury or disease within the cells and tissues of the body may trigger specific changes in gene expression at a micro-level within the blood cells. These changes can capitalized on as biosensors for diagnostics purposes. An increasing number of clinical pharmacogenomic (PG) studies are employing gene expression profiling PBMCs for the identification of novel transcriptional biomarkers of disease, drug activity, drug efficacy/toxicity, and even markers predictive of clinical outcomes. Comprehensive tumor profiling has become a field of intensive research aiming at identifying biomarkers relevant for improved diagnostics and therapeutics (35). A major challenge in development of cancer biomarkers will be the integration of proteomics with genomics and metabolomics data and their functional interpretation in conjunction with clinical data and epidemiology. Proteomics-based technologies enable to distinguish the healthy patient from the tumor patient with high sensitivity and specificity and could greatly improve common classification systems and diagnostics. However, this progress has not yet been transferred from bench to bedside but could open the door to a more accurate and target specific personalized medicine with improved patient survival (36).

Conclusions

This paper follows the broad definition of a genomic biomarker as a characteristic that can be objectively measured and evaluated as an indicator of normal biological or pathogenic processes as well as pharmacological responses to a therapeutic intervention. Tests based on "classical" biomarkers have been around for more than half a century, but interest in their application for diagnostics and drug discovery as well as development has increased remarkably since the beginning of the 21st century with the discovery of GBs. GBs are different types of biomarkers originating from various "-omics" technologies and combining genomics, proteomics and metabolomics. Currently the most important applications of GBs are in drug discovery and development. The role of GBs in various therapeutic areas particularly cancer, cardiovascular diseases and disorders of the central nervous system, is expected to change in the near future the modern medicine. In fact, GBs are useful not only for diagnosis of some of these diseases but also for understanding the pathomechanism as well as a basis for development of therapeutics. GBs will facilitate the combination of therapeutics with diagnostics through pharmacogenetics, pharmacogenomics and pharmacoproteomics, and will thus play an important role in the development of personalized medicine. The Regulatory Authorities in Europe and in US have already open the dialogue with the stakeholders to ensure that the benefits of the new technologies are conveyed in a timely manner to patients with the provisions of better information on the use of both old and innovative medicines.

Acknowledgements. This work was supported by a grant of the Italian Ministry of Health "Commissione per la vigilanza ed il controllo sul doping e per la tutela della salute nelle attività sportive" to G.N.

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