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Knowledge and skills needs for health professions about pharmacogenomics testing field

R. DI FRANCIA[1], D. VALENTE[2], O. CATAPANO[2], M. RUPOLO[3], U. TIRELLI[3], M. BERRETTA[3]

[1]Hematology-Oncology and Stem Cell Transplantation Unit, National Cancer Institute, Fondazione "G. Pascale" IRCCS, Naples (Italy)
[2]Italian Association of Pharmacogenomics and Molecular Diagnostics, Caserta (Italy)
[3]Department of Medical Oncology; National Cancer Institute, Aviano, Pordenone (Italy)

Abstract. – *Background:* **Promise in the future, a disease could be ranked into genetic categories, allowing bespoke tailoring of medicine to maximize therapeutic effects and to reduce the potential for adverse drug response. This new feature requires for health professionals to have competencies not only for the basic skills of their discipline, but also for the understanding on why, when, and how that knowledge should be applied to improve personalized therapies for their patients. Current opinion on basic competences of health professions includes knowledge and skills on two fundamental features: (1) genetics of disease, to allow the understanding and the identification of diseases associated to genetic variations, and to facilitate the development of new genomic tests; and (2) ethical, social and economical implications that are fundamental to identify those factors that might contribute to a successful integration of pharmacogenomics into international health and public policy.**

Aim: **Briefly, we described (1) current knowledge on genetic variations that interact with therapies and the need to detect them; (2) the most common available methods for detecting mutations; and (3) ethical, social and economic issues related to pharmacogenetic testing and recording of genetic information (e.g., critical evaluation of the development of new tests, privacy, the current absence of public reimbursement, etc).**

Conclusions: **These could be useful recommendations for academic institutions and educational programs to prepare health professionals with the necessary abilities for their future practice.**

Key Words:

Pharmacogenomics, Health profession education, Genotyping methods, Genetic knowledge base.

Introduction

Advances in genetics and molecular biology technologies have led to many changes in pharmaceutical sciences. In particular, with the developments in pharmacogenetics and pharmacogenomics (PGx), detailed information about human genome made available and the genetic basis for success/failure of pharmacotherapy have being studied[1].

Pharmacogenetic knowledge is rapidly developing and changing; it is imperative that healthcare professionals keep abreast of advances and clinical indications. The current knowledge of health professions regarding PGx is still low. There exists an acute lack of education of both physicians and pharmacists regarding pharmacogenetics and personalized care[2]. Academic curricula are slowly including teaching of this field in their courses. Healthy institutions and academic organizations must play a central role in educating health professionals on the best use for applications of advancing pharmacogenomics research, and in articulating on the role of physicians and pharmacists in the development and use of gene-based therapies, as well as in making treatment choices as the result of available patient-specific genetic information[3].

The large number of drug options also means that physicians are often spoilt for choice, and have a low threshold to consider alternative therapies when toxicity becomes unmanageable. The need to evaluate the genetic basis for side effects becomes less clinically relevant in such circumstances.

However, it is often forgotten that genetic testing is not only predictive for treatment related toxicity

Corresponding Author: Raffaele Di Francia, Ph.D.; e-mail: rdifrancia@iapharmagen.org

or allows for dose adjustment, but also determines response or lack thereof. It is frequently imperative that must be done before treatment, as giving inappropriate treatment may result in an outcome poorer than the alternative[1]. A "treat-and-see" approach has ethical and legal implications in this era where genetic testing is readily available. It delays and even potentially deprives patients of appropriate treatment, and deterioration is often rapid without it. Moreover, we think that genetic testing could have a key role for the treatment choice in the so called frail patients (i.e. elderly and HIV-positive patients) for whom the efficacy and especially the toxicity profile are important aspects[4,5].

However, one should keep in consideration that it will not be feasible to conduct randomized trials on each and every diagnostic test, and the economic value of such tests can be modelled using decision analysis techniques

The goal of this review is to provide information (in terms of knowledge-base in genetics, ethical, social and economic) for the health profession about the genetic variations implicate in pharmacotherapy and the most commonly available methods for their molecular detection.

Genetics Competencies

Needs to Detect Genetic Variations in Pharmacotherapy

Pharmacogenomic approaches have been applied to many existing therapeutic agents in an effort to identify relevant inherited variations that may better predict patients' response to treatment. Genetic variations, which can alter the protein expressions and/or amino acid sequence of the encoded proteins, include nucleotide repeats, insertions, deletions, translocations and Single Nucleotide Polymorphisms (SNPs).

Such genetic polymorphisms in drug metabolizing enzymes like the Cytochrome P450 family[6]; transporters like Multidrugs Receptors-1[7]; and molecular targets, have been actively explored with regard to functional changes in phenotype (altered expression levels and/or activity of the encoded proteins) and their contribution to variable drug response. The following Table I describes some clinically relevant examples of genetic defects illustrating the relevance of PGx in optimizing pharmacotherapy, as a way to enhance efficacy and safety. For example the new generation of anticancer drugs have high specificity toward tumour cells, provide a broader therapeutic window with less/low toxicity in comparison with conventional chemotherapies; therefore, these drugs represent a new and promising approach to targeted cancer therapy. These new drugs are designed to interfere with a specific molecular target, usually a protein with a critical role in tumour growth or progression (i.e. tyrosine kinase). There are multiple types of targeted therapies available, including monoclonal antibodies, antisense inhibitors, and inhibitors of tyrosine kinase. Obviously, many of these new drugs set up a selective pressure for tumour cells that can survive and proliferate in its presence. The same basic principle seems to be true for protein kinase inhibitors. The best understanding of this problem at a molecular level comes from studies on imatinib resistance in Chronic Myelogeneous Leukaemia (CML) patients carrying BCR/ABL fusion gene. These imatinib-resistant clones, consisting a single nucleotide mutation in ABL Kinase domain (with consequent amino acid substitution), are successfully suppressed by second-generation Tyrosine kinase inhibitors (i.e. Dasatinib, Nilotinib), still active on almost all imatinib-resistant mutants[8].

Similarly to imatinib, other two biological drugs (Gefitinib and Erlotinib) showed clinical activity in a subset of patients affected by Non Small Cell Lung Cancer (NSCLC). The mechanism of action for both drugs is the selective inhibitions of the kinase activity of epidermal growth factor receptor (EGFR)[9]. Recently, it has been reported in NSCLC patients that specific point mutation of EGFR gene in tumour cells select Gefitinib-responders' patients (EGFR mutated), from non-responders (EGFR wild type)[10]. The availability of this kind of biomarkers is currently useful tool for predicting resistance to specific drug therapy.

Current Genotyping Methodology

The technology platform needed for genotyping is different; it does depend from type of mutation, acquired genetic change, or the analysis of inherited SNPs. This is due to the heterogeneity of the sample source. Either the tumour itself or the sample may contain a large excess of wild type DNA, therefore, highly specific and sensible techniques are required to detect mutant tumour genomes in a background of normal DNA. Whereas, for inherited SNPs there is a copious of suitable methods for genotyping able to detect mutant allele either in heterozygosis or homozygosis cells. Rational selection of the best methods to detect them is dependent from the specifics aims of different laboratories[11].

Table I. Most significative known genetic variants and their effect in pharmacotherapy.

GENE[a]	Polymorphism (nucleotide translation)	Molecular effect	Drug	Effect on therapy
Cytochrome P450 family	Various polymorphism	Decreased enzyme activity	Various	Inter-individual variability inpharmacokinetics
TPMT2, 3A, 3C	Various polymorphism	Decreased enzyme activity	6-MP Thioguanine	Hematopoietictoxicity
UGT1A 28	TA repeats in 5' promoter	Decreased enzyme activity	Irinotecan	Neutropenia toxicity
MDR1	(C3435T)	Low expression	Various	Drug resistance
TYMS	3 tandem repeats	Increased enzyme activity	5-FU, Metatrexate	Drug resistance
DPYD	IVS14+1G	Decreased enzyme activity	5-FU, Metatrexate	Neutropenia toxicity
DHFR	T91C	Increased enzyme activity	Metatrexate	Drug resistance
MTHFR	(C677T) (A1298C)	Decreased enzyme activity	5-FU, Metatrexate	Toxicity
c-KIT	D860 N567K	Constitutive signal activation	Imatinib	desensitizes activity in GIST
K-RAS	G12x G13D	Inhibition of the tyrosine kinase domain-binding drug	Cetuximab Panitumomab	Desensitizes activity in colon-rectum carcinoma
B-RAF	V600E	Inhibition of the tyrosine kinase domain-binding drug	Vemurafenib	Good response in melanomas
EGFR	L858R	Inhibition of the tyrosine kinase domain-binding drug	Gefitinib Erlotinib	Good response in NSCLC
BCR/ABL fusion gene	t(9;22) BCR/ABL	Constitutive signal activation	Imatinib Dasatinib Nilotinib	Good response in CML
ABL	T315I, M351T	Inhibition of the tyrosine kinase domain-binding drug	Imatinib	Drug resistance in CML
PML/RARα fusion gene	t(15;17) PML/RARα	Block of myeloid lineage cells	All Trans Retinoic acid (ATRA)	Good response in AML-M3 subtypes
ADRB1 ADRB2	R389G	G-protein altered	beta-bloccants	Desensitizes activity
MHC class B 1	Several SNPs including codon K751Q	HLA-B~5701 aplotype	Abacavir	Hypersensitivity r
VKORC1	Many, VKORC1 haplotypes including codon G3673A	Associated with a higher/low warfarin dose	Warfarin	Variable anticoagulant effect

Abbreviations: TPMT: thiopurine methyltransferase; UGT1A1: UDP-glucuronosyltransferase 1A1; MDR1: multidrug resistance 1; TYMS: thymidylate synthase; DHFR: Dihydrofolate reductase; MTHFR = 5,10-methylene tetra hydrofolate reductase; EGFR: Epidermal Growth Factor Receptor; 5-FU: 5-fluorouracil; 6-MP: 6-mercaptopurine; AML:Acute Myeloid Leukemia; NSCLC: Non-Small Cell Lung Cancer; CML: Chronic Myeloid Leukemia; ADRB: adrenergic b-receptors; VKORC1: Vitamin K epoxide reductase complex 1; The present list is not meant to be whole comprensive. [a]Genes are available for genotyping test or under consideration for clinical diagnostics.

Current genotyping technologies can be divided into two major categories, depending upon their ability to screen for new mutations or to identify known mutations. In general, all genotyping platforms must fulfill two requirements: 1) discrimination between alternative alleles (i.e. mutant *vs.* wild type) and 2) detection of both alleles (i.e. mutant *and* wild type) in a DNA sample. The only platform able to fulfill both tasks in a single step assay is the Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI TOF). However, the technological platforms may be "homogeneous" if based on a single tube reaction, or "heterogeneous" when involving both a liquid and a solid phase (i.e. DNA Chip). The most-used platforms for the detection of known SNPs can be operatively classified into two major categories (Table II): 1) homogeneous; 2) heterogeneous.

The advantages of homogeneous methods include reduced risk of cross-contamination, time-effectiveness and practicability. Traditional techniques for SNPs genotyping detection such as Single-Strand Conformational Polymorphism (SSCP) and Restriction Fragment Length Polymorphism (RFLP) have now been largely replaced by high-throughput methods that are able to generate higher numbers of data, and are easier to automate. High-throughput processing is achievable by fully integrated systems using 96- or 384-well plate robotic processing such as 1G Genetic analyzer by Illumina/Solexa (Cambridge, UK), SOLiD by Applied Biosystems (Foster City, CA, USA) and Genome Sequencer FLX by Roche Diagnostics (Brandfort, CT, USA). These new types of ultrafast DNA sequencers provide a consensus base accuracy of 99.99%. In almost all homogeneous assays, DNA amplification is required. Non-PCR-based technologies such as the Ligase chain reaction, Rolling Circle Amplification (RCA) and Invader® assays (Third Wave Technologies, Madison, WI, USA) are able to genotype directly from genomic DNA.

Heterogeneous methods such as DNA chip-based microarray, Golden Gate® assay and mass spectrometry genotyping technologies, are the latest development in the genotyping arena, but these newer technologies are currently less widely used in the clinical laboratory setting than cheaper PCR-based methods. Additionally, costs for DNA chip assay are projected to be high and results interpretation will remain strictly dependent on the availability of highly qualified and well-trained personnel. Here, we highlight some of the most popular technologies currently used in specialized laboratories, focus on the transition from research setting to clinical laboratory as previously discussed by other Authors[12].

In conclusion, no single genotyping platform stands out as ideal; the methods listed in Table II show that many overlaps and attempts to compare them may result difficult and unproductive.

Table II. Current methods for genotyping at molecular level.

Methods for detection and screening for unknown mutations
Detection and screening
Conventional sequencing
Denaturing-HPLC
Denaturing gradient gel electrophoresis (DGGE)
Heteroduplex DNA assay (melting curve)
High throughput sequencing
Single strand conformation polymorphism (SSCP)
Methods for detection of known mutations
Homogenous
Fluorescent probe by Allelic Discrimination (Hyb Probe® TaqMan®, Beacons® Scorpions®)
High Resolution Melting (HRM)
Invader® assay
Locked Nucleic Acid (LNA) probe
Peptide nucleic acid-mediated Clamping PCR
Pyrosequencing
Heterogenous
Gene Chip technology
Golden Gate® assay
Maldi-TOF Mass Spectroscopy
Oligo ligation assay (SNPlex®)

Ethical, Social and Economics Competencies

As genomics-based technologies are widely introduced in clinical laboratories testing setting, the risks of mishandling or misinterpreting data from patient's sample analyses becomes a significant consideration with especially dramatic consequences where the test becomes commercially available to the public[13]

Generally, genotyping is performed either by custom service laboratories or academic referenced laboratories, as well as by using commercial kits (when available). In the USA, diagnostics products are regulated by the *Food and Drug Administration* (FDA), whereas diagnostic services are under the rules of the *Clinical Laboratory Improvement Act* (CLIA). In Europe this field is covered by *in vitro Diagnostic* (IVD) directive, without a distinction

Table III. Basic competencies (in terms of knowledge and skills) in Pharmacogenomics for health profession.

	Knowledge	Skills
Genetics	• Basic concepts • Nomencalature (genetic glossary) • Most recently SNPs causing intervariability respons to drugs • The role of genetic factors in preventing disease • The difference between clinical diagnosis of disease and identification of genetic predisposition • Genomic tests available in specialized laboratories • Current methods available to detect pharmacogenomics tests • Know where/how to find information about Pharmacogenomics • Understand how association between genomic variation and drug response are investigate and uncovered • Understand that pharmacogenetic testing is like all other clinical testing it will not have 100 percent reliability, but rather is used along with other clinical information	• Current information about pharmacogenomics for self, clients and colleagues • Explain basic concepts of probabilities of genetic factors in maintenance of health and development of disease • Seek coordination and collaboration with an interdisciplinary team of health professionals • Identify patients who have undergone pharmacogenetic testing in the past so that a specific test is not repeated unnecessarily • Identify drug therapy problems that may be related to genetic variability, even when a pharmacogenetic test has not been done • Critically evaluate information obtained from pharmacogenetic/genomic clinical trials and identify limitations in study design, technology, and data interpretation that will influence patient care • identify the epidemiologic implications of pharmacogenetic/genomic studies and its impact at the societal level as well as that of the individual patient • Interpret the results of pharmacogenetic testing, and make drug therapy recommendations based on the results
Ethical, social and economic implications	• Understand the potential physical and/or psychosocial benefits, limitations, and risks of pharmacogenetic information for individuals, family members, and communities • Appreciate the ethical, legal and social issues related to pharmacogenetic testing and recording of genetic information (e.g., privacy, the potential for genetic discrimination in health insurance and employment) • Understand the increased liability that accompanies access to detailed patient information • Maintain the confidentiality and security of patient health records	• Discuss costs of pharmacogenetic services, benefits and potential risks of using health insurance for payment of pharmacogenetic services, including potential risks of discrimination • Tailor information and services to patient culture, education, and language • Adopt a code of conduct in patient treatment that is free of racial, ethnic, and religious bias • Identify appropriate resources offered by professional organizations, disciplines, or institutions

Appendix to Table III: Issues listed derives, in part, from National Coalition for Health Professional Education in Genetics (//wikigenetics.org/index.php/NCHPEG-Principles_of_Genetics_for_Health_Professionals) and in part from, databases available to the genetic community with a wide range of aims and scopes including: i) those presenting guidelines on pharmacogenomics related to government policy such as Food and drug Administration (www.fda.gov/cder/genomics/default.htm) and European Medicine Agency (www.emea.europa.eu/pdfs/human/ich/43798606en.pdf); ii) those provide a genetic row data such as Genbank (www.ncbi.nlm.nih.gov/Genbank/index.html); and iii) those providing higher level structure and annotation such as Pfam (www.sanger.ac.uk/Software/Pfam). Other types of large-scale data resource used in Pharmacogenetic testing include publication databases such as Pharmacogenomics Knowledge Base (www.pharmgkb.org/index.jsp) and disease/gene information resources and tools such as OMIM (www.ncbi.nlm.nih.gov/sites/entrez?db=omim/) or Orphanet. (www.orpha.net). The table lists of FDA-approved drugs with pharmacogenomic information in their labels are available at www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm. Clearly there are overlaps between many of these database and an attempt at categorize them to any detailed degree may be difficult and unproductive.

between commercial products (used by laboratories) and diagnostics service. In both circumstance, a voluntary list of international laboratories (with CLIA certification in the US and CE mark in Europe) that perform genetic tests can be found on the National Institute of Health-funded website named GeneTests™ [http://www.ncbi.nlm.nih.gov/sites/GeneTests/lab?db=GeneTests], although only a small minority of genetic tests listed on this site are PGx tests. Clinical laboratories may develop and validate tests in-house ("home-brew") and perform them as a laboratory service; which may further reduce the cost of analysis[11,14].

Pharmaceutical and Biotech companies frequently develop their own clinical pharmacokinetic and pharmacodynamic tests for new drug studies. They are required to have validated assays for human clinical phase III trials complying with current Good Clinical Practice guidelines for FDA or EMEA submission purposes. This involves testing patients as a potential recipients before the administration of the drug. This difference poses an ethical dilemma for pharmaceutical companies, especially if inadequate testing excludes some patients who might benefit from receiving the drug or, conversely, long-term dosing continues with a treatment that does not have good clinical efficacy[15]. Pharmaceutical companies should be involved with the initial development of PGx assays because they have the primary data and information necessary for this stage of assay development. However, this assay development activity should be transferred to outside referenced laboratories, clinical core laboratories in academic health centers, or established Clinical Research Organizations when research and development transit into clinical application because these independent external sites are able to handle this function[16].

Drug selection based on genetic assessment may be considered confidential information. Currently, PGx testing may provide detailed genetic information necessary for health professions to prescribe the correct drug and its dose. The skills of health operator must be orientated to maintain the confidentiality and security of patient's health records. In this field, the clinical laboratories could be the most proficient means to protect patient/physician confidentiality.

Reimbursement or payment for genetic testing is another topic of considerable consequence that is already creating controversy among health maintenance organizations, healthcare providers, and the patients themselves. One can predict, however, that health insurance companies will be very interested in patient PGx testing to document the proper dosing of expensive prescription drugs and hence reduce the occurrence or risks of adverse drug reactions. It will be interesting to see whether insurers will consider PGx testing to be a cost-effective alternative to the current trial-and-error approach to dosage regulation. However, if the detection of these genetic variants is routinely incorporated either into clinical practice or large clinical trials, knowledge concerning the predictive value of PGx which will eventually enable the individualization of optimized therapy could be gained[17]. However, we still need a precise demonstration that PGx tests offer an added value, in terms of relative cost and benefit.

Furthermore, trials evaluating the pharmacoeconomic impact of genotyping testing before therapy will likely provide answers for policy making in the merging of PGx testing into clinical practice. The primary aim of a cost-effectiveness analysis is to provide sufficiently robust information for decision-makers to allocate resources to healthcare interventions. Overviews of cost-effectiveness studies on PGx technologies are now available[18]. A relevant example is the National Institute for Health and Clinical Excellence (NICE). NICE forms a Diagnostic Advisory Committee, which is willing to stimulate Pharma and Academic communities to produce a robust set of data, including design and data source in economic models of healthcare[19]. Only few studies have addressed the cost-effectiveness of pharmacogenomics testing implication in clinical practice[18]. For example *van den Akker et al*, included thiopurine S-methyltransferase (TPMT) genotyping prior to 6-mercaptopurine treatment in paediatric Acute Lymphoblastic Leukaemia (ALL); the mean calculated cost from 4 European countries was € 2100,00 per life-year considering low myelosuppression-related hospitalization; the cost for genotyping of TMPT mutation averaged around € 150,00[20]. Early outline of genotyping cost for "home brew" pharmacogenomic tests averaged about € 20,00 per SNP[14].

Conclusions

The potential is enormous for pharmacogenomics to yield a powerful set of molecular diagnostics that will become routine tools by which pharmacists and physicians select the proper

medications and doses for each individual patient. Instead of starting patients on the "average dose" that was found to be safe and effective in most patients in large clinical trials, pharmacogenomics has the potential to provide patient-specific data upon which the selection of drugs and doses can be individualized and optimized. Using the amount of DNA that can be isolated from just few milliliters of blood, it is possible to determine thousands of genotypes, even with current technology, described above. So, taken together, the process will be to collect a single blood sample from each patient, submit an aliquot of the sample to a reference laboratory for analysis of a panel of genotypes, and test for those established to be important determinants of drug disposition and effects. The results of this specific panel of genetic variants would be electronically deposited into a secured database, into and out of which data can be accessed only with the patient's authorization (to her/his health care professionals). The results of these tests will not be simply a list of gene SNPs, but rather a report formatted and interpreted according to the patient's diagnosis and treatment options. For example, the report could be a recommended algorithm for the selection of anticoagulation, starting with those most likely to be effective and well tolerated, based on the patient's genotypes for the panel of genes known to be significant determinants of the disposition and effects of coagulation medications (i.e. VKORC1 mutation and warfarin administration). As patients experience additional illnesses, additional genotypes will be characterized and the data added to the same secured database, to which the patient's future physicians and pharmacists would be granted access as needed to make treatment decisions. Of course, these new tools will not replace the more conventional biochemical tests that are now routinely used to assess organ function and disease progression; rather they will complement these contemporary tests and provide additional tools for selecting medications that are optimal for each patient. Furthermore, genotyping will not obviate the need for follow up assessment of response, adherence to treatment, or drug interactions, which will continue to be important clinical responsibilities of health professionals.

Over the next few years, the emergence of molecular detection as results of the genomic alterations in therapy will drive diagnostics companies to develop new tests able to produce results for tailoring patient's treatment. Hopefully, the future implementation of the methods for genotyping of variants influencing therapy will result in personalized treatments and eventually, in shifting the clinical benefit from disease relapse towards disease eradication. Therefore, it is fundamental that pharmaceutical and biotechnology companies join together, in order to develop an extensive study the standardization method to validated tests suitable for routine diagnostics in pharmacogenomics.

In summary, with the increasing number of novel genetic markers being identified and validated, pharmacogenomics will make the practice of health profession and medicine should be less an art and more a science.

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