Early Outline Evaluation of Genotyping Costs of Pharmacogenomics

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Keywords: Pharmacogenomics; Genotyping methods; Molecular diagnostics; Pharmacoeconomy; Clinical laboratory

Abbreviations: PG: Pharmacogenetics and pharmacogenomics; NICE: National Institute for Health and Clinical Excellence; SNPs: Single Nucleotide Polymorphisms; SSCP: Single-Strand Conformational Polymorphism; FRET: Fluorescent Resonance Energy Transfer; MALDI TOF: Matrix-Assisted Laser Desorption/Ionization Time of Flight; ASA-PCR: Allele Specific Amplification-Polymerase Chain Reaction; RFLP: Restriction Fragment Length Polymorphism; D-HPLC: Denaturing-High Performance Liquid Chromatography; FDA: Food and Drug administration; CE: European Community; UGT1A1: Uridinediphosphate Glucuronosyl Transferase IA1

Introduction

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.

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 [1].

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 between commercial products (used by laboratories) and diagnostics service. In both case, a voluntary list of international laboratories (with CLIA certification in the US) or established Clinical Research Organizations when research and development transit into clinical application because these independent external sites are able to handle this function [3].

Several methods to assess the quality of cost-effectiveness, cost-utility and cost-benefit of PG tests have become available. A relevant example is the National Institute for Health and Clinical Excellence (NICE). NICE forms a Diagnostic Advisory committee, which stimulates Pharma and Academic communities to produce a robust set of data, including the design and data source, for economic models of healthcare [5].

Relative costs of PG tests, here, were evaluated by "manually cured criteria" due to lack of specific guidelines.

We wish to stress that the cost-effectiveness, cost-utility and cost-benefit analysis (i.e. toxicity-related hospitalization) are not considered here.

The goal of this issue is to provide information on the advantages and limitations, in terms of costs, of the most common available methods for molecular detection of polymorphisms related to drug therapy.

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Received September 26, 2013; Accepted January 31, 2014; Published February 07, 2014


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We believe that retrospective and prospective trials evaluating the pharmacoeconomic impact of genotyping testing will provide answers on the possibility to incorporate PG testing into routine clinical practice.

### Current Genotyping Methods for Known Mutations

The most popular platforms for the detection of known SNPs can be classified in two major groups: i) platforms discriminating alternative alleles (mutant vs. wild type) and ii) platforms detecting mutant alleles in a given DNA. The only platform able to detect and discriminate the alleles is MALDI TOF. There are also other allele discrimination methods, although not as powerful as MALDI-TOF, including: i) PCR-based without fluorescent probe (ASA, SSCP and RFLP); ii) methods combining PCR with hybridization probes (FRET-based platforms, Invader assay and LNA Probes); iii) PCR-based with intercalating fluorescent dye as High Resolution Melting (HRM); iv) PCR required only for sample pre-treatment as Denaturing-High Performance Liquid Chromatography (D-HPLC).

The principal methods used to detect mutant alleles in given DNA, are: i) Pyrosequencing (require a PCR pre-treatment); ii) PNA mediated clamping-PCR; iii) Gene chip technologies; and iv) Sequencing methods.

New high-throughput sequencing technologies are incorporated in new platforms such as: 1G Genetic analyzer by Illumina/Solexa (Cambridge, UK), SOLiD by Life technologies (Foster City, CA, USA) and Genome Sequencer FLX by Roche Diagnostics (Brandford CT USA). These “Next Generation DNA Sequencers” provide a consensus base accuracy of 99.99%.

Non-PCR-based technologies, such as the Golden Gate® Assay and DNA chip-based microarray are the latest development in the genotyping arena. These technologies are able to directly genotype genomic DNA without PCR amplification. These new technologies are not widely used in the general clinical laboratory setting compared to PCR-based methods.

The qualitative assessments of the polymorphic traits in genes are performed, without a necessary gold standard for the daily diagnostic routine (Table 1).

### Genotyping Costs

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 [5]. A relevant example is the NICE: 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 [6].

Only few studies have addressed the cost-effectiveness of pharmacogenomics testing implication in clinical practice. For example, Aquilante et al. [7] performed a study to compare the accuracy, the rapidity, and the cost of two methodologies used for genotyping a single variant in the cytochrome P450 (CYP) 2C9 metabolizing enzyme gene: the cost/sample for 1 SNP detection was (US dollars) $1.90 by PCR-Pyrosequencing and $ 3.14 by RFLP. Other Authors, 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 myelosupression-related hospitalization; the cost for genotyping of TMPT mutation averaged around €150,00 [8]. In addition, Gold et al. [9], concluded that pharmacogenetic testing for UGT1A1*28 variant homozygosity before Iromotecan treatment may be cost effective. The genotype testing cost is averaged $103.00z.

Early outline of genotyping cost for “home brew” pharmacogenomic tests is averaged about €20,00 per SNP.

### Conclusion and Future Outlook

It is well known that PG tests performed before drug treatment, lower overall medical costs and provide higher quality of life and longer life expectancy. However, we still need a precise demonstration that PG tests offer an added value, in terms of relative cost and benefit.

The usefulness of the variants in clinical practice depends on improving the diagnostic prediction or fostering changes in prevention

<table>
<thead>
<tr>
<th>Genotyping methods to detect known SNP</th>
<th>Instrument mean costs§</th>
<th>Approximate reagent costs per SNP$</th>
<th>Approxim time-labour per SNP#</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-HPLC</td>
<td>++</td>
<td>moderate</td>
<td>very fast</td>
</tr>
<tr>
<td>SSCP</td>
<td>+</td>
<td>Low</td>
<td>very laborious</td>
</tr>
<tr>
<td>Allele Specific Amplification (ASA)</td>
<td>+</td>
<td>very low</td>
<td>moderate</td>
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<tr>
<td>Restriction Fragment Length Polymorphism (RFLP)</td>
<td>+</td>
<td>very low</td>
<td>very laborious</td>
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<tr>
<td>FRET probe Allelic Discrimination (Hyb Probe® TaqMan®, Beacons® Scorpions®)</td>
<td>++</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td>Locked Nucleic Acid (LNA) probe</td>
<td>++</td>
<td>moderate</td>
<td>moderate</td>
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<tr>
<td>Oligo ligation assay (SNPlex®)</td>
<td>+++</td>
<td>high</td>
<td>Fast</td>
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<tr>
<td>PCR-Invader® Assay</td>
<td>+++</td>
<td>high</td>
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<tr>
<td>High resolution melting (HRM)</td>
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<td>low</td>
<td>moderate</td>
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<tr>
<td>Pyrosequencing</td>
<td>+++</td>
<td>high</td>
<td>Fast</td>
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<tr>
<td>Peptide nucleic acid-mediated Clamping PCR*</td>
<td>+</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td>Gene Chip technology (LabOnChip)*</td>
<td>++++/++++</td>
<td>very high</td>
<td>moderate</td>
</tr>
<tr>
<td>Maldi-TOF</td>
<td>+++</td>
<td>very high</td>
<td>moderate</td>
</tr>
<tr>
<td>Conventional sequencing*</td>
<td>+</td>
<td>low</td>
<td>moderate</td>
</tr>
</tbody>
</table>

*Approximate instrumentation list price were scored as ± (<10000€); ±± (<50000€); ±±± (<100000€); ±±±± (>100000€).
$Reagent costs were scored as very low (<5€), low (<10€), moderate (<30€), high (<50€), very high (>50€).
#Time-labour refers input needed to perform a single test of multiple samples. It were scored as very fast (<1 hour), fast (<4 hours), moderate (<1 day), laborious (<2 day) very laborious (>2 working day).

Table 1: Most common platforms used for genotyping at molecular level.
or treatment strategies. There is a compelling need for detailed and extensive studies to establish the cost and effectiveness of genotyping. Although there are still unanswered questions, PG researchers have generated and improved specific tools for novel treatment and handling of cancer patients. With new PG markers being identified and validated, physicians will have ways and means to tailor specific drugs to individual genetic profiles.

Over the next few years, the emergence of molecular resistance to new therapies as a result of genomic alterations in cancer will drive diagnostics companies to develop new tests for individualized therapy. The future implementation of the methods for genotyping will lead to personalized treatment and eventually will shift the balance from disease relapse toward disease eradication. Therefore, it is essential that pharmaceutical and biotechnology companies join the efforts to develop accurate and cheap tests for routine diagnostics in pharmacogenomics.

Authors’ Conflict of Interest Disclosure

The authors stated that there are no conflicts of interest regarding the publication of this article.

References