AmpliChip CYP450®: genotipaje del citocromo P450 en pacientes psiquiátricos

AmpliChip CYP450®: Cytochrome P450 Genotyping in Psychiatric Patients. Full text.
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Key points

- Pharmacogenetics may offer enormous potential in providing clinical benefits to patients (personalised medicine), as well as clear financial advantages for Healthcare Services.
- However, unless the variants under study are relatively common among the population (prevalence > 30%) and there is a clear effect in terms of response to medication, large-scale population studies will be required to determine whether genetic variants alter patient prognosis.
- Among the most interesting allelic gene variants of cytochrome P450 which define the main phenotypes – i.e. poor, intermediate, extensive and ultra rapid – (1), are those that identify poor metabolisers which are associated with a greater incidence of adverse drug reactions (ADRs) and costly treatments as a result of prolonged hospitalisation (2).
- In theory, genotyping tests should identify most genetic variants functionally capable of modifying the expression or function of the proteins responsible for drug metabolism, transportation and/or reception. To choose the best suited method requires knowledge on the main mutations or polymorphisms to be studied, along with the sensitivity/specificity of the procedure, sample requirements and cost.
- The AmpliChip CYP450® test is heralded as one of the most practical and comprehensive methods for analysing a large number of the genetic variants of genes CYP2D6 and CYP2C19 and for identifying pharmacogenetic profiles in psychiatric patients (3). However, before this technology is introduced in the National Health Service prospective studies must be conducted on the AmpliChip CYP450® test in order to address crucial issues such as genotype veracity, correct patient identification, benefits derived from any treatment changes suggested by outcomes, presence of other genetic and environmental factors that may influence metabolism, the usefulness of the intervention in patients treated with drugs that are metabolised by CYP450 and, finally, how the data compiled may be applied to the prescription of drug therapy.
- In addition, structures, professional training and education will need to be adapted as well as decision-making with regard to candidate drugs for pharmacogenetic testing to ensure the successful introduction of pharmacogenetics in clinical practice; both cost-effectiveness studies and laboratory monitoring must be conducted by pharmaceuticals and specialists in the field, while possible ethical and legal issues also need to be fully addressed.
• Existing evidence on the sensitivity/specificity of the AmpliChip CYP450® test in determining CYP2D6 and CYP2C19 genotypes is poor. The only study that we were able to assess was conducted by industry, although research on CYP450 microarrays and the experience of research groups warrant the consistency of the genotypes obtained using AmpliChip CYP450®.

• There are some indications that microarrays – including AmpliChip CYP450®) – are sometimes a good alternative to sequencing although there are more requirements to be met in obtaining pharmacogenetic profiles for psychiatric patients.
Description of the technology

Name of the technology

AmpliChip CYP450® (DNA arrays based on the Affymetrix microarray platform).

Description of the technology

The clinical use of drugs frequently highlights therapeutic ineffectiveness or pharmacological toxicity which invariably appears in the case of psychiatric patients under drug therapy. This may be due to varying plasma levels for the drug which depend, among other factors, on the dosage and pharmacogenetics of the prescribed medication.

In humans, the cytochrome P450 (CYP) enzyme superfamily plays a pivotal role in xenobiotic oxidation and/or reduction which render them more hydrophilic and, hence, facilitate their excretion.

To date, 18 CYP450 families have been described. These have been divided into 43 sub-families (indicated in alphabetical order) and a total of 59 genes (identified numerically) located mainly in the endoplasmatic reticulum of hepatocytes (4).

Moreover, we can identify gene variants which code for cytochrome P450 enzymes. These variants – mutations and polymorphisms – enable sub-groups of individuals with varying degrees of metabolic reaction to be distinguished among the general population. Sub-groups with a poor phenotype for metabolism of a given drug are known as poor metabolisers compared to intermediate, extensive, and ultra rapid metabolisers (5;6).

In general terms, medication has an exacerbated effect on subjects classified as poor metabolisers and they may show increased adverse drug reactions. This is due to impaired metabolism which in turn increases plasma drug concentration (7;8). However, ultra rapid metabolisers may not achieve the usual therapeutic concentrations as a result of increased metabolism, and hence therapeutic response to standard dosage is inadequate (Annex 5).

Gene CYP2D6 has at least 70 allelic variants responsible for four kinds of phenotypes whereas the two main variants of CYP2C19 yield reduced metabolic capacity (9).

25% of the drugs commonly used in clinical practice are metabolised by CYP2D6 and CYP2C19 (10). Some groups of drugs, such as anti-depressants,
anti-psychotics, anti-arrhythmic drugs, betablockers and narcotics, are metabolised by CYP2D6 while CYP2C19 metabolises proton pump inhibitors, benzodiazepines, anti-convulsants, anti-coagulants and anti-infective drugs (Annex 6).

The AmpliChip CYP450® test is an alternative procedure to automatic sequencing – cheaper in theory – capable of genotyping 31 genetic variations for two of the most important and well-known CYP450 genes, CYP2D6 and CYP2C19, in one single assay. Patients – who should not be on medication at the time of the test - perceive this as a non-aggressive method since samples for analysis are obtained via venous puncture.

Not all patients treated with drugs metabolised by CYP2D6 and CYP2C19 are eligible for genotyping with AmpliChip CYP450®. Use of this technology is especially suited to drugs characterised by a narrow therapeutic range or drugs causing severe ADRs.

A large amount of evidence suggests that psychotropic drugs are among the most frequently and inappropriately used (3). The AmpliChip CYP450® test was conceived as yet another tool to assist in providing personalised therapy for psychiatric patients based on knowledge of the subject’s metabolising profile. This will avoid delays in the administration of effective therapy, adverse drug reactions and excessive expenditure on ineffective treatments.

AmpliChip CYP450® contains 15,000 oligonucleotide probes synthesised in situ on a silicon chip or slide, thereby enabling testing of CYP2D6 and CYP2C19 genes to subsequently establish the metaboliser phenotype for each patient with the aim of determining drug dosage requirements for drugs metabolised by these CYPs. Essentially, it is based on five processes which combine Roche PCR technology and Affymetrix microarray systems (11;12).

1. PCR amplification from a purified DNA taken from a whole blood sample (13). Tests can also be performed on plasma, serum and epithelial cell samples, obtained from buccal swabs (14).
2. Fragmentation and labelling of the amplified product using a fluorescent substance.
3. Hybridisation of the labelled product on a DNA microarray.
4. The resulting fluorescence, which highlights hybridised areas, is visualised using a laser system.
5. Determining the CYP450 genotype and subsequent prediction of metaboliser phenotype.

wild-type or polymorphic sequence (Appendix 4). Combining the enzyme activity codified by both alleles allows for determination of the overall enzyme activity of each cytochrome.

In the AmpliChip CYP450® test, predicted phenotypes – known as such since genetic and environmental factors may affect the way in which an organism metabolises CYP2D6 and CYP2C19-dependent drugs – include the following:

- **CYP2D6:** poor (no enzyme activity), intermediate (reduced enzyme activity), extensive (“normal” enzyme activity) and ultra rapid (enzyme activity is higher than normal).
- **CYP2C19:** poor (no enzyme activity) and extensive (“normal” enzyme activity).

**Development status of the Technology**

The AmpliChip CYP450® test is considered by the US FDA (Food and Drug Administration) as a Class II device (subject to special controls) and has received the CE seal of approval which grants approval for its use for diagnostic purposes within the European Union (13). The Instituto de Medicina Legal at Santiago de Compostela University (Spain) is currently running a trial with AmpliChip CYP450® to test CYP2D6 in psychiatric patients. Concoradance between AmpliChip CYP450® and automatic sequencing and quantitative PCR (for duplications) results has been found in 140 patients (100%) (data not published).

**Distribution**

Distributed by Roche Molecular Diagnostics.

**Alternative Technologies**

- DNA enzyme amplification by Polymerase Chain Reaction (PCR) and subsequent automatic sequencing or Restriction Fragment Length Polymorphism analysis (RFLP) using restriction enzymes. These options require a high number of reactions to characterise a minimum recommended number of CYP2D6 alleles (15).
- In addition, there are a number of allelo-specific technologies for analysing genome mutations and polymorphisms (SNPs), such as Taqman and Pyrosequencing (small-medium scale genotyping technologies), SNPlex and Sequenom (medium-large scale genotyping technologies).
technologies) and Illumina (large-scale genotyping technology). The use of these is only cost-effective in studies which include a large number of samples.
Clinical features

Type of Technology

Diagnostic test.

Scope for Application of the Technology

Hospital.

Indications

Genotyping of certain CYP2D6 and CYP2C19 gene variants, which have a significant role in drug metabolism. The main recipients of these interventions are subjects with a poor metaboliser phenotype – fairly infrequent among Caucasians (8;16-20) - given that a decline in CYP450 enzyme activity, and its associated increase in plasma drug concentrations, may trigger and multiply adverse drug reactions. In general terms, poor CYP2D6 and CYP2C19 metabolisers present poor tolerance to treatment with certain tricyclic antidepressants and antipsychotics. As a result, it is recommended that these patients be treated with other psychiatric drugs which are not dependent on these particular P450 cytochromes. Moreover, although clinical relevance still remains unclear, co-medication with CYP2D6 and CYP2C19 inhibiting drugs (e.g. paroxetine, quinidine, fluoxetine, bupropion, fluvoxamine, mirtazapine) must be monitored with care since these drugs can “transform” an extensive metaboliser into a poor metaboliser.

Number of patients

Mental disorders are one of the most prevalent diseases in the developed world and they take centre stage with respect to other chronic conditions. Their repercussion on healthcare is significant, given the economic impact of related pharmaceutical expenditure and as a result of the disabilities and dysfunctions they entail.

The use of psychiatric drugs requires strict control – both clinical and in the laboratory. Their therapeutic range is overall very narrow, so patients below or
above that range will be exposed to non-therapeutic effects or to the onset of adverse drug reactions. The side effects that may arise from treatment frequently account for treatment discontinuation or failure, calling for other measures to be taken, including continuous dosage adjustment.

Adverse drug reactions appear ever more frequently in daily medical practice. Roughly 6.5% of hospital admissions are related to adverse reactions to drug treatment. The impact of ADR is manifold and highly significant; ADRs reduce life expectancy, increase the number of complications and substantially raise social and healthcare costs. The more than two million severe ADRs that have been recorded are one of the main causes of death, accounting for 100,000 deaths/year (21) in the US, and totalling over 100 billion dollars (22) a year in expenditure for the US Health Service.
Aims

The overall objectives of the technical reports on emerging technologies are to:

- Pinpoint new technologies – or changes in existing technologies - that may have a potential impact on the Healthcare System as early as possible.
- Summarise the information available on detected technologies.
- Draw up recommendations for different decision-making levels within the Healthcare System.

In these cases, the specific aims focus on evaluating the efficacy of AMPLICHIP CYP450®. Cytochrome P450 Genotyping in Psychiatric Patients.
Methodology

The method used entails a structured search in pre-determined data bases, a critical review of the literature retrieved, summary of the outcomes, and evaluation of results within the context of the National Health System.

The search focused on finding diagnosis test assessment reports and data bases used are the following: Medline, EMBASE and the Cochrane Library Clinical Trials Register. A search was also run on the European Agency for the Evaluation of Medicinal Products (EMEA), Food and Drug Administration (FDA), The International Network of Agencies for Health Technology Assessment (INAHTA), The European Network for New and Changing Technologies (EuroScan) and the North American clinical trials registry, ClinicalTrials.gov (http://clinicaltrial.gov/).

The search strategy used is shown in Appendix 1.

A critical analysis was performed by using CASP scale (Critical Appraisal Skills Programme).
Efficacy, effectiveness and safety

Clinical effectiveness

No studies were found that addressed the sensitivity and specificity of the AmpliChip CYP450® test compared to other existing technologies, such as allelo-specific PCR, or PCR followed by automatic sequencing or treatment with restriction enzymes, save for the study provided by the manufacturer. The same applies to studies relating the use of AmpliChip CYP450® with prognosis in psychiatric patients. However, one study did report on the association between the CYP2D6 poor metaboliser phenotype (determined by AmpliChip CYP450® and/or allelo-specific PCR) and the onset of adverse reactions to risperidone, along with treatment discontinuation as a result of ADRs (23).

The manufacturer provided information assessing, among other parameters, detection limit, specificity and reproducibility of the AmpliChip CYP450® genotyping array.

- To establish the detection limit, we analysed three dilutions of two DNA samples (2.5 ng, 25 ng and 50 ng). The lowest amounts of DNA yielding correct determination of the genotype were 25 ng for CYP2D6, and 2.5 ng for CYP2C19.
- The specificity of AmpliChip CYP450® was evaluated using 100 DNA samples with two CYP2D6 alleles showing normal predicted enzyme activity, along with 270 samples for CYP2C19. When comparing the result with the genotype studied using other methods, the estimated specificity for wild-type samples was 100% for genotype detection of both genes.
- To assess reproducibility, we designed a panel with seven cell lines representing eleven CYP2D6 alleles and the three CYP2C19 known alleles. Trials were carried out five times in triplicate, at three different laboratories, using three batches of reagents. For both genes, reproducibility was 99.99%.

Prior to marketing of the AmpliChip CYP450® test, two methods for CYP2D6 genotyping were compared, namely Affymetrix GeneChip CYP450® versus allele-specific PCR. For the five alleles tested (1) the concordance was
This methodology is effective but fails to analyse 22 of the alleles covered by the AmpliChip CYP450® test.

**Systematic Reviews**

We selected two reports released by the US and Canadian Agencies for Health Technology Assessment in 2004 and 2006 respectively, namely the Technology Evaluation Center (TEC) of the BlueCross BlueShield Association (BCBS) and the Canadian Co-ordinating Office for Health Technology Assessment (CCOHTA) (Appendix 3). The first study reports on a general assessment of the clinical practice applications of P450 cytochrome genotyping, using several strategies, and their cost-effectiveness (24). The second report discusses whether genotyping of CYP2D6 and CYP2C19 (using AmpliChip CYP450®) and subsequent prediction of metaboliser enzyme activity may have an impact on the prognosis of patients treated with drugs that are related to cytochrome P450 (25). Both reviews conclude that further studies are required to address the potential benefits and risks associated with this technology, although it may be used in tandem with other approaches for selection of drugs and dosage adjustments.

**Clinical Practice Guidelines**

We identified a Clinical Practice Guideline which made positive recommendations in terms of implementing pharmacogenetics in psychiatry. However, the Guideline does not explicitly outline the methodology adopted and provides no evidence regarding the analytical validity of the AmpliChip CYP450® test (26).

**Risks and safety**

The phenotype of patients with infrequent alleles may not be predicted correctly by AmpliChip CYP450®, since this microarray does not test for all CYP2D6 and CYP2C19 alleles.

Therapeutic response to drugs is a multifactor process, and hence other factors may exert an influence on drug metabolism in psychiatric patients. These factors include age, gender, nutrition, smoking, co-medication with other drugs, liver and kidney function, and hereditary factors which are not considered by this technology.

As an incorrect phenotype prediction may lead to inadequate therapeutic decisions, the data obtained from AmpliChip CYP450® must be seen as complementary to the information compiled from routine monitoring and
interpreted by professionals. Detecting the levels of tricyclic antidepressants and antipsychotics, among other drugs, may contribute to characterize poor metabolisers. As a consequence, this procedure may cost less than genetic analyses.

Other Technologies

There are other microarray-based technology tests for CYP450 such as CodeLink Human P450 SNP Bioarray® (GE Healthcare, USA), Signature Genetics® (Seryx-Signature Genetics, USA), DrugMet Genotyping Test® (Jurilab LTD., Finland) and Tag-It Mutation Kits® (Tm Bioscience Corp, Canada) (Annex 7).
Economic issues

Cost per unit and price

The retail value of each microarray (valid for one experiment only) is roughly 400-500$. Moreover, the AmpliChip CYP450® test requires laboratory staff with adequate training in molecular biology, as well as the Affymetrix GeneChip System 3000Dx equipment, which costs 200,000$.