Research Highlights

Highlights from the latest articles in personalized medicine

Evaluation of the AmpliChip™ CYP450 Test

Evaluation of: Rebsamen MC, Desmeules J, Daali Y *et al.*: The AmpliChip CYP450 test: cytochrome P450 2D6 genotype assessment and phenotype prediction. *Pharmacogenomics J.* 9(1), 34–41 (2009).
– Monique J Bijl & Ron HN van Schaik

In December 2004, the US FDA approved the AmpliChip™ CYP450 test (Roche Diagnostics [IN, USA]) as the first pharmacogenetic test that uses microarray technology. This test analyzes genetic variants in the cytochrome P450 2D6 and 2C19 genes (*CYP2D6* and *CYP2C19*) [101]. *CYP2D6* is involved in the metabolism of approximately 25% of all drugs metabolized by CYPs. The *CYP2D6* gene is highly polymorphic with more than 80 variant alleles having been described to date [102], some of which have consequences on drug metabolism as certain variant alleles encode decreased *CYP2D6* enzyme activity. A total of four phenotypic groups can be identified in the population: ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs) and poor metabolizers (PMs). The AmpliChip test can predict a patient's phenotype according to specific allele combinations.

In the study by Rebsamen *et al.* [1], the genotype results of the AmpliChip CYP450 test for the *CYP2D6* nonfunctional alleles (*3, *4, *5, *6) and gene duplication (*xN) were verified with existing methods (real-time PCR). In addition, the predicted patient's phenotype was compared with results using the classical dextromethorphan-phenotyping method, a specific *CYP2D6* probe drug. The AmpliChip test genotyping accuracy was 100% compared with routine genotyping methods for the five variant alleles analyzed. In fact,

the AmpliChip test improved genotyping, since additional rare variant alleles were also detected. A perfect correlation was observed between genotype results, phenotype prediction and measured phenotype for *CYP2D6* PMs. Among 114 measured EMs, 108 were correctly predicted (95% sensitivity). The AmpliChip test defines individuals with either one non-functional allele and one decreased activity allele or two decreased activity alleles as an IM. The sensitivity of the AmpliChip test in predicting IMs was 42%, although some measured IMs were close to the EM border. The current paper clearly demonstrates that the majority of individuals with one active and one inactive allele (EM/PM) have an EM rather than an IM phenotype.

The AmpliChip test measures the gene duplication of active genes, taking variant alleles into account. However, the sensitivity of the UM phenotype prediction was low (6%), with a low positive predictive value (50%) but a high specificity (99%). Earlier reports indicate that only a limited number of phenotypic UMs are correlated with gene duplications, but the probe drug may influence this correlation. Screening for *CYP2D6* UMs might be insufficient, but in cases of toxicity or treatment inefficacy, genotyping may be helpful.

Discussions are ongoing as to whether the classification of PMs, IMs, EMs and UMs is the most accurate approach. Gaedigk *et al.* and Steimer *et al.* proposed another classification system based on the presumed activity of individual *CYP2D6* alleles [2,3]. In this *CYP2D6* activity score system, nonfunctional alleles are assigned an activity score of 0, functional alleles are assigned a score of 1 and decreased activity alleles (e.g., *10 and *41) are assigned a score between 0 and 1. This system was demonstrated to be more accurate in predicting the *in vivo* activity than the traditional

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phenotype system. Further research in large groups of patients using CYP2D6 probe drugs is needed to validate these scoring systems.

The AmpliChip CYP450 test is a useful tool to predict a patient's phenotype and may help clinicians to optimize pharmacotherapy by improving drug efficacy and reducing adverse drug reactions.

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Websites

- 101 Roche Diagnostics: AmpliChip CYP450 for *in vitro* diagnostic use www.amplichip.us/
- 102 The Human Cytochrome P450 (CYP) Allele Nomenclature Committee www.cypalleles.ki.se/

Impact of the *CYP2D6* genotype on breast cancer recurrence and hot flashes in women receiving tamoxifen

Evaluation of: Ramón y Cajal T, Altés A, Paré L *et al.*: Impact of CYP2D6 polymorphisms in tamoxifen adjuvant breast cancer treatment. *Breast Cancer Res. Treat.* (2009) (Epub ahead of print);

Lynn Henry N, Rae JM, Li L, Azzouz F *et al.*: Association between CYP2D6 genotype and tamoxifen-induced hot flashes in a prospective cohort. *Breast Cancer Res. Treat.* (2009) (Epub ahead of print).
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Tamoxifen is widely used as an antiestrogen in the treatment of estrogen-receptor-positive breast cancer. Tamoxifen undergoes extensive hepatic metabolism, being broken down into more potent metabolites, including 4-hydroxy-tamoxifen and endoxifen (4-hydroxy-N-desmethyl-tamoxifen). Endoxifen and 4-hydroxy-tamoxifen have a 50-fold higher affinity for the estrogen receptor than tamoxifen. Plasma concentrations of endoxifen are, on average, 5–10 times higher than those of 4-hydroxy-tamoxifen, making endoxifen the most active substance.

Cytochrome P450 2D6 (CYP2D6) plays an important role in the formation of endoxifen from tamoxifen. The CYP2D6 gene is highly polymorphic with more than 70 variant alleles having been identified to date. In Caucasians, 5–10% of the population lack CYP2D6 enzyme activity owing to the inheritance of two nonfunctional alleles of the CYP2D6 gene (e.g., CYP2D6*3, *4, *5 and *6). These individuals are classified as PMs. CYP2D6*4 is the most common variant allele leading to the PM phenotype in Caucasians. Other genetic variants, including disfunctional alleles (e.g., *10 and *41), occur less frequently. Subjects with two functional alleles (e.g., *1 and *2) are classified as EMs, whereas heterozygous carriers of CYP2D6 polymorphisms demonstrate large variability in their phenotype. UM have more than two functional copies of the CYP2D6 gene and exhibit extremely high enzyme activity [1].

Homozygosity for CYP2D6 non-functional alleles has been associated with lower plasma concentrations of endoxifen. Recently, Ramón y Cajal *et al.* studied the impact of CYP2D6 polymorphisms in tamoxifen-adjuvant breast cancer treatment. In this study, a significant

difference in disease-free survival was observed between CYP2D6 PMs (*4/*4, *4/*41, *1/*5 and *2/*5) and EMs plus IMs (95 vs 119 months; $p = 0.016$) [2]. Although this study was small ($n = 91$), the results support the previous findings of Goetz *et al.* and Schroth *et al.* [3,4]. Goetz *et al.* described a higher risk of breast cancer recurrence and a lower incidence of hot flashes in women using adjuvant tamoxifen with decreased CYP2D6 enzyme activity (owing to the presence of the CYP2D6*4/*4 genotype or co-administration of CYP2D6 inhibitors) [3].

In addition, Lynn Henry *et al.* prospectively studied the incidence and severity of hot flashes in tamoxifen users between CYP2D6 genotypes [4]. At 4 months, IMs reported higher weekly hot-flash scores than EMs ($p = 0.011$) and PMs ($p = 0.038$). PMs were less likely to develop severe hot flashes than EMs and IMs combined ($p = 0.062$) [5]. The association between IMs and hot-flash scores in this study is surprising but can reflect poor adherence in EMs, who experienced more severe hot flashes and discontinued tamoxifen therapy before the 4th month assessment. The authors recommend that the presence, or absence, of hot

flashes should not be used to determine tamoxifen's efficacy. Adverse events, with the exception of hot flashes, were evaluated in the study by Ramón y Cajal *et al.* They did not find a significant association between *CYP2D6* genotype and toxicity, but severe and mild toxicities were more frequent among PMs than in patients with a normal metabolizing pattern.

In conclusion, several studies demonstrated that *CYP2D6* PMs using tamoxifen have an increased risk of breast cancer recurrence and mortality or worse disease-free survival. These patients probably benefit more from aromatase inhibitors, which lower the amount of estrogen by inhibiting the conversion to estrogen. The metabolism of these compounds does not depend on *CYP2D6* enzyme activity. Genotyping

of the *CYP2D6* gene before the start of endocrine treatment in breast cancer could identify PMs who may respond better to aromatase inhibitors than to tamoxifen therapy.

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Role of cytochrome P450 polymorphisms in pulmonary drug toxicity

Evaluation of: Wijnen PA, Drent M, Nelemans PJ *et al.*: Role of cytochrome P450 polymorphisms in the development of pulmonary drug toxicity: a case–control study in The Netherlands. *Drug Saf.* 31(12), 1125–1134 (2008).
– Monique J Bijl

The cytochrome P450 (CYP450) enzymes are a family of enzymes that metabolize xenobiotics, including most drugs, and some endogenous compounds. SNPs in the genes encoding the CYP450 enzymes can affect drug metabolism by altering enzyme activity. Sometimes, CYP metabolism may cause the formation of toxic metabolites or reactive oxygen species which may damage cellular function. As well as residing in the liver, CYP450 enzymes can also be found in the human lung where they might be involved in pulmonary toxicity [1].

Drugs can induce interstitial lung disease (ILD) [2]. The mechanism of drug-induced ILD is poorly understood, but inflammation initiated by drugs, drug metabolites or free radical formation may be an underlying factor.

In a case–control study by Wijnen *et al.*, they used a candidate gene approach to establish whether variation in *CYP450* genes contributes to pulmonary toxicity by comparing variant allele frequencies in drug-induced ILD cases with healthy volunteers and patients with idiopathic pulmonary fibrosis [3]. The prevalence of *CYP2D6* and *CYP2C19* variant alleles was significantly higher among ILD cases than in healthy volunteers or idiopathic pulmonary fibrosis patients. This study has several limitations. First, it is assumed that the pulmonary toxicity is drug induced. The percentage of drugs metabolized by CYP450 enzymes was higher in the ILD cases than in both control groups. Although the evidence is weak, this study demonstrates that the presence of *CYP450* variant alleles appears to be a susceptibility risk factor for the development of drug-induced pulmonary adverse events. Pharmacogenetic testing could identify patients who are at increased risk of developing adverse reactions. However, further studies are necessary to evaluate the benefit of pharmacogenetic testing and the cost–effectiveness, especially in pulmonary diseases.

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