

# The AmpliChip CYP450 test: cytochrome P450 2D6 genotype assessment and phenotype prediction

MC Rebsamen<sup>1</sup>, J Desmeules<sup>2</sup>,  
Y Daali<sup>2</sup>, A Chiappe<sup>1</sup>,  
A Diemand<sup>1</sup>, C Rey<sup>1</sup>, J Chabert<sup>2</sup>,  
P Dayer<sup>2</sup>, D Hochstrasser<sup>1</sup>  
and MF Rossier<sup>1</sup>

<sup>1</sup>Service of Laboratory Medicine, University Hospitals, Geneva, Switzerland and <sup>2</sup>Service of Clinical Pharmacology and Toxicology, University Hospitals, Geneva, Switzerland

## Correspondence:

Dr MC Rebsamen, Services of Laboratory Medicine, University Hospitals of Geneva, 24 Rue Micheli-du-Crest, 1211 Geneva 14, Switzerland.

E-mail: michela.rebsamen@hcuge.ch

Polymorphisms of the cytochrome P450 2D6 (CYP2D6) gene affecting enzyme activity are involved in interindividual variability in drug efficiency/toxicity. Four phenotypic groups are found in the general population: ultra rapid (UM), extensive (EM), intermediate (IM) and poor (PM) metabolizers. The AmpliChip CYP450 test is the first genotyping array allowing simultaneous analysis of 33 CYP2D6 alleles. The main aim of this study was to evaluate the performance of this test in CYP2D6 phenotype prediction. We first verified the AmpliChip CYP450 test genotyping accuracy for five CYP2D6 alleles routinely analysed in our laboratory (alleles 3,4,5,6, × N;  $n=100$ ). Results confirmed those obtained by real-time PCR. Major improvements using the array are the detection of CYP2D6 intermediate alleles and identification of the duplicated alleles. CYP2D6 phenotype was determined by assessing urinary elimination of dextromethorphan and its metabolite dextrorphan and compared to the array prediction ( $n=165$ ). Although a low sensitivity of UM prediction by genotyping was observed, phenotype prediction was optimal for PM and satisfying for EM and IM.

*The Pharmacogenomics Journal* (2009) 9, 34–41; doi:10.1038/tpj.2008.7; published online 1 July 2008

**Keywords:** CYP2D6; genotyping; phenotyping; AmpliChip CYP450 test

## Introduction

Cytochrome P450 2D6 (CYP2D6) mediates the metabolism of almost 25% of drugs in common clinical use, including analgesics such as opiates, anti-arrhythmic agents, anti-psychotic, antidepressant and  $\beta$ -blockers.<sup>1</sup> A high degree of interindividual variability in drug pharmacokinetic exists, mainly due to genetic polymorphisms, to co-medications or environmental factors affecting enzyme activity and to the physiological and/or pathological status of the individual.<sup>2</sup> CYP2D6 is the only cytochrome P450 enzyme which is not inducible and therefore genetic variation contributes largely to the variability of its activity. In clinical therapy, this variability has important consequences including the lack of response to a treatment and/or adverse drug reactions for a given therapeutic regimen.<sup>3</sup> In order to reduce these potentially unwanted events possibly leading to severe adverse reactions or even death,<sup>4,5</sup> it might therefore be of decisive interest to be able to assess an individual's CYP2D6 activity.

Indeed, besides the therapeutic drug monitoring that allows quantification of the circulating drug concentration in an individual during a treatment, two complementary approaches may be used to assess CYP2D6 activity

Received 14 December 2007; revised 16 May 2008; accepted 21 May 2008; published online 1 July 2008

independently of a specific treatment and even before starting it. On one hand, CYP2D6 phenotyping allows determination of the actual enzymatic activity by administering a specific probe drug to the individual and measuring the concentration of the drug and its metabolite in the urine. Based on this method four groups of metabolizers, displaying gradually decreasing CYP2D6 activity, can be identified in the population: ultra rapid (UMs), extensive (EMs), intermediate (IMs) and poor metabolizers (PMs). On the other hand, genotyping allows precise determination of the individual's *CYP2D6* DNA sequence and the possibility to predict a phenotype based on the alleles identified.

Over 80 *CYP2D6* allelic variants have been discovered so far and are associated to the absence, a decrease or an increase in enzyme activity according to the Human Cytochrome P450 Allele Nomenclature Committee (<http://www.cypalleles.ki.se/>). Although the association between a given *CYP2D6* allele and enzyme activity is well defined, phenotype prediction according to allele combination is more complex and very different predictive powers are observed depending on the group of metabolizers considered. Indeed, almost 100% of PMs are identified by genotyping, whereas only 20% of UMs are correlated to an increased number of *CYP2D6* gene copies.<sup>6–8</sup> Less information is available concerning IMs given the fact that classical *CYP2D6* genotyping has been more frequently employed to identify the major *CYP2D6* alleles associated to a loss of enzyme activity and to detect gene duplication. However, as much as 10–15% of Caucasians are phenotypically IMs (compared to 6–8% PMs and 5–10% UMs) therefore displaying impaired enzyme function and potentially benefiting of a drug dose adjustment to improve treatment efficacy and/or safety.<sup>9</sup>

A large number of molecular biology methods exist to analyse *CYP2D6* alleles. The vast majority of these methods identify one allele at the time implying that only a small subset of variants are usually analysed, possibly reducing the phenotype prediction power of genotyping for certain groups of metabolizers.

We routinely perform genotyping of *CYP2D6* by real-time PCR to detect four defective mutations of *CYP2D6* (\*3, \*4, \*5, \*6) that allows the detection of over 80% of *CYP2D6* dysfunction in a Caucasian population and one variant leading to increased enzymatic activity. Recently, we introduced the microarray technology in our laboratory and performed genotyping on the AmpliChip CYP450 test from Roche. This test identifies 33 *CYP2D6* alleles, including variants associated to impaired enzyme activity and seven gene duplications, as well as two *CYP2C19* mutations. We first aimed to verify the genotype determination accuracy of the AmpliChip CYP450 test for *CYP2D6* alleles \*3, \*4, \*5, \*6 and \* $\times$ N by genotyping 100 individuals with both our routine methods and the microarray. Secondly, *CYP2D6* phenotype was determined for 165 individuals using dextromethorphan (DEM) as a probe drug and phenotype prediction performance of the AmpliChip CYP450 test was evaluated.

## Results

### Genotyping

A total of 165 individuals' DNA samples were genotyped using the AmpliChip CYP450 test. Genotype distribution and allele frequency are shown in Tables 1 and 2. The five more frequent alleles in our population were \*1 (35.5%), \*4 (20.6%), \*2 (15.5%), \*41 (7.3%) and \*35 (7.3%). Among the 11 individuals resulting positive for a *CYP2D6* gene duplication, 5 had a duplicated allele \*2, 3 had a duplication of allele \*41, 2 of allele \*1 and 1 of allele \*4. As illustrated in Table 3, most of the 33 *CYP2D6* allelic variants identified by the AmpliChip CYP450 test are associated with a predicted enzyme activity (absent, decreased, normal or increased). Among the 13 non-functional alleles tested, 6 were found in

**Table 1** *CYP2D6* genotype distribution ( $n = 165$ )

Genotype	Number of individuals	Frequency (%)
*1/*2	22	13.3
*1/*1	21	12.7
*1/*4	18	10.9
*1/*41	11	6.7
*4/*4	9	5.5
*4/*35	8	4.8
*2/*4	7	4.2
*1/*35	7	4.2
*2/*41	6	3.6
*4/*5	5	3.0
*2/*2	5	3.0
*4/*41	4	2.4
*1/*9	4	2.4
*2/*35	4	2.4
*1/*10	3	1.8
*1/*5	2	1.2
*1/*3	2	1.2
*4/*9	2	1.2
*4/*10	2	1.2
*4/*2 $\times$ N	2	1.2
*1/*2 $\times$ N	2	1.2
*41/*1 $\times$ N	1	0.6
*1/*41 $\times$ N	1	0.6
*10/*1 $\times$ N	1	0.6
*1/*4 $\times$ N	1	0.6
*41/*2 $\times$ N	1	0.6
*41/*41 $\times$ N	1	0.6
*4/*41 $\times$ N	1	0.6
*2/*10	1	0.6
*9/*10	1	0.6
*5/*35	1	0.6
*9/*35	1	0.6
*17/*35	1	0.6
*35/*35	1	0.6
*2/*7	1	0.6
*1/*29	1	0.6
*1/*6	1	0.6
*4/*6	1	0.6
*6/*9	1	0.6
*6/*41	1	0.6

our population (\*3, \*4, \*4 × N, \*5, \*6, \*7) accounting for 25.4% of the total alleles. Six alleles associated with decreased activity were observed (\*41, \*9, \*10, \*17, \*29, \*41 × N) corresponding to 14.2 % of the total alleles frequency. Gene duplication alleles accounted for 3.3% of total alleles, but only 2.1% was associated to an increased enzyme activity considering the nature of the duplicated allele.

In order to confirm genotyping performance of the AmpliChip CYP450 test for the five CYP2D6 alleles routinely analysed in our laboratory, 100 samples were also tested for CYP2D6 alleles \*3,\*4,\*5,\*6 by real-time PCR using fluorescent probes. In addition, the presence or absence of CYP2D6 gene duplication, without further identification of the duplicated allele, was assessed by long template PCR. Genotype distribution of CYP2D6, as determined by these classical PCR methods, is summarized in Table 4. A total of 41 individuals carried at least one of the five tested alleles and the results obtained with the AmpliChip CYP450 test were 100% concordant.

In addition, the AmpliChip CYP450 test allowed precise genotype identification of the 59 individuals negative for

the four null alleles tested by classical methods, the 22 \*1/\*4 individuals and the 9 positive for CYP2D6 gene duplication (Table 5). Interestingly, within the subgroup of 59 individuals, approximately 24% carried one allele associated with an impaired enzyme activity and 1 individual was a compound heterozygous for two of these defective alleles (\*9/\*10). Similarly, for \*1/\*4 individuals the AmpliChip CYP450 test revealed that 18% of the \*1 attributed by default by classical methods were actually impaired alleles. Concerning gene duplication, four individuals out of nine carried at least one allele associated to a reduced CYP2D6 activity according to the classical genotyping method. Moreover, the AmpliChip CYP450 test allowed identification of the duplicated allele showing that for three individuals out of nine the duplicated allele was not the one associated with normal enzyme activity. Taken together, these results indicate that, compared to our routine tests, genotype information is strongly improved by the AmpliChip CYP450 test in a relevant number of cases.

#### From genotype to phenotype

According to the conventional classification system, the AmpliChip CYP450 test predicts individuals as PM if they carry two non-functional alleles; IM if they carry one non-functional allele and one associated with reduced activity or

**Table 2** Frequency of CYP2D6 allele (n = 330)

Allele	Occurrence	Frequency (%)
*1	117	35.5
*4	68	20.6
*2	51	15.5
*41	24	7.3
*35	24	7.3
*9	9	2.7
*10	9	2.7
*5	8	2.4
*6	4	1.2
*3	2	0.6
*7	1	0.3
*17	1	0.3
*29	1	0.3
*2 × N	5	1.5
*41 × N	3	0.9
*1 × N	2	0.6
*4 × N	1	0.3

**Table 4** CYP2D6 genotype distribution as assessed by classical PCR methods (n = 100)

Genotype	Number of individuals
*1/*1	59
*1/*4	22
*4/*4	5
*1/*3	1
*1/*6	1
*4/*6	1
*1/*5	1
*4/*5	1
*1 and Dupli	5
*4 and Dupli	4

Using these methods \*1 is assigned by default when none of the five alleles tested is present and Dupli indicates the presence of a duplication without identification of the allele.

**Table 3** Predicted enzymatic activity of the CYP2D6 allelic variants detected by the array

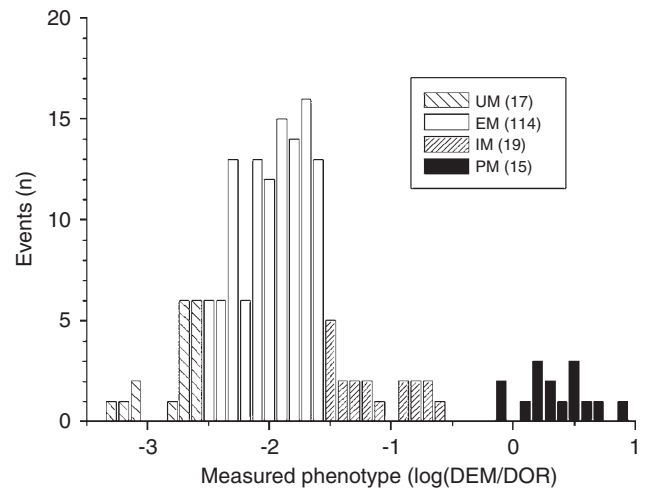
Predicted CYP2D6 activity				
Absent	Decreased	Normal	Increased	Unknown
Allele				
*3,*4,*5, *6,*7,*8, *11,*14A, *15,*19,*20, *40,*4 × N	*9,*10,*17, *29,*36,*41, *10 × N,*17 × N, *41 × N	*1,*2,*35	*1 × N,*2 × N, *35 × N	*14B,*25, *26,*30,*31

**Table 5** CYP2D6 genotype assignment by AmpliChip for the \*1/\*1 (*n*=59), the \*1/\*4 (*n*=22) and the Dupli positive (*n*=9) individuals assessed by classical PCR methods

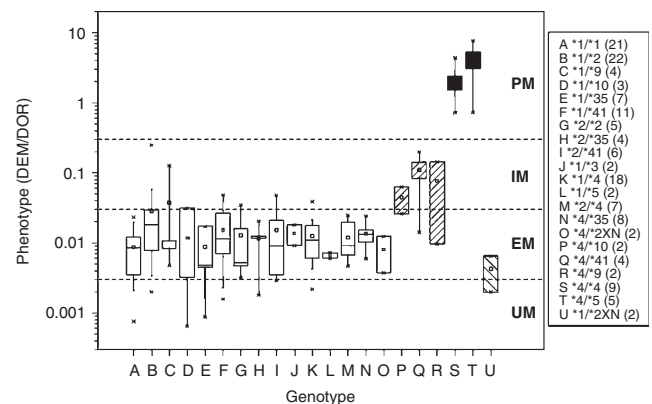
Genotype by PCR	Genotype by AmpliChip	Number of individuals
*1/*1	*1/*2	18
	*1/*1	15
	*1/*41	5
	*1/*35	4
	*1/*9	3
	*2/*35	3
	*2/*2	2
	*2/*41	2
	*1/*10	1
	*9/*10	1
	*1/*29	1
	*2/*7	1
	*9/*35	1
	*17/*35	1
	*35/*35	1
*1/*4	*1/*4	8
	*35/*4	7
	*2/*4	3
	*10/*4	1
	*41/*4	3
*1 and Dupli	*1/*2 × <i>N</i>	2
	*10/*1 × <i>N</i>	1
	*41/*41 × <i>N</i>	1
	*41/*2 × <i>N</i>	1
*4 and Dupli	*4/*2 × <i>N</i>	2
	*4/*41 × <i>N</i>	1
	*1/*4 × <i>N</i>	1

two reduced activity alleles; EM if they carry at least one functional allele and UM if they carry at least three copies of a functional allele. In order to evaluate the phenotype prediction performance of the AmpliChip CYP450 test, CYP2D6 enzyme activity was determined by assessing the metabolic ratio (MR) between DEM and its metabolite dextrophan (DOR) in the urine collected during 8 h after ingestion of a single 25 mg oral dose of DEM.

Using this method individuals are classified as UM when the MR is below 0.003, EM when the MR is between 0.003 and 0.03, IM between 0.03 and 0.3 and PM for an MR above 0.3; increasing MR reflecting decreasing CYP2D6 activity.<sup>10</sup> This classification is based on previous studies using either debrisoquine<sup>11</sup> or DEM.<sup>12</sup> For debrisoquine, four phenotype groups have been defined regarding the MRs (>12.6 for PM, 2.0–12.6 for IM, 0.1–2.0 for EM and <0.1 for UM). Recently, Gaedigk *et al.*<sup>13</sup> proposed a comparable classification for DEM as the one we suggest in our study, except for UM (one order of magnitude lower). Figure 1 illustrates the distribution of the measured CYP2D6 phenotype corresponding to the DEM/DOR ratio. As expected, four distinct phenotypic groups were observed displaying mean DEM/DOR ratios of 0.0019 (0.0005–0.0029) for UM, 0.0128 (0.0031–0.0299) for EM, 0.089 (0.0311–0.2488) for IM and 2.71 (0.72–7.68) for PM. The frequency of the four phenotypic groups was as



**Figure 1** Distribution of the measured cytochrome P450 2D6 (CYP2D6) phenotypes corresponding to the dextromethorphan/dextrophan (DEM/DOR) ratio. Four phenotypic groups are defined: ultra rapid (UM), extensive (EM), intermediate (IM) and poor (PM) metabolizers, displaying increasing DEM/DOR ratios and therefore decreasing CYP2D6 activity. Numbers in brackets indicate the number of individuals in each phenotype category.



**Figure 2** Comparison of the cytochrome P450 2D6 (CYP2D6) measured phenotypes (y axis) to those predicted by the AmpliChip CYP450 array (□ EM, ▨ IM, ■ PM, ▩ UM) depending on the genotype described on the right panel (x axis, A–U). Boxes indicate the interquartile ranges and numbers in brackets the number of individuals for each genotype. Genotypes observed only once are not included.

follows: 10% UM, 69% EM, 12% IM and 9% PM. It has to be noted that our population consisted exclusively of women. Although the literature concerning the effect of gender on CYP2D6 activity remains controversial, we cannot formally exclude a potential influence of this parameter on phenotype distribution. We next compared the CYP2D6-measured phenotypes to those predicted by the AmpliChip CYP450 test according to the genotype (A–U, Figure 2). Phenotype prediction by AmpliChip CYP450 test indicates genotypes A–O as EM, P–R as IM, S–T as PM and U as UM. Excepted for UM, we observed an overall good phenotype prediction by the AmpliChip CYP450 test. When comparing for all type of

Measured phenotype					n	Sensitivity	Specificity	Pos. Pred. Value	Neg. Pred. Value
	UM	EM	IM	PM					
UM	0	0	16	1	UM 17	6% (1/17)	99% (147/148)	50% (1/2)	90% (147/163)
EM	0	5	108	1	EM 114	95% (108/114)	47% (24/51)	80% (108/135)	80% (24/30)
IM	0	8	11	0	IM 19	42% (8/19)	97% (141/146)	61% (8/13)	93% (141/152)
PM	15	0	0	0	PM 15	100% (15/15)	100% (150/151)	100% (15/15)	100% (150/150)
	PM	IM	EM	UM	165				
Predicted phenotype									

**Figure 3** Evaluation of the AmpliChip cytochrome P450 2D6 (CYP450) test performance in phenotype prediction. (a) For each type of metabolizer the number of individuals for whom the predicted phenotype corresponded to the measured (DEM/DOR) is indicated in the shadowed boxes. The number of observed discrepancies is in white boxes. (b) Sensitivity, specificity, and positive and negative predictive values of phenotype prediction by the AmpliChip CYP450 test are calculated for each type of metabolizer.

metabolizers the number of individuals for whom the predicted phenotype corresponded to the measured DEM/DOR ratio a coherence of 80% was obtained (Figure 3a, shadowed boxes). Some discrepancies were observed, especially for the UM group (Figure 3a, white boxes). To further evaluate the performance of the AmpliChip CYP450 test, sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of phenotype prediction were calculated for each type of metabolizer (Figure 3b).

We observed an optimal phenotype prediction performance of the AmpliChip CYP450 test for the 15 PMs present in our population. Indeed, sensitivity, specificity, PPV and NPV were all 100%.

Among 114 measured EMs, 108 were correctly predicted by the AmpliChip CYP450 test resulting in a sensitivity of 95%. One individual with a  $*1/*2 \times N$  genotype was predicted UM. The five remaining were predicted IM by the AmpliChip CYP450 test according to the following genotypes:  $*4/*9$ ,  $*9/*10$ ,  $*4/*41 \times N$ ,  $*4/*41$ ,  $*4/*10$ . In order to try to understand these discrepancies, we looked whether these genotypes were present in other subgroups of our whole population. We found another  $*4/*9$ , three other  $*4/*41$  and one additional  $*4/*10$  for whom, as expected, a measured IM phenotype was observed. Therefore, among the eight individuals carrying one of these three genotypes predicted IM, five were actually measured IM. No additional individuals carrying the  $*9/*10$  or  $*4/*41 \times N$  were present in our population. Given the fact that for gene duplication the AmpliChip CYP450 test allows identification of the duplicated allele but no information is obtained concerning the number of copies, we can hypothesize that for the  $*4/*41 \times N$  genotype, a high number of  $*41$  are present resulting in a EM phenotype. We found a specificity of 47% for EM phenotype prediction by the AmpliChip CYP450 test. Indeed, among the 51 individuals measured non-EM only 24 were correctly predicted non-EM, whereas 11 measured IM and 16 measured UM were wrongly predicted EM. Concerning these 16 measured UM, as shown in Table 6,

**Table 6** DEM/DOR ratios and CYP2D6 genotype distribution of wrongly EM-predicted measured UM ( $n = 16$ )

Measured UM DEM/DOR	Genotype by AmpliChip	Predicted Phenotype
0.00050	$*1/*41 \times N$	EM
0.00065	$*1/*10$	EM
0.00076	$*1/*1$	EM
0.00088	$*1/*35$	EM
0.00158	$*1/*41$	EM
0.00182	$*2/*35$	EM
0.00200	$*1/*2$	EM
0.00210	$*1/*1$	EM
0.00220	$*1 \times N/*41$	EM
0.00220	$*1/*4$	EM
0.00225	$*1/*1$	EM
0.00230	$*1/*41$	EM
0.00230	$*2/*10$	EM
0.00270	$*1/*35$	EM
0.00284	$*1/*1$	EM
0.00290	$*2/*41$	EM

Abbreviations: DEM, dextromethorphan; DOR, dextrophan; EM, extensive metabolizer; UM, ultra rapid metabolizer.

only 2 carried a duplication and could potentially have been wrongly predicted EM due to a high number of gene copies. For the 14 remaining, looking at the DEM/DOR distribution we did not find a specific trend that could explain the discrepancies between measured and predicted phenotype. Indeed, identical allele combinations are associated to very different DEM/DOR values within the range defined for UM (Table 6). Therefore, factors independent of CYP2D6 genotype are potentially involved in the UM phenotype. Overall, in our study 17 individuals were measured UM and only 2 were predicted UM by the AmpliChip CYP450 test presenting a  $*1/*2 \times N$  genotype. For one of them, as expected, an UM phenotype was measured, for the other an

EM phenotype was measured. These data resulted in a PPV of 50% and a very low sensitivity (6%) of UM phenotype prediction by the AmpliChip CYP450 test. Nevertheless, a specificity of 99% and a NPV of 90% were obtained.

When considering IM, the sensitivity of the AmpliChip CYP450 test in phenotype prediction was 42%. Indeed, among the 19 measured IM, 8 were correctly predicted IM and 11 EM. Among these 11 individuals, 4 were \*1/\*2, 1 \*2/\*2, 1 \*1/\*10, 1 \*1/\*4, 1 \*1/\*6, 1 \*1/\*41, 1 \*2/\*41, 1 \*1/\*9. It has to be noted that the measured DEM/DOR values for 6 of these 11 individuals were between 0.0312 and 0.0397, therefore being at the very beginning of the IM range close to the EM-measured phenotype classification. The observed PPV was 61% given the fact that five individuals predicted IM by the AmpliChip CYP450 test were measured EM by DEM/DOR ratio (details above in the EM paragraph). A high specificity and NPV in IM phenotype prediction were observed, 97% and 93%, respectively.

## Discussion

*CYP2D6* gene polymorphisms and their implication in drug efficacy and toxicity have been known for a long time. Although a large number of publications point for a real potential benefit for the patient of knowing its *CYP2D6* genotype, the conditions for an efficient systematic practical application of this knowledge remains to be determined. One of the issues that remain to be clearly defined is the adequate procedure to associate a *CYP2D6* genotype to the corresponding phenotype. Technical improvement in genotype determination is potentially valuable to address this complex issue.

### Genotyping improvement by the AmpliChip CYP450 test

A first evaluation of this microarray test concluded that this technology is rapid, reliable, accurate and very easy to perform.<sup>14</sup> We compared *CYP2D6* genotyping using the AmpliChip CYP450 test to our routine methods and found 100% concordance for the five tested alleles. Besides the practical advantage to analyse 33 *CYP2D6* allelic variants in one experiment, the AmpliChip CYP450 test improves genotyping, compared to our classic tests, by detecting relevant alleles for a number of ethnic groups not often analysed in routine as well as alleles associated with a decreased CYP2D6 activity predicting an IM phenotype. Concerning gene duplication, of the five individuals that would have been predicted UM using our routine tests (see Table 5, \*1 and Dupli) only two are actually considered UM according to the AmpliChip CYP450 test. Although this is not a large number of individuals, in our opinion the adequate technology should be used to detect UM in order to avoid false positives, especially if considering drug dose adjustment according to the genotype.

### Genotype–phenotype association

The efficacy of genotype–phenotype association is considerably different depending on the group of metabolizers considered.

*Poor metabolizers.* In agreement with previous published data using various genotyping methods, for PMs we observed a perfect correlation between genotype results, phenotype prediction and measured phenotype.<sup>15,16</sup> In this study, our classical methods detecting \*3,\*4,\*5,\*6 were as performing as the AmpliChip CYP450 test to predict a PM phenotype. However, the AmpliChip CYP450 test offers the possibility to identify additional rarer null alleles. Taken together, these results clearly indicate that genotyping alone is definitely sufficient to predict PM when considering a population devoid of identified organic pathology or of using drugs that could interfere with CYP2D6 activity.

*Intermediate, ultra rapid and extensive metabolizers.* Overall, we found a satisfying IM phenotype prediction performance by the AmpliChip CYP450 test. The major type of misclassification in the measured IM group was an EM prediction according to genotype. This could be explained by non-genetic factors having been shown to reduce CYP2D6 expression and/or activity.<sup>17,18</sup> On the other hand, limitations in phenotype measurement could also contribute to these discrepancies. Indeed, in contrast to the PM phenotype, where a DEM MR antinode can be precisely defined, the definition for the IM and UM phenotype is less clear. A continuum between the IM and the EM as well as between the EM and the UM phenotypes is observed (Figure 1).<sup>13,19</sup> Taking into account the analytical variability and the large intra-individual variability of baseline DEM/DOR MRs previously reported (CV of approximately 50%),<sup>20</sup> correct classification of individuals with DEM/DOR ratios close to the arbitrary limit between these groups remains difficult. In this context, more than half of the measured IMs incorrectly predicted EM by the array had DEM/DOR values closed to the EM range (<0.04). Moving these borderline IMs to the EM group would increase the sensitivity of IM prediction from 42% to approximately 62%. Given the fact that the reduced enzyme activity associated to this genotype has been reported to be of potential clinical relevance,<sup>21,22</sup> we consider the ability of the AmpliChip CYP450 to predict IM phenotype valuable although the sensitivity is not optimal.

Concerning UM, our results showed that sensitivity of UM phenotype prediction by genotype is not satisfying and confirmed that gene duplication explains only a small minority of UMs.<sup>7,23</sup> As discussed above for IMs and previously published, phenotyping studies measuring urine metabolites may overestimate the number of UMs.<sup>24</sup> In this context, in our study among the 16 measured UMs wrongly predicted EM by genotype, 9 had a DEM/DOR ratio close to the EM range (>0.002; Table 6). Moreover, Gaedigk *et al.*<sup>13</sup> recently published phenotyping data using DEM and defined a lower cut-off for UMs (<0.0003). Under these conditions no UM would be present in our population. Given all these limitations, the use of genotyping to screen for UM in the general population might be insufficient in our opinion. However, in individual cases of toxicity or treatment inefficacy clinically pointing to a potential UM, genotyping is a rapid and easy approach to exclude or confirm an UM resulting from gene duplication.

Considering EM, we found a good phenotype prediction by the AmpliChip. Excluding gene duplication the following allele combinations were found in this predicted group: homozygous for normal alleles (em/em,  $n = 60$ ), heterozygous carrying a normal and a decreased activity allele (em/im,  $n = 28$ ) and heterozygous carrying one normal allele and one non-functional allele (em/pm,  $n = 40$ ). In each of these three subgroups the large majority of individuals are measured EM, supporting the idea that one functional allele is sufficient to have normal CYP2D6 activity in the absence of other potential drug interactions. In addition, DEM/DOR values were comparable in these three groups indicating that em/em, em/im as well as em/pm individuals are equally efficient in metabolizing DEM and that no gene dose effects were detectable with this phenotyping method. However, depending on the probe drug used to phenotype, differences in exact determination of CYP2D6 enzymatic activity may be observed.<sup>25</sup> Moreover, using therapeutic drugs to define genotype–phenotype association was also shown to be very promising. Indeed, as reported by Steimer *et al.*,<sup>22</sup> for patients receiving amitriptyline, the concentrations of its metabolite nortriptyline were significantly associated to the different genotype combinations within the EM group according to the functional gene dose. If this observation is further confirmed and considering the high precision in gene dose assessment of the AmpliChip CYP450 test compared to classical methods,<sup>14</sup> this microarray technology could be an excellent tool to improve phenotype prediction.

## Materials and methods

### Population

A population of 165 Caucasian women participating in a study on fibromyalgia at the University Hospitals of Geneva (Swiss National Foundation NRP53) was selected to evaluate CYP2D6 genotype–phenotype relationships. One of the selection criteria for the present study was the absence of any medication or pathology potentially affecting CYP2D6 activity and/or expression. The protocol was approved by the local ethics committee and written informed consent was obtained from all participants.

### DNA extraction and CYP2D6 genotyping

Genomic DNA was extracted from 200 µl of whole blood using the QIAamp DNA blood mini kit (QIAGEN, Hombrechtikon, Switzerland). Each sample was genotyped by the AmpliChip CYP450 test (Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's instructions. Briefly, CYP2D6 and CYP2C19 genes are amplified in two separate PCR reactions using two specific Primer Mixes and the AmpliChip CYP450 Master Mix containing a blend of AmpliTaq Gold DNA polymerases, AmpErase and dNTPs. For each individual, the DNA amplicons from the two independent reactions are pooled, fragmented using DNase I and labelled with biotin at the 3'-end using Terminal

Transferase and AmpliChip TdT Labelling Reagent. The resulting sample is then hybridized to the AmpliChip CYP450 Microarray using the Affymetrix GeneChip Fluidics Station 450Dx and an AmpliChip-specific protocol. The hybridized array is washed and stained with streptavidin-conjugated phycoerythrin. After staining, the array is scanned by an Affymetrix GeneChip Scanner 3000Dx and data analysis is performed by the GeneChip Operating Software and the AmpliChip CYP450 Data Analysis Software to obtain the individual's CYP2D6 genotype and predicted phenotype.

In parallel, for 100 DNA samples, five CYP2D6 allelic variants were also determined by real-time PCR assays using fluorescent probes routinely used in our laboratory for diagnostic purposes. Briefly, commercially available Pre-Developed Taqman Assay Reagents (Applied Biosystems, Warrington, UK) including specific primers, FAM and VIC-labelled TaqMan MGB probes complementary to wild-type and variant sequences respectively, were used for CYP2D6\*3, \*4 and \*6 detection (assay no. 4312564 for CYP2D6\*4; no. 4312563 for CYP2D6\*3 and no. 4312565 for CYP2D6\*6). Reactions were performed according to the manufacturer's instructions in Taqman Universal PCR Master Mix (Applied Biosystems) using an iCycler iQ detection system allowing discrimination between sequence variants (Bio-Rad, California, USA).

The presence of CYP2D6 gene deletion or duplication was assessed by real-time long PCR using SYBR Green and specific primers on a LightCycler (Roche Diagnostics) followed by melting temperature analysis as previously described.<sup>26</sup> The identity of the duplicated allele cannot be determined by this method.

### CYP2D6 phenotyping

According to a standardized protocol, a single 25 mg oral dose of the CYP2D6 substrate, DEM was administered to the individual and phenotype was evaluated following HPLC by calculating MR between deconjugated DEM and its metabolite DOR in the urine (8 h collection). A validated chemical hydrolysis method using HCl was used to allow detection of both free and conjugated DOR as described elsewhere.<sup>10</sup>

The chromatographic equipment consisted of an Agilent 1100 Series LC system (Agilent, Palo Alto, USA). DEM and DOR separation was carried out on a phenyl column (150 × 4.6 mm i.d., particle size 5 µm) coupled with a guard column with the same stationary phase. Mobile phase consisted of a mixture of acetonitrile and orthophosphoric acid 50 mM (18/82) adjusted to pH 3.0 with sodium hydroxide 4 N and was delivered at 0.8 ml min<sup>-1</sup>. Fluorescence was measured with emission and excitation wavelengths set at 280 and 310 nm, respectively. As previously described for CYP2D6 phenotyping a logarithmic scale is used to classify the different CYP2D6 metabolizers. Accordingly, the DEM/DOR ratio is below 0.003 for UM, between 0.003 and 0.03 for EM, between 0.03 and 0.3 for IM and above 0.3 for PM.

## Acknowledgments

This study was supported by a grant from the Swiss National Science Foundation (NRP53-4049-405340-104645/1). Roche Diagnostics provided the AmpliChip CYP450 test at no cost to the study.

## References

- Gardiner SJ, Begg EJ. Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. *Pharmacol Rev* 2006; **58**: 521–590.
- Lin JH. Pharmacokinetic and pharmacodynamic variability: a daunting challenge in drug therapy. *Curr Drug Metab* 2007; **8**: 109–136.
- Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA* 1998; **279**: 1200–1205.
- Gasche Y, Daali Y, Fathi M, Chiappe A, Cottini S, Dayer P et al. Codeine intoxication associated with ultrarapid CYP2D6 metabolism. *N Engl J Med* 2004; **351**: 2827–2831.
- Koren G, Cairns J, Chitayat D, Gaedigk A, Leeder SJ. Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother. *Lancet* 2006; **368**: 704.
- Chou WH, Yan FX, Robbins-Weilert DK, Ryder TB, Liu WW, Perbost C et al. Comparison of two CYP2D6 genotyping methods and assessment of genotype-phenotype relationships. *Clin Chem* 2003; **49**: 542–551.
- Dahl ML, Johansson I, Bertilsson L, Ingelman-Sundberg M, Sjoqvist F. Ultrarapid hydroxylation of debrisoquine in a Swedish population. Analysis of the molecular genetic basis. *J Pharmacol Exp Ther* 1995; **274**: 516–520.
- Griese EU, Zanger UM, Brudermanns U, Gaedigk A, Mikus G, Morike K et al. Assessment of the predictive power of genotypes for the in-vivo catalytic function of CYP2D6 in a German population. *Pharmacogenetics* 1998; **8**: 15–26.
- Raimundo S, Fischer J, Eichelbaum M, Griese EU, Schwab M, Zanger UM. Elucidation of the genetic basis of the common 'intermediate metabolizer' phenotype for drug oxidation by CYP2D6. *Pharmacogenetics* 2000; **10**: 577–581.
- Daali Y, Cherkaoui S, Doffey-Lazeyras F, Dayer P, Desmeules JA. Development and validation of a chemical hydrolysis method for dextromethorphan and dextrophan determination in urine samples: application to the assessment of CYP2D6 activity in fibromyalgia patients. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008; **861**: 56–63.
- Dalen P, Dahl ML, Bernal Ruiz ML, Nordin J, Bertilsson L. 10-Hydroxylation of nortriptyline in white persons with 0, 1, 2, 3, and 13 functional CYP2D6 genes. *Clin Pharmacol Ther* 1998; **63**: 444–452.
- Schmid B, Bircher J, Preisig R, Kupfer A. Polymorphic dextromethorphan metabolism: co-segregation of oxidative O-demethylation with debrisoquin hydroxylation. *Clin Pharmacol Ther* 1985; **38**: 618–624.
- Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. *Clin Pharmacol Ther* 2008; **83**: 234–242.
- Heller T, Kirchheiner J, Armstrong VW, Luthe H, Tzvetkov M, Brockmoller J et al. AmpliChip CYP450 GeneChip: a new gene chip that allows rapid and accurate CYP2D6 genotyping. *Ther Drug Monit* 2006; **28**: 673–677.
- Sachse C, Brockmoller J, Hildebrand M, Muller K, Roots I. Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolizers of debrisoquine. *Pharmacogenetics* 1998; **8**: 181–185.
- McElroy S, Sachse C, Brockmoller J, Richmond J, Lira M, Friedman D et al. CYP2D6 genotyping as an alternative to phenotyping for determination of metabolic status in a clinical trial setting. *AAPS PharmSci* 2000; **2**: E33.
- McMasters DR, Torres RA, Crathern SJ, Dooney DL, Nachbar RB, Sheridan RP et al. Inhibition of recombinant cytochrome P450 isoforms 2D6 and 2C9 by diverse drug-like molecules. *J Med Chem* 2007; **50**: 3205–3213.
- Hellum BH, Nilsen OG. The *in vitro* inhibitory potential of trade herbal products on human CYP2D6-mediated metabolism and the influence of ethanol. *Basic Clin Pharmacol Toxicol* 2007; **101**: 350–358.
- de Leon J. The crucial role of the therapeutic window in understanding the clinical relevance of the poor versus the ultrarapid metabolizer phenotypes in subjects taking drugs metabolized by CYP2D6 or CYP2C19. *J Clin Psychopharmacol* 2007; **27**: 241–245.
- Chladek J, Zimova G, Martinkova J, Tuma I. Intra-individual variability and influence of urine collection period on dextromethorphan metabolic ratios in healthy subjects. *Fundam Clin Pharmacol* 1999; **13**: 508–515.
- Furman KD, Grimm DR, Mueller T, Holley-Shanks RR, Bertz RJ, Williams LA et al. Impact of CYP2D6 intermediate metabolizer alleles on single-dose desipramine pharmacokinetics. *Pharmacogenetics* 2004; **14**: 279–284.
- Steimer W, Zopf K, von Amelunxen S, Pfeiffer H, Bachofer J, Popp J et al. Allele-specific change of concentration and functional gene dose for the prediction of steady-state serum concentrations of amitriptyline and nortriptyline in CYP2C19 and CYP2D6 extensive and intermediate metabolizers. *Clin Chem* 2004; **50**: 1623–1633.
- Lovlie R, Daly AK, Matre GE, Molven A, Steen VM. Polymorphisms in CYP2D6 duplication-negative individuals with the ultrarapid metabolizer phenotype: a role for the CYP2D6\*35 allele in ultrarapid metabolism? *Pharmacogenetics* 2001; **11**: 45–55.
- de Leon J. AmpliChip CYP450 test: personalized medicine has arrived in psychiatry. *Expert Rev Mol Diagn* 2006; **6**: 277–286.
- Frank D, Jaehde U, Fuhr U. Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. *Eur J Clin Pharmacol* 2007; **63**: 321–333.
- Muller B, Zopf K, Bachofer J, Steimer W. Optimized strategy for rapid cytochrome P450 2D6 genotyping by real-time long PCR. *Clin Chem* 2003; **49**: 1624–1631.