Relation between CYP2D6 Genotype, Phenotype and Therapeutic Drug Concentrations among Nortriptyline and Venlafaxine Users in Old Age Psychiatry

Key words
depression
genetics
cytochrome P450 (CYP) enzymes
venlafaxine
nortriptyline

Abstract

Objectives: To determine relations between drug concentrations and the cytochrome P450-CYP2D6 genotype or phenotype among elderly patients treated with nortriptyline or venlafaxine.

Methods: A post-hoc analysis of a clinical trial was performed. Patients were grouped into phenotypes according to the metabolite/mother compound ratio. Genotypes were assessed by the CYP2D6 *3 and *4 alleles.

Results: Data was available from 81 patients (41 nortriptyline, 40 venlafaxine) with a mean age of 72 years. No phenoconversion from poor metabolizers (PM) to extensive metabolizers (EM), or vice versa, was found. However, we did find phenoconversion from PM to intermediate metabolizers (IM), IM to EM, or vice versa in 36% of observations. Among nortriptyline users, patients with a PM or IM genotype had more supra-therapeutic blood levels, although this did not reach statistical significance. In exploratory analyses we found men were more likely (RR: 2.4; 95% CI: 1.14–5.07) to display phenoconversion from an IM genotype to EM phenotype. In addition, compared to non-PMs, PMs were found to have higher risk (RR: 1.56; 95% CI: 1.03–2.37) on non-response, although this was only significant when response was measured on the Hamilton Rating Scale for Depression and not on the Montgomery Åsberg Depression Rating Scale.

Conclusion: Patients phenoconverted, but we did not observe phenoconversion from PM to EM or vice versa. Genotype information could be used as a valuable tool, in addition to therapeutic drug monitoring, to prevent supratherapeutic drug levels of nortriptyline or venlafaxine in elderly patients with a PM genotype.

Background

The relationship between genotype and phenotype of the polymorphic cytochrome P450 2D6 enzyme (CYP2D6) has been intensively studied over the last decade. The function of this enzyme can be classified into 4 different drug metabolizer types: the extensive metabolizer (EM), which corresponds with normal enzyme functionality and includes the majority of the patient population; the poor metabolizer (PM) characterized by almost no enzyme activity; the intermediate metabolizer (IM) characterized by a decreased, but usually still active, enzyme; and finally the ultra-rapid metabolizer (UM) characterized by increased enzyme activity. These different patient types can be distinguished by assessing the metabolic ratio of a drug and its metabolite after administration of a CYP2D6-specific substrate. This is usually referred to as phenotyping. At the same time, these differences can also be predicted by pharmacogenetics tests, which is referred to as genotyping.

Nortriptyline and venlafaxine are primarily metabolized by CYP2D6 [1,2]. To apply knowledge about differences in CYP2D6 capacity, guidelines have been formulated for both nortriptyline and venlafaxine to adapt the dosage based on the CYP2D6 genotype [3,4]. However, in a study among 865 venlafaxine users, 24% of the patients who were genotyped as EM appeared to have a PM phenotype [5]. This can be due to co-administration of strong CYP2D6 inhibitors, like paroxetine, which is known to induce PM phenotypes among genetically EMs [6]. This phenomenon of discrepancy between genotype and phenotype has been referred to as phenoconversion and has provoked discussion as to whether genotyping can accurately predict the phenotype [5,7,8]. In addition, practitioners often refer to therapeutic drug monitoring (TDM) as an accurate and sufficient indicator of the phenotype.
which renders additional genotyping superfluous. An additional concern is the ageing of the patient population. Although in general it is reported that CYP2D6 function does not decrease in the elderly, other CYP enzymes like CYP3A4 or CYP2C19 are associated with a decreased function among old-aged patients [9]. In line with this, a decreased clearance of CYP2D6 substrates like flecainae or desipramine has been described in older patients which might be related to a decreased function of these other secondary metabolizing CYP enzymes [10, 11]. Relatively little is known about the genotype-phenotype relationship in elderly populations, and due to demographic ageing, this population will increase in the West.

In this post-hoc analysis we tried to address 2 research questions: (1) do we observe the phenomenon of phenoconversion in an elderly population, and (2) which genotypes among older patients experience sub- or supratherapeutic drug levels? In addition, 2 exploratory analyses were performed: first to study if gender or age were related to phenoconversion, and second to study if non-response was related to phenotype and/or genotype. In this way, greater insight is gained into the possible added value of genotyping as well as TDM to optimize treatment with nortriptyline and venlafaxine in elderly populations.

Methods

We performed a post-hoc analysis with data from a randomized controlled trial among 81 starters of nortriptyline or venlafaxine aged 60 years or older [12]. In the trial, efficacy and safety of nortriptyline and venlafaxine was compared among inpatients diagnosed with major depressive disorder (according to the DSM-IV criteria). Only patients with a score of ≥ 20 on the Montgomery Asberg Depression Rating Scale (MADRS) were included [13]. Patients with or without a prior episode of depression (treated with an antidepressant) were included. Patients diagnosed with dementia according to the DSM-IV or a mini-mental state examination score of < 15 were excluded [14]. Patients were intensively monitored by TDM and blood samples were collected after 3, 5, and 12 weeks. Both the mother compounds, nortriptyline (NTP) or venlafaxine (VFX) as well as the main CYP2D6 metabolites, OH-nortriptyline and O-desmethylvenlafaxine (ODV), were measured. To test for any significant differences in the occurrence of (sub- or supra-) therapeutic levels between the genotypes, Fisher’s exact test for binomial data was used. To test if phenoconversion was related to gender or age (groups: ≤ 75 years; > 75 years), data was analyzed with logistic regression. In addition relative risk ratios were calculated. To assess the relation between non-response (yes/no) and genotype, genotypes or phenotypes were collapsed into dichotomous outcomes, normal (EM and IM) or poor (PM) metabolizers. Relative risk ratios were calculated. Analyses were performed with IBM SPSS Statistics, version 23. P-values of < 0.05 were considered significant.

Results

For the first TDM measurement (week 3), complete data from 75 out of 81 participants (40 NTP; 35 VFX) was available. Demographics of included patients are shown in Table 1. After 5 weeks and 12 weeks, no TDM data was available for 13 and 26 participants, respectively. This resulted in 33 NTP and 35 VFX patient samples after 5 weeks and after 12 weeks in 27 NTP and

Phenotyping

We determined the phenotypes according to the metabolite/mother compound ratio after 3, 5 and 12 weeks. A ratio between 0 and 0.5 was categorized as a PM, and between 1.5 and 10 as EM [15]. To determine the IM phenotype a ratio between 0.5 and 1.5 was used.

Phenoconversion

The predicted phenotype and the measured phenotype were compared for each TDM sample and the corresponding clinical sensitivity and specificity for CYP2D6 testing was calculated. Patients who had a different phenotype compared to their genotype in the majority of their TDM samples were considered to experience phenoconversion. Phenoconversion could occur in 2 directions, being towards more (i.e., IM→EM; PM→IM) or less (EM→IM; IM→PM) metabolic capacity then predicted based on genotype. In an exploratory analysis, relations between gender, age, and phenoconversion were analyzed.

Therapeutic drug monitoring

We translated measured plasma concentrations into clinically relevant outcomes being therapeutic (within therapeutic range), subtherapeutic (below lowest level of therapeutic range) or supratherapeutic (above highest level of therapeutic range). For NTP we used a therapeutic reference range of 50–150 µg/L. For VFX a range of 100–400 µg/L was used for the sum of VFX and ODV [16]. Note that in the trial the TDM data of VFX was not considered leading in pharmacotherapy since the dose-effect relation during the time of the trial was highly debated [12].

Response to antidepressant

12 weeks after start of treatment response was measured with the MADRS and the Hamilton Rating Scale for Depression (HAM-D; 17-item version) [13, 17]. For both instruments, a reduction of at least 50% from baseline score was considered as a response. In an exploratory analysis, relationships between plasma concentrations and response as well as genotype and response were assessed.

Statistics

To test for any significant differences in the occurrence of (sub- or supra-) therapeutic levels between the genotypes, Fisher’s exact test for binomial data was used. Used to test if phenoconversion was related to gender or age (groups: ≤ 75 years; > 75 years), data was analyzed with logistic regression. In addition relative risk ratios were calculated. To assess the relation between non-response (yes/no) and genotype, genotypes or phenotypes were collapsed into dichotomous outcomes, normal (EM and IM) or poor (PM) metabolizers. Relative risk ratios were calculated. Analyses were performed with IBM SPSS Statistics, version 23. P-values of < 0.05 were considered significant.

Genotyping

Patients with a *3 or *4 allele were classified as IM and patients with 2 dysfunctional alleles as PMs (\(^*3/3\); \(^*4/4\); \(^*3/4\)). Patients with no dysfunctional alleles were classified as EM. For the determination of the UM genotype, information about gene duplication is needed. This genetic information was not available, and therefore UMs could not be identified.

Table 1: After 5 weeks and 12 weeks, no TDM data was available for 13 and 26 participants, respectively. This resulted in 33 NTP and 35 VFX patient samples after 5 weeks and after 12 weeks in 27 NTP and
27 VFX patient samples. For 2 patients using nortriptyline (corresponding to 6 TDM samples) genotype information was missing. Therefore, in total 98 TDM samples were available for NTP and 95 for VFX to compare phenotype with genotype.

**Phenoconversion**

No phenoconversion from the PM to the EM genotype, or vice versa, was observed in patients treated with NTP or VFX (Table 2). Phenoconversion from PM to IM, IM to EM, or vice versa was found in 36% (69 out of 193 comparisons). Among the NTP group this was 28% (27 out of 98 comparisons) and among the VFX group 44% (42 out of 95 comparisons). Most phenoconversion towards a phenotype with more enzyme activity was found (i.e., from IM→EM; PM→IM). Phenoconversion towards less enzyme activity (i.e., from EM→IM or PM; IM→PM) was not related to either gender or age (Table 3).

**Clinical outcomes**

Patients with an IM or PM genotype were more frequently exposed to blood levels above the therapeutic range compared to patients with an EM genotype, although differences were not significant. After 5 weeks, differences for NTP were on the borderline of significance (p < 0.07). Patients who had blood levels outside the therapeutic range after 12 weeks were more frequently non-responders, compared to patients who had blood levels within the therapeutic range. Based on the HAM-D scale results were significant (Fisher’s exact test: p < 0.01), but not on the MADRS scale (Fisher’s exact test: p = 0.16). Interestingly, patients with a drug concentration below the therapeutic range were mostly responders and patients with blood concentrations above the therapeutic range were more frequently non-responders. In line with these findings, we found patients with a PM genotype, who are more likely to experience high drug concentrations, had a higher risk of non-response. In contrast, patients with an IM genotype displayed a response similar to EMs. When PM genotypes were compared to non-PMs, differences in response were again only significant when measured on the HAM-D scale. In addition, comparable outcomes were found for NTP phenotypes. Relative risk ratios for non-response of PMs and EM phenotypes among old-aged depressed inpatients for nortriptyline (A) and venlafaxine (B).
was found for this type of phenoconversion (i.e., IM→EM), indicating patients with an IM genotype we did observe phenoconversion to PM or EM and vice versa. Among the VFX users we observed most patients with an IM genotype had an EM phenotype. A gender difference was found for this type of phenoconversion (i.e., IM→EM), indicating male subjects have a higher risk. This is evidence that women have a lower venlafaxine clearance and as such less metabolic reserve to compensate for the reduced CYP2D6 function [19,20]. On the other hand, these findings indicate that among VFX users, lowering the dosage based on the IM genotype is not adequate. Differences were not significant, which indicates a larger sample size is needed. We found no such difference for VFX users during the first 5 weeks of pharmacotherapy. However, at week 12 most patients had VFX blood levels above the therapeutic range which were relatively more PMs compared to EMs. These numbers are too small to draw any firm conclusions, however our results indicated that not at the start of VFX treatment but 3 months thereafter, patients with less metabolizing capacity become more vulnerable to a relatively high VFX exposure. The PM genotype and phenotype related to a significantly higher risk of non-response based on the HAM-D scale, but not on the MADRS scale, although a similar effect was observed. These differences are likely related to the small number of PMs, which made the outcomes highly dependent on a different classification of one or 2 PMs. Although both the MADRS and HAM-D scales are suitable for measuring depression response among the elderly, there are some differences in the weight given to certain symptoms of depression. Compared to the MADRS, the HAM-D is influenced more by items rating sleep, anxiety and somatic symptoms and less by mood changes [22]. These items are more sensitive to somatic symptoms, which could occur due to side effects of the antidepressant like diarrhea, dizziness and dry mouth, which are reported to be related to high plasma concentrations of NTP or VFX PMs [23,24]. It is important that future studies among old-aged patients be conducted using a larger sample size to replicate the findings of this exploratory analysis.

The main limitation of this study was the limited genetic information available. The assessed mutations (∗3 and ∗4) are the most frequently found mutations in Caucasians, however numerous other mutations have been identified [25]. These mutations can also cause decreased enzymatic activity, which we did not test for. In addition, we did not have information about duplications of the gene, which can cause increased metabolic activity and can be used to classify UM. However, in Caucasians, these duplications explain only one third of the UM phenotypes [23,24]. The missed mutations for decreased enzymatic activity could explain unjustified classification of genotyped EMs which were phenotyped as IMs, as was observed among the NTP users. Nevertheless, it cannot explain the observed phenoconversion among VFX users (i.e., IM→EM), since missed mutation will lead to a decreased enzyme activity instead of increased activity. Although this could theoretically be related to an UM genotype, which we did not have the genetic information for, the proportion of UMs in Caucasian populations is very small (1–2%) and it is unlikely this would explain our results [25]. After 12 weeks, 26 patients did not complete TDM because 27 patients were lost to follow-up in the trial [12]. Loss to follow-up was often related to insufficient improvement, which could be related to insufficient plasma levels that could be related to the genotype. In this analysis we did not assess such influences.

### Table 3
Proportions of patients within, below or above the therapeutic range for each genotype after 3, 5, and 12 weeks of treatment.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Week 3 Within range (%)</th>
<th>Below (%)</th>
<th>Above (%)</th>
<th>p-value</th>
<th>Week 5 Within range (%)</th>
<th>Below (%)</th>
<th>Above (%)</th>
<th>p-value</th>
<th>Week 12 Within range (%)</th>
<th>Below (%)</th>
<th>Above (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nortriptyline</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>23 (60)</td>
<td>6 (16)</td>
<td>9 (24)</td>
<td>0.18</td>
<td>22 (67)</td>
<td>5 (15)</td>
<td>6 (18)</td>
<td>0.07</td>
<td>16 (59)</td>
<td>4 (15)</td>
<td>7 (26)</td>
<td>0.64</td>
</tr>
<tr>
<td>PM</td>
<td>1 (33)</td>
<td>0 (0)</td>
<td>2 (67)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td></td>
<td>1 (50)</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>7 (70)</td>
<td>0 (0)</td>
<td>3 (30)</td>
<td></td>
<td>6 (86)</td>
<td>0 (0)</td>
<td>1 (14)</td>
<td></td>
<td>2 (40)</td>
<td>1 (20)</td>
<td>2 (40)</td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>15 (60)</td>
<td>6 (24)</td>
<td>4 (16)</td>
<td></td>
<td>16 (67)</td>
<td>5 (21)</td>
<td>3 (12)</td>
<td></td>
<td>13 (65)</td>
<td>3 (15)</td>
<td>4 (20)</td>
<td></td>
</tr>
<tr>
<td>Venlafaxine</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>32 (86)</td>
<td>1 (3)</td>
<td>4 (11)</td>
<td></td>
<td>23 (66)</td>
<td>4 (11)</td>
<td>8 (23)</td>
<td></td>
<td>9 (33)</td>
<td>1 (4)</td>
<td>17 (63)</td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.00</td>
<td>2 (67)</td>
<td>0 (0)</td>
<td>1 (33)</td>
<td>0.32</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td>0.72</td>
</tr>
<tr>
<td>IM</td>
<td>14 (82)</td>
<td>2 (12)</td>
<td>1 (6)</td>
<td></td>
<td>12 (80)</td>
<td>2 (13)</td>
<td>1 (7)</td>
<td>0.43</td>
<td>4 (29)</td>
<td>1 (7)</td>
<td>9 (64)</td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>15 (88)</td>
<td>2 (12)</td>
<td>0 (0)</td>
<td></td>
<td>9 (53)</td>
<td>2 (12)</td>
<td>6 (35)</td>
<td></td>
<td>5 (45)</td>
<td>0 (0)</td>
<td>6 (55)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4
Risk estimates for non-response based on PM genotype or phenotype compared to non-PM genotype or phenotype. Bold relative risk ratios were found significant (based on 95% confidence interval).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Relative risk ratio</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-responder when</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM genotype</td>
<td>MADRS 1.28</td>
<td>0.70–2.35</td>
<td>0.43</td>
</tr>
<tr>
<td>HAM-D 1.56</td>
<td>1.03–2.37</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Non-responder when</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM phenotype</td>
<td>MADRS 1.43</td>
<td>0.85–2.41</td>
<td>0.18</td>
</tr>
<tr>
<td>HAM-D 1.67</td>
<td>1.14–2.43</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>
because of the small sample size. However, such influences would be interesting and should be studied in larger datasets. Genotyping was found to reliably predict PM phenotypes of CYP2D6. For a reliable prediction of PM phenotypes, inclusion of an IM group and usage of potent CYP2D6 inhibitors need to be taken into account. Uncertainty was found among IM-genotyped patients, and for VFX the IM genotype did not influence antidepressant response. Surprisingly, not subtherapeutic, but supratherapeutic drug concentrations were found to be related to non-response. Consequently, PM genotype was found to be related to a higher risk on non-response, although results should be interpreted with caution due to the post-hoc nature and small sample size of this study. In conclusion, dose adaptation of VFX based on the IM genotype is unlikely to improve clinical outcomes among male patients, which could be related to the frequently observed EM phenotype. Nevertheless, genotype information could be used as a valuable tool, in addition to TDM, to prevent supratherapeutic drug levels of NTP or VFX in elderly patients with a PM genotype.

Conflict of Interests

The authors declare no conflict of interest.

References

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