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ABCB1 (P-Glycoprotein/MDR1) Gene G2677T/A Sequence Variation (Polymorphism): Lack of Association with Side Effects and Therapeutic Response in Depressed Inpatients Treated with Amitriptyline

To the Editor:

Amitriptyline belongs to the class of tricyclic antidepressants (TCAs), which have been a cornerstone of antidepressive therapy for more than 4 decades. Despite being replaced by
newer drugs in the United States, TCAs, and amitriptyline in particular, are still widely used in Europe and many parts of the world, where prescriptions for TCAs far outnumber those for newer, more expensive drugs.

Recently, we reported significant correlations between sequence variations in the genes that encode the metabolizing enzymes cytochrome P450 2D6 (CYP2D6) and 2C19 (CYP2C19), serum amitriptyline/nortriptyline concentrations, and adverse drug reactions in a population of 50 depressed inpatients (1, 2).

However, genetic variations in drug-metabolizing enzymes do not sufficiently explain the wide interindividual variations commonly observed in drug response and clinical outcome. Obviously, other genetic and environmental factors must be considered as well. The influence of sequence variations in the gene ATP-binding cassette, subfamily B (MDR/TAP), member 1 (ABCB1) on the disposition and efficacy of P-glycoprotein (P-gp) substrates (e.g., TCAs) is currently being discussed (3).

As part of the blood–brain barrier, P-gp actively exports significant amounts of antidepressant from the brain. Recent studies showed that penetration of amitriptyline and its metabolites (including nortriptyline) into the brain is enhanced in mice lacking P-gp (4, 5). Therefore, dysfunctional sequence variants of the \( \text{ABCB1} \) gene might lead to diminished P-gp activity and higher bioavailability of P-gp substrates in the central nervous system. The nonsynonymous \( \text{G2677T/A} \) sequence variation (Ala893Ser/Thr) in \( \text{ABCB1} \) can be used to differentiate between the 2 most common haplotypes found in Caucasians. We investigated whether this sequence variation contributes to therapeutic response or adverse effects. This study was performed in a population treated with amitriptyline, in whom we had previously found a highly significant correlation between sequence variations in the CYP2D6 and CYP2C19 genes and side effects (1, 2). The study included 50 Caucasian psychiatric inpatients in 2 centers, with at least medium-grade depressive disorder, who received amitriptyline at a fixed dose of 75 mg twice a day over 3 weeks.

Blood samples for monitoring concentrations of amitriptyline and nortriptyline were taken weekly, along with evaluations of depression (Hamilton Depression Scale and Clinical Global Impression Scale) and side effect [Dosage Record and Treatment Emergent Symptoms Scale (DOTES)] scores.

Genotyping was performed by fluorescence resonance energy transfer using specific probes and the LightCycler\textsuperscript{TM} system (Roche Molecular Biochemicals). We validated the procedure by checking the results with a published method for restriction-fragment-length polymorphism analysis (6) and found complete concordance. Patients were classified as wild-type homozygous (GG genotype), variant homozygous/combined heterozygous (TT or TA genotype), or heterozygous (GT or GA genotype). The genotype distribution was similar to that obtained with other Caucasian samples (3): 15 GG homozygotes (30%), 11 TT homozygotes (22%), 22 GT heterozygotes (44%), 1 GA heterozygote (2%), and 1 TA heterozygote (2%).

We used regression analysis and ANOVA, performed with SPSS 13.0 (SPSS), to test for significant differences among the different genotypes. We analyzed 3 \( \text{ABCB1} \) genotype groups (homozygous, heterozygous, and variant) and 4 CYP risk groups as factor variables. CYP side effect risk groups had been previously defined based on nortriptyline concentrations and genotype-dependent increased adverse effects (1). Clinical response and side effects scores were examined as dependent variables. No significant effect of the P-gp polymorphism on therapeutic response (Hamilton Depression Scale and Clinical Global Impression Scale scores on day 21) could be identified (data not shown).

The ANOVA results regarding side effects obtained for the \( \text{ABCB1} \) genotype and the CYP risk groups, as well as for the combined factor variables, are given in Table 1. Beyond the known associations for the CYP risk groups, no significant additional effect of the P-gp polymorphism on the DOTES side effects total sum score on day 21 could be identified. Analysis of the 5 adverse events clusters of the DOTES score separately yielded the same result for 4 of the 5 clusters (Table 1). We found a significant difference for anticholinergic/gastrointestinal symptoms \( (P = 0.026) \) that was nullified by post hoc testing for multiple comparisons.

To summarize, in contrast to sequence variations in CYP2D6 and CYP2C19, the \( \text{ABCB1} \) \( \text{G2677T/A} \) sequence variation did not significantly influence side effects in a group of depressed inpatients treated with amitriptyline, nor was it associated

<table>
<thead>
<tr>
<th>Variables</th>
<th>( \text{ABCB1} ) genotype</th>
<th>CYP side effects risk group</th>
<th>( \text{ABCB1} ) genotype × CYP side effects risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean DOTES sum score (day 21)</td>
<td>0.247</td>
<td>&lt;0.001\textsuperscript{a}</td>
<td>0.043\textsuperscript{a}</td>
</tr>
<tr>
<td>Mean DOTES scores on day 21</td>
<td>0.113</td>
<td>0.279</td>
<td>0.002\textsuperscript{a}</td>
</tr>
<tr>
<td>Cluster a, mental side effects</td>
<td>0.267</td>
<td>0.028\textsuperscript{a}</td>
<td>0.814</td>
</tr>
<tr>
<td>Cluster b, neuromuscular symptoms</td>
<td>0.079</td>
<td>0.418</td>
<td>0.141</td>
</tr>
<tr>
<td>Cluster c, anticholinergic/GI symptoms</td>
<td>0.390</td>
<td>0.013\textsuperscript{a}</td>
<td>0.206</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Significant effect.
\textsuperscript{b} GI, gastrointestinal; AT, amitriptyline; NT, nortriptyline.

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Table 1. Results of univariate analysis of variance for the fixed factors \( \text{ABCB1} \) genotype and CYP side effects risk groups.
How Reliable Are Critical Error Calculations?

To the Editor:
The critical systematic error ($\Delta SE_C$) and the critical random error ($\Delta RE_C$) are indicators used in quality-control planning (1). $\Delta SE_C$ is calculated with the following formula:

$$\Delta SE_C = \frac{TE_A - B}{s} - z \quad (1)$$

where $TE_A$ is the total error allowable, $B$ is the method bias, $s$ is the standard deviation of the sample, and $z$ is a factor specifying the one-tailed significance. $\Delta RE_C$ is calculated with the formula:

$$\Delta RE_C = \frac{TE_A - B}{s \times z_2} \quad (2)$$

where $z_2$ is a factor specifying the two-tailed significance.

$\Delta SE_C$ and $\Delta RE_C$ allow the selection of appropriate quality-control procedures through power function graphs (2).

In practice, critical errors are only estimators of the true $\Delta SE_C$ and $\Delta RE_C$ values, which are unknown. These estimators are calculated based on a limited number of results, which are assumed to be representative of the total population of results that could be obtained by stable performance measurement methods in the same material. The reliability of these estimations determines the practical value of decisions about an appropriate quality-control strategy.

Confidence intervals (CIs) should be used to express the reliability of an estimated statistic (3). In the above formulas, $TE_A$, $z_1$, and $z_2$ are constant values. $B$ and $s$ are the difference and standard deviation, respectively, with individual CIs that can be determined by traditional parametric statistical methods. However, obtaining CIs for $\Delta SE_C$ and $\Delta RE_C$ is not straightforward because these estimators depend jointly on $B$ and $s$.

To find the solution for this problem, we can refer to the mathematical equivalence of critical errors and $C_{pk}$, a process capability index that has been thoroughly studied and is well known in industrial quality management (4). In medical laboratory settings, $C_{pk}$ may be calculated with the following formula:

$$C_{pk} = \frac{TE_A - B}{3 \times s} \quad (3)$$

By rearranging the above equations, Chesher and Burnett (4) derived:

$$C_{pk} = \frac{\Delta SE_C + z}{3} = \frac{\Delta RE_C \times z_2}{3} \quad (4)$$

Several methods for determining CIs for $C_{pk}$ have been proposed (5). In 1990, Bissell (6) described an approximate two-sided CI for $C_{pk}$ by assuming that the distribution of $C_{pk}$ is gaussian. In Bissell’s approach, this CI is given by:

$$C_{pk} = \pm z_2 \sqrt{\frac{1}{n} + \frac{C_{pk}^2}{2n - 2}} \quad (5)$$

where $n$ is the number of measurements used in calculating $s$ and $B$.

Kushler and Hurley (7) tested Bissell’s method and concluded that it is easily computed and gives reasonably accurate results.

Taking into account the mathematical equivalence of $\Delta SE_C$, $\Delta RE_C$, and $C_{pk}$, it is possible to find approximate two-sided CIs for $\Delta SE_C$ and $\Delta RE_C$. By rearranging Eqs. 4 and 5, we can derive the confidence interval for $\Delta SE_C$:

$$\Delta SE_C = \pm z_2 \sqrt{\frac{1}{n} + \frac{C_{pk}^2}{2n - 2}} \quad (6)$$

and the confidence interval for $\Delta RE_C$:

$$\Delta RE_C = \pm z_2 \sqrt{\frac{1}{n} + \frac{C_{pk}^2 \times z_2^2}{2n - 2}} \quad (7)$$

CIs calculated for critical errors depend on the number of measurement results used in calculating them. Decisions on the adequacy of quality-control algorithms may be highly uncertain when based on a small number of measurement results. Such a situation might occur in a medical laboratory; for example, when a measurement method is newly introduced into routine practice. A minimum of 20 results is typically used to form an initial esti-

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