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ORIGINAL ARTICLE

A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression

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ABSTRACT

The multi-drug resistance gene *ABCB1* (or *MDR1*) encodes a P-glycoprotein (P-gp) that regulates passage of many substances across the blood–brain barrier. The antidepressant amitriptyline and its metabolites (including nortriptyline) are substrates for P-gp, and in mice lacking P-gp, penetration of amitriptyline, but not fluoxetine, into the brain is enhanced. We reasoned that polymorphic variation of P-gp may contribute to differing responses of patients to antidepressant drugs. A single nucleotide polymorphism (SNP) of *ABCB1* (3435C>T) was recently correlated with expression levels and *in vivo* function of P-gp. We examined this SNP in patients with major depression enrolled in a randomized antidepressant treatment trial of nortriptyline and fluoxetine, and observed a significant association between nortriptyline-induced postural hypotension and 3435C>T ($\chi^2 = 6.78$, df = 2, $P = 0.034$). Our results suggest that homozygosity for 3435T alleles of *ABCB1* is a risk factor for occurrence of nortriptyline-induced postural hypotension (OR = 1.37, $P = 0.042$, 95% CI 1.01–1.86).
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Keywords: ABC transporters; *ABCB1*; antidepressants; affective disorders; pharmacogenetics; multidrug resistance gene *MDR1*; fluoxetine

INTRODUCTION

Tricyclic antidepressants (TCAs) have been widely used to treat unipolar major depression since their development in the 1950s. Despite their efficacy in relieving depressive symptoms, TCAs are associated with a significant and clinically relevant side effect burden. Most TCA side effects such as blurred vision, constipation, impaired psychomotor coordination, excessive sedation and postural hypotension arise through muscarinic cholinoceptor, histamine H1 receptor, and α_1-adrenoceptor blockade.[1–4] The most common cardiovascular complication of TCAs is postural hypotension, believed to be due to blockade of α_1-adrenoceptors.[3] Postural hypotension is especially problematic in elderly patients, who are prone to injury through falls.[5,6]

Interindividual variability in predisposition to TCA-induced postural hypotension could conceivably be genetically determined. One candidate gene in which variability might modulate antidepressant drug responses is *ABCB1*, which encodes the multidrug resistance (MDR1) P-glycoprotein (P-gp).[7,8] This protein is expressed on the luminal surface of cerebral capillary endothelial cells, and it is implicated as a critical component of the blood–brain barrier (BBB) that regulates the entry or elimination of drugs and other substances from the CNS.[9–11] Uhr *et al* (2000)[1] observed that mice with targeted inactivation of the P-glycoprotein gene *Abcb4* (formerly known as *Mdr1a*) displayed greater brain penetration of the TCA amitriptyline, but not the serotonin selective reuptake inhibitor (SSRI) fluoxetine, compared with normal mice. This study demonstrated that amitripty-

line and its metabolites were substrates of P-gp, and that brain concentrations of this drug are dependent on the activity of this protein. This effect was attributed to the ability of P-gp to act as a pump which transports the drug from the brain back into circulation at the BBB.[1]

On the basis of these findings, we reasoned that polymorphisms in *ABCB1* that alter P-gp function may influence individual responses to antidepressant drugs. Several variants of *ABCB1* are known.[2,8,12,13] Of particular interest was the single nucleotide polymorphism (SNP) 3435C>T in exon 26 of *ABCB1* that was significantly correlated with expression levels and function of P-gp.[2] Analysis of duodenal expression of P-gp showed two-fold lower levels (as measured by Western blots) in patients who were homozygous for the T allele of this SNP, compared to those who were homozygous for the C allele.

Heterozygous individuals showed an intermediate phenotype. A similar correlation was observed between the T allele of 3435C>T and *in vivo* activity of P-gp as measured by plasma levels of the P-gp substrate digoxin after oral administration.[2] A subsequent study[14] demonstrated that efflux of the P-gp substrate rhodamine 123 from natural killer cells was significantly less efficient in individuals homozygous for 3435T compared with 3435C. This adds further support to the association of a functional effect with this SNP.

In this study we investigated whether the *ABCB1* polymorphism 3435C>T influenced the response of patients to the TCA nortriptyline, an active metabolite of amitriptyline that is an antidepressant in itself, or the SSRI fluoxetine, in a randomized trial for antidepressant treatment of major depression, in terms of both efficacy and adverse drug effects.

RESULTS

Of 195 patients with depression who entered the clinical trial, we had *ABCB1* (3435C>T) genotypes on 160 patients. Table 1 shows the clinical characteristics of the 82 who were randomized to fluoxetine and the 78 who were randomized to nortriptyline. From this table it is apparent that 72 (88%) completed an adequate 6-week trial of fluoxetine and 54 (69%) completed an adequate trial of nortriptyline. Of patients not completing an adequate trial of the antidepressant to which they had been randomized, over three quarters discontinued in the first week and over 90% within 3 weeks. For fluoxetine the most problematic side effects were agitation, restlessness, and akathisia; while for nortriptyline postural hypotension and then weight gain were most problematic. However, one patient randomized to nortriptyline (who took the drug for 6 weeks) was judged to have not had an adequate trial, as on a dosage of 50 mg postural hypotension limited the achievement of adequate blood levels and she showed minimal improvement. From Table 1 it can also be seen that the improvement in depressive symptoms, measured by the MADRS or HDRS was comparable across drugs, among those completing an adequate 6-week trial. There was also no relationship between response to treatment and genotype (nortriptyline data in Table 3).

Table 1 Clinical characteristics of the 160 depressed patients randomized to fluoxetine or nortiptyline treatment

Clinical characteristics	*Fluoxetine*	*Nortriptyline*
Number	82	78
Age[a]	33.3 (11.6)	30.3 (10.9)
% Female	54%	63%
Baseline severity[a]		
MADRS (all patients)	31.8 (6.3)	29.4 (6.7)
MADRS (trial completers)	31.9 (6.4)	29.3 (6.7)
HDRS (all patients)	20.4 (4.5)	19.0 (4.4)
HDRS (trial completers)	20.5 (4.7)	18.7 (4.0)
6-Week outcome		
% (Number) completing adequate 6-week trial	88 (72)	69 (54)
MADRS (trial completers)[a]	12.5 (10.9)	13.3 (11.4)
HDRS (trial completers)[a]	7.7 (6.7)	7.6 (6.7)

[a]Figures expressed as mean (SD).

We developed a PCR-RFLP assay for the 3435C>T SNP of *ABCB1*, based on the observation that the C allele creates a *Dpn*II restriction site (Figure 1). All patients were genotyped using this assay (Table 2), and allele frequencies were observed to be 47.2% for 3435C and 52.8% for 3435T. The ethnicity of individuals in this study was predominately New Zealand Caucasian, and these allele frequencies are comparable to those reported for German and United Kingdom Caucasian populations (Table 2).[2,15]

Of the 78 patients randomized to nortriptyline treatment, 24 did not complete the 6-week trial (Table 3). Eight of these patients experienced significant postural hypotension. Of these eight patients, one discontinued nortriptyline during the 6 weeks due to the side effects, one continued the antidepressant for 6 weeks but the side effect of postural hypotension prevented an adequate dose or blood level being achieved, and six had an adequate 6-week trial, but experienced postural hypotension. Analysis of 3435C>T genotypes in this group illustrated a markedly biased distribution: four were homozygous T, four were heterozygous, and none were homozygous C (Table 3). When the data from all nortriptyline-treated patients were analyzed by the three genotypes there was a significant association with postural hypotension while on nortriptyline ($\chi^2 = 6.78$, df = 2, $P = 0.034$). The odds ratio for risk of postural hypotension for the presence of one T allele was not significant. However, homozygote 3435T subjects were more likely than those with one or more C alleles to develop postural hypotension, with an odds ratio of 1.37, $P = 0.042$, and 95% CI of 1.01–1.86.

Of the 82 patients randomized to fluoxetine, none experienced postural hypotension during the 6-week antidepressant trial. Furthermore, the 3435C>T genotypes of the 10 patients who withdrew from fluoxetine treatment showed no particular bias: two were homozygous C, four were homozygous T, and four were heterozygous (data not shown).

The mean nortriptyline dose taken by each genotype group was very similar, and measured blood concentrations

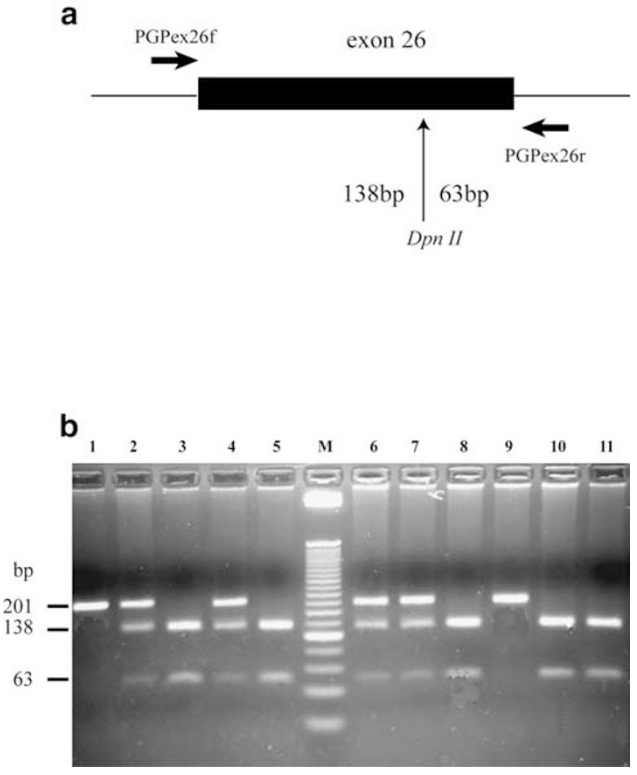

Figure 1 PCR-RFLP assay for the detection of the *ABCB1* polymorphism 3435C>T. (a) PCR amplification of exon 26 of *ABCB1* is carried out using the primers PGPex26f and PGPex26r. The resulting 201-bp PCR product is digested with the restriction endonuclease *Dpn*II, which cuts only at the C allele to yield 138-bp and 63-bp fragments. (b) DNA fragments were separated on 3% agarose-TBE gels consisting of 3:1 NuSieve GTG (Seakem) and UltraPure agarose (Life Technologies), and sized using a 25-bp ladder (M). Lanes 1 and 9 are homozygous T, lanes 2, 4, 6 and 7 are heterozygotes; and lanes 3, 5, 8, 10 and 11 are homozygous C.

of the drug were also similar between groups (Table 3). In fact, the mean concentration in the group with postural hypotension was lower than that without, although this difference was not significant. Because metabolism of nortriptyline is mediated by the polymorphic enzyme cytochrome 2D6, we carried out DNA tests for poor metaboliser status as previously described.[16] Nine of the patients in this study were poor metabolisers, but *CYP2D6* genotype was not associated with postural hypotension (data not shown).

DISCUSSION

Our hypothesis that *ABCB1* genotype might influence side effects caused by nortriptyline was stimulated by the observation that knockout mice lacking P-gp differentially accumulate amitriptyline and its metabolites in the brain but not fluoxetine.[1] The proposed mechanism of this differential accumulation was diminution of active transport of amitriptyline back into the circulation at the BBB, due to loss of functional P-gp in cerebral capillary endothelial cells. We reasoned that polymorphic variants of P-gp might, in humans, similarly lead to differential accumulation of antidepressant drugs in the brain and that this could manifest as a genotype-dependent difference in propensity to side effects. Two descriptions of clear *in vivo* functional differences in P-gp attributed to the 3435C>T polymorphism of *ABCB1*[2,14] led us to test whether this SNP influenced patient response to a tricyclic antidepressant in the setting of a randomized trial.

The major outcome of this study was the finding of a significant association between the *ABCB1* single nucleotide polymorphism 3435C>T and the risk of developing postural hypotension when treated with nortriptyline. Postural hypotension is defined as an excessive fall in blood pressure (typically >20/10 mmHg) on assuming the upright posture.[17] This cardiovascular phenomenon is a side effect of TCA medication that occurs in 10–50% of patients on therapeutic dosages,[18] and is characterized by dizziness, visual disturbances, palpitations, headache and fainting.[18,19] In extreme cases a sustained loss of blood perfusion of vital organs can result in heart attack or stroke.[20] Postural hypotension resulting from TCA treatment is usually attributed to α-adrenoceptor blockade, at both autonomic[21] and central[22,23] sites. We propose that an *ABCB1* genotype-dependent effect on function of P-gp leads to a greater relative accumulation of nortriptyline or its metabolites in the brain, thus increasing the risk of postural hypotension for a given dose.

It is conceivable that other factors may have led to an apparent association between the presence of homozygous 3435T alleles and an increased risk of developing TCA-induced postural hypotension. However, neither nortriptyline dose nor blood concentrations of drug differed signifi-

Table 2 Distribution of *ABCB1* 3435C>T genotypes in depressed patients enrolled in this study (predominantly Caucasian) compared with other studies of Caucasian groups

Genotype	*Frequency (%)*				
	This study (n = 160)	*Hoffmeyer et al (2000)[2] (n = 188)*	*Ameyaw et al (2001)[15] (n = 190)*	*Cascorbi et al (2001)[24] (n = 461)*	*Schaeffeler et al (2001)[25] (n = 537)*
C/C	21.3	27.8	24	20.8	25.9
C/T	51.9	48.3	48	50.5	47.7
T/T	26.9	23.9	28	28.6	26.4

Table 3 Rates of postural hypotension, completion of adequate 6-week antidepressant trial, nortriptyline dosage at 6 weeks, and nortiptyline blood concentrations at 6 weeks, by genotype, in patients randomized to nortriptyline

Genotype	*Rate % (n) of postural hypotension*	*% (n) Completing an adequate antidepressant trial*	*6-Week nortriptyline dose (mg)*	*6-Week nortriptyline blood concentration (nmol l^{-1})*	*MADRS (completers)*	
					Baseline	*6 Weeks*
C/C	0% (0/18)	67% (12)	103 (±25)	345 (±211)	30.3 (10.1)	12.7 (9.9)
C/T	11% (4/44)	80% (35)	97 (±39)	306 (±193)	28.9 (5.9)	13.7 (11.9)
T/T	25% (4/16)	50% (8)	97 (±28)	293 (±95)	29.8 (5.5)	12.5 (12.2)

cantly by genotype group. In addition, we established that there was no relationship between *CYP2D6* poor metaboliser genotype and the occurrence of postural hypotension.

The functional significance of the 3435C>T SNP is unclear. The C to T substitution is silent as it is located in the third (wobble) position of a codon.[2] The SNP could conceivably affect splicing of *ABCB1* transcripts or their translation by modulating codon preference. Alternatively, it is possible that the association between nortriptyline-induced postural hypotension and this SNP occurs because the T allele is in linkage disequilibrium with some other polymorphism that alters *ABCB1* function. Tanabe *et al* (2001)[40] observed linkage disequilibrium between the 3435C>T SNP and a missense SNP (2677G>A, T) in Japanese individuals, although it is unclear whether this SNP occurs at an appreciable frequency in other ethnic groups.

Because of the risk of type I error in association studies such as this, it will be important to confirm these findings in other studies. In addition, it would be of interest to determine whether the association can be generalized to TCAs other than nortriptyline. In this study postural hypotension was only assessed symptomatically and not by actual measurement. In future attempts to confirm this finding blood pressure measurement would be desirable, as symptomatic assessment may underestimate the true extent of postural hypotension.

Despite the introduction of SSRIs and other newer antidepressants with more favourable side effect profiles, TCAs are still widely used in the treatment of major depression. Furthermore, 3435T is very common, with an allele frequency of about 0.5 in Caucasians.[2,15,24] Recent studies describe considerable ethnic variation in occurrence of 3435T, with allele frequencies ranging from 0.1 in Ghanians to 0.66 in Southwest Asians.[15,25] These data, and our finding that homozygous 3435T is a risk allele for postural hypotension, lead to the prediction that the prevalence of TCA-induced postural hypotension will vary by ethnicity.

The rate of symptomatic postural hypotension in physically healthy but depressed patients is approximately 4% on nortriptyline, but is considerably higher on some other TCA drugs.[26,27] A retrospective study of 148 depressed patients treated with imipramine found just under 20% of patients had severe hypotensive symptoms that necessitated the alteration or withdrawal of TCA medication.[28] Some 4% of affected patients in this study sustained physical injuries from falls attributed to imipramine-induced postural hypotension. Although drug-induced postural hypotension can occur at any age, older patients are at the greatest risk of sustaining serious, even life-threatening injury from falls caused by this blood pressure dysfunction.[17,18,26,29] Clearly, the ability to predict risk of TCA-induced postural hypotension in this group would be of considerable value. It is of note that the mean age of the depressed patients in this study is only 31 years, and only five patients were over the age of 55 years. The four homozygous T patients with postural hypotension had a mean age of 26 years (range 20–42 years).

The PCR-RFLP assay described in this paper will be a valuable aid for further studying the *ABCB1* 3435C>T SNP and its possible relevance to the prescribing of antidepressants. If the association between the T allele and increased risk of postural hypotension is replicated in other studies, then this PCR-RFLP assay may be applied prospectively to identify newly diagnosed patients at greater risk of suffering this side effect. These patients may then be preferentially prescribed antidepressants other than TCAs. This pharmacogenetic approach may significantly reduce the risk of physical injury from postural hypotension associated falls, especially in the elderly and in osteoporosis sufferers. Finally, the possibility that the 3435C>T polymorphism is associated with postural hypotension induced by other classes of drug should be explored.

MATERIALS AND METHODS

Subjects

The depressed patients for this study were recruited for a long-term clinical trial examining patterns and predictors of remission, response, recovery, relapse and recurrence following an initial randomization to either fluoxetine or nortriptyline as their first antidepressant.[30–32] To be included in the study, depressed patients over the age of 18 years must have provided written informed consent, be judged to have a principal current diagnosis of a major depressive episode and required treatment with antidepressant medication. Exclusion criteria were minimized but included a diagnosis of schizophrenia or bipolar I disorder, a principal current diagnosis of severe alcohol or drug dependence, severe antisocial personality disorder which was judged likely to interfere with cooperation with the research protocol, or major physical illness. At the baseline assessment patients were also required to have been free of all psychotropic medication for at least 2 weeks or five drug half-lives, except for

an occasional hypnotic for sleep. This study was approved by the Canterbury Ethics Committee (Christchurch, New Zealand).

At the baseline assessment patients were assessed using the Structured Clinical Interview for Depression (SCID),[33] and rated for depression severity on the Hamilton (HDRS)[34] and Montgomery & Asberg (MADRS)[35] rating scales. Patients were also assessed for axis II disorders, completed a range of self report questionnaires, and had a detailed neurobiological assessment which included neuroendocrine, amino acid, and plasma protein measures. The collection of blood for DNA analysis was added after the clinical study began, and thus some of the first patients in the study did not provide DNA for genotyping. Of 195 patients in the clinical study, this paper reports on 160 for whom genotypes were available.

After the baseline assessment, patients were initially randomized to treatment with either fluoxetine or nortriptyline for a period of 6 weeks. During this time clinicians saw patients at least weekly and had flexibility to adjust dosages of drugs to try and achieve optimal clinical outcomes. For patients on nortriptyline this included blood levels after one week and as clinically indicated. All patients on nortriptyline at 6 weeks, when levels would have achieved steady-state, had blood concentrations measured for research purposes. During these weekly visits, and systematically at 3 and 6 weeks, clinicians enquired about side effects and recorded details of these. The side effect of postural hypotension was assessed by clinical history, and was not systematically checked by the measurement of supine and standing blood pressure. When the side effects were noted, clinicians were blind to all genotype data.

The HDRS and MADRS were repeated after 3 weeks and 6 weeks on the initially prescribed antidepressant medication.

Nortriptyline Measurement

Nortriptyline concentrations in plasma were measured by gas-chromatography using a thermionic specific detector.[36] The internal standard was clomipramine. The standard curves were linear ($r^2 > 0.99$) up to 833 nmol l^{-1}. The limit of detection (signal:noise 3:1) was 50 nmol l^{-1} from a 3.0-ml sample. Intra- and inter-day coefficients of variation were $< 10\%$.

DNA Extraction From Peripheral Blood

Genomic DNA was extracted from peripheral blood essentially as described by Ciulla *et al* (1988).[37] Peripheral blood (10 ml in an EDTA tube) was mixed with 40 ml of lysis buffer (0.32 M sucrose, 10 mM Tris pH 7.5, 5 mM $MgCl_2$ 1% Triton X-100) soon after it was drawn. Leukocytes were recovered by centrifugation and resuspended in lysis solution (4 M guanidine isothiocyanate, 25 mM sodium acetate, 0.84% β-mercaptoethanol) to release DNA. An equal volume of isopropanol was added, and the DNA was recovered by centrifugation then washed three times in cold 70% ethanol. Genomic DNA was resuspended in 10 mM Tris pH 8.0 1 mM EDTA (0.5 ml) and stored at −20°C.

Genotyping

We developed a polymerase chain reaction (PCR) – restriction fragment length polymorphism (RFLP) assay to detect 3435C>T in genomic DNA. Oligonucleotide primers were designed using the primer selection program Primer3.[38] The forward primer PGPex26f was 5′-CAAAGAAATAAA GCGACTGAATG-3′, and the reverse primer PGPex26r was 5′TTATTAGGCAGTGACTCGATGAA-3′. These spanned nucleotides 3294 to 3316, and 3469 to 3491 respectively, of *ABCB1* cDNA sequence (GenBank accession No. M14758)[39] with the first base of the ATG start codon designated 1.

PCR reaction volumes were 25 μl, and contained 2 mM $MgCl_2$, 0.625 μM of each primer, 200 μM dNTPs, 1 U of *Taq* DNA polymerase (Roche Biochemicals, Mannheim, Germany), and approximately 100 ng of genomic DNA. Thermal cycling was performed with an initial denaturation of 30 s at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 58°C, 30 s at 72°C, and a terminal extension of 2 min at 72°C. Reaction products were digested with *Dpn*II for 2 h at 37°C and resolved by electrophoresis on 3% agarose – TBE gels consisting of 3:1 NuSieve GTG (FMC, Rockland, ME, USA) and UltraPure agarose (Gibco BRL, Gaithersburg, MD, USA), containing ethidium bromide (Figure 1). A 25-base pair ladder (Gibco BRL, Gaithersburg, MD, USA) was used as a size marker.

Statistical Analysis

All data were entered into the relational database PARADOX, and then transferred to the statistics programme SYSTAT for statistical analyses. The first question addressed was whether the *ABCB1* genotype was related to therapeutic efficacy of nortriptyline. This was addressed by ANOVA. We then identified that the only specific side effect of nortriptyline which caused major clinical problems for more than five patients was postural hypotension. The association of postural hypotension with genotype was assessed by chi-square and logistic regression analysis.

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DUALITY OF INTEREST

None declared.

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