

# Genetic predictors of response to antidepressants in the GENDEP project

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The objective of the Genome-based Therapeutic Drugs for Depression study is to investigate the function of variations in genes encoding key proteins in serotonin, norepinephrine, neurotrophic and glucocorticoid signaling in determining the response to serotonin-reuptake-inhibiting and norepinephrine-reuptake-inhibiting antidepressants. A total of 116 single nucleotide polymorphisms in 10 candidate genes were genotyped in 760 adult patients with moderate-to-severe depression, treated with escitalopram (a serotonin reuptake inhibitor) or nortriptyline (a norepinephrine reuptake inhibitor) for 12 weeks in an open-label part-randomized multicenter study. The effect of genetic variants on change in depressive symptoms was evaluated using mixed linear models. Several variants in a serotonin receptor gene (*HTR2A*) predicted response to escitalopram with one marker (rs9316233) explaining 1.1% of variance ( $P=0.0016$ ). Variants in the norepinephrine transporter gene (*SLC6A2*) predicted response to nortriptyline, and variants in the glucocorticoid receptor gene (*NR3C1*) predicted response to both antidepressants. Two *HTR2A* markers remained significant after hypothesis-wide correction for multiple testing. A false discovery rate of 0.106 for the three strongest associations indicated that the multiple findings are unlikely to be false positives. The pattern of associations indicated a degree of specificity with variants in genes encoding proteins in serotonin signaling influencing response to the serotonin-reuptake-inhibiting escitalopram, genes encoding proteins in norepinephrine signaling influencing response to the norepinephrine-reuptake-inhibiting nortriptyline and a common pathway gene influencing response to both antidepressants. The single marker associations explained only a small proportion of variance in response to antidepressants, indicating a need for a multivariate approach to prediction.

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## Introduction

A clinician presented with a case of depression has to make a choice among more than 20 available antidepressant drugs. Although the efficacy of each antidepressant is modest on average, the response varies widely from patient to patient. For an individual, the effect of switching from one antidepressant to another is often dramatic, but clinical characteristics are poor predictors of which drug will work for whom and the search for an effective antidepressant usually proceeds by trial and error. Pharmacogenetics explores the potential of genetic

measurements to inform the individualized choice of treatment. Family studies suggest that response to antidepressants is heritable,<sup>1</sup> and over the past decade, molecular pharmacogenetic studies have started to explore the function of specific genetic variants in antidepressant response. The knowledge of molecular mechanisms of antidepressant action enables selection of plausible candidate genes and allows focused analyses that are more powerful than hypothesis-free genome-wide exploration.

Previous molecular pharmacogenetic studies have identified variants in several genes as associated with response to one or several antidepressants. Associations reported by the larger pharmacogenetic studies include genes encoding the serotonin transporter,<sup>2</sup> norepinephrine transporter,<sup>3</sup> serotonin receptor *HTR2A*,<sup>4</sup> brain-derived neurotrophic factor (*BDNF*),<sup>5</sup> glucocorticoid receptor<sup>6</sup> and glucocorticoid-receptor-associated co-chaperone *FKBP5*.<sup>7</sup> With the notable exception of the length polymorphism in the serotonin transporter gene promoter,<sup>2</sup> these associations have not yet been replicated.

Most molecular pharmacogenetic studies have been limited by small sample size or lack of comparators. Owing to small sample size, many studies lack power to detect moderately strong associations and subsequently it is difficult to distinguish between true results and type I and type II errors. The STAR\*D study had a large sample, but the use of a single antidepressant precluded differentiation between specific moderation of antidepressant action and general prognostic indicators.<sup>4</sup> The few studies that compared different antidepressants suggest that genetic variants differentially affect response to drugs with different modes of action.<sup>3,8</sup>

The mechanisms of action of commonly used antidepressants include serotonin reuptake inhibition and norepinephrine (noradrenaline) reuptake inhibition. The Genome-based Therapeutic Drugs for Depression (GENDEP) is a comparative study that aims to explore the function of clinical and genetic variables in determining the response to a serotonin-reuptake-inhibiting and a norepinephrine-reuptake-inhibiting drug. Clinical predictors including severity of depression, number of previous episodes, duration of episode, age, gender and antidepressant explained one-third of variability in response to the antidepressants with most of the unexplained variance being due to unknown individually stable factors.<sup>9</sup> This large proportion of variance in response to the antidepressants presents an opportunity for pharmacogenetic exploration.

In the present paper, we explore the function of genetic variation in 10 candidate genes, selected on the basis of known molecular mechanisms of antidepressant action and results of previous pharmacogenetic studies. The candidates include genes involved in serotonin signaling (encoding serotonin receptors *HTR1A* and *HTR2A*, and tryptophan hydroxylases *TPH1* and *TPH2*) and genes involved in norepinephrine signaling (norepinephrine transporter *SLC6A2* and norepinephrine receptor *ADRA2A*). We also included four genes that have been shown to be involved in action of different types of antidepressants, and are likely to

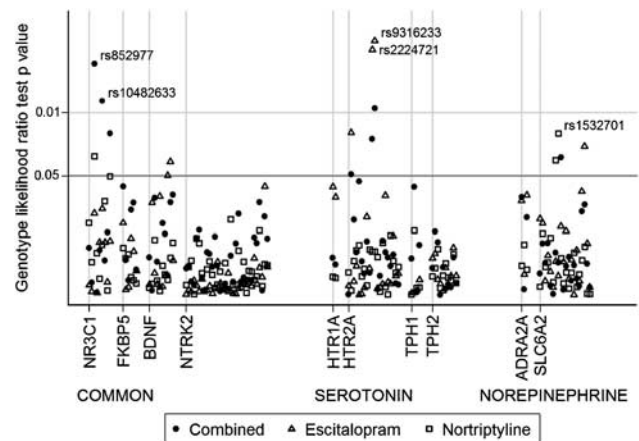
be involved in the final common pathway of antidepressant effect (*BDNF*, its receptor *NTRK2*, the glucocorticoid receptor *NR3C1* and its associated co-chaperone *FKBP5*). The present report excludes the serotonin transporter as the large body of available data led us to formulate and test more specific hypotheses regarding markers in this gene.<sup>10</sup>

Three hypotheses were tested. First, variants in the genes involved in serotonin signaling (*HTR1A*, *HTR2A*, *TPH1* and *TPH2*) will primarily influence response to the serotonergic antidepressant escitalopram. Second, variants in the genes involved in norepinephrine signaling will primarily influence response to the norepinephrine-reuptake-inhibiting antidepressant nortriptyline. Third, variants in the common pathway genes (*BDNF*, *NTRK2*, *NR3C1* and *FKBP5*) will affect response to both antidepressants. The word 'primarily' in the first two hypotheses reflects the fact that the mechanisms of action of escitalopram and nortriptyline are related and the selectivity of the antidepressants is relative. Therefore, we did not expect any polymorphism to have the opposite effect on response to the two drugs. The relative selectivity of the two antidepressants led us to expect effect concentration in the group treated by each antidepressant.

## Results

### Common pathway genes and antidepressant response

Of the 57 markers in the common pathway genes, 3 *NR3C1* markers were associated with response to antidepressants in the entire GENDEP sample (Figure 1; Table 1). Two of these associations remained significant after correction for the effective number of tests across the *NR3C1* gene but none survived correction for hypothesis-wide effective number of comparisons ( $M_{\text{eff}}$ ).



**Figure 1** Pharmacogenetic associations with response on Montgomery-Asberg Depression Rating Scale (MADRS). On the x axis, genes are ordered according to hypothesis, and markers within a gene are ordered according to chromosomal position. The y axis represents the *P*-value of single marker association, based on likelihood ratio tests of mixed linear models with and without additive genetic term, plotted on a log scale. The labels on the bottom of the graph indicate which comparison is hypothesized for which gene. Results of non-hypothesized tests are plotted for comparison.

**Table 1** Pharmacogenetic associations with response on MADRS

Gene	Marker	Minor allele	MAF	n	$\beta$	R <sup>2</sup> (%)	P	M <sub>eff</sub> corrected P	FDR q
<i>Common pathway</i>									
NR3C1	rs852977	G	0.30	742	0.066	0.49	0.0029	0.1114	0.1060
NR3C1	rs10482633	C	0.17	743	0.077	0.64	0.0075	0.2862	0.2044
NR3C1	rs10052957	A	0.18	746	0.054	0.27	0.0171	0.6517	0.2660
<i>Serotonergic</i>									
HTR2A	rs7324218	T	0.06	415	-0.199	0.63	0.0167	0.4007	0.2660
HTR2A	rs2224721	A	0.20	422	-0.136	1.22	0.0020	0.0492	0.1060
HTR2A	rs9316233	G	0.18	422	-0.146	1.10	0.0016	0.0393	0.1060
<i>Noradrenergic</i>									
SLC6A2	rs36029	C	0.42	335	-0.080	0.75	0.0337	0.5500	0.4589
SLC6A2	rs1532701	G	0.46	312	0.092	0.78	0.0170	0.2830	0.2660

The table shows hypothesized associations significant at the nominal significance level ( $P < 0.05$ ) with minor allele frequency (MAF), number of subjects included in each analysis ( $n$ ), standardized regression coefficient under additive genetic model ( $\beta$ ), proportion variance explained ( $R^2$ ), probability of null hypothesis being true ( $P$ ),  $P$ -value corrected for the hypothesis-wide effective number of comparisons ( $M_{\text{eff}}$  corrected  $P$ ) and experiment-wide false discovery rate  $q$ -value (FDR  $q$ ). The  $\beta$  coefficients indicate the difference on the MADRS scale in units of standard deviations associated with each minor allele, so a positive  $\beta$  means that minor allele is associated with worse outcome and a negative means that the minor allele is associated with better outcome.

#### Serotonergic genes and response to escitalopram

Of the 36 markers in serotonergic genes, three *HTR2A* markers, showed nominally significant associations ( $P < 0.05$ ) with response to escitalopram (Figure 1; Table 1). The two strongest associations (rs9316233 and rs2224721) explained 1.1 and 1.2% of variance in response to escitalopram and remained significant after corrections for the gene-wide and hypothesis-wide  $M_{\text{eff}}$ . The two markers were in linkage disequilibrium (LD,  $r^2 = 0.78$ ) and were located in a polymorphic region between two conserved blocks in intron 2 of the *HTR2A* gene.

#### Noradrenergic genes and response to nortriptyline

Of the 23 markers in noradrenergic genes, two *SLC6A2* markers, showed nominally significant associations with response to nortriptyline (Figure 1; Table 1). These associations were identified with a modest probability and neither of them survived correction for gene-wide or hypothesis-wide  $M_{\text{eff}}$ .

#### False discovery rate across hypotheses

As multiple positive findings were expected and found, the false discovery rate  $q$ -value was calculated to quantify the joint probability of multiple findings reflecting true associations as opposed to false positives, taking into account all comparisons performed to test the three hypotheses. The top three of the 116 hypothesized associations (rs9316233, rs2224721 and rs852977) were attributed a  $q$ -value of 0.106. Thus, taking into account the number of comparisons performed in this study, each of these three associations is more than eight times likely to be correct than a false positive. Although, after correction for multiple tests across the three hypotheses, it was not possible to reject any of the null hypotheses at a conventional level of statistical significance, it is highly unlikely that all of them represent false positives: the posterior probability that the three

strongest findings were all false positives was 0.0012. The posterior probability of all eight reported findings being false positive was  $2.1 \times 10^{-6}$ . In comparison, the lowest  $q$ -value among the 232 non-hypothesized tests was 0.986, consistent with a chance level, and the distribution of  $q$ -values in the hypothesized and non-hypothesized tests was entirely distinct (Supplementary Figure 1).

#### Exploration of symptom dimensions

To explore the effects of genetic polymorphisms on change in specific symptoms, all analyses were repeated with Montgomery–Asberg Depression Rating Scale (MADRS) score replaced by observed mood, cognitive and neurovegetative symptom dimensions. The results for observed mood dimension closely resembled those for MADRS, with variants in the *NR3C1* gene predicting response to both antidepressants, markers in the *HTR2A* gene predicting response to escitalopram and variants in the *SLC6A2* gene predicting response to nortriptyline (Supplementary Figure 2a). The differences between hypothesized and non-hypothesized comparisons were even more marked for the observed mood dimension than for the total MADRS score. Analysis of cognitive symptoms showed several drug-specific associations that were also found with MADRS and, in addition, a non-hypothesized association between escitalopram response and variation in the *BDNF* gene (rs10835210; Supplementary Figure 2b). There appears to be little relationship between variants in the 10 candidate genes and change in neurovegetative symptoms in response to antidepressants (Supplementary Figure 2c).

#### Replication of previously reported pharmacogenetic associations

Table 2 shows the GENDEP findings for five markers that were previously reported to be associated with antidepressant response in large studies or in multiple smaller studies. As many previous studies have used a categorical responder/

**Table 2** Previously reported pharmacogenetic associations

Gene	Marker	Antidep	Previous reports				GENDEP				Replicated?	
			Reported	n	OR	P	n	P (MMR)	OR	P (OR)	Marker	Gene
<i>HTR2A</i>	rs7997012	SRI	McMahon <i>et al.</i> <sup>4</sup>	1953	2.26	0.000002	439	0.4188	1.07	0.6373	No	Yes
<i>SLC6A2</i>	rs5569	NRI	Kim <i>et al.</i> <sup>3</sup>	105	7.54	0.001	330	0.2789	1.28	0.1831	No	Yes
<i>FKBP5</i>	rs1360780	All	Binder <i>et al.</i> <sup>7</sup>	294	14.96	0.00048	733	0.7112	1.05	0.6479	No	No
<i>NR3C1</i>	rs6190	All	van Rossum <i>et al.</i> <sup>6</sup>	367	5.17	0.008	737	0.2992	1.07	0.5742	No	Yes
<i>BDNF</i>	rs6265	All	Kato and Seretti <sup>5</sup>	490	1.63	0.02	754	0.2914	0.87	0.2828	No	No

Abbreviations: GENDEP, Genome-based Therapeutic Drugs for Depression; MMR, mixed model regression; NRI, norepinephrine reuptake inhibitors; OR, odds ratio; SRI, serotonin reuptake inhibitors.

The table compares previous reports of associations of specific markers with GENDEP results for the same markers. Data for 'previous reports' were extracted from the first paper reporting the association with the exception of *BDNF* where the numbers were extracted from a recent meta-analysis. The GENDEP *P*-values are reported for the mixed effect linear regression (MMR) of continuous MADRS and for OR for the dichotomous response on the 17-item Hamilton scale. The ORs are calculated in the same direction as in the original reports. The threshold for replication was set at 0.05, uncorrected.

nonresponder outcome, we report results of logistic regression with a standard categorical response outcome (>50% improvement on 17-item Hamilton Depression Rating Scale (HDRS-17) in addition to the results of linear mixed effects models. Both approaches showed negative findings for all previously reported markers. As a different definition of outcome was used in the STAR\*D study, we repeated the analysis for rs7997012, following the procedure reported by McMahon *et al.*<sup>4</sup> (a categorical definition of response, but excluding subjects with 'borderline' improvement of 40–45%, dropouts before week 3 and subjects who discontinued the drug because of intolerance from the analysis). This approach also brought negative results (odds ratio (OR)=1.14, 95% CI 0.80–1.63, *P*=0.4583). As Binder *et al.*<sup>7</sup> have reported rs1360780 in the *FKBP5* gene to be associated with response in a recessive fashion with TT homozygotes having a better response, we repeated the analyses for rs1360780 under a recessive T model. The results remained negative (OR=1.20, 95% CI 0.75–1.91, *P*=0.4425). In summary, none of the previously reported markers was replicated in GENDEP, but in three cases, other associations were found within the same gene.

## Discussion

The present study found a number of associations between candidate gene variants and response to antidepressant drugs. The distribution of multiple positive findings supported the hypothesis that pharmacogenetic associations are specific to antidepressant mode of action. As expected, variants in a serotonergic gene predicted response to the serotonin-reuptake-inhibiting antidepressant escitalopram, polymorphisms in a noradrenergic gene predicted response to the norepinephrine-reuptake-inhibiting antidepressant nortriptyline and markers in a common pathway gene (*NR3C1*) predicted response to both antidepressants. In comparison, the distribution of findings from non-hypothesized associations did not depart from chance level and there was little or no association between serotonergic genes and response to nortriptyline and between noradrenergic

genes and response to escitalopram. The specificity in pharmacogenetic associations combined with the number of positive results and low false discovery rate provide support for the hypotheses that genetic sequence variations genes in serotonin and norepinephrine signaling influence response to antidepressants with corresponding modes of action and genes in the glucocorticoid signaling pathway influence response to both types of antidepressants.

The strongest detected association was between markers in the gene encoding the serotonergic 5-HT<sub>2A</sub> receptor and response to escitalopram. This finding is of interest, as desensitization of 5-HT<sub>2A</sub> receptors appears to be a crucial element in the response to serotonin-reuptake-inhibiting antidepressants<sup>11</sup> and pharmacogenetic association of this gene with response to citalopram has been reported in the STAR\*D sample.<sup>4</sup> However, the rs7997012 marker reported in STAR\*D has not been significantly associated with continuous or categorical outcomes in GENDEP. Possible reason for the difference between the two large studies include the winner's curse<sup>12</sup> and inflation of effect by population stratification due to the inclusion of Hispanic participants in the STAR\*D study. The *HTR2A* markers associated with response in GENDEP and STAR\*D are in weak LD (*D'*=0.09). However, as they are located relatively near to each other in intron 2 of *HTR2A* and are not known to be functional, it is possible that these multiple associations reflect the influence of unidentified functional variants in this region, which are imperfectly tagged through population-variable LD with the genotyped markers. Therefore, this region is an important candidate for in-depth analysis and sequencing. This finding provides compelling evidence for a predictive value of this genetic region for response to serotonin-reuptake-inhibiting antidepressants and raises hope of finding functional markers with stronger effects.

Response to nortriptyline was associated with several variants in *SLC6A2*. This gene encodes the norepinephrine transporter, which is the molecular target of nortriptyline action, and therefore represents a candidate with high prior probability of association. Pharmacogenetic association between rs5569 in *SLC6A2* and response to nortriptyline

has been reported in a Korean sample.<sup>3</sup> However, the association with rs5569 was not replicated in GENDEP. The markers found to be associated with response to nortriptyline in GENDEP are in a different region of the gene and are not in LD with rs5569.

Among the common pathway genes encoding for proteins in glucocorticoid and neurotrophic signaling, several markers in the glucocorticoid receptor gene *NR3C1* were associated with response to both antidepressants in GENDEP. Given the important function that the glucocorticoid receptor has in the response to antidepressants,<sup>13</sup> these associations merit further exploration. The previously reported association of response to antidepressants with rs6190 in *NR3C1*<sup>6</sup> was not replicated. Similarly, the previously reported association between rs1360780 in *FKBP5* and response to antidepressants<sup>7</sup> did not replicate in GENDEP. The latter remained negative when we repeated the analyses with the outcome (50% reduction in HDRS) and genetic model (recessive T) as in the original report.<sup>7</sup> Although the nonsignificant results pointed in the same direction, the OR of 1.20 for response in TT homozygotes versus other genotypes was not comparable to the ORs of 14.96 and 5.4 in the discovery and replication samples of the original report.

The exploration of specific symptom dimensions<sup>14</sup> has shown that the reported pharmacogenetic associations are driven by change in core mood symptoms and are therefore unlikely to be due to side effects of antidepressants or nonspecific effects on neurovegetative symptoms. However, the detected associations explained only a small proportion of variance in antidepressant response. No single association was sufficiently robust to have clinical utility for prediction of response in an individual patient. For example, individuals carrying two minor alleles of rs9316233 in the *5HTR2A* gene achieved an improvement that was on average 3.1 MADRS points more than individuals carrying two common alleles during escitalopram treatment: a difference that is barely appreciable in an individual patient, corresponding to half the difference between 'no change' and 'slight improvement' on clinical global impression.<sup>15</sup> Therefore, in the short term, the present findings have heuristic rather than clinical implications. In the longer term, clinically useful pharmacogenetic prediction could be achieved either by finding genetic markers with much stronger effects or by combining a number of weakly predictive markers. Replication of numerous findings of weak-to-moderate strength in several large studies will be required to establish multivariate prediction of response to specific antidepressants. The negative results of attempts to replicate previously reported findings<sup>4,6,7</sup> in GENDEP indicate that the clinical benefits of pharmacogenetic research may be long way ahead. The fact that GENDEP and other studies tend to identify other variants near to the previously reported markers rather than replicate the exact marker–response association points to the importance of fine-grained population stratification and other population-specific factors.

Although GENDEP is the largest comparative study of a serotonin-reuptake-inhibitor and a norepinephrine-reup-

take-inhibiting antidepressant to date, it was only powered to detect moderate-to-strong associations with a limited number of markers. A more powerful and comprehensive investigation will require combination of samples across studies at the cost of increased heterogeneity. This phase of GENDEP concentrated on common variants in a limited number of functional candidate genes. However, the coverage was incomplete, especially for two large genes (*NTRK2* and *HTR2A*), due to regions of low LD and failure of several single nucleotide polymorphisms (SNPs) in quality check procedures. Although known functional variants and previously reported markers were preferentially targeted, unknown functional variants may have been missed. Detailed exploration and sequencing of the identified genetic regions may detect functional variants with much stronger associations than the tagging markers, as has been the case in other diseases.<sup>16</sup>

To minimize the influence of population stratification, recruitment of GENDEP participants was restricted to subjects of white European parentage, known ancestry-informative markers were used to detect population stratification and center of recruitment was included as a random effect in all analyses. Analysis of population stratification using Structure was limited by a relatively small number of genotyped markers. We also used the genomic control approach, for which it has been shown that as few as 30 markers are sufficient to detect an inflation of a test statistic due to population stratification.<sup>17</sup> There was no inflation of test statistic in the GENDEP sample, indicating that false positives due to population stratification are unlikely. However, fine-grained population stratification and heterogeneity due to relatively liberal inclusion criteria may have reduced the power to detect true associations. On the other hand, the inclusive criteria and rigorous statistical procedures accounting for center effects increase the generalizability of the GENDEP findings.

The comparison of two active antidepressants with different modes of action allowed testing the specificity of pharmacogenetic associations. However, as placebo was not used, we were unable to distinguish between features common to the two antidepressants and nonspecific factors associated with illness course, placebo-response and spontaneous recovery. The related modes of action of the two antidepressants and lack of statistical power precluded exploration of drug-by-gene interactions. Meaningful tests of such interactions will require large samples treated by drugs with unrelated modes of action.

In conclusion, GENDEP provided compelling evidence for a specific function of serotonergic genes in response to a serotonin reuptake inhibitor and noradrenergic genes in response to a norepinephrine reuptake inhibitor in depression. The glucocorticoid receptor gene may be involved in the response to antidepressants irrespective of the mode of action. Previously reported pharmacogenetic associations were not replicated, but other associations were found in the same genes. The single marker associations explained only a small proportion of variance in response to antidepressants, indicating a need for a multivariate approach to prediction.

## Materials and methods

### Study design and sample

GENDEP is a part-randomized multicenter open-label pharmacogenetic study with two active pharmacological treatment arms.<sup>9</sup> Nine psychiatric centers in eight European countries recruited 811 adult outpatients (296 men and 514 women) aged between 19 and 72 (mean age 42.5, s.d. 11.8) suffering from unipolar depression of at least moderate severity according to International Classification of Diseases 10/*Diagnostic and Statistical Manual of Mental Disorders*, fourth edition and established in the semi-structured SCAN interview.<sup>18</sup> To minimize the effect of population stratification, we restricted the recruitment to white individuals of European ancestry for at least two generations. Personal and family history of schizophrenia or bipolar affective disorder and current dependence on alcohol or drugs constituted exclusion criteria. The average participant was in his or her second episode of depression and scored 28.7 (s.d. 6.7) on the MADRS, 21.8 (s.d. 5.3) on the 17-item HDRS and 28.2 (s.d. 9.7) on the Beck Depression Inventory (BDI). The recruitment procedure, sample and clinical outcomes have been described in detail elsewhere.<sup>9</sup> The retention rate was comparable to other studies with 628 (77%) participants completing 8 weeks and 527 (65%) completing 12 weeks on the originally allocated antidepressant. The outcome data were 92.9% complete.<sup>9</sup> GENDEP is registered at EudraCT (no. 2004-001723-38, <http://eudract.emea.europa.eu>) and ISRCTN (no. 03693000, <http://www.controlled-trials.com>). The study was approved by ethics boards of all the participating centers. All participants provided written informed consent.

### Interventions

Two antidepressants were selected that represent the two most common mechanisms of action among commonly used antidepressants and have a good efficacy record. Escitalopram is a highly selective inhibitor of the serotonin transporter with no direct effect on norepinephrine reuptake.<sup>19</sup> Nortriptyline is a tricyclic antidepressant with a hundred times higher affinity for the norepinephrine transporter than for the serotonin transporter.<sup>20</sup> Nortriptyline was used in preference to the even more selective reboxetine as it has better established efficacy and was considered to be clinically at equipoise with escitalopram.

Patients with no contraindications were randomly allocated to receive nortriptyline (50–150 mg daily) or escitalopram (10–30 mg daily) for 12 weeks. Patients with contraindications for one of the drugs were allocated nonrandomly to the other antidepressant. The titration was guided by a protocol with a degree of flexibility to accommodate individual differences in tolerability and dose requirement.<sup>9</sup> Other psychotropic medication was not allowed with the exception of occasional use of hypnotics. Compliance with antidepressants was monitored weekly by self-reported pill count and plasma levels of antidepressants were measured at week 8.

### Phenotype definition

The response to antidepressant medication is a complex phenotype that includes changes in various symptoms occurring over a period of up to 12 weeks following the initiation of an antidepressant. In the GENDEP study, response to escitalopram and nortriptyline was assessed by weekly administration of three established measures of depression severity: the clinician-rated 10-item MADRS,<sup>21</sup> the HDRS-17<sup>22</sup> and the self-reported 21-item BDI;<sup>23</sup> administered at week 0 (baseline) and then weekly for 12 weeks. The MADRS proved to be the most internally consistent and informative of the three scales.<sup>14</sup> The depressive symptoms were even better described by three symptom dimensions derived by categorical data factor analysis: observed mood, cognitive symptoms and neurovegetative symptoms.<sup>14</sup> Therefore, we used the MADRS as a primary continuous outcome variable and the three symptom dimensions for a secondary exploration of the data. All available data across the 12-week study were used in analyses to provide an optimized description of outcome (see Statistical analysis).

### Genotyping and quality control

DNA was extracted using a standard procedure from blood collected in EDTA.<sup>24</sup> DNA samples were available for 795 of the 811 participants.

The selection of candidate genes was made by the principal investigators of the GENDEP Consortium (<http://gendep.iop.kcl.ac.uk>) based on known mechanisms of antidepressant action and pharmacogenetic literature available at the time of study planning (2003) and updated in 2006, when the genotyping protocol was finalized. A total of 140 SNPs were selected to tag common DNA sequence variation in the 10 candidate genes. Tagging SNPs were selected using the SNPTagger program and HapMap data on the CEPH CEU population, NCBI Build 35/UCSC hg17/May 2004<sup>25</sup> that tag common variants with minor allele frequency of 0.05 or more with a pair-wise  $r^2 \geq 0.8$  within the genes' coding sequences and 1000 bp upstream and downstream flanking regions, to cover the core and proximal promoter sequences.<sup>26</sup> For two large genes (*NTRK2* and *HTR2A*), a very large number of markers (> 50 per gene) would have been required to tag the entire sequence, and therefore a set of SNPs were selected to target the intron-exon boundaries and proximal regulatory sequences where functional variants are more likely to be located. Known functional polymorphisms were always included. The SNPs were genotyped using the SNPLEX method, which includes oligonucleotide ligation assay and PCR technology for ligation product amplification and allelic discrimination. The assay products were run on an ABI 3130 sequencer and data were exported and analyzed using GeneMapper software, version 4.0. Ten percent of samples were genotyped in duplicate and the agreement was 100%.

As recent association studies have highlighted the importance of tight quality control of genotype data,<sup>27</sup> we applied the following multiple quality control criteria: SNPs were omitted from analysis if poor genotype clustering prevented GeneMapper from making calls or there was a

genotype mismatch between CEPH genotypes deposited in HapMap and those derived using in-house genotyping of the GENDEP sample. For each marker, individual genotypes were omitted if their peak heights were <25% of the average for that genotypic group across the entire sample to avoid false heterozygosity assignment due to background noise in poor quality samples. As low call rates may indicate inaccurate genotyping, markers were omitted if the call rate after the previous exclusions was less than 90%. Although moderate departures from the Hardy–Weinberg equilibrium are expected in a case-only sample,<sup>28</sup> gross departures may reflect genotyping errors. Therefore, eight markers with major departures from Hardy–Weinberg equilibrium ( $P < 0.001$ ) were excluded. A total of 116 markers satisfied all the above quality control criteria. Finally, 36 individuals were excluded as their call rate across the 116 markers was <75%.

The clean data set retained for analyses comprised 116 markers in 760 individuals (424 treated with escitalopram and 336 treated with nortriptyline). The genotype data in the final sample were 94.2% complete. The included markers tagged 100% of common DNA sequence variation in *BDNF* and *HTR1A*, 94% in *NR3C1*, 76% in *SL6A2*, 60% in *TPH1*, 50% in *ADRA2A*, 46% in *NTRK2* and 42% in *HTR2A* with a pair-wise  $r^2 \geq 0.8$ . The 51 participants with missing or inadequate genotype data did not differ on demographic or clinical variables from the remaining participants.

#### Statistical analysis

All available weekly data on response to antidepressants were included in the analyses and effects of marker genotypes were tested using linear mixed models fitted with maximum likelihood. The mixed models account for the clustering of individuals within centers, allow use of all repeated measurements and provide unbiased estimates in the presence of dropout without imputation.<sup>29,30</sup> It has been repeatedly demonstrated that mixed models are preferable to end-point analyses with last-observation-carried-forward procedure.<sup>29,30</sup> All models included fixed effects of time (linear and quadratic), baseline depression severity, drug, age and sex, and random effects of individual and recruitment center. To account for correlations between repeated observations, the intercept and slope of time were allowed to vary randomly between subjects. For each genetic marker, nested models with and without the genetic term were compared using likelihood ratio tests to assess the contribution of the genetic information to the prediction of symptom change over and above the relevant clinical and demographic variables. An additive genetic model was used for all analyses with each genotype coded as the number of minor alleles. Models were fitted using Stata 10.<sup>31</sup>

Three different hypotheses were tested for non-overlapping subsets of the genetic markers: response to escitalopram was hypothesized to be influenced by variation in serotonin-related genes (*5HTR1A*, *5HTR2A*, *TPH1* and *TPH2*), response to nortriptyline was expected to be related to variation in norepinephrine-related genes (*ADRA2A* and *SLC6A2*) and response to both antidepressants was

hypothesized to be associated with polymorphisms in the common pathway genes (*NR3C1*, *FKBP5*, *BDNF* and *NTRK2*). A total of 116 tests were performed to probe the three hypotheses: 36 tests examined the effect of markers in serotonergic genes on response to escitalopram, 23 markers in noradrenergic genes were tested for their effect on response to nortriptyline and 57 tests related the markers in the common pathway genes to response in the combined group of patients treated with either antidepressant (Supplementary Table 1). The results of the other possible 232 non-hypothesized comparisons (for example, between markers in noradrenergic genes and response to escitalopram) are shown to demonstrate the specificity of hypothesized associations and are not intended to directly test any hypothesis.

Several approaches were applied to correct for multiple non-independent comparisons. First, the effective number of comparisons ( $M_{\text{eff}}$ ) was computed from the correlation matrix of genotyped markers, using a procedure described by Li and Ji,<sup>32</sup> which takes into account higher-order correlations between markers, and was applied in the web-based SNPSPD software.<sup>33</sup> The gene- and hypothesis-wide multiple comparison-corrected significance thresholds were then calculated as  $\alpha_{\text{corr}} = 0.05/M_{\text{eff}}$  (Supplementary Table 1). This procedure provides an effective control of type I error rate in the context of multiple correlated tests and has good agreement with the permutation test.<sup>32,33</sup>

Second, false discovery rate was applied to quantify the uncertainty across the three hypotheses tested in the 116 single marker tests. As all genotyped markers were in functional candidate genes, multiple positive findings were expected and the pattern of multiple positive findings was potentially of more interest than any single result. This is reflected in the false discovery rate  $q$ -value, which denotes the expected proportion of false negatives among multiple findings. Based on the mixed linear model results, we calculated the  $q$ -value for each of these 116 non-independent tests using the step-up procedure described by Benjamini and Hochberg.<sup>34</sup> The  $q$ -value calculated in this way has been shown to retain desirable properties for multiple related tests in genetic association studies<sup>35,36</sup> and can be intuitively interpreted in terms of posterior error probability.<sup>37</sup>

#### Power analysis

To facilitate the interpretation of both positive and negative results, a power analysis was performed using the program Quanto.<sup>38</sup> We aimed to detect genetic effects explaining at least 2% of variance in antidepressant response, which corresponds to an effect size of 0.22 (Cohen's  $d$ ) or a difference of 2 MADRS points per allele under an additive genetic model for a polymorphism with a minor allele frequency of 0.3. Power for detecting such effect was calculated for the nominal significance level  $\alpha = 0.05$  and for the  $\alpha_{\text{corr}}$  corrected for hypothesis-wide effective number of independent comparisons ( $M_{\text{eff}}$ , Supplementary Table 1).<sup>32,33</sup>

The sample of 760 individuals provides power of 0.97 to detect an effect of a marker in a common pathway gene explaining 2% of variance in antidepressant response at  $\alpha = 0.05$ . Power is reduced to 0.76 if the same effect is to be detected at  $\alpha_{\text{corr}} = 0.0013$ . The sample of 424 escitalopram-treated individuals provides power of 0.83 to detect an effect of a marker in a serotonin-related gene explaining 2% of variance in response to escitalopram at  $\alpha = 0.05$ . Power is reduced to 0.44 for  $\alpha_{\text{corr}} = 0.0021$ . The sample of 336 nortriptyline-treated individuals provides power of 0.74 to detect an effect of a marker in the norepinephrine-related genes on response to nortriptyline at  $\alpha = 0.05$  and power of 0.36 at  $\alpha_{\text{corr}} = 0.0030$ . The power increases steeply with effect size: thus the nortriptyline-treated sample gives a power of 0.88 to detect the effect of a polymorphism explaining 5% of variance at  $\alpha_{\text{corr}} = 0.0030$ . In summary, GENDEP is powered to detect clinically significant effects of genes on response to antidepressants at a nominal level of significance, but only strong effects can be detected after correction for multiple comparisons.

#### Population stratification

To minimize confounding by population stratification, the recruitment of GENDEP participants was restricted to subjects of European ancestry for at least two generations. However, as uniform self-reported ethnicity does not exclude stratification, we further investigated the genetic structure of the sample. In addition to the candidate genes markers, a panel of 35 SNP markers selected as being most informative of structure in European population<sup>39</sup> were genotyped in all individuals to establish the presence of and allow effective control for population stratification. These ancestry-informative markers were analyzed using 10 000 burn-ins and 10 000 repetitions under the admixture model with correlated allele frequencies in Structure.<sup>40</sup> This analysis found no significant clustering within the sample. Stratification was further estimated using the genomic control approach.<sup>41</sup> Based on the same set of ancestry-informative SNPs and all candidate gene markers, we estimated the genomic inflation factor to be less than 1 ( $\lambda_{\text{mean}} = 0.83$ ), confirming that reported GENDEP results are unlikely to be inflated by population stratification.

#### Duality of interest

Uher, Huezo-Diaz, Perroud, Smith, Rietschel, Mors, Hauser, Maier, Kozel, Barreto, Placentino, Dernovsek, Schulze, Zobel, Czerski, Larsen, Souery, Giovannini, Gray, Lewis, Craig have no competing interests. Henigsberg and Kalember participated in clinical trials sponsored by pharmaceutical companies including GlaxoSmithKline and Lundbeck. Aitchison, Farmer and McGuffin have received consultancy fees and honoraria for participating in expert panels for pharmaceutical companies including Lundbeck and GlaxoSmithKline.

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