Genomewide interaction and enrichment analysis on antidepressant response

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Background. Genomewide association studies (GWASs) on antidepressant efficacy have yielded modest results. A possible reason is that response is influenced by other factors, which possibly interact with genetic variation. We used a GWAS model to predict antidepressant response, by including predictors previously known to affect response, such as quality of life (QoL). We also evaluated the association between genes, previously implicated in gene–environment (G×E) interactions, and response using an enrichment analysis.

Method. We examined a sample of 1426 depressed patients from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial: 774 responders, 652 non-responders and 418865 single nucleotide polymorphisms (SNPs) were analysed. First, in a GWAS model, we investigated whether genetic variations interact with patients' levels of QoL to predict response, after controlling for demographic characteristics, severity and population stratification. Second, we conducted an enrichment analysis exploring whether candidate genes that have emerged from prior G×E interaction studies on depression are associated with treatment response.

Results. The GWAS model, with QoL as a moderator, yielded one SNP (rs520210) associated with response in the *NEDD4L* gene ($p = 3.64 \times 10^{-8}$). In the Caucasian sample only, we observed a drop in significance for this SNP. The enrichment analysis showed that SNPs within serotonergic genes contained more significant markers that predicted response, compared with a random set of genes in the genome.

Conclusions. Our findings point to possible target genes, which are proposed for further independent replication. Our enrichment analysis provides further support, in a genomewide context, of the role of serotonergic genes in influencing antidepressant response.

Introduction

Major depressive disorder (MDD) is a highly recurrent and prevalent disorder with societal costs. Despite the increased amount of available pharmacological treatments, many patients do not respond with the first-line treatment, while others experience costly delays until they find which treatment is efficacious for them. Whereas it is estimated that about 50% of patients with uncomplicated MDD will respond to any single antidepressant (Papakostas & Fava, 2008, 2009), other reports show lower response rates (Souery *et al.* 2007). For example, in a large, multicentre trial, the Sequenced Treatment Alternatives to Relieve Depression (STAR*D), approximately one in three patients with MDD achieved remission after being

treated with the selective serotonin reuptake inhibitor (SSRI) citalopram (Trivedi *et al.* 2006*a*).

There is substantial evidence that antidepressant response is to a certain extent heritable (O'Reilly *et al.* 1994; Franchini *et al.* 1998). Pharmacogenetic research seems to be a promising path toward personalized treatment; however, the link between genetic risk variants and response to treatment is far from direct. To date, genomewide association studies (GWASs) on treatment response of antidepressants (Ising *et al.* 2009; Garriock *et al.* 2010; Uher *et al.* 2010) provide modest results, which do not reach genomewide significance and are hard to replicate, thereby making any translation into potential clinical utility difficult. On the other hand, there is a substantial research showing that candidate polymorphisms of target proteins may be involved in antidepressant drug action (Serretti *et al.* 2005; Kato & Serretti, 2010; Porcelli *et al.* 2011). Environmental factors, both early and late in life, also influence treatment response (Uher, 2011). It has been recently observed that the same genes,

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environments, and gene–environment (G×E) interactions implicated in aetiological pathways to depression may also play a role in predicting treatment response (Keers & Uher, 2012). Genetic variation, as on the serotonin-transporter-linked polymorphic region (5-HTTLPR) for example, may increase the risk for MDD (Uher & McGuffin, 2010) or may lead to a worse response to the SSRI treatment (Mandelli et al. 2009; Keers et al. 2011) under unfavourable psychosocial environmental conditions. Although there is accumulating evidence of G×E interactions on the aetiology of depression emerging from candidate gene studies, statistical and methodological challenges hinder GWASs to examine the potential moderating role of life stressors (Khoury & Wacholder, 2009). However, considering the complexity of the phenotype, it has been suggested that alternative pharmacogenetic study designs should be considered, in particular those that take into account the manifold sociodemographic and environmental variables that make an impact on treatment response in the clinical setting (Malhotra, 2010). Such factors may interact with genetic variation to influence antidepressant response. For example, the patient's quality of life (QoL) has been previously found to be an independent predictor of acute antidepressant treatment response (Pyne et al. 2001) and low QoL was associated with lower remission scores in the STAR*D trial (Trivedi et al. 2006a).

The aim of the present study was twofold. First, in an attempt to improve the predictive model of treatment response, we examined the possible moderating role of the patient's level of QoL, in a GWAS model of treatment response to citalopram, in a large group of depressed patients from the STAR*D trial. Other sociodemographic and clinical characteristics, such as age, sex and symptom severity, known to influence response to treatment (Drago & Serretti, 2011), were also included in the model. Second, since a number of candidate genes have emerged from prior G×E interaction studies on depression, we were interested in investigating whether this specific and possibly putative cluster of genes is likely to represent a causal pathway in predicting treatment response. We therefore constructed an enrichment analysis to examine whether those genes are associated with antidepressant treatment response.

Method

Participants

The STAR*D trial has been previously described in detail elsewhere (Fava et al. 2003; Rush et al. 2003). In brief, we focused on data collected at level 1 of the trial, wherein all participants were treated with citalopram. Genotypic and phenotypic data are available from the National Institute of Mental Health (NIMH) Center for Collaborative Genetic Studies on Mental Disorders.

Genotypic information was available for 1939 STAR*D samples. Genotyping was performed for 964 samples from the STAR*D discovery project using the Human Mapping 500 K Array set (Affymetrix Inc., USA). An additional 975 samples was genotyped using the Genome-Wide Human SNP Array 5.0 (Affymetrix Inc., USA). This sample and genotyping techniques have been described in detail by Garriock et al. (2010). The two groups were balanced in terms of variables such as ethnicity, sex and responders and non-responders (Garriock et al. 2010).

Quality control was performed using PLINK v1.07 (Purcell et al. 2007). A total of 496 415 markers were initially included and the following single nucleotide polymorphisms (SNPs) were subsequently removed: 10 144 SNPs with minor allele frequencies (MAFs) <1%; 44 483 SNPs that failed the missingness per marker test (>0.05); 23 121 SNPs on the basis of the Hardy–Weinberg equilibrium test (10×10^{-5}); and 74 SNPs that existed in duplicate. The remaining 418 865 SNPs were used for further analyses.

Population heterogeneity

The –GENOME function was used in PLINK and independence was computed by the –INDEP function on the basis of 50 SNPs in the window, five SNP window shift, and a variance inflation factor of 2. A total of 140 313 independent SNPs were yielded after frequency and genotype pruning. Clusters were computed using a forced entry of 10 clusters using the K function in PLINK. This was done to simulate the 10 clusters used by Garriock et al. (2010) and as previously suggested (Price et al. 2006). Quantile–quantile (Q-Q) plots of expected and observed p values were constructed for the phenotype and adjustment for clusters significantly improved inflation ($\lambda = 1.03$; for Q-Q plot, see online Supplementary Fig. S1).

Phenotype

The phenotype was constructed on the basis of the Garriock et al. (2010) study. In brief, the responders group was comprised of participants that showed a 50% or greater reduction, from baseline to final visit, in the self-report 16-item Quick Inventory of Depressive Symptomatology (QIDS-SR). Participants who did not meet this criterion were non-responders. Only participants who completed a 6-week window of treatment with citalopram were included. Due to different versions of the data files in the STAR*D,

in our study there were available phenotypic data for 1947 participants; 969 showed non-response, whereas 978 responded to antidepressant treatment. Participants who belonged to ethnic groups that comprised only a small proportion of the sample were excluded: Asian ancestry (*n*=21), Pacific Island ancestry (*n*=10), Native American (*n*=16) or mixed (*n*=68) or unknown (*n*=1). Subsequently, there were 1279 non-Hispanic Caucasians, 305 African-Americans and 247 Hispanic Caucasians available for analyses. Of those 1831, 189 had not completed 42 days of treatment and were excluded. Another 88 participants were removed because they had more than 5% of their genotypes missing; 30 participants were shown to be cryptically related to others in the sample [assessed using an identity by descent (IBD) >0.1875 in PLINK; Anderson *et al.* 2010]. Of the remaining participants, 40 participants were coded with ambiguous sex (genetic–clinical sex disagreement assessed in PLINK) and therefore excluded. Covariate data, including the QoL measurement, were not available for 64 participants. This yielded a final sample size of 1426 participants for analyses on the response phenotype.

Assessment of QoL and covariates

The Quality of Life Enjoyment and Satisfaction Questionnaire was used to have an estimate of a person's functioning and QoL upon entering the trial (Daly *et al.* 2010). The measure was administered using an interactive voice response telephone interview, within 72 h of the baseline visit (Daly *et al.* 2010). It is a self-report 16-item questionnaire, which measures specific domains such as quality of social relationships, living or housing situation and physical health (Endicott *et al.* 1993). Each item is scored on a five-point Likert scale (1=very poor to 5=very good) indicating the degree of satisfaction achieved during the past week.

Covariates

We included age and sex as covariates since sociodemographic variables were associated with antidepressant response in the STAR*D (Drago & Serretti, 2011) and it has been found that sex mediates the effects of experimental manipulation of serotoninergic levels on mood (Booij *et al.* 2002). Patients' symptom severity was previously associated with QoL levels, in the STAR*D trial (Trivedi *et al.* 2006*b*), so we included the severity of depressive symptoms at baseline as another covariate (continuous variable). We also controlled for genetic stratification (population clusters).

Statistical analysis

Part A, GWASs

(i) A genomewide interaction model was examined in PLINK, using logistic regression, including the –INTERACTION, and –PARAMETERS functions, with QoL as the interaction factor and treatment response as the outcome, while controlling for covariates sex, age, depression severity and population clusters. Interaction was assessed using the additive model, and QoL was binary (median split: high >43 *v.* low ⩽43). The log odds ratio (OR) ($\beta_{\mathrm{Gadd} \times \mathrm{QoL}}$) of the interaction effect was the main outcome. This model was adopted from Cornelis *et al.* (2012) who suggested that dichotomizing the interaction variable results in a model that is less subject to inflation (λ=about 1.01). Although some information may be lost due to this dichotomization, this saturated model may eliminate some mis-specification under the null hypothesis and decrease type I error (Cornelis *et al.* 2012). We also run an exploratory analysis with response as a continuous trait (percentage change on the QIDS from baseline).

(ii) A genomewide logistic regression model on treatment response, including all covariates (and QoL) and omitting the interaction term, was tested. This analysis was conducted in order to control for a possible lack in reliability due to the inclusion of the interaction term in the first model (Purcell *et al.* 2007). The WGAViewer program (http://compute1.lsrc.duke.edu/softwares/WGAViewer/) was used for the inspection of the GWAS results (Ge *et al.* 2008).

Part B, enrichment analysis

Genes were selected on the basis of their prior implication in G×E interaction studies. G×E studies were searched via published articles and reviews in the National Center for Biotechnology Information (NCBI) PubMed database. Screening of the resulting studies resulted in a list of 15 genes that have been previously examined together with environmental factors in predicting depression status or severity, or antidepressant response. The list constructed for the enrichment analysis involved the following 15 genes: *SLC6A4* (serotonin transporter); *TPH1* (tryptophan hydroxylase 1); *TPH2* (tryptophan hydroxylase 2); *MAOA* (monoamine oxidase A); *HTR1A* (serotonin receptor 1A); *HTR2A* (serotonin receptor 2A); *HTR2C* (serotonin receptor 2C); *CRHR1* (corticotropin-releasing hormone receptor 1); *NR3C1* (glucocorticoid receptor subfamily 3, group C, member 1); *FKBP5*

(FK506 binding protein 5); *BDNF* (brain-derived neurotrophic factor); *SLC6A3* (dopamine transporter); *DRD2* (dopamine receptor D$_2$); *COMT* (catechol-*O*-methyltransferase); and *GABRA2* (GABA A receptor α_2). The enrichment analysis was conducted using the following steps. The genomic sections that contained the genes were identified (PLINK annotation); these sections were imputed (using the CEU HapMap 1000 genomes), checked for quality (imputation quality score >0.9) and pruned (r^2 >0.2); SNPs with low MAF were excluded. Then, the association between the phenotype and the list of SNPs (original and imputed) harboured by those genes was tested. From the remaining part of the genome a random selection of variations was taken 10^{-5} times (the number of total SNPs being the same as in the 'gene list of interest', and all of them belonging to genes randomly selected throughout the genome). The association of this random list of variations with the phenotype was tested. Then, the number of variations significantly ($p<0.05$) associated with the outcome in the 'gene list of interest' and the 'random list' were tested for a significant differential distribution (Fisher's exact test). The permuted *p* was then extracted from the 10^5 Fisher tests, as suggested previously (Phipson & Smyth, 2010). Subsequently, the GeneMANIA program (University of Toronto; http://www.genemania.org/) was used to produce networks of the top pathway list genes on the basis of their genetic interaction, physical interaction, co-expression or co-localization with the identified proteins, as used by other investigators (Siddiqui *et al.* 2012).

Results

The GWAS analysis involved 418 865 SNPs for association with treatment response and moderation by QoL. Of the 1426 participants included in the analysis, 774 participants were responders and 652 were non-responders to treatment. Sociodemographic characteristics are shown in Table 1. Responders had a lower mean age compared with non-responders. There were no significant sex differences on the response outcome, although it can be observed that males were slightly over-represented in the non-responder group ($\chi_1^2=3.47$, $p=0.06$). QoL scores were normally distributed in the sample. In the whole sample, QoL scores correlated with severity of depression: the higher the symptoms, the lower the QoL (Pearson's $r=-0.54$, $p<0.001$). Responders were more likely to report a higher QoL compared with non-responders ($\chi_1^2=33.26$, $p<0.001$). Responders also had lower symptom severity upon entering the trial ($t_{1424}=4.51$, $p<0.001$). There were also differences in ethnicity between responders and non-responders ($\chi_2^2=23.92$, $p<0.001$);

Table 1. *Sociodemographic characteristics of the sample (n=1426)*

	Responders (*n*=774)	Non-responders (*n*=652)
Sex, *n* (%)		
Males	289 (37.3)	275 (42.2)
Females	485 (62.7)	377 (57.8)
Mean age, years (S.D.)*	42.54 (13.40)	45.02 (13.06)
Mean depressive symptoms, QIDS (S.D.)*	14.80 (5.06)	16.01 (5.03)
QoL, *n* (%)*		
High	451 (58.3)	280 (42.9)
Low	323 (41.7)	372 (57.1)
Ethnicity, *n* (%)*		
White non-Hispanic	591 (76.3)	426 (65.3)
African-American	98 (12.7)	140 (21.5)
Hispanic	85 (11.0)	86 (13.2)

S.D., Standard deviation; QIDS, Quick Inventory of Depressive Symptomatology; QoL, quality of life.
* $p<0.001$.

African-Americans were over-represented in the non-responder group compared with the responder group.

Genomewide association analysis

Genomewide interaction

The results of this model are presented in Table 2, where *p* values of the interaction effect are reported. We found one SNP (rs520210) within the *NEDD4L* (neural precursor cell expressed, developmentally down-regulated 4-like E3) gene that was associated with treatment response. Responders were most likely to be carriers of the minor allele (A), compared with non-responders, especially those reporting low QoL upon entering the trial. Visual inspection of the results showed that for all top 10 resulting SNPs, responders had higher frequencies of the minor allele, compared with non-responders. Non-responders with high QoL were more likely to have low minor allele frequencies for the top SNPs (online Supplementary Fig. S2). The Manhattan plot of this analysis is reported in online Supplementary Fig. S3. For purposes of ethnic specificity, and since African-Americans were over-represented in the non-responder group, we repeated the model including only Caucasian individuals. Of the 1426 patients, 238 were excluded, who were of African-American ancestry; this group was too small for a separate GWAS analysis. The results in the Caucasian sample (*n*=1188; 171 Hispanics included) correlate with those of the whole sample; however,

Table 2. *Top 15 SNPs of the genomewide interaction model on citalopram treatment response*

SNP	Rank	p	CHR	Position	Type	Allele[a]	Closest gene	Distance to gene[d]	OR[b]	t
rs520210	1	3.64×10^{-8}	18	55 808 073	Intron variant	A	*NEDD4L*	0	0.76	−5.51
rs4650956	2	1.05×10^{-7}	1	176 996 052	Intron variant	G	*ASTN1*	0	0.77	−5.32
rs6682507	3	2.06×10^{-7}	1	177 000 203	Intron variant	T	*ASTN1*	0	0.77	−5.19
rs156466	4	2.12×10^{-7}	5	8 743 390	Intergenic variant	G	*LOC100505738, SEMA5A*	291 748	0.70	−5.19
rs11701162	5	3.22×10^{-7}	21	44 026 143	Intergenic variant	A	*SLC37A1; PDE9A*	24 593	0.76	−5.11
rs10913287	6	3.31×10^{-7}	1	176 969 316	Intron variant	C	*ASTN1*	0	0.78	−5.11
rs6672689	7	4.16×10^{-7}	1	177 009 394	Intron variant	A	*ASTN1*	0	0.78	−5.06
rs11775176	8	4.20×10^{-7}	8	22 893 034	Intron variant[c]	A	*TNFRSF10B*	0	0.78	−5.06
rs408465	9	4.75×10^{-7}	21	44 006 207	Intergenic variant	T	*SLC37A1, PDE9A*	4657	0.79	−5.04
rs6131468	10	4.89×10^{-7}	20	1 387 687	Intergenic variant	T	*FKBP1A, NSFL1C*	−13 881	0.79	−5.03
rs9534442	11	9.34×10^{-7}	13	47 197 754	Intron variant	T	*LRCH1*	0	0.79	−5.07
rs12427491	12	1.01×10^{-6}	13	47 205 810	Intron variant	T	*LRCH1*	0	0.79	−5.06
rs4814229	13	1.01×10^{-6}	20	1 388 219	Intergenic variant	G	*FKBP1A, NSFL1C*	−14 413	0.79	−5.05
rs10798494	14	1.07×10^{-6}	1	176 964 273	Intron variant	T	*ASTN1*	0	0.79	−4.87
rs449888	15	2.27×10^{-6}	21	44 003 992	Intergenic variant	G	*SLC37A1, PDE9A*	2442	0.79	−4.86

SNP, Single nucleotide polymorphism; CHR, chromosome; OR, odds ratio; *NEDD4L*, neural precursor cell expressed, developmentally down-regulated 4-like E3; *ASTN1*, astrotactin 1; *LOC100505738*, uncharacterized LOC100505738; *SEMA5A*, *TNFRSF10B*, tumour necrosis factor receptor superfamily, member 10b; *SLC37A1*, solute carrier family 37 (glycerol-3-phosphate transporter), member 1; *PDE9A*, phosphodiesterase 9A; *FKBP1A*, FK506 binding protein 1A; *NSFL1C*, NSFL1 (p97) cofactor (p47); *LRCH1*, leucine-rich repeats and calponin homology domain containing 1; NMD, nonsense-mediated decay.

[a] Minor allele.
[b] Odds ratio for minor allele.
[c] NMD transcript variant.
[d] Base pairs.

lower significance values were noted for the top SNP rs520210 ($p = 1.87 \times 10^{-6}$). The rs520210 (*NEDD4L*), rs11775176 (tumour necrosis factor receptor superfamily, member 10b; *TNFRSF10B*), rs156466 (intergenic: *LOC100505738; SEMA5A*), rs408465 (*SLC37A1; PDE9A*) and rs11701162 (*SLC37A1; PDE9A*) were consistently among the top 20 SNPs in both analyses (Table 2; online Supplementary Table S1). Running the model in Caucasians with Hispanics excluded ($n = 1017$), we observed that the rs12427491 (*LRCH1*), rs156466 (intergenic: *LOC100505738; SEMA5A*) and rs408465 (*SLC37A1; PDE9A*) variants were the only ones resulting among the top 15 (online Supplementary Table S2), as in the original model with the whole sample. The rs520210 SNP did not reach genomewide significance ($p = 6.98 \times 10^{-5}$), but the effect was in the same direction with an OR of 0.79 for minor allele carriers. Finally, in an exploratory analysis we investigated response to treatment by using the continuous quantitative trait as an outcome, namely the percentage change in QIDS from baseline. The rs520210 SNP did not reach genomewide significance ($p = 1.35 \times 10^{-5}$) and it did not result among the top 15

SNPs, but the effect remained in the same direction (online Supplementary Table S3). The top SNPs of the latter analysis did not correlate with the ones with response using a dichotomous outcome.

A logistic regression model on treatment response, including all covariates

We performed another logistic regression model on treatment response, entering the QoL factor simply as a covariate (no interaction factor), together with the rest of the covariates (age, sex, symptom severity, population clusters). Results were very similar to the interaction model: the top SNP was also rs520210 ($p = 7.01 \times 10^{-7}$). The top eight SNPs of this analysis were also found within the top SNPs of the interaction analysis. Top SNPs for this analysis are shown in online Supplementary Table S4, while a Manhattan plot is shown in online Supplementary Fig. S4. Results for the two models are very similar; however, higher p values result from the interaction model. From the latter regression model, we also examined each main effect of the covariates separately, including

QoL. No SNP reached genomewide significance; however, QoL and population-cluster variables showed the most significant moderations; Manhattan plots for each covariate are presented in online Supplementary Fig. S5.

Enrichment analysis

10×10^5 Fisher's exact tests served for extrapolating the permutated p value describing the enrichment in the original lists of genes compared with 10×10^5 random ones, with respect to the association of single SNPs with the phenotype under analysis. Each of the 10×10^5 random groups was created as to meet two criteria: (1) being as large as the original one (same number of SNPs); and (2) enlisting SNPs that only belong to genes selected randomly in the genome. We found that the number of SNPs significantly ($p<0.05$) associated with the phenotype in the original list was four times as large as what was expected by chance (21% compared with 5%, permutated (10^5) p value: 0.01976). Further exploration of the original gene list showed that significant SNPs were mainly found within the serotonergic genes (Table 3). The *MAOA, GABRA2, CREB1, CRHR1, NR3C1* and *BDNF* genes did not contain SNPs that were significantly associated with response. A full list of SNPs of the investigated genes and their level of significance is reported in online Supplementary Table S5.

The association between the genes in the form of a biological network is presented in Fig. 1. According to functional enrichment analysis in the GeneMANIA program, most network genes are involved in the regulation of neurotransmission and drug binding, which appear as top functions of the network. Furthermore, the *SLC6A4, SLC6A3* and *DRD2* genes are involved in monoamine transport; the *HTR2A* and *DRD2* genes are involved in G-protein coupled amine receptor activity, and the *SLC6A3* and *COMT* genes are involved in the neurotransmitter biosynthetic process. A more detailed account of the functionality of the network of genes can be found in online Supplementary Table S6.

Discussion

The present study undertook two approaches in order to predict antidepressant treatment response. First, we examined whether genetic variations interact with the patients' levels of QoL to predict antidepressant response, after controlling for demographic characteristics, depression severity and population stratification. Second, we conducted an enrichment analysis, which explored whether candidate genes (that have emerged from prior gene–environment interaction studies) are associated with treatment response, in a genomewide context.

Genomewide interaction

Our model for predicting response to citalopram in the STAR*D sample yielded interesting candidate genetic loci. The top finding that survived genomewide significance involves a SNP (rs520210) of the *NEDD4L* gene, which has not been previously reported. We found that responders were most likely to be carriers of the minor allele (A), compared with non-responders, especially those reporting low QoL upon entering the trial. QoL probably explains a certain degree of variance in treatment response, since more pronounced genetic effects were observed in the interaction model and since QoL was shown to be among the most (statistically) relevant covariates (online Supplementary Fig. S5). However, a lower significance level (not reaching genomewide significance) was observed for the rs520210 SNP in the analysis with the Caucasian sample only, where it was among the top SNPs, when Hispanics were included, and it did not result among the top SNPs when Hispanics were excluded ($n = 1017$). Furthermore, few SNPs and genes were consistent across all models. Decreased statistical power due to the lower sample size could explain these discrepancies; however, other factors may also play a role. African-Americans were over-represented in the non-responder group, which may have influenced the results observed in the whole sample. Although statistically controlled for, effects of population stratification cannot be excluded. For this reason, we have also reported results in Caucasians separately (see online Supplementary Tables S1 and S2). Several SNPs showed a trend of association in the whole sample, but not in the smaller ethnic homogeneous samples, whereas a number of genes or intergenic regions (*NEDD4L, LRCH1, SLC37A1, TNFRSF10B, SEMA5A*) appeared among the top hits across models. The relative lack of consistency raises the possibility that some observations are false positive, largely dependent on sample size, or ethnic group, and easily disappear when group sizes change.

Nevertheless, our analyses yielded some interesting loci that can be further investigated in future studies. The *NEDD4L* (18q21.31) gene is the gene for encoding the ubiquitin protein ligase enzyme. Ubiquitins are small regulatory proteins that direct protein recycling and are then functional to the housekeeping activities of cells. Our result is consistent with the one reported by Garriock *et al.* (2010) who found another ubiquitin E3 protein ligase (*UBE3C*) gene involved in antidepressant response in the same sample. Both genes are involved in the ubiquitin-dependent protein catabolic

Table 3. *Significant SNPs within significant genes of the pathway*[a]

Gene	SNP	CHR	Position	Major allele	Minor allele	Non-responders MAF	Responders MAF	OR[b]	(95% CI)	p
HTR2A	rs9562683	13	46298746	A	C	0.288	0.353	1.354	(1.155–1.588)	0.00033
	rs977003	13	46313002	A	C	0.465	0.526	1.278	(1.102–1.482)	0.00200
	rs4942577	13	46295455	A	G	0.275	0.328	0.776	(0.660–0.913)	0.00389
	rs985934	13	46353726	A	G	0.359	0.411	0.802	(0.689–0.934)	0.00633
	rs9526245	13	46343968	G	T	0.197	0.240	1.289	(1.077–1.542)	0.00868
	rs2770297	13	46344666	C	T	0.252	0.297	1.255	(1.063–1.482)	0.01054
	rs1928040	13	46345237	A	G	0.501	0.454	0.828	(0.714–0.961)	0.01852
	rs1328683	13	46356875	C	T	0.187	0.223	0.803	(0.668–0.965)	0.02191
	rs6313	13	46367941	A	G	0.423	0.378	0.830	(0.714–0.965)	0.02214
	rs985933	13	46353864	A	G	0.370	0.414	1.202	(1.034–1.399)	0.02470
	rs6316	13	46369224	C	T	0.025	0.013	0.522	(0.297–0.917)	0.02967
	rs582854	13	46343878	G	T	0.371	0.411	0.845	(0.725–0.984)	0.03653
	rs7335733	13	46294464	G	T	0.015	0.007	0.483	(0.229–1.019)	0.04095
SLC6A3	rs2550948	5	1503444	C	T	0.504	0.442	0.780	(0.672–0.904)	0.00077
	rs2937639	5	1496728	A	G	0.475	0.424	0.811	(0.700–0.941)	0.00326
	rs2937649	5	1510317	C	T	0.021	0.008	0.356	(0.180–0.703)	0.00375
	rs2963242	5	1490726	C	T	0.028	0.012	2.345	(1.342–4.097)	0.00461
	rs16877324	5	1428977	A	G	0.039	0.023	1.753	(1.132–2.712)	0.01372
SLC6A4	rs8076005	17	25571336	A	G	0.290	0.243	1.275	(1.079–1.506)	0.00696
TPH1	rs10832879	11	18027232	C	T	0.030	0.009	3.214	(1.730–5.973)	0.00032
	rs7937368	11	18030005	A	T	0.475	0.423	1.232	(1.062–1.429)	0.01148
	rs211135	11	17980543	A	C	0.494	0.445	0.822	(0.709–0.952)	0.01172
	rs1079785	11	18028849	A	G	0.475	0.424	0.814	(0.702–0.944)	0.01240
	rs10741734	11	18001224	A	G	0.360	0.402	0.837	(0.718–0.975)	0.04109
	rs1800532	11	18004392	G	T	0.349	0.388	0.844	(0.725–0.984)	0.04373
TPH2	rs10879355	12	70699276	C	T	0.426	0.382	1.200	(1.033–1.395)	0.01777
	rs1386485	12	70698634	A	C	0.440	0.396	1.200	(1.032–1.395)	0.01971
	rs1386483	12	70698761	A	G	0.443	0.400	0.838	(0.721–0.974)	0.02071
	rs11179052	12	70698968	C	T	0.404	0.364	1.187	(1.020–1.381)	0.02788
	rs11179065	12	70707768	A	G	0.109	0.084	0.751	(0.585–0.965)	0.04181
	rs11179003	12	70629554	C	T	0.048	0.034	1.461	(1.004–2.125)	0.04773
COMT	rs5993889	22	18335375	G	T	0.026	0.011	0.414	(0.230–0.745)	0.00336
	rs9332377	22	18335692	A	G	0.206	0.170	0.791	(0.654–0.957)	0.01578
FKBP5	rs16878806	6	35677097	A	G	0.075	0.047	1.642	(1.201–2.244)	0.00364
	rs9394312	6	35780308	C	G	0.409	0.460	1.235	(1.061–1.437)	0.00600
	rs1475774	6	35727532	C	T	0.050	0.032	1.608	(1.101–2.348)	0.01840
	rs9470056	6	35630123	A	G	0.167	0.198	1.224	(1.010–1.484)	0.04310
DRD2	rs4581480	11	112829684	C	T	0.189	0.147	0.738	(0.605–0.900)	0.00546
	rs17529477	11	112822277	C	T	0.261	0.298	0.830	(0.703–0.979)	0.01911
	rs2245805	11	112795909	A	C	0.310	0.269	0.821	(0.698–0.966)	0.02054
	rs2002453	11	112794508	A	G	0.309	0.269	0.823	(0.699–0.968)	0.02199
	rs11214606	11	112815079	A	G	0.035	0.052	1.505	(1.036–2.187)	0.03297
	rs4421776	11	112866861	A	G	0.265	0.302	1.200	(1.019–1.414)	0.03708
	rs4611239	11	112869481	C	T	0.266	0.302	0.838	(0.711–0.987)	0.04428

SNP, Single nucleotide polymorphism; CHR, chromosome; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; *HTR2A*, serotonin receptor 2A; *SLC6A3*, dopamine transporter; *SLC6A4*, serotonin transporter; *TPH1*, tryptophan hydroxylase 1; *TPH2*, tryptophan hydroxylase 2; *COMT*, catechol-*O*-methyltransferase; *FKBP5*, FK506 binding protein 5; *DRD2*, dopamine receptor D2.

[a] Total number of SNPs examined in: *HTR2A*, 39; *SLC6A3*, 18; *SLC6A4*, 9; *TPH1*, 9; *TPH2*, 32; *COMT*, 14; *FKBP5*, 9; *DRD2*, 19. For a full list of SNPs and genes within the pathway please refer to online Supplementary Table S5.
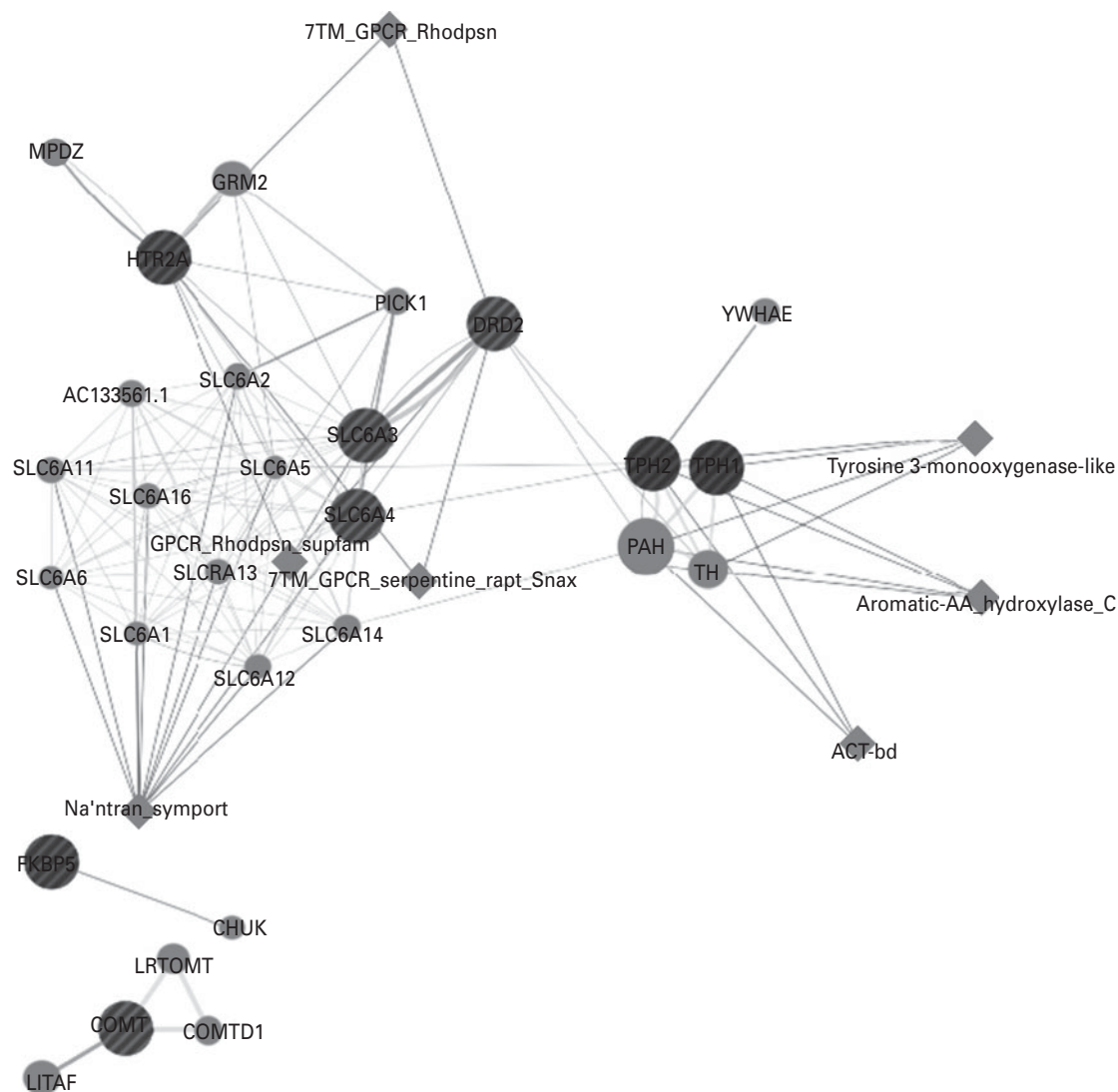
[b] OR for minor allele.

Fig. 1. Network of the enriched genes. Network analyses showed that the genes involved in monoamine transport and regulation of neurotransmitter levels (*DRD2, SLC6A3, SLC6A4*; dopamine receptor D2, dopamine transporter, serotonin transporter, respectively) are interrelated; *HTR2A* (serotonin receptor 2A) is primarily involved in drug binding modulation and is interconnected with other serotonergic genes. *MPD2*, monophosphate deaminase 2; *GRM2*, glutamate receptor metabotropic 2; *PICK1*, protein interacting with PRKCA 1; *AC133561.1*, uncharacterized protein; *YWHAE*, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide; *TPH2*, tryptophan hydroxylase 2; *TPH1*, tryptophan hydroxylase 1; *PAH*, phenylalanine hydroxylase; *TH*, tyrosine hydroxylase; *ACT-bd*, amino acid binding ACT domain protein; *FKBP5*, FK506 binding protein 5; *CHUK*, conserved helix-loop-helix ubiquitous kinase; *LRTOMT*, leucine rich transmembrane and *0*-methyltransferase domain containing; *COMT*, catechol-*O*-methyltransferase; *LITAF*, lipopolysaccharide-induced tumour necrosis factor (TNF) factor; *COMTD1*, catechol-*O*-methyltransferase domain containing 1. Further functions are shown in online Supplementary Table S6. The network was designed using GeneMANIA (University of Toronto; http://www.genemania.org/).

process, and inhibitor-SMAD (I-SMAD) binding. Ubiquitin proteasome pathway components (*PSMD9, USP36*) were recently observed in a pathway analysis exploring the risk for MDD (Wong *et al.* 2012). Furthermore, the area where *NEDD4L* is located (namely, area 18q21–23) has been previously shown to contain significant loci in a GWAS of bipolar II disorder (Nwulia *et al.* 2007) and in a linkage study of

bipolar I and bipolar II (Baron, 2002). In addition, considering the role of the *NEDD4L* gene in processes involving sodium imbalances, polymorphisms in *NEDD4L* have been implicated extensively in hypertension (Kristjansson *et al.* 2002; Araki *et al.* 2008), as well as in treatment efficacy of *β*-blockers in hypertensive patients (Svensson-Färbom *et al.* 2011). Association between this gene and hypertension is particularly

interesting, since it has been recently shown that depressed patients under serotonergic antidepressant treatment have an increased risk of hypertension (Licht *et al.* 2009). Future studies may want to further investigate this gene in G×E models of treatment response, with hypertension or related illness as the 'E' parameter.

From the top findings of the GWAS in the whole sample, several SNPs belong to the astrotactin (*ASTN1*) gene. The neuronal protein astrotactin (ASTN1) is a well-known receptor for glial-guided neuronal migration (Fishell & Hatten, 1991; Zheng *et al.* 1996), a fundamental process in the development of the laminar structure of cortical regions of the brain (Wilson *et al.* 2010). Interestingly, a pharmaco-genetic GWAS on antidepressant response in an independent sample (GENome-based therapeutic drugs for DEPression; GENDEP) located a gene [*UST* (uronyl-2-sulfotransferase) gene, 6q25.1)], which also plays a role in neuronal migration (Uher *et al.* 2010). The *TNFRSF10B* gene is another interesting gene that resulted among the top 10 SNPs in the whole sample and in Caucasians (including Hispanics). This gene binds to the TRAIL protein [tumour necrosis factor (TNF)-related apoptosis-inducing ligand, a ligand that induces the process of cell death], and plays a major role in the apoptotic process and activation of nuclear factor-κB (NF-κB)-inducing kinase activity. NF-κB has been reported as a mediator of stress-impaired neurogenesis and depressive-like phenotypes (Koo *et al.* 2010), and mediator of stress response (LaPlant *et al.* 2009), in animal models.

Furthermore, other genes that were consistently observed in the Caucasian, as well as in the whole, sample were an intergenic region near *SEMA5A*, which has been previously associated with autism (Weiss *et al.* 2009), intergenic regions near *SLC37A1* (which is a glycerol-3-phosphate transporter), and the *LRCH1* gene; the latter two genes have not been associated yet with psychiatric disorders. Finally, intergenic variations near *FKBP12* arose as promising loci. *FKBP12* is the prototype of the FKBP family and binds immunosuppressive drugs, such as FK506 and rapamycin (Harding *et al.* 1989). From the FKBP group, *FKBP5* has been previously associated with the recurrence of depressive episodes and antidepressant response in an independent study (Binder *et al.* 2004), and in the STAR*D trial using a candidate gene approach (Lekman *et al.* 2008). Furthermore, the *FKBP5* modulated effects of childhood trauma in predicting major depression (Appel *et al.* 2011; Zimmermann *et al.* 2011). The *FKBP5* gene was also one of the significant genes implicated in our enrichment analysis.

Enrichment

We found that serotonergic genes implicated in previously published G×E interaction studies are part of a network, which is likely to affect antidepressant treatment response. Variation in the *HTR2A* gene has been associated with antidepressant response or remission in a number of studies (Uher *et al.* 2009; Horstmann *et al.* 2010; Kishi *et al.* 2010; Lucae *et al.* 2010). The *HTR2A* gene was previously studied in the STAR*D sample, using a candidate gene approach, by McMahon *et al.* (2006), who examined 68 candidate genes and found significant associations with only two intronic variants in the *HTR2A* and treatment response. *HTR2A* influence on citalopram response was reproduced by Paddock *et al.* (2007) who used a larger STAR*D sample and examined another large number of candidate markers. To date, several variants within *HTR2A* have been proposed to affect anti-depressant response, but failures to replicate findings also exist (Drago *et al.* 2009). By using a different methodological approach from the above studies, our observations from the enrichment analysis further support the role of the serotonergic genes in influencing antidepressant response to citalopram. A variant in the *TPH1* gene also showed a strong effect (OR=3.2), which could be worthy of further investigation. Furthermore, several significant SNPs were found within the *FKBP5* gene, which has been previously associated with treatment outcome in depression in two independent studies (Binder *et al.* 2004; Kirchheiner *et al.* 2008). In the STAR*D trial, it was previously examined using a candidate gene approach (only two SNPs) and an association was found with remission (Lekman *et al.* 2008).

Challenges and limitations

Our sample size has insufficient power to detect interaction effects (Murcray *et al.* 2011). Nevertheless, we hypothesize that by considering a multifactorial, or more individualized, model of antidepressant response, genetic effects may become more detectable. There is increasing evidence emerging from GWASs that there are no genetic variants that contribute with a large effect (Flint & Munafo, 2013). Hence, apart from the large sample sizes that would provide sufficient power to observe small effects, designing multi-variate models of prediction with the tools available in each study could assist in unravelling variants of risk (Cohen-Woods *et al.* 2013).

Several limitations need to be acknowledged. A major limitation with regard to the QoL measure is that the level of depression severity of the patient could have biased responses and might be intertwined with the measure itself. Exploring the relationship

between depression severity and QoL in our sample, we found a moderate correlation ($r = 0.54$); hence, approximately 29% ($r^2 = 0.29$) of a patient's QoL can be explained by his/her levels of depressive symptoms upon entering the trial. Consequently, we statistically controlled for the severity of depression in our analyses. Depression severity is an inherent part of the disorder; in fact, we can diagnose the disorder due to the level of severity. QoL, however, also contains aspects on physical health and quality of social relationships that could be independent of (or not affected by) the individual's symptomatology. This is supported by a factor analysis on the QoL measure showing distinct factors 'physical health', 'leisure time activities', 'social relationships' and 'subjective feelings', the latter being more likely to overlap with variance explained by depressive symptoms (Ritsner *et al.* 2005). Interestingly, a recent study in the STAR*D sample showed that QoL was a factor that contributed significantly to the patients' Individual Burden of Illness Index, and the variance explained by QoL was additional to that explained by depressive symptoms in a principal component analysis (Cohen *et al.* 2013). These additional aspects (satisfaction with work, health issues, social relationships, family relationships, economic condition, and leisure time) represented by the QoL measure could be considered as 'external' conditions, possibly approaching a proxy of an environmental contributor. Apart from depressive symptoms, several other factors that may have affected reports on QoL could be the duration of the episode, education, family status and other variables. Within the STAR*D trial, factors related to QoL have been previously examined and only age and depression severity were shown to have an important contribution (Daly *et al.* 2010), both factors being taken into account in the present analysis. Nevertheless, future studies would benefit from more robust and independent assessments. Ultimately, the assessment of important environmental adversities, such as childhood maltreatment, would be an important step in genomewide environment interactions (Karg & Sen, 2012), since such events play a significant role in the onset of depression and response to treatment (Uher, 2011). It would be interesting, therefore, to explore these G × E interactions in large samples, such as the GENDEP or MARS (Munich Antidepressant Response Signature), where environmental variables may be available. Finally, other factors that are likely to moderate the relationship between genes and antidepressant response could not be reported. For example, epigenetic factors possibly conceal some important effects in such investigations and are worthy of future research. Our study also suffers from other limitations of the STAR*D trial, as already reported previously (Laje *et al.* 2009; Garriock *et al.* 2010).

Our study provides new insights for interesting genetic markers and mechanistic pathways; however, the variance explained is still modest. In addition, our enrichment analysis revealed that certain genes implicated in prior G × E studies are likely to affect antidepressant treatment response. These findings provide further incentives for research into the mechanisms of the proposed gene-related proteins in relation to antidepressant action.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0033291713001554.

Declaration of Interest

A.S. is or has been a consultant/speaker for Abbott, Angelini, AstraZeneca, Clinical Data, Boheringer, Bristol–Myers Squibb, Eli Lilly, GlaxoSmithKline, Italfarmaco, Janssen, Lundbeck, Pfizer, Sanofi and Servier.

References

Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT (2010). Data quality control in genetic case–control association studies. *Nature Protocols* **5**, 1564–1573.

Appel K, Schwahn C, Mahler J, Schulz A, Spitzer C, Fenske K, Stender J, Barnow S, John U, Teumer A, Biffar R, Nauck M, Volzke H, Freyberger HJ, Grabe HJ (2011). Moderation of adult depression by a polymorphism in the *FKBP5* gene and childhood physical abuse in the general population. *Neuropsychopharmacology* **36**, 1982–1991.

Araki N, Umemura M, Miyagi Y, Yabana M, Miki Y, Tamura K, Uchino K, Aoki R, Goshima Y, Umemura S, Ishigami T (2008). Expression, transcription, and possible antagonistic interaction of the human *Nedd4L* gene variant: implications for essential hypertension. *Hypertension* **51**, 773–777.

Baron M (2002). Manic-depression genes and the new millennium: poised for discovery. *Molecular Psychiatry* **7**, 342–358.

Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B, Papiol S, Seaman S, Lucae S, Kohli MA, Nickel T, Kunzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Modell S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Kohnlein O, Dabitz H, Bruckl T, Muller N, Pfister H, Lieb R, Mueller JC, Lohmussaar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B (2004). Polymorphisms in *FKBP5* are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nature Genetics* **36**, 1319–1325.

Booij L, Van der Does W, Benkelfat C, Bremner JD, Cowen PJ, Fava M, Gillin C, Leyton M, Moore P, Smith KA, Van der Kloot WA (2002). Predictors of mood response to acute tryptophan depletion. A reanalysis. *Neuropsychopharmacology* **27**, 852–861.

Cohen RM, Greenberg JM, Ishak WW (2013). Incorporating multidimensional patient-reported outcomes of symptom severity, functioning, and quality of life in the individual burden of illness index for depression to measure treatment impact and recovery in MDD. *Journal of the American Medical Association Psychiatry* **70**, 343–350.

Cohen-Woods S, Craig IW, McGuffin P (2013). The current state of play on the molecular genetics of depression. *Psychological Medicine* **43**, 673–687.

Cornelis MC, Tchetgen EJ, Liang L, Qi L, Chatterjee N, Hu FB, Kraft P (2012). Gene-environment interactions in genome-wide association studies: a comparative study of tests applied to empirical studies of type 2 diabetes. *American Journal of Epidemiology* **175**, 191–202.

Daly EJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Gaynes BN, Warden D, Morris DW, Luther JF, Farabaugh A, Cook I, Rush AJ (2010). Health-related quality of life in depression: a STAR*D report. *Annals of Clinical Psychiatry* **22**, 43–55.

Drago A, De Ronchi D, Serretti A (2009). Pharmacogenetics of antidepressant response: an update. *Human Genomics* **3**, 257–274.

Drago A, Serretti A (2011). Sociodemographic features predict antidepressant trajectories of response in diverse antidepressant pharmacotreatment environments: a comparison between the STAR*D study and an independent trial. *Journal of Clinical Psychopharmacology* **31**, 345–348.

Endicott J, Nee J, Harrison W, Blumenthal R (1993). Quality of Life Enjoyment and Satisfaction Questionnaire: a new measure. *Psychopharmacology Bulletin* **29**, 321–326.

Fava M, Rush AJ, Trivedi MH, Nierenberg AA, Thase ME, Sackeim HA, Quitkin FM, Wisniewski S, Lavori PW, Rosenbaum JF, Kupfer DJ (2003). Background and rationale for the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study. *Psychiatric Clinics of North America* **26**, 457–494.

Fishell G, Hatten ME (1991). Astrotactin provides a receptor system for CNS neuronal migration. *Development* **113**, 755–765.

Flint J, Munafo MR (2013). Candidate and non-candidate genes in behavior genetics. *Current Opinion in Neurobiology* **23**, 57–61.

Franchini L, Serretti A, Gasperini M, Smeraldi E (1998). Familial concordance of fluvoxamine response as a tool for differentiating mood disorder pedigrees. *Journal of Psychiatric Research* **32**, 255–259.

Garriock HA, Kraft JB, Shyn SI, Peters EJ, Yokoyama JS, Jenkins GD, Reinalda MS, Slager SL, McGrath PJ, Hamilton SP (2010). A genomewide association study of citalopram response in major depressive disorder. *Biological Psychiatry* **67**, 133–138.

Ge D, Zhang K, Need AC, Martin O, Fellay J, Urban TJ, Telenti A, Goldstein DB (2008). WGAViewer: software for genomic annotation of whole genome association studies. *Genome Research* **18**, 640–643.

Harding MW, Galat A, Uehling DE, Schreiber SL (1989). A receptor for the immunosuppressant FK506 is a *cis-trans* peptidyl-prolyl isomerase. *Nature* **341**, 758–760.

Horstmann S, Lucae S, Menke A, Hennings JM, Ising M, Roeske D, Muller-Myhsok B, Holsboer F, Binder EB (2010). Polymorphisms in *GRIK4*, *HTR2A*, and *FKBP5* show interactive effects in predicting remission to antidepressant treatment. *Neuropsychopharmacology* **35**, 727–740.

Ising M, Lucae S, Binder EB, Bettecken T, Uhr M, Ripke S, Kohli MA, Hennings JM, Horstmann S, Kloiber S, Menke A, Bondy B, Rupprecht R, Domschke K, Baune BT, Arolt V, Rush AJ, Holsboer F, Muller-Myhsok B (2009). A genomewide association study points to multiple loci that predict antidepressant drug treatment outcome in depression. *Archives of General Psychiatry* **66**, 966–975.

Karg K, Sen S (2012). Gene x environment interaction models in psychiatric genetics. *Current Topics in Behavioral Neurosciences* **12**, 441–462.

Kato M, Serretti A (2010). Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. *Molecular Psychiatry* **15**, 473–500.

Keers R, Uher R (2012). Gene–environment interaction in major depression and antidepressant treatment response. *Current Psychiatry Reports* **14**, 129–137.

Keers R, Uher R, Huezo-Diaz P, Smith R, Jaffee S, Rietschel M, Henigsberg N, Kozel D, Mors O, Maier W, Zobel A, Hauser J, Souery D, Placentino A, Larsen ER, Dmitrzak-Weglarz M, Gupta B, Hoda F, Craig I, McGuffin P, Farmer AE, Aitchison KJ (2011). Interaction between serotonin transporter gene variants and life events predicts response to antidepressants in the GENDEP project. *Pharmacogenomics Journal* 11, 138–145.

Khoury MJ, Wacholder S (2009). Invited commentary: from genome-wide association studies to gene–environment-wide interaction studies – challenges and opportunities. *American Journal of Epidemiology* 169, 227–230.

Kirchheiner J, Lorch R, Lebedeva E, Seeringer A, Roots I, Sasse J, Brockmöller J (2008). Genetic variants in *FKBP5* affecting response to antidepressant drug treatment. *Pharmacogenomics* 9, 841–846.

Kishi T, Yoshimura R, Kitajima T, Okochi T, Okumura T, Tsunoka T, Yamanouchi Y, Kinoshita Y, Kawashima K, Naitoh H, Nakamura J, Ozaki N, Iwata N (2010). *HTR2A* is associated with SSRI response in major depressive disorder in a Japanese cohort. *Neuromolecular Medicine* 12, 237–242.

Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS (2010). Nuclear factor-κB is a critical mediator of stress-impaired neurogenesis and depressive behavior. *Proceedings of the National Academy of Sciences USA* 107, 2669–2674.

Kristjansson K, Manolescu A, Kristinsson A, Hardarson T, Knudsen H, Ingason S, Thorleifsson G, Frigge ML, Kong A, Gulcher JR, Stefansson K (2002). Linkage of essential hypertension to chromosome 18q. *Hypertension* 39, 1044–1049.

Laje G, Perlis RH, Rush AJ, McMahon FJ (2009). Pharmacogenetics studies in STAR*D: strengths, limitations, and results. *Psychiatric Services* 60, 1446–1457.

LaPlant Q, Chakravarty S, Vialou V, Mukherjee S, Koo JW, Kalahasti G, Bradbury KR, Taylor SV, Maze I, Kumar A, Graham A, Birnbaum SG, Krishnan V, Truong HT, Neve RL, Nestler EJ, Russo SJ (2009). Role of nuclear factor κB in ovarian hormone-mediated stress hypersensitivity in female mice. *Biological Psychiatry* 65, 874–880.

Lekman M, Laje G, Charney D, Rush AJ, Wilson AF, Sorant AJ, Lipsky R, Wisniewski SR, Manji H, McMahon FJ, Paddock S (2008). The *FKBP5*-gene in depression and treatment response – an association study in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) cohort. *Biological Psychiatry* 63, 1103–1110.

Licht CM, de Geus EJ, Seldenrijk A, van Hout HP, Zitman FG, van Dyck R, Penninx BW (2009). Depression is associated with decreased blood pressure, but antidepressant use increases the risk for hypertension. *Hypertension* 53, 631–638.

Lucae S, Ising M, Horstmann S, Baune BT, Arolt V, Muller-Myhsok B, Holsboer F, Domschke K (2010). *HTR2A* gene variation is involved in antidepressant treatment response. *European Neuropsychopharmacology* 20, 65–68.

Malhotra AK (2010). The pharmacogenetics of depression: enter the GWAS. *American Journal of Psychiatry* 167, 493–495.

Mandelli L, Marino E, Pirovano A, Calati R, Zanardi R, Colombo C, Serretti A (2009). Interaction between SERTPR and stressful life events on response to antidepressant treatment. *European Neuropsychopharmacology* 19, 64–67.

McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, Sorant AJ, Papanicolaou GJ, Laje G, Fava M, Trivedi MH, Wisniewski SR, Manji H (2006). Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *American Journal of Human Genetics* 78, 804–814.

Murcray CE, Lewinger JP, Conti DV, Thomas DC, Gauderman WJ (2011). Sample size requirements to detect gene–environment interactions in genome-wide association studies. *Genetic Epidemiology* 35, 201–210.

Nwulia EA, Miao K, Zandi PP, Mackinnon DF, DePaulo Jr JR, McInnis MG (2007). Genome-wide scan of bipolar II disorder. *Bipolar Disorders* 9, 580–588.

O'Reilly RL, Bogue L, Singh SM (1994). Pharmacogenetic response to antidepressants in a multicase family with affective disorder. *Biological Psychiatry* 36, 467–471.

Paddock S, Laje G, Charney D, Rush AJ, Wilson AF, Sorant AJ, Lipsky R, Wisniewski SR, Manji H, McMahon FJ (2007). Association of GRIK4 with outcome of antidepressant treatment in the STAR*D cohort. *American Journal of Psychiatry* 164, 1181–1188.

Papakostas GI, Fava M (2008). Predictors, moderators, and mediators (correlates) of treatment outcome in major depressive disorder. *Dialogues in Clinical Neuroscience* 10, 439–451.

Papakostas GI, Fava M (2009). Does the probability of receiving placebo influence clinical trial outcome? A meta-regression of double-blind, randomized clinical trials in MDD. *European Neuropsychopharmacology* 19, 34–40.

Phipson B, Smyth GK (2010). Permutation *P*-values should never be zero: calculating exact *P*-values when permutations are randomly drawn. *Statistical Applications in Genetics and Molecular Biology* 9, 39.

Porcelli S, Drago A, Fabbri C, Gibiino S, Calati R, Serretti A (2011). Pharmacogenetics of antidepressant response. *Journal of Psychiatry and Neuroscience* 36, 87–113.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics* 38, 904–909.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* 81, 559–575.

Pyne JM, Bullock D, Kaplan RM, Smith TL, Gillin JC, Golshan S, Kelsoe JR, Williams DK (2001). Health-related quality-of-life measure enhances acute treatment response prediction in depressed inpatients. *Journal of Clinical Psychiatry* 62, 261–268.

Ritsner M, Kurs R, Gibel A, Ratner Y, Endicott J (2005). Validity of an abbreviated quality of life enjoyment and satisfaction questionnaire (Q-LES-Q-18) for schizophrenia, schizoaffective, and mood disorder patients. *Quality of Life Research* 14, 1693–1703.

Rush AJ, Trivedi M, Fava M (2003). Depression, IV: STAR*D treatment trial for depression. *American Journal of Psychiatry* **160**, 237.

Serretti A, Benedetti F, Zanardi R, Smeraldi E (2005). The influence of serotonin transporter promoter polymorphism (SERTPR) and other polymorphisms of the serotonin pathway on the efficacy of antidepressant treatments. *Progress in Neuro-psychopharmacology and Biological Psychiatry* **29**, 1074–1084.

Siddiqui NU, Li X, Luo H, Karaiskakis A, Hou H, Kislinger T, Westwood JT, Morris Q, Lipshitz HD (2012). Genome-wide analysis of the maternal-to-zygotic transition in Drosophila primordial germ cells. *Genome Biology* **13**, R11.

Souery D, Oswald P, Massat I, Bailer U, Bollen J, Demyttenaere K, Kasper S, Lecrubier Y, Montgomery S, Serretti A, Zohar J, Mendlewicz J (2007). Clinical factors associated with treatment resistance in major depressive disorder: results from a European multicenter study. *Journal of Clinical Psychiatry* **68**, 1062–1070.

Svensson-Färbom P, Wahlstrand B, Almgren P, Dahlberg J, Fava C, Kjeldsen S, Hedner T, Melander O (2011). A functional variant of the NEDD4L gene is associated with beneficial treatment response with β-blockers and diuretics in hypertensive patients. *Journal of Hypertension* **29**, 388–395.

Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K, Biggs MM, Balasubramani GK, Fava M (2006a). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *American Journal of Psychiatry* **163**, 28–40.

Trivedi MH, Rush AJ, Wisniewski SR, Warden D, McKinney W, Downing M, Berman SR, Farabaugh A, Luther JF, Nierenberg AA, Callan JA, Sackeim HA (2006b). Factors associated with health-related quality of life among outpatients with major depressive disorder: a STAR*D report. *Journal of Clinical Psychiatry* **67**, 185–195.

Uher R (2011). Genes, environment, and individual differences in responding to treatment for depression. *Harvard Review of Psychiatry* **19**, 109–124.

Uher R, Huezo-Diaz P, Perroud N, Smith R, Rietschel M, Mors O, Hauser J, Maier W, Kozel D, Henigsberg N, Barreto M, Placentino A, Dernovsek MZ, Schulze TG, Kalember P, Zobel A, Czerski PM, Larsen ER, Souery D, Giovannini C, Gray JM, Lewis CM, Farmer A, Aitchison KJ, McGuffin P, Craig I (2009). Genetic predictors of response to antidepressants in the GENDEP project. *Pharmacogenomics Journal* **9**, 225–233.

Uher R, McGuffin P (2010). The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update. *Molecular Psychiatry* **15**, 18–22.

Uher R, Perroud N, Ng MY, Hauser J, Henigsberg N, Maier W, Mors O, Placentino A, Rietschel M, Souery D, Zagar T, Czerski PM, Jerman B, Larsen ER, Schulze TG, Zobel A, Cohen-Woods S, Pirlo K, Butler AW, Muglia P, Barnes MR, Lathrop M, Farmer A, Breen G, Aitchison KJ, Craig I, Lewis CM, McGuffin P (2010). Genome-wide pharmacogenetics of antidepressant response in the GENDEP project. *American Journal of Psychiatry* **167**, 555–564.

Weiss LA, Arking DE, Daly MJ, Chakravarti A (2009). A genome-wide linkage and association scan reveals novel loci for autism. *Nature* **461**, 802–808.

Wilson PM, Fryer RH, Fang Y, Hatten ME (2010). *Astn2*, a novel member of the astrotactin gene family, regulates the trafficking of ASTN1 during glial-guided neuronal migration. *Journal of Neuroscience* **30**, 8529–8540.

Wong ML, Dong C, Andreev V, Arcos-Burgos M, Licinio J (2012). Prediction of susceptibility to major depression by a model of interactions of multiple functional genetic variants and environmental factors. *Molecular Psychiatry* **17**, 624–633.

Zheng C, Heintz N, Hatten ME (1996). CNS gene encoding astrotactin, which supports neuronal migration along glial fibers. *Science* **272**, 417–419.

Zimmermann P, Bruckl T, Nocon A, Pfister H, Binder EB, Uhr M, Lieb R, Moffitt TE, Caspi A, Holsboer F, Ising M (2011). Interaction of FKBP5 gene variants and adverse life events in predicting depression onset: results from a 10-year prospective community study. *American Journal of Psychiatry* **168**, 1107–1116.