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ORIGINAL ARTICLE

Genome-wide association study of increasing suicidal ideation during antidepressant treatment in the GENDEP project

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Suicidal thoughts during antidepressant treatment have been the focus of several candidate gene association studies. The aim of the present genome-wide association study was to identify additional genetic variants involved in increasing suicidal ideation during escitalopram and nortriptyline treatment. A total of 706 adult participants of European ancestry, treated for major depression with escitalopram or nortriptyline over 12 weeks in the Genome-Based Therapeutic Drugs for Depression (GENDEP) study were genotyped with Illumina Human 610-Quad Beadchips (Illumina, San Diego, CA, USA). A total of 244 subjects experienced an increase in suicidal ideation during follow-up. The genetic marker most significantly associated with increasing suicidality (8.28×10^{-7}) was a single-nucleotide polymorphism (SNP; rs11143230) located 30 kb downstream of a gene encoding guanine deaminase (*GDA*) on chromosome 9q21.13. Two suggestive drug-specific associations within *KCNIP4* (Kv channel-interacting protein 4; chromosome 4p15.31) and near *ELP3* (elongation protein 3 homolog; chromosome 8p21.1) were found in subjects treated with escitalopram. Suggestive drug by gene interactions for two SNPs near structural variants on chromosome 4q12, one SNP in the apolipoprotein O (*APOO*) gene on chromosome Xp22.11 and one on chromosome 11q24.3 were found. The most significant association within a set of 33 candidate genes was in the neurotrophic tyrosine kinase receptor type 2 (*NTRK2*) gene. Finally, we also found trend for an association within genes previously associated with psychiatric phenotypes indirectly linked to suicidal behavior, that is, *GRIP1*, *NXPH1* and *ANK3*. The results suggest novel pathways involved in increasing suicidal ideation during antidepressant treatment and should help to target treatment to reduce the risk of this dramatic adverse event. Limited power precludes definitive conclusions and replication in larger sample is warranted.
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Introduction

Since the issuing of a 'black box warning' by the regulatory bodies in the US and Europe,[1,2] there has been an ongoing controversy about the issue of emergence and/or worsening of suicidal ideation during antidepressant treatment. In an attempt to predict and reduce the risk of these adverse events, several teams of researchers have sought to identify subjects at risk of suicidality during antidepressant treatment based on clinical and demographic data.[3–5] More

recently, there has been a focus on genetic markers in order to explain the susceptibility to treatment-related emergence and worsening of suicidal ideation.[6–9] Recently, in the Genome-Based Therapeutic Drugs for Depression (GENDEP) study, which comprises subjects treated with escitalopram and nortriptyline, we have reported an association between treatment-increasing suicidal ideation and two candidate genes, namely, the brain-derived neurotrophic factor (*BDNF*) and its receptor (neurotrophic tyrosine kinase receptor type 2, *NTRK2*) genes.[9] Other candidate genes such as the cyclic adenosine monophosphate response element-binding (*CREB1*) gene and the glutamate receptor (*GRIA3* and *GRIK2*) genes have also been reported to be associated with treatment-emergent suicidal ideation (TESI) in citalopram-treated subjects.[7,8] In addition, Laje *et al.*,[6] in the first genome-wide association study of TESI, found evidence of association with markers within *PAPLN*, encoding papilin, and *IL28RA*, encoding an interleukin receptor.

Despite significant advances in our understanding of the genetic etiology of TESI, the genetic determinants underlying this complex trait are mostly unknown. The GENDEP study offers a unique opportunity to search, on a genome-wide basis, for genetic variants involved in increasing suicidal ideation during antidepressant treatment and to identity whether common and/or drug-specific pathways are involved in this phenomenon.

Materials and methods

Study design and sample

A total of 811 adult patients of European ethnicity, suffering from unipolar depression of at least moderate severity according to ICD-10 (International Classification of Diseases, 10th revision) or DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) criteria, established in the semi-structured Schedules for Clinical Assessment in Neuropsychiatry interview[10] and aged between 19 and 72 years, were recruited in nine European centers.[11] Exclusion criteria have been described previously.[12] GENDEP is a partially randomized multicenter clinical and pharmacogenomic study. Participants with no contraindications were randomly allocated to receive, for 12 weeks, either escitalopram (10–30 mg daily), a selective inhibitor of the serotonin transporter,[13] or nortriptyline (50–150 mg daily), a tricyclic antidepressant with a hundred times higher affinity for the norepinephrine transporter than for the serotonin transporter.[14] Patients with contraindications for one drug were nonrandomly allocated to the other antidepressant. Each participant was evaluated weekly using the Hamilton Rating Scale for Depression (HDRS-17), the Beck Depression Inventory and the Montgomery–Asberg Depression Rating Scale. The study protocol was approved by the ethics committee of each center, and a written informed consent was obtained from all subjects. GENDEP is registered under the following references: EudraCT No. 2004-001723-38 (http://eudract.emea.europa.eu); current controlled trials ISRCTN03693000 (http://www.controlled-trials.com).

Phenotype definition

As previously described,[3] suicidal ideation was assessed using the third item of the HDRS-17, the ninth item of the Beck Depression Inventory and the tenth item of the Montgomery–Asberg Depression Rating Scale. Item response theory was used to calculate a composite score allowing the use of all available data and providing unbiased estimates in the presence of missing values.[15] An increase of at least 0.5 standard deviation (s.d.) and reaching a level of at least 1 s.d. above the minimum score on the standardized item response theory-derived suicidality scale was considered as increasing suicidal ideation. Individuals with either emergence or worsening of suicidal ideation at any time point in the 12 weeks of follow-up were considered as cases.[3] Controls were those without increasing suicidal ideation during this period. To compare our results with previously published studies,[6] we also considered, in a secondary analysis, individuals with only TESI ($N=48$), compared with individuals without suicidal ideation either at baseline or during treatment ($N=230$).

Genotyping

Genotyping was performed as previously described.[12] Briefly, DNA was extracted from blood collected in EDTA provided by 795 participants, and 727 samples available in sufficient quantity and quality were sent to the Centre National de Génotypage, France and genotyped using the Illumina Human610-quad bead chip (Illumina).

Quality control and population stratification

Quality control procedures on the 727 genotyped samples using PLINK[16] have previously been described in detail in Uher *et al.*[12] Markers were retained if they had a minor allele frequency (MAF) of $\geqslant 0.01$, a genotyping completeness of 99% and no significant departures from Hardy–Weinberg equilibrium ($P>0.0001$). A total of 539 199 single-nucleotide polymorphism (SNP) markers were retained for the analyses. At the individual level, samples with ambiguous sex ($N=3$), outliers on autosomal heterozygosity ($N=3$) and one of each pair of related individuals ascertained through estimation of identity by descent ($N=6$) were excluded. Finally, genotyping completeness was assessed for each individual, and outliers with genotyping completeness $<95\%$ were excluded from further analyses ($N=4$).

Principal component analysis was applied in EIGENSTRAT[17] based on a linkage disequilibrium (LD)-pruned data set of SNPs in low LD and excluding known regions of long-range LD.[18] The first four principal components were used as covariates in the analyses (see Uher *et al.*[12] for details). Five individuals with non-European ancestry were excluded from the analyses.

Statistical analysis

A total of 706 individuals were included in the present analysis. Logistic regression under an additive genetic model

adjusting for age, gender and center of recruitment, was used to investigate the association between genotypes and the outcome. The association was first tested in the whole sample, irrespective of the type of antidepressant. We then tested for association separately within subjects treated with escitalopram and nortriptyline. Interaction between genotype and drug was also tested. As gender has been suggested to be a modifier of the association between SNPs and suicidal behavior,[19,20] we finally investigated genotype-by-gender interaction in the whole sample, in escitalopram- and in nortriptyline-treated subjects. As mentioned above, TESI was also considered in a secondary analysis.

To account for multiple testing, genome-wide significance was set at $P<5\times10^{-8}$.[21] Associations detected at $P<5\times10^{-6}$ were reported as suggestive findings of interest.

Imputation

All HapMap phase II SNPs within 100 kb upstream and downstream of significant or suggestive SNPs were imputed and tested for association. Imputation was performed using proxy association in PLINK.[16] The confidence threshold for making a call was set to 0.95, and only SNPs imputed in at least 95% of individuals were tested for association.

Power analysis

Power calculations were performed using QUANTO.[22] The sample of 706 subjects provides a power of 0.85 to detect an effect with an odds ratio (OR) of at least 2, assuming a MAF = 0.25 at the suggestive significance threshold of $\alpha=5\times10^{-6}$. The study has a power of 0.56 to detect an association with an OR of 2 at the genome-wide significant threshold of $\alpha=5\times10^{-8}$, assuming a MAF = 0.25. The sample of 394 individuals treated by escitalopram (120 cases and 274 controls) has a power of 0.32 at the suggestive significance threshold of $\alpha=5\times10^{-6}$. The corresponding value for the 312 individuals treated by nortriptyline (124 cases and 188 controls) was 0.22.

Exploration of candidate genes

A total of 846 markers within 33 candidate genes and 20 kb upstream and downstream, identified as being involved in suicidality, were investigated in this study (Supplemental Table S1 gives a list of candidate genes and rationale for their inclusion). For each candidate gene, the effective number of comparisons (M_{eff}) was computed using the web-based SNPSpd software (http://gump.qimr.edu.au/general/daleN/SNPSpD/) with the method described by Li and Ji.[23,24] The threshold for significance was then calculated as $\alpha_{corr}=0.05/M_{eff}$ for each candidate gene. Results that remained significant after correction for the M_{eff} within a gene and within each analysis are reported.

Results

Genotyping quality control and sample

Table 1 displays the 706 unrelated subjects who passed quality control and were included in genome-wide analyses. As previously reported,[3] subjects with increasing suicidal ideation had higher baseline severity of depression, as measured by the Montgomery–Asberg Depression Rating Scale and the Beck Depression Inventory. Moreover, the nortriptyline-treated sample included more individuals with treatment-increasing suicidal ideation (39.70%) than the escitalopram-treated sample (30.50%; $\chi^2=6.64$; $P=0.01$; Table 1). As previously reported, the rates of treatment-increasing suicidal ideation peaked in the fifth week of treatment and were relatively evenly distributed over the 12 weeks of follow-up.[3]

Genome-wide analysis: whole sample

Figure 1 shows the quantile–quantile plot with a uniform distribution of *P*-values, suggesting no inflation of the test statistics (genomic control coefficient, $\lambda=1.013$). After adjustment for age, gender, center of recruitment and ancestry principal components, no association was identified at a genome-wide level of significance. The most significant association with increasing suicidal ideation ($P=8.28\times10^{-7}$) across the whole sample was detected at SNP rs11143230 located on chromosome 9q21.13. This SNP minor allele was associated with an increased risk of treatment-increasing suicidal ideation, with an OR of 1.88 (95% CI 1.46–2.42; Figure 2). We reanalyzed the data using the proxy association function implemented in PLINK[16] to assess the haplotype background around SNP rs11143230. This analysis provided evidence for association of a similar magnitude, with haplotypes constructed using adjacent SNPs in the absence of rs11143230 (omnibus haplotype test; $P=1.54\times10^{-6}$ considering all haplotypes and with $P=1.08\times10^{-6}$ for the best single haplotype). These analyses strongly support association of this genomic region with treatment-increasing suicidal ideation.

To better define the extent of the associated genomic region, all HapMap phase II SNPs within 100 kb upstream and downstream of rs11143230 were imputed and tested for association. Three other SNPs within the region, all with a

Table 1 Sample description

	Increasing suicidal ideation			
	Yes (N = 244)		*No (N = 462)*	
	Mean	*s.d.*	*Mean*	*s.d.*
Age	42.8	11.8	41.5	11.6
MADRS score at baseline	30.1	6.4	28.3	6.7
HDRS-17 score at baseline	22.1	5.2	21.6	5.3
BDI at baseline	31.9	8.7	26.4	9.5
	Number	*Percentage*	*Number*	*Percentage*
Female	160	65.60	284	61.50
Escitalopram treated	120	30.50	274	69.50
Nortriptyline treated	124	39.70	188	60.30

Abbreviations: BDI, beck depression inventory; HDRS-17, hamilton rating scale for depression; MADRS, montgomery–asberg depression rating scale.

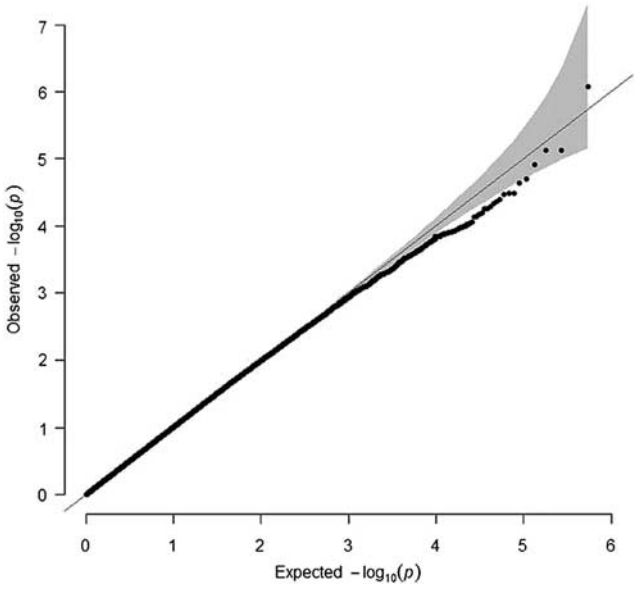

Figure 1 Quantile–quantile (Q–Q) plot for association between treatment-increasing suicidal ideation and genotypes in the whole sample. The gray shaded area represents the approximate 95% confidence interval (CI) of expected $-\log_{10}$ (*P*-values). Black dots represent the observed *P*-values. The red line is the expected line under the null distribution. The color reproduction of this figure is available on the html full text version of the paper.

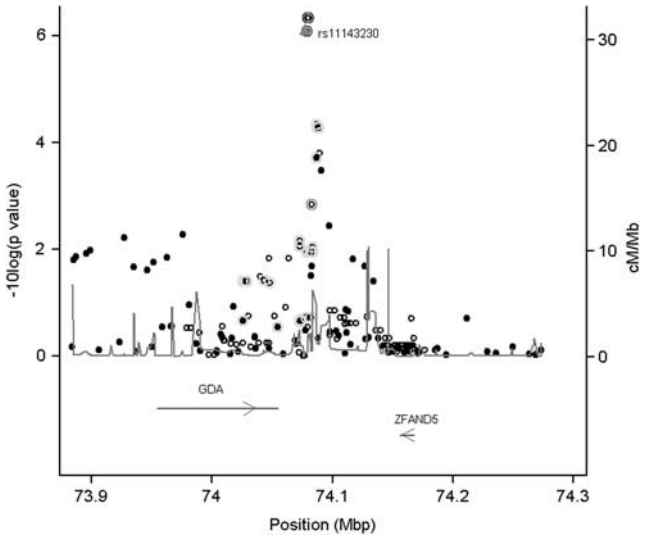

Figure 2 Genome-wide analysis of increasing suicidal ideation: whole sample. Genotyped (full circles) and imputed (hollow circles) single-nucleotide polymorphisms (SNPs) plotted by position on chromosome 9 against association with increasing suicidal ideation ($-\log_{10}$ (*P*-value)). The panel shows 200 kb upstream and downstream of rs11143230 (highlighted in a red triangle with black outline color) on chromosome 9. Estimated recombination rates (cM/Mb) (from HapMap) are plotted in gray to describe the local linkage disequilibrium (LD) structure. The SNPs surrounding the strongest associated genotyped SNP are color-coded to reflect their LD with this SNP (Green: $r^2 \geqslant 0.8$; Orange: $r^2 = 0.5$–0.8 and Yellow: $r^2 = 0.2$–0.5). Genes and the direction of transcription are noted below the plots. The color reproduction of this figure is available on the html full text version of the paper.

MAF of around 0.35, were imputed with high certainty and associated with treatment-increasing suicidal ideation at a similar level of significance as rs11143230 (Figure 2). These three imputed SNPs (rs17628566, rs17552729 and rs17628494) were in complete LD with rs11143230 ($r^2 = 1.0$) and mapped to the same haplotype block. This LD block is ~88 kb in size and extends from intron 2 to around 30 kb downstream of a gene encoding a guanine deaminase (*GDA*) between two recombination hotspots (position 73 987 129 and 74 130 184) (Figure 2).

No significant association was found in the secondary TESI analysis. The most significantly associated SNP was rs227870, with a $P = 1.21 \times 10^{-5}$ in an intergenic region on chromosome 14 (Supplementary Table S2). Interestingly, a trend of association was observed for rs12782806 within the ankyrin3 (*ANK3*) gene on the chromosome 10q21.2 (OR of 3.6; 95% CI 1.95–6.66; $P = 4.53 \times 10^{-5}$).

Genome-wide analysis: escitalopram-treated subjects

The quantile–quantile plot (Supplementary Figure S1A) ($\lambda = 1.018$) showed no inflation of test statistics. Two SNPs showed suggestive levels of significance for association. One, rs358592, is located on chromosome 4p15.31 in the gene encoding Kv channel-interacting protein 4 (*KCNIP4*). This SNP's minor allele was associated with a reduced risk of increasing suicidal ideation during treatment with escitalopram, with an OR = 0.39 (95% CI 0.26–0.58; $P = 2.50 \times 10^{-6}$) (Figure 3a). Although the omnibus haplotype test (proxy association) did not provide further support for association in this region, one haplotype of three SNPs met proxy criteria with $P = 4.12 \times 10^{-6}$. Seven other SNPs with a MAF of around 0.30 and high LD with rs358592 ($r^2 >= 0.8$), could be imputed with high confidence and were associated at a similar level of significance as rs358592 (Figure 3a). The second SNP (rs4732812) showing a suggestive association with a $P = 3.35 \times 10^{-6}$ was located on chromosome 8p21.1, 24 kb upstream of a gene encoding for elongation protein 3 homolog (*ELP3*) (Figure 3b). The minor allele was associated with reduced risk of increasing suicidal ideation in escitalopram-treated subjects (OR = 0.39; 95% CI 0.26–0.58). No proxies were found and no imputed SNPs were associated with the outcome at similar level of significance as rs4732812 (Figure 3b). No interaction between the two SNPs was found.

Genome-wide analysis: nortriptyline-treated subjects

The quantile–quantile plot (Supplementary Figure S1B) ($\lambda = 1.016$) showed no inflation of test statistics. No marker was associated at a genome-wide or suggestive level of significance. The most significantly associated marker was rs6812841 located on chromosome 4q21.23 with a $P = 7.70 \times 10^{-6}$.

Genome-wide analysis: genotype–drug interaction

The quantile–quantile plot ($\lambda = 1.015$) showed no inflation of test statistics (Supplementary Figure S1C). Two markers on chromosome 4q12 interacted with drug at a suggestive

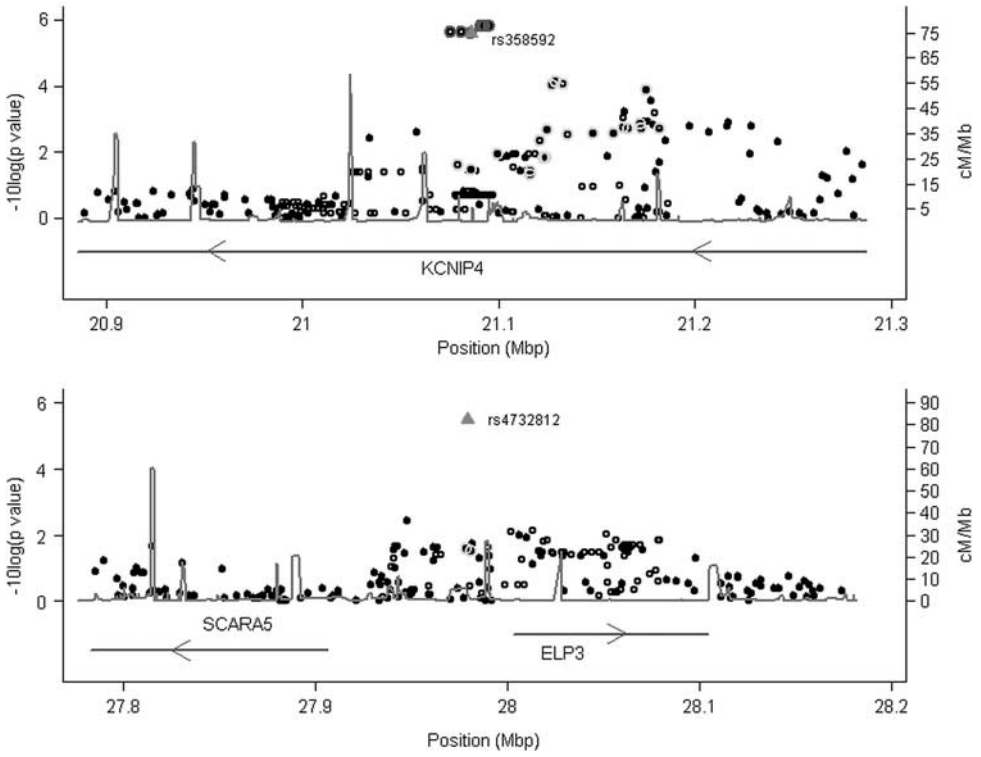

Figure 3 Genome-wide analysis of increasing suicidal ideation: escitalopram-treated subjects. Genotyped (full circles) and imputed (hollow circles) single-nucleotide polymorphisms (SNPs) plotted by position on chromosome 4 (upper panel) and chromosome 8 (lower panel) against association with increasing suicidal ideation ($-\log_{10}$ (*P*-value)) in escitalopram-treated subjects. The two panels show 200 kb upstream and downstream of rs358592 on chromosome 4 and rs4732812 on chromosome 8, both highlighted in red triangles. Estimated recombination rates (cM/Mb) (from HapMap) are plotted in gray to describe the local linkage disequilibrium (LD) structure. The SNPs surrounding the most significant genotyped SNP are color–shape coded to reflect their LD with this SNP (Green: $r^2 \geqslant 0.8$; Orange: $r^2 = 0.5$–0.8 and Yellow: $r^2 = 0.2$–0.5). Genes and the direction of transcription are noted below the plots. The color reproduction of this figure is available on the html full text version of the paper.

level of significance: rs1368607 and rs1433412 both near two copy number variants (CNV). Minor alleles in both SNPs were associated with risk of treatment-increasing suicidal ideation among nortiptyline-treated subjects, with an OR = 2.66 (95% CI 1.64–4.31) and 2.61 (95% CI 1.61–4.24) for rs1368607 and rs1433412, respectively, and an opposite effect in escitalopram-treated subjects (Table 2). After an imputation of HapMap SNPs 100 kb upstream and downstream of rs1368607, nine other SNPs, all with a MAF of around 0.18 and $r^2 \geqslant 0.8$ with rs1368607, were imputed with high confidence and interacted with drug at a level of significance comparable with rs1368607 (Figure 4a).

rs2707159 in the apolipoprotein O (*APOO*) gene on chromosome Xp22.11 also interacted with drug at a suggestive level of significance ($P = 4.50 \times 10^{-6}$), with increased risk of treatment-increasing suicidal ideation in carriers of the minor allele among nortriptyline-treated subjects (OR = 3.01; 95% CI 1.72–5.26) and an opposite effect in escitalopram-treated ones (Table 2) (Figure 4b). Finally, rs2846685 on chromosome 11q24.3 interacted with drug with a *P*-value of 4.71×10^{-6} (Table 2). This SNP is located 2.5 kb upstream of the p53-regulated apoptosis-inducing protein 1 (*p53AIP1*) gene and 22 kb downstream of the Rho GTPase-activating protein isoform 2 (*RICS*) gene. No other genotyped or imputed SNP reached similar level of significance as rs2846685 (Figure 4c). Proxy associations were not performed for genotype–drug interaction analyses, as the proxy option is not available for interaction analyses in PLINK.

Genome-wide analysis: genotype–gender interaction

There was no inflation of the test statistics ($\lambda = 1.009$) for this analysis. One marker in an intergenic region on chromosome 9q34.3 interacted with gender at a suggestive level of significance, with a *P*-value of 3.81×10^{-6} in escitalopram-treated subjects (Supplementary Table S3). Female carriers of the minor allele had an increased risk of increasing suicidal ideation (OR = 1.72; 95% CI 1.11–2.65), whereas the opposite effect was observed in males (OR = 0.28; 95% CI 0.34–0.55). No other SNPs interacted with gender at a suggestive level of significance.

For all the above findings, significance remained in the suggestive range after adjusting for baseline severity of depressive disorder.

Pharmacogenetic analysis: candidate genes

Among the 846 markers in the 33 candidate genes, none was significantly associated with outcome after M_{eff} correction across all candidate gene markers (M_{eff} corrected $P = 0.05/577.26 = 8.66 \times 10^{-5}$). Associations that remained significant

Table 2 Associations with increasing suicidal ideation identified at suggestive level of significance and candidate gene results

Chromosome	Locus	N markers per gene	M_{eff}	Corrected M_{eff} P-value	SNP	Position	MAF	Minor allele	Whole sample		Escitalopram		Nortriptyline		Genotype × drug		HWE
									OR	P-value	OR	P-value	OR	P-value	OR	P-value	P-value
Genome-wide analysis: whole sample																	
9	*GDA*	—	—	—	rs11143230	74 077 523	0.35	C	1.88	8.28E−07	2.22	7.49E−06	1.57	0.0150	0.77	0.3075	1.000
Genome-wide analysis: escitalopram-treated subjects																	
4	*KCNIP4*	—	—	—	rs358592	21 086 088	0.3	C	0.67	0.0036	0.39	2.50E−06	1.08	0.7173	2.16	0.0074	0.858
8	Intergenic	—	—	—	rs4732812	27 979 307	0.27	T	0.58	0.0001	0.39	3.35E−06	0.81	0.2859	1.89	0.026.3	0.340
Genome-wide analysis: genotype–drug interaction																	
4	Intergenic	—	—	—	rs1368607	58 734 992	0.18	G	1.12	0.4714	0.66	0.0393	2.66	7.71E−05	4.87	1.76E−06	0.104
4	Intergenic	—	—	—	rs1433412	58 730 152	0.19	G	1.10	0.5172	0.65	0.0373	2.62	9.71E−05	4.76	2.22E−06	0.082
X	*APOO*	—	—	—	rs2707159	23 827 244	0.17	C	1.12	0.5110	0.56	0.0162	3.01	0.0001	5.92	4.50E−06	0.742
11	Intergenic	—	—	—	rs2846685	128 320 571	0.33	T	0.92	0.5293	0.57	0.0018	1.66	0.0056	3.38	4.71E−06	0.128
Candidate genes																	
12	*SCN8A*	29	13.01	0.0039	rs1905248	50 293 270	0.23	A	1.22	0.1604	1.73	0.0051	0.75	0.1632	0.38	0.0009	0.292
12	*SCN8A*	29	13.01	0.0039	rs12424271	50 301 235	0.13	A	1.26	0.1971	1.94	0.0072	0.67	0.1318	0.34	0.0032	0.733
3	*CCK*	12	8.01	0.0064	rs4533619	42 264 816	0.14	G	0.74	0.0977	0.45	0.0041	0.98	0.9340	2.33	0.0288	0.875
3	*CCK*	12	8.01	0.0064	rs747455	42 280 968	0.24	T	0.77	0.0659	0.55	0.0032	0.88	0.5350	1.93	0.0329	1.000
7	*CRHR2*	22	13.11	0.0039	rs2240401	30 639 870	0.34	C	0.85	0.1764	1.14	0.4393	0.55	0.0010	0.47	0.0026	0.006
17	*YWHAE*	22	13.17	0.0039	rs17625475	1 226 859	0.12	T	1.59	0.0072	2.00	0.0027	1.51	0.1269	0.81	0.5638	0.117
9	*NTRK2*	97	43.81	0.0012	rs1822420	86 657 096	0.14	T	0.75	0.1019	1.24	0.3571	0.38	0.0004	0.30	0.0012	0.148
9	*NTRK2*	97	43.81	0.0012	rs10868235	86 683 575	0.47	C	1.33	0.0185	1.77	0.0008	0.95	0.7558	2.68	0.0120	0.880

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.
For candidate-gene analyses, associations are listed that remain significant after correction for the effective number of comparisons (M_{eff}) within each gene. Odds ratio (OR) indicates the effect of each minor allele.

after M_{eff} correction for multiple comparisons within each gene are listed in Table 2. The strongest association with treatment-increasing suicidal ideation was observed for rs1822420 within the *NTRK2* gene among notriptyline-treated subjects with a *P*-value of 0.0004, the minor allele being protective against increasing suicidal ideation with an OR of 0.38 (95% CI 0.22–0.65). Four other genes were associated with treatment-increasing suicidal ideation: the cholecystokinin (*CCK*) and the tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide (*YWHAE*) genes in escitalopram-treated subjects; the corticotropin-releasing hormone receptor 2 (*CRHR2*) gene in nortriptyline-treated subjects and in an interaction with drug; and the sodium channel, voltage-gated, type VIII α-polypeptide (*SCN8A*) gene in an interaction with drug (Table 2).

Discussion

In a genome-wide search for genetic determinants of increasing suicidal ideation, the strongest associated marker with suggestive significance was in the vicinity of a functionally plausible gene. Its association was supported by a strong independent signal in the region after removing the strongest associated marker. We have also identified a genomic region that appears to be associated with suicidality in a drug-dependent manner. Although limited statistical power means that most true associations remain below the level of detection, this study suggests new candidate regions to be pursued in further investigations and, if replicated, to serve as predictors of adverse events and inform individualized prescription of antidepressants.

We found a suggestive association with polymorphisms near the *GDA* gene that encodes cypin, a protein with GDA activity. *GDA* is a functional candidate based on its function as a regulator of dendrite patterning and synaptic formation between hippocampal neurons[25,26] and its involvement in dopamine and glutamate signaling.[27–29] Tannu *et al*.[29] showed significant decrease in GDA in Rhesus monkeys following cocaine self-administration. Moreover, cypin interacts with the postsynaptic density protein-95 (PSD-95), a protein that has a role in glutamate signaling[27,28] and therefore may influence synaptic development and plasticity.[28] Interestingly, PSD-95 has been involved in an experimental rodent model of depression and shown to be modulated by the antidepressant fluoxetine.[30,31] In addition, reduced levels of PSD-95 were found in postmortem prefrontal cortex of depressed subjects and in the striatum of bipolar disorder subjects.[27,32] These data warrant further investigation of the *GDA* gene in treatment-increasing suicidal ideation. Interestingly, rs10748045 within a gene encoding for the glutamate receptor-interacting protein 1 (*GRIP1*) was among the 10 strongest associated SNPs with treatment-increasing suicidal ideation (see Supplementary Table S2). GRIP1, which is a protein composed of seven PDZ (PSD-95/Discs large/zona occludens-1) domains, has been associated with several psychiatric disorders.[33–35] This

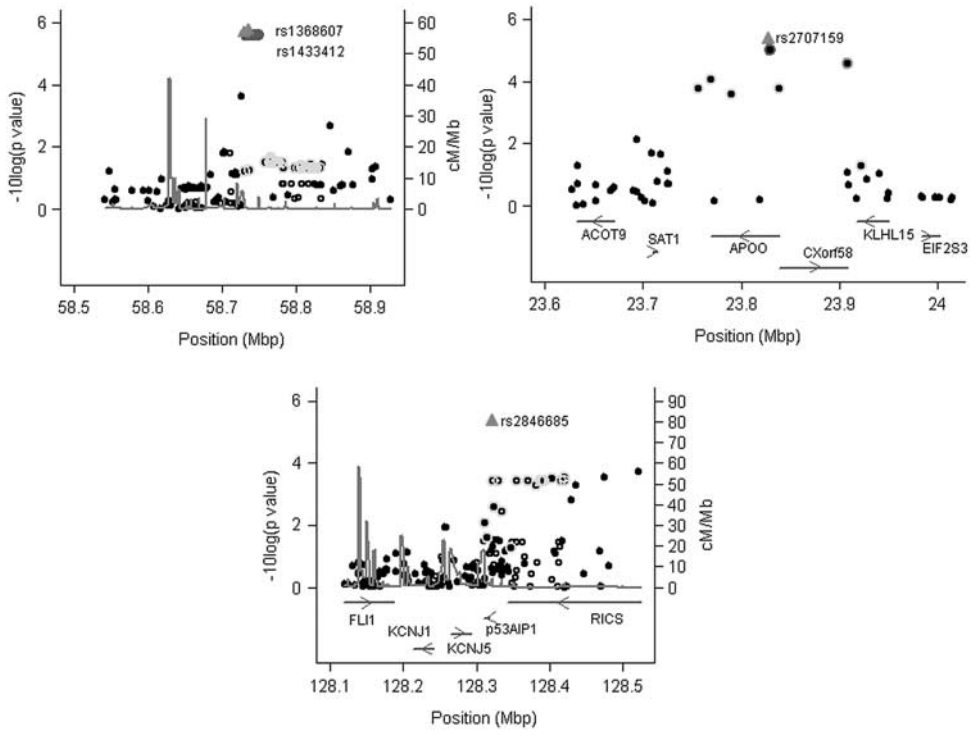

Figure 4 Genome-wide analysis of increasing suicidal ideation: genotype–drug interaction. Genotyped (full circles) and imputed (hollow circles) single-nucleotide polymorphisms (SNPs) plotted by position on chromosome 4 (upper left panel), chromosome X (upper left panel) and chromosome 11 (lower panel). −10log (*P*-value) reflects the significance of the interaction with drug. The three panels show 200 kb upstream and downstream the most significant SNPs (highlighted in red triangles) on each chromosome. Estimated recombination rates (cM/Mb) (from HapMap) are plotted in gray to describe the local linkage disequilibrium (LD) structure (not available for chromosome X). The SNPs surrounding the most significant genotyped SNP are color–shape coded to reflect their LD with this SNP (Green: $r^2 \geqslant 0.8$; Orange: $r^2 = 0.5$–0.8 and Yellow: $r^2 = 0.2$–0.5). Genes and the direction of transcription are noted below the plots. The color reproduction of this figure is available on the html full text version of the paper.

association further supports the role of glutamatergic systems in treatment-increasing suicidal ideation. Finally and also of interest among the 10 strongest associated SNPs, was rs12531005 within *NXPH1* gene encoding neurexophilin 1 binding α-neurexins, which promote adhesion between dendrites and axons. Recently, this gene showed some evidence of association with neuroticism in a genome-wide association study (rs2349775 with an $r^2 = 0.17$ with rs12531005), and given the major role of neuroticism in suicidal behaviors, this result is notable.[36,37]

Two suggestive drug-specific associations were found when looking at subjects treated with escitalopram, one within the *KCNIP4* gene and the other near the *ELP3* gene. Despite their involvement in neuronal functions, to our knowledge, these genes have never been investigated in psychiatry, and further research is needed to corroborate their involvement in increasing suicidal ideation.

A region on chromosome 4 showed a differential association, with increasing suicidality as a function of which antidepressant was used. Interestingly, the two most significant SNPs were located near two structural variants (CNV-51422 and CNV-56877) that may influence the expression of genes at longer distances. This highlights the importance of investigating the whole genome in order to find potential associations. Two other suggestive associations were found when looking at gene by drug interaction; one within the *APOO* gene and the other near the *P53AIP1* gene. It is of interest that rs2707159 in the *APOO* gene is located only 113 kb downstream of one of the candidate genes, the spermidine/spermine *N*1-acetyltransferase (*SAT1*) gene. *SAT1* has previously been associated with suicide and is located in a region that has recently been suggested to be involved in suicidal behavior (Xp22.11).[38,39] However, none of the *SAT1* SNPs remained significant after M_{eff} correction in the candidate gene analysis. Several other SNPs showed a differential effect depending on the drug used (see Table 2). This highlights the specificity of the effect of SNPs to a particular drug. If replicated, this finding would strongly suggest that depending on the pattern of SNPs association, one drug should be used over another in order to avoid side effects such as increasing suicidality. This kind of moderating effect of SNPs, conditional on the type of antidepressant used, is a 'well-known' mechanism for antidepressant response, as in the case of the common length polymorphism in the serotonin transporter promoter.[40]

When looking at the small sample of individuals with TESI, we found a nonsignificant trend of association with polymorphism within the *ANK3* gene (see Supplementary

Table S2). As this gene, in a recent genome-wide association study, has been associated with bipolar disorder,[41] a mental illness with very high risk of suicide,[42] this result is worth pursuing.

Despite a larger sample size in our case–control design, we were not able to replicate the previously reported associations between TESI and *PAPLN* and *IL28RA* genes6. Both rs11628713 in the *PAPLN* gene and rs10903034 in the *IL28RA* gene that were reported as significant in the Laje *et al.*'s[6,7] study were given a *P*-value of 0.6 or above in our study. Several explanations could be given for the discrepancies between the two studies. The first is the difference in sample size and coverage of the genome. The present GENDEP sample has a larger number of individuals and comprehensive genomic coverage. A second consideration is a different definition of the outcomes: TESI vs treatment-increasing suicidal ideation. However, we previously showed that the two phenotypes share similar risk factors and should represent the same phenomenon.[3] A third explanation pertains to the questionnaire used to measure suicidal ideation. Indeed, Valtonen *et al*.[43] highlighted how the inconsistencies across studies in measuring suicidal ideation may lead to discrepant results. We decided to use a composite score based on self- and clinician-rated scales to obtain the most reliable estimate based on all available data. GENDEP is the largest study comparing two different classes of antidepressants with different modes of action, and until another study is able to replicate our findings, we are unable to suggest whether or not these results are relevant for other medications.

All the above differences between studies should also explain why we did not replicate, in a comprehensive list of 33 candidate genes, the previous reported association with TESI in citalopram-treated subjects (*CREB1*, *GRIA3* and *GRIK2*).[7,8] Even when looking at a closely comparable phenotype, TESI in escitalopram-treated subjects, none of the SNPs within these genes remained significantly associated after genome-wide M_{eff} correction. However, concordant with our previous report in the same sample,[9] we found a significant association with polymorphisms within the *NTRK2* gene. Markers in several other candidate genes, including *SCN8A*, *CCK*, *YWHAE* and *CRHR2*, showed a genome-wide M_{eff}-corrected significant association with treatment-increasing suicidal ideation that would have been reported as significant in candidate gene association studies. *SCN8A* has been associated with suicide attempts.[44] *CCK* and *YWHAE* were found to be associated with suicides in Japanese samples.[45,46] Finally, *CRHR2* was associated with suicidal behaviors in bipolar disorder families.[47] The implication of these genes in treatment-increasing suicidal ideation raises question about the possible link with more severe form of suicidal behaviors such as suicide attempt and suicide completion.

The strengths and limitations of GENDEP have been highlighted before.[9,11,12] In brief, the strengths include a specifically recruited sample for pharmacogenetic study without inflation of test statistics. The main limitation is the absence of a placebo group, which does not allow us to distinguish between genetic and nonspecific effects on treatment-increasing suicidal ideation. Statistical power limits our ability to detect weak or moderate effects. Another limitation is the assessment of suicidal ideation using suicide items of scales designed to measure overall depression severity. However, and this is one of the strengths of this study, the evaluation of suicidal ideation did not rely on a single rating scale and integrated self-report with clinician evaluation.

In conclusion, these data suggest novel loci and pathways involved in increasing suicidal ideation during treatment with antidepressants. Investigations in other large studies and meta-analyses are needed to confirm or refute associations between these variants and treatment-related suicidal behaviors. In the future, validated genetic markers may be used to inform personalized treatment of depression.

Conflict of interest

Perroud, Uher, Ng, Hauser, Guipponi, Maier, Mors, Gennarelli, Rietschel, Dernovsek, Stamp, Lathrop, Breen, Craig and Lewis declare no competing interests. Henigsberg and Souery participated in clinical trials sponsored by pharmaceutical companies including GlaxoSmithKline and Lundbeck. Henigsberg has received honoraria for participating in expert panels for pharmaceutical companies. 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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Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)