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ORIGINAL INVESTIGATION

Separate and combined effects of genetic variants and pre-treatment whole blood gene expression on response to exposure-based cognitive behavioural therapy for anxiety disorders

Jonathan R.I. Coleman[a], Kathryn J. Lester[a,b], Susanna Roberts[a], Robert Keers[a,c], Sang hyuck Lee[a,d], Simone De Jong[a,d], Héléna Gaspar[a,d], Tobias Teismann[e], André Wannemüller[e,f], Silvia Schneider[e], Peter Jöhren[f], Jürgen Margraf[e], Gerome Breen[a,d*] and Thalia C. Eley[a,d*]

[a]King's College London, Institute of Psychiatry, Psychology and Neuroscience, MRC Social, Genetic and Developmental Psychiatry (SGDP) Centre, London, UK; [b]School of Psychology, University of Sussex, UK; [c]School of Biological and Chemical Sciences, Queen Mary University of London, London, UK; [d]National Institute for Health Research Biomedical Research Centre, South London and Maudsley National Health Service Trust, London, UK; [e]Mental Health Research and Treatment Center, Ruhr-Universität Bochum, Bochum, Germany; [f]Dental Clinic Bochum, Bochum, Germany

ABSTRACT

Objectives: Exposure-based cognitive behavioural therapy (eCBT) is an effective treatment for anxiety disorders. Response varies between individuals. Gene expression integrates genetic and environmental influences. We analysed the effect of gene expression and genetic markers separately and together on treatment response.

Methods: Adult participants ($n \leq 181$) diagnosed with panic disorder or a specific phobia underwent eCBT as part of standard care. Percentage decrease in the Clinical Global Impression severity rating was assessed across treatment, and between baseline and a 6-month follow-up. Associations with treatment response were assessed using expression data from 3,233 probes, and expression profiles clustered in a data- and literature-driven manner. A total of 3,343,497 genetic variants were used to predict treatment response alone and combined in polygenic risk scores. Genotype and expression data were combined in expression quantitative trait loci (eQTL) analyses.

Results: Expression levels were not associated with either treatment phenotype in any analysis. A total of 1,492 eQTLs were identified with $q < 0.05$, but interactions between genetic variants and treatment response did not affect expression levels significantly. Genetic variants did not significantly predict treatment response alone or in polygenic risk scores.

Conclusions: We assessed gene expression alone and alongside genetic variants. No associations with treatment outcome were identified. Future studies require larger sample sizes to discover associations.

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Introduction

Anxiety disorders are the most common group of mental illnesses, with lifetime prevalence estimates ranging between 10 and 30% (Kessler et al. 2007; Michael et al. 2007). They are an economic burden on society and the sixth largest cause of disability globally (Fineberg et al. 2013; Baxter et al. 2014). Suffering from an anxiety disorder is distressing, with affected individuals reporting adverse effects on quality of life comparable to sufferers of major depressive disorder, and in excess of the population norm (Mendlowicz and Stein 2000).

Treatment of anxiety disorders uses a variety of pharmacological and psychological modalities (National Collaborating Centre for Mental Health 2011). Exposure-based cognitive behavioural therapy (eCBT) is a common treatment, and shows large effect sizes across the anxiety disorders, comparable to or better than those obtained by anxiolytic medication (Norton and Price 2007; Stewart and Chambless 2009; Barlow et al. 2013; Cuijpers et al. 2013; Margraf and Zlomuzica 2015). During eCBT, participants confront the object of their anxiety (whether literally, referred to as in vivo, or through imagination or virtual reality, referred to as in sensu), within a carefully managed and supportive environment. They identify the cognitive and behavioural processes underlying their anxious response, and develop strategies to mitigate against these negative schema and to cope with their anxiety (Otto et al.

CONTACT Thalia Eley thalia.eley@kcl.ac.uk Social, Genetic and Developmental Psychiatry Centre (MRC), Institute of Psychiatry, Psychology and Neuroscience – PO80, DeCrespigny Park, Denmark Hill, London SE5 8AF, UK
*G. Breen and T.C. Eley have contributed equally to the work.

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2004). Rates of response (in terms of a reduction in symptom severity) and of remission (no longer meeting diagnostic criteria) vary between specific disorders and studies, but are invariably less than 100% (Ballenger 1999; Olatunji et al. 2010; Hofmann et al. 2012; Loerinc et al. 2015).

Numerous influences have been proposed to lead to poorer treatment outcome, including high initial severity, Axis I and Axis II comorbidity, illness duration, low expectancy of treatment success, poor treatment compliance and therapeutic alliance, and general interpersonal difficulties (Newman et al. 2013). However, studies disagree on the importance and validity of such predictors (Taylor et al. 2012; Olatunji et al. 2013; Schneider et al. 2015). The success of any specific treatment for a given participant is difficult to predict. This is relevant given the high costs (both economic and emotional) of pursuing unsuccessful treatment (Otto et al. 2000). It is of clear interest to develop reliable predictors of treatment response.

Genetic variants represent a potential source of predictors. The study of such variants (termed therapygenetics) has largely been confined to candidate gene studies (Eley et al. 2012; Lester and Eley 2013). However, these findings have proven difficult to replicate, and the direction of effect found has been inconsistent between studies (Lester et al. 2016). Recently, we published a genome-wide association study (GWAS) of therapy response in a cohort of children with anxiety disorders (Coleman et al. 2016). Although underpowered to identify the small-effect variants typical of behavioural phenotypes, sufficient power was available to test some effect sizes reported in the therapygenetics literature. No variants were found at conventional genome-wide significance, and candidate variants were not replicated. Therefore, the effects of individual genetic variants on response to CBT are likely to be small, and the predictive effects of such variants are likely to be negligible when used alone.

Studying the differential expression of gene transcripts may be more useful for predicting treatment response. Multiple factors affect gene expression, potentially including genetic variants and environmental influences. Gene expression represents a biologically relevant means of combining genetic and environmental variation to predict response to CBT for anxiety disorders. Two studies have found an association between increased expression of FKBP5 and response to CBT for post-traumatic stress disorder (PTSD; Levy-Gigi et al. 2013; Yehuda et al. 2013). A recent analysis of change across treatment including a subset of the cohort presented within this paper showed no association between treatment response and individual gene expression, nor when expression was clustered according to similarities in expression in the data (Roberts et al. under review).

This investigation combines genetic and gene-expression approaches to predict response to eCBT. It assesses the interaction of differential gene expression at baseline (both of individual transcripts, and using data- and literature-driven clustering methods) and genetic variation to assess the outcome of eCBT for panic disorder (PD) and specific phobias (SPs).

Method

Participants and therapeutic procedure

Two hundred and forty-four participants diagnosed with PD or a SP completed one of four eCBT treatment programmes at the Mental Health Research and Treatment Center, Ruhr-Universität Bochum, Germany as part of standard care. In all programmes, diagnoses were made according to DSM-IV criteria using the Diagnostisches Interview bei psychischen Störungen (DIPS) and Mini-DIPS, structured interviews with well-established reliability, validity and patient acceptance (Margraf 1994; In-Albon et al. 2008; Suppiger et al. 2008; Suppiger et al. 2009; Bruchmuller et al. 2011; Schneider and Margraf 2011). All treatment programmes featured core elements of exposure therapy, including psychoeducation, applied relaxation and exposure (in vivo or in sensu). Specifics of each treatment programme are described below. All treatments were regularly supervised by experienced senior clinicians using audio-visual recordings in order to ensure treatment protocol integrity.

Individuals diagnosed with a SP of receiving dental treatment, not secondary to a separate diagnosis (such as PTSD or injection phobia), were treated in a dental anxiety-specific (DA) programme (Wannemuller and Johren 2015). Treatment was given in five weekly sessions comprising an initial diagnostic and psychoeducation session, a session developing relaxation techniques, and three in sensu exposure sessions related to dental treatment. Participants were not excluded on the basis of concurrent treatment with anxiolytic medication.

Participants with a SP not primarily associated with dental fear were treated in a longer-term programme covering up to 30 sessions, split into five initial sessions of diagnosis and psychoeducation, and 25 sessions of in vivo exposure (relevant to their SP) with elements of cognitive restructuring. Participants were excluded from the study if they were using anxiolytic medication.

Participants with a primary diagnosis of PD with agoraphobia, or agoraphobia alone, were randomised

either to eCBT (PD-CBT; akin to the SP group) or to an exposure-alone condition without any element of cognitive restructuring (PD-exposure [EXP]; Clinical Trials: NCT01680327). Participants in both conditions were excluded if they were using anxiolytic medication. Bodily sensation was used as the specific exposure stimulus for participants suffering from PD. However, as there were no patients with PD without agoraphobia in the trial, interoceptive exposure was always combined with in vivo exposure.

Prior to receiving exposure, immediately following completion of the treatment programme, and at a follow-up assessment approximately 6 months after treatment completion, all participants completed a range of questionnaire measures. In addition, peripheral blood was drawn for DNA and RNA extraction.

Phenotype definition

Treatment response was defined as percentage improvement in the clinician-rated severity scale of the Clinical Global Impression-Severity (CGI-S) rating, and was examined pre-treatment to post-treatment, and pre-treatment to follow-up. The CGI-S ranges from 1 to 7, with a score of 1 representing no symptoms of concern and a score of 7 representing extremely severe illness requiring hospitalisation (Guy 1976). The scale was chosen as it was used in all treatment groups, and was expected to capture severity in a disorder-independent fashion.

The CGI-S was rescaled to range from 0 to 6 to allow outcome to be defined as percentage decrease in severity across time (as this has previously been used successfully in pharmacogenetic GWAS) with 100% indicating full remission (Uher et al. 2010). For both phenotypes, correlations were calculated between percentage improvement and a variety of covariates: age, gender, severity at baseline, presence of comorbid mental disorders, number of treatment sessions attended, treatment period (days between pre-treatment and post-treatment assessment), follow-up period (days between post-treatment assessment and follow-up assessment), use of psychoactive medication at pre-treatment, use of any other medication pre-treatment, body mass index (BMI) and whether the participant smoked. Although the use of concurrent anxiolytic medication was an exclusion criterion for the SP, PD-CBT and PD-EXP groups, some participants were using other medications which may have a psychotropic effect, so this covariate was not restricted to the DA group (Table 1).

Of these covariates, severity at baseline, presence of comorbid mental disorders, use of psychoactive medication and follow-up period were correlated with at least one phenotype in the whole cohort (Table 2). In secondary examinations within each treatment group, treatment period was associated with at least one phenotype in both the PD-CBT ($P = 0.014$) and SP groups ($P = 0.012$). BMI was weakly associated ($P = 0.0424$) with response at post-treatment in the PD-CBT group; however, as this effect was not seen in any other group nor in the whole cohort, BMI was not included as a covariate.

Table 2. Correlations between clinical covariates and treatment response phenotypes for the whole cohort ($N = 187$).

	Post-treatment		Follow-up	
Variable	*r*	*P*	*r*	*P*
Age (years)	−0.0536	0.469	0.0212	0.816
Gender	0.045	0.543	0.0497	0.585
Baseline CGI severity	0.112	0.128	0.303	**$7x10^{-4}$**
Treatment sessions	0.129	0.0818	0.106	0.255
Treatment duration (days)	0.117	0.111	0.0189	0.835
Follow-up duration (days)	−0.178	**0.0409**	−0.0411	0.6614
Psychoactive medication (use)	−0.205	**0.00521**	−0.203	**0.0243**
Other medication (use)	−0.0483	0.514	−0.117	0.200
Mental comorbidity (yes/no)	−0.210	**0.00407**	−0.188	**0.0379**
Body mass index	0.0572	0.440	0.0008	0.993
Smoker (yes/no)	−0.0317	0.687	0.0146	0.880

Correlations with nominal significance ($P < 0.05$) are highlighted in bold.

Table 1. Demographic and treatment information on participants with genotype and/or expression data.

Variable	WC	DA	SP	PD-CBT	PD-EXP	Test	Stat	*P*
N	187	95	38	25	29	–	–	–
Age in years (Mean [SD])	39.2 [11.4]	40.5 [10.4]	37.8 [13.2]	38.4 [11.9]	37.4 [11.9]	ANOVA	0.831	0.478
Gender (N male [%])	67 [35.8]	35 [36.8]	9 [23.7]	13 [52.0]	10 [34.5]	Chi square	5.35	0.148
Baseline CGI-S (Mean [SD])	4.70 [1.13]	4.83 [1.27]	4.16 [0.973]	4.80 [0.707]	4.86 [0.915]	ANOVA	3.80	**0.0112**[a]
Treatment duration in days (Mean [SD])	200 [184]	47.6 [32.8]	340 [152]	351 [118]	383 [137]	ANOVA	151	**3.15×10^{-49}**[b]
Follow-up duration in days (Mean [SD])	215 [62.7]	249 [72]	191 [41.4]	190 [35.4]	191 [44.7]	ANOVA	11.4	**4.32×10^{-7}**[c]
Psychoactive medication at baseline (*N* taking [%])	20 [10.7]	18 [18.9]	1 [2.63]	1 [4.00]	0 [0.00]	Fisher's exact test		**0.00247**[d]
Mental disorder comorbidities (*N* [%])	72 [38.5]	46 [48.4]	8 [21.1]	9 [36.0]	9 [31.0]	Chi square	9.58	**0.0225**[e]

Post hoc *t*-tests (variances assumed unequal; Bonferroni corrected significance threshold = 0.00834; significant results in bold).
[a]SP lower: vs. DA: $t = -3.29$, $P = 0.00143$; vs. PD-CBT: $t = -3.03$, $P = 0.00361$; vs. PD-EXP: $t = -3.04$, $P = 0.00350$.
[b]DA shorter: vs. SP: $t = -11.7$, $P = 2.79 \times 10^{-14}$; vs. PD-CBT: $t = -12.8$, $P = 1.93 \times 10^{-12}$; vs. PD-EXP: $t = -13.0$, $P = 1.19 \times 10^{-13}$.
[c]DA longer: vs. SP: $t = 4.96$, $P = 3.19 \times 10^{-6}$; vs. PD-CBT: $t = 4.86$, $P = 6.93 \times 10^{-6}$; vs. PD-EXP: $t = 4.48$, $P = 2.74 \times 10^{-5}$.
[d]Higher rate in DA: vs. SP: $t = 3.38$, $P = 9.47 \times 10^{-4}$; vs. PD-CBT: $t = 2.63$, $P = 0.0103$; vs. PD-EXP: $t = 4.69$, $P = 9.33 \times 10^{-6}$.
[e]Higher rate in DA: vs. SP: $t = 3.24$, $P = 0.00174$; vs. PD-CBT: $t = 1.12$, $P = 0.269$; vs. PD-EXP: $t = 1.71$, $P = 0.0930$.
WC, whole cohort; DA, dental anxiety; SP, specific phobia; PD-CBT, panic disorder CBT; PD-EXP, panic disorder exposure.

The phenotypes for analysis were defined as the residuals from two linear mixed regressions investigating change in severity between pre-treatment and post-treatment, and pre-treatment and follow-up. Percentage decrease in severity was regressed on fixed effects of baseline severity, presence of comorbid mental disorders, use of psychoactive medication, treatment period (and follow-up period in the analysis pre-treatment to follow-up), and a higher-order random effect of treatment group (to account for differences between treatment groups).

Genotyping

DNA was extracted from peripheral blood drawn pre-treatment using FlexiGene DNA Kits, following the protocol provided by the manufacturer (QIAGEN, Manchester, UK). DNA concentration was quantified using spectrophotometry (NanoDrop 1000, NanoDrop, Wilmington, DE, USA), and samples diluted to 40 µl at a concentration of 75 ng/µl for genotyping. Genotyping was performed using the Illumina PsychChip microarray (Illumina, San Diego, CA, USA), a modified version of the Illumina HumanCoreExome microarray with additional content of interest in psychiatric genomics. All laboratory procedures were performed at the Institute of Psychiatry, Psychology and Neuroscience, King's College London.

Genotype quality control

Quality control was performed following a previously published protocol (Coleman et al. 2015). In brief, genotype data were called using Illumina GenomeStudio software, with manual recalling where appropriate. Rare variants were recalled using ZCall (Goldstein et al. 2012). Variants were removed from the analysis if they were rare (minor allele frequency <0.05), present in $<99\%$ of individuals, or deviated substantially from Hardy–Weinberg equilibrium (Hardy–Weinberg test $P < 1 \times 10^{-5}$). Individuals were excluded if they had genotype calls for $<99\%$ of variants, where reported gender differed from that indicated by the genotypes, or if genome-wide estimates of heterozygosity >3 standard deviations from the sample mean. Additional exclusions were made if the individual showed cryptic relatedness to other individuals in the study (identity by descent [IBD] > 0.1875) or had an average proportion of variants shared IBD with the cohort as a whole >6 standard deviations above the cohort mean.

Following quality control, variants were imputed to the Phase 3 release from the 1000 Genomes Project, using IMPUTE2 with concurrent phasing (1000GenomesConsortium 2012; Howie et al. 2012). X chromosome variants were imputed using the March 2012 Phase 1 release (1000GenomesConsortium 2012; Howie et al. 2012). Imputed variants were imported into PLINK2 for analysis, and filtered to remove uncertain variants (posterior-probability <0.8) and poorly imputed variants (info <0.8) (Chang et al. 2015). Following hard-calling, variants present in $<98\%$ of the cohort were dropped from analysis.

Gene expression

Whole blood samples were drawn at pre-treatment using PAXgene blood RNA tubes. Blood RNA was isolated and purified using the PAXgene Blood miRNA Kit according to the manufacturer's protocol using the QIAcube (QIAGEN). RNA quality was measured using spectrophotometry (NanoDrop 1000, NanoDrop) and integrity using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Genome-wide expression levels were measured from 750 ng total RNA using the Illumina HumanHT-12v4 Expression BeadChip (Illumina).

Gene expression quality control

Raw expression data were processed following internal pipelines (available at https://github.com/snewhouse/BRC_MH_Bioinformatics). Samples with detection rates dissimilar from the rest of the cohort were identified and removed in GenomeStudio (Illumina). Raw data were imported into R for quality control primarily using the lumi package (Du et al. 2008; Team 2012). Expression data were background corrected using module-based background correction for BeadArrays (Ding et al. 2008). Probes with an expression level >2 standard deviations above the background mean were defined as detected. *XIST* gene expression (specific to females) and Y chromosome gene expression (specific to males) was compared to reported gender and gender inferred from genotyping, and discordant samples removed. Expression data were log2 transformed and normalised using robust splines normalisation from the lumi package (Du et al. 2008; Schmid et al. 2010). Sample co-expression relationships were assessed, and samples with connectivity <2 standard deviations from the cohort mean were excluded (Oldham et al. 2012). Associations between covariates and the first principal component of the expression data were assessed using stepwise linear regression bootstrapped 100 times, with randomised order of covariates in the regression. Covariates included batch variables (expression microarray, sample position on microarray, date of RNA extraction, date of expression measurement, machine used in RNA isolation, RNA integrity (RIN)

value, RNA yield, amplified concentration of RNA, whether the sample required additional treatment to remove DNA and whether the blood sample was the first or second drawn) and demographic covariates (BMI and smoking). The effect of associated covariates was regressed out of the expression data, using the sva ComBat package in the case of categorical variables (extraction date) and linear regression (with RcppArmadillo) in the case of continuous variables (RIN value, RNA yield, amplified concentration of RNA) (Johnson et al. 2007; Eddelbuettel and Sanderson 2014). Probes detected in <80% of the sample were removed. As expression data were generated from whole blood without assessment of cellular composition, deconvolution methods implemented in CellMix were used to assess the origin of RNA transcripts before and after differentially expressed probes were selected (Gaujoux and Seoighe 2013). Correlations between the estimated final proportions of leukocytes (neutrophils, lymphocytes and monocytes) and the two CBT response phenotypes were calculated. Additional exclusion of probes was performed to allow combined analysis with genotyped variants. Specifically, probes were excluded if they were not annotated in the ENSEMBL hg19 build, if they contained any genetic variant genotyped in the cohort, or if they did not map to a unique site on the genome. Probes were identified using nucleotide universal identifiers (nuIDs), which are unique to the DNA sequence of the probe (Du et al. 2007).

Statistical analysis

Following quality control, the association of genome-wide genotyping data with both response phenotypes was assessed in GWAS. The participants in the study were of Central or Eastern European ancestry. Genomic estimation of ancestry was established using principal components analysis performed in EIGENSOFT (Price et al. 2006). No principal component was correlated with either of the phenotypes at a level greater than chance. To account for finer-scale population stratification, analyses were run using a linear mixed model incorporating a random effect of gross genetic similarity between individuals (the *mlma-loco* option in GCTA; Yang et al. 2011). Results were clumped in PLINK2, pruning all variants in linkage disequilibrium ($r^2 > 0.25$, ± 250 kb) of a variant with a lower *P* value.

Genotype information was used as a target dataset in polygenic risk scoring. Specifically, the results of a previous GWAS of CBT response in children were used to predict both phenotypes in the whole cohort using PRSice, which performs high-resolution polygenic risk scoring to identify the most predictive risk score (Euesden et al. 2015; Coleman et al. 2016). Further GWAS were performed on the cohort minus individuals treated for DA, and the results from these subset GWAS were used to predict response to treatment in the DA subgroup.

Probe-level expression data were imported into R, and analysed using weighted gene correlation network analysis (WGCNA; Langfelder and Horvath 2008). Data-driven clustering of co-expressed probes was performed using an automatically-constructed signed network from the *blockwiseModules* function in WGCNA (details on this procedure are provided in the Supplemental Material available online; Langfelder and Horvath 2008). Correlations between individual probes and both response phenotypes, and between WGCNA module eigengenes and response phenotypes, were calculated. Local false discovery rates were calculated to account for multiple testing using the qvalue package in R (Dabney et al. 2004).

Probe-level correlations were mapped to HUGO gene names, ranked according to significance and used in gene ontology (GO) enrichment analysis in GOrilla (Eden et al. 2009). Where multiple probes mapped to the same gene, the highest-ranked was retained. Details of the enrichment analysis performed by GOrilla are provided in the Supplemental Material. Significance was set as the Bonferroni correction for the 8746 GO terms tested ($P = 5.72 \times 10^{-6}$), with results reported below $P = 5 \times 10^{-4}$. Results were pruned for redundancy in REViGO, with results with >50% dispensability dropped (Supek et al. 2011).

Probe-level expression data were combined using a machine-learning approach in WEKA, to assess the viability of prediction from expression probe data alone (Hall et al. 2009). Classical machine learning algorithms were used to predict outcome using the full dataset (3,233 expression probes, 166 participants for baseline to post-treatment analysis, 110 participants for baseline to follow-up analysis). Five approaches were used: mean prediction with ZeroR; inverse distance weighting with a nearest neighbours algorithm (kNN), with and without subset evaluation; linear kernel-based regression with regression SVM (SMOReg) and a 500-tree Random Forest algorithm. Multiple algorithms were chosen as they optimise different aspects of the learning process. All analyses were performed using 10-fold cross-validation (splitting the cohort into 80% training and 20% test subsamples), repeated 5 times.

Probe-level expression data and genotype data were imported into R for eQTL analyses using the MatrixEQTL package (Shabalin 2012). All transcripts

captured by the assessed probes were mapped to the hg19 build of the human genome. Analyses were performed using a two-stage design. C*is*-eQTLs were calculated independent of the phenotype, using the *modelLINEAR* option and genotypes from a window ± 100 kb of the transcript. Linkage-independent results were obtained by clumping using PLINK2 (250 kb window, $r^2 < 0.25$), and by performing conditional eQTL analyses (Chang et al. 2015). Both techniques identified the same sentinel SNPs (data not shown). Clumped results were retained for the second, phenotype-dependent, stage. SNP-by-treatment response interactions predicting expression change were assessed for both phenotypes, using the *modelLINEAR_CROSS* option in MatrixEQTL, to investigate whether the effect of eQTLs in the data differed in relation to treatment response.

Power analyses for the expression analyses were performed using the pwr package in R.

Ethics

Ethics approval for this study was received from the Ethics Committee at the Faculty of Psychology, Ruhr-Universität Bochum, from the London-Bentham NRES Committee and from the King's College London Psychiatry, Nursing and Midwifery Research Ethics Sub-Committee. All participants provided informed consent. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Results

Phenotype data were available on 187 participants (185 at post-treatment; 122 at follow-up). Following quality control, genotype data were available on 3,343,497 variants (267,037 genotyped) for 181 participants for the post-treatment analysis (122 were available for the analysis at follow-up). Data from 3,233 expression probes were available on 166 (110) participants. Both data types were available on 162 (110) participants.

Demographics and clinical covariates

Demographic data on the cohort are displayed in Table 1. Individuals in the SP group had lower baseline severity than all other groups. Groups also differed by mental disorder comorbidity, with individuals in the DA group exhibiting more comorbidity than other groups, and significantly more than the SP group (details of mental comorbidities are provided in Supplemental Table 1, available online). As expected, there was a higher rate of psychoactive medication use in the DA group compared to all others. Treatment duration also differed significantly across the groups, with shorter treatment in the DA group than in all others. Follow-up duration was significantly longer in the DA group. All covariates showing inter-group differences were included as covariates when defining the treatment response phenotypes (as was a random effect of treatment group; Table 2).

Changes in CGI from pre-treatment to post-treatment and to follow-up are described in Table 3. All treatments were generally effective, with most participants improving on the CGI-S between pre-treatment and post-treatment, and between pre-treatment and follow-up. However, there was considerable variance in the percentage change shown between individuals. Demographic differences between response groups following treatment are described for the whole cohort in Supplemental Table 2 (available online). Significantly lower baseline severity and higher comorbidity was observed in those deteriorating compared to those improving. No other significant differences were observed.

GWAS and polygenic risk score analysis

Results from both GWAS are shown in Supplemental Table 3 and Supplemental Figures 1 and 2 (available online). No variants passed the threshold for genome-wide significance ($P = 5 \times 10^{-8}$), but three independent

Table 3. Treatment response as percentage change in CGI-S, and grouped by improvement (percentage change positive), no change, and deterioration (percentage change negative), in the whole cohort and each treatment.

	Whole cohort	DA	SP	PD-CBT	PD-EXP	Test	Stat	*P*
Response post-treatment								
N	185	95	37	24	29	–	–	–
% change in CGI (Mean [SD])	67.4 [34.6]	62.0 [32.1]	77 [39.5]	67.1 [37.1]	72.9 [31.8]	ANOVA	2.01	0.115
Improved (*N* [%])	165 [89.2]	84 [88.4]	33 [89.2]	21 [87.5]	27 [93.1]	Fisher's exact test		0.613
No change (*N* [%])	16 [8.65]	10 [10.5]	2 [5.41]	2 [8.33]	2 [6.90]			
Deteriorated (*N* [%])	4 [2.16]	1 [1.05]	2 [5.41]	1 [4.17]	0 [0.00]			
Response at follow-up								
N	122	54	32	17	19	–	–	
% change in CGI (Mean [SD])	59.7 [46.2]	52.3 [54.5]	71.4 [37.9]	71.5 [30.7]	49.8 [41.2]	ANOVA	1.90	0.134
Improved (*N* [%])	101 [82.8]	42 [77.8]	28 [87.5]	16 [94.1]	15 [78.9]	Fisher's exact test		0.641
No change (*N* [%])	11 [9.02]	5 [9.26]	3 [9.38]	1 [5.88]	2 [10.5]			
Deteriorated (*N* [%])	10 [8.20]	7 [13.0]	1 [3.13]	0 [0.00]	2 [10.5]			

Groups did not differ on treatment response by either measure.

loci in the analysis to post-treatment and four loci in the analysis to follow-up reached a suggestive level of significance ($P < 5 \times 10^{-6}$). Quantile-quantile plots indicated no substantial genomic inflation in either analysis.

Polygenic risk score analysis from an independent GWAS of response to CBT in children failed to predict response in the whole cohort with $P < 0.001$ (Supplemental Table 4a, available online, threshold adjusted for multiple testing; Euesden et al. 2015). Prediction between the DA treatment group and all other treatment groups explained more variance in outcome than the analysis using the independent GWAS, but predictors were not significant (Supplemental Table 4b, available online). Further discussion of the GWAS and PRSice analyses are included in the Supplemental Material available online.

Individual expression probes

No probes were significantly associated with either phenotype after correcting for multiple testing (all $q > 0.05$; Table 4). The probes with the lowest q values in this analysis showed no overlap with those reported in a parallel analysis of this cohort, examining change in expression over the course of treatment (Roberts et al. under review).

Power analyses indicated the analyses have 80% power to detect associations capturing at least 14.8% (post-treatment), and 19.6% (follow-up) of variance respectively, where $\alpha = 1.55 \times 10^{-5}$ (Bonferroni correction for 3,233 tests).

Data-driven network-based analyses

Clustering by co-expression patterns yielded eight network modules ranging from 750 to 63 probes and a further "grey" module of 459 probes that did not fall into any cluster. Although different clusters showed associations with a variety of sample characteristics, no cluster was associated with either treatment response phenotype (all $P > 0.05$; Figure 1).

Literature-driven GO analysis

HUGO gene names were assigned to 2,652 probes associated with at least one GO term (process, function or component). No significant pathways were found after correction for multiple testing (all $P > 5.72 \times 10^{-6}$). Following removal of redundant GO terms, five processes and one function were associated with $P < 5 \times 10^{-4}$ in the analysis from baseline to post-treatment. From baseline to follow-up, eight processes and two functions were associated with $P < 5 \times 10^{-4}$ (Supplemental Table 5, available online).

Classical machine learning analyses

Classical machine learning methods did not outperform the null model in either analysis. The most effective model was random forest classification (root-mean-square error [RMSE]: 31.3, post-treatment; 42.7, follow-up) but this did not outperform ZeroR, which predicts the mean (RMSE: 30.6, post-treatment; 42.3, follow-up).

Expression quantitative trait loci

Expression quantitative trait loci (eQTL) analysis identified 42,868 *cis*-eQTLs with $q < 0.05$, independent of phenotype. Following the removal of variants in linkage disequilibrium with more strongly associated eQTLs, 1,492 variants were present with $q < 0.05$ (Table 5, Supplemental Table 5, available online). Phenotype-dependent analyses of the interaction between these variants and treatment response predicting expression levels yielded no associations with $q < 0.05$ (Table 6). One interaction was identified with $q < 0.2$ (rs10498246 × treatment response baseline to follow-up, predicting *SP110* (probe nuID:

Table 4. Largest correlations between individual expression probes and the treatment response phenotypes.

Associations between expression probes and treatment outcome					
Probe nuID	Gene	WGCNA module	Pearson's *r*	*P*	*q*
Baseline – post-treatment					
TkiT0uUa.K4LZ5M7h4	*FDFT1*	blue	0.282	2.34×10^{-4}	0.756
0Z7unqF.KAuA5K4ggU	*FDFT1*	grey	0.241	0.00175	1
Eqx.SxEEVcl.VLrWJI	*IL18RAP*	grey	0.237	0.00211	1
Te4VV0giY1VcQvr17E	*RNASE6*	grey	−0.216	0.00515	1
QuynqD354KD6IAXvnk	*YIPF4*	grey	−0.214	0.00550	1
Baseline – follow-up					
TXm4UjVovoAQ4ApVQo	*MYC*	grey	−0.346	2.17×10^{-4}	0.702
Krrborr9LqDhB.rPoo	*HNRNPA1P33*	brown	−0.294	0.00180	1
Ew_iK7UunWqIb0nFeE	*AIF1*	grey	0.265	0.00518	1
6dFQSN.UitTrolYwV4	*MAL*	grey	−0.240	0.0115	1
T0upGOh1A5dC87MXtU	*PPP6C*	turquoise	0.235	0.0136	1

WGCNA modules refer to the data-driven clusters to which each probe belongs.

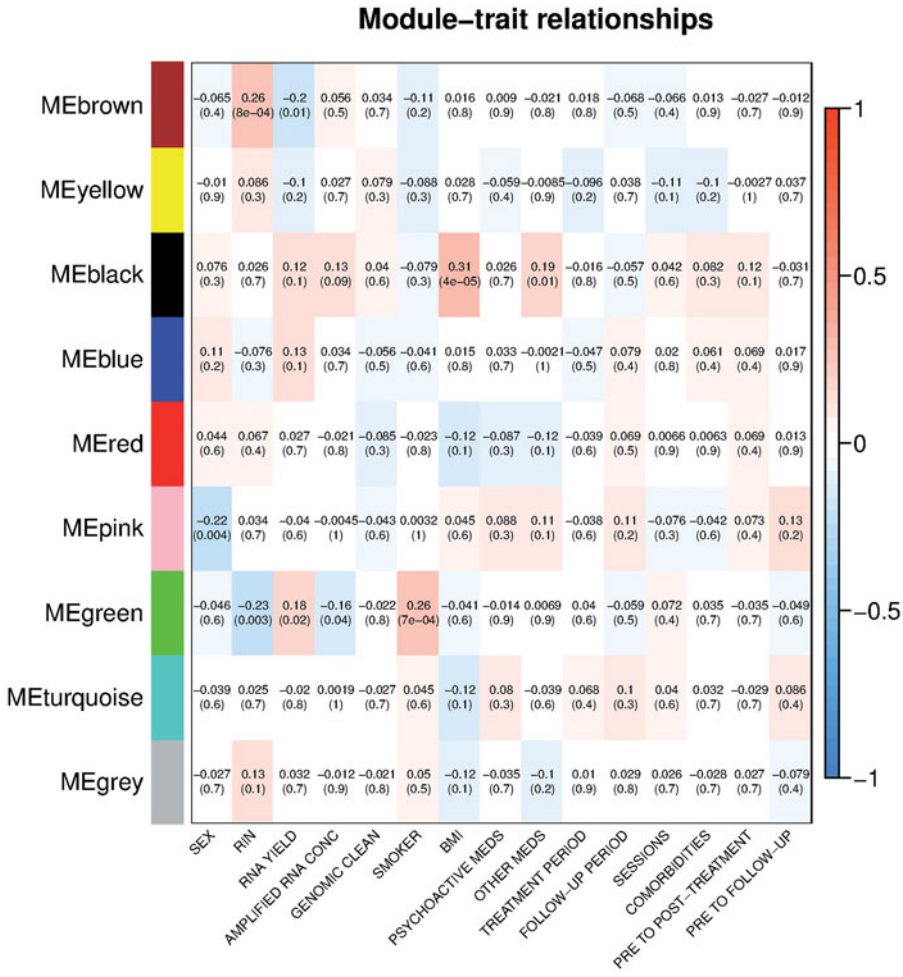

Figure 1. Correlations between expression profiles of module eigengenes from WGCNA and treatment phenotypes (and covariates). Positive correlations are shown in red, negative correlations in blue, with colour intensity indicating strength of correlation. No module expression profile is associated with a treatment response phenotype (all $P > 0.05$).

Table 5. Raw and clumped results from the expression QTL analysis, by false discovery rate.

Linkage-dependent and -independent blood eQTLs		
FDR q threshold	No. of variants	No. of linkage-independent variants
0.01	26,566	788
0.05	42,868	1492
0.1	54,795	2159
0.5	61,799	2503

fcV3S0U75lf1e3op0U) expression, $B = -0.0041$, $P = 2.23 \times 10^{-5}$, $q = 0.103$).

Discussion

We performed genome-wide analysis of genetic variation and pre-treatment gene expression to assess independent and combined effects on response to CBT for anxiety disorders in a cohort of adult participants. This is the first analysis to integrate this data in studying psychological treatment response, and (together with a companion paper; Roberts et al. under review) is an analysis of the largest psychological treatment cohort in which gene expression analyses have been performed. Despite this, no variants or expression profiles were associated (at a genome-wide level of significance) with treatment response across the treatment period or at a 6-month follow-up.

The cohort is larger than previous studies of the effect of gene expression on response to CBT in anxiety disorder (Levy-Gigi et al. 2013; Yehuda et al. 2013). However, it is clear that this study is underpowered to detect all but the largest effects on response, and that robust prediction requires larger cohort sizes. Integrating data from two different approaches (that is, genotyping and gene expression) increases power, but requires two sets of quality control, resulting in fewer samples with full data available (Ritchie et al. 2015). Obtaining a large sample size for a study such as this is non-trivial. Prospective recruitment results in a high rate of attrition as participants withdraw from treatment or are lost to follow-up. Furthermore, this attrition is likely to be related to poor treatment response.

Table 6. Top six results from eQTL-outcome interactions predicting expression level.

eQTL x treatment response predicting gene expression					
SNP	Probe nuID	Gene	β	*P*	*q*
Baseline – post-treatment					
rs11260538	94gYDdn0tHeWCmeGk0	*SDF4*	0.00394	7.86×10^{-4}	1
rs3129996	llGfH57t5ug93Xe1XU	*KIAA1949*	−0.00431	0.00126	1
rs16965033	onsnvop.hKDoejReHU	*HERPUD1*	−0.00528	0.00155	1
rs3743888	9Sft35eUe7g2mGiR5E	*AXIN1*	0.00270	0.00163	1
rs11850781	NoXN6F3SR7AMv_v_6Q	*NIN*	−0.00577	0.00164	1
Baseline – follow-up					
rs10498246	fcV3S0U75lf1e3op0U	*SP110*	−0.00414	2.23×10^{-5}	0.103
rs6701295	cXl3ddwDJC3qA16ri4	*SMG5*	0.00249	2.26×10^{-4}	0.523
rs1737046	Tt5huq2hqZcdZqzRSc	*HCG4*	−0.00318	3.99×10^{-4}	0.616
rs4602357	6olooHit00T3Imfo5U	*CEP63*	0.00251	5.94×10^{-4}	0.688
rs12343854	fpnvXlHteCO4OrrGP0	*SEMA4D*	0.00231	9.24×10^{-4}	0.784

No interactions are significant at $q < 0.05$.

The aim of this investigation was to study genetic and transcriptomic correlates of response to exposure-based therapy, which may act across diagnostic boundaries. We sought to increase power by recruiting from treatment studies for two disorders with differing treatment procedures. Studying anxiety disorders as a heterogeneous group has been effective in genomics (Otowa et al. 2016). However, combining groups increases heterogeneity, partially negating the increased power from the enlarged sample size. The disorders studied are conceptually distinct, and treatment is tailored to the needs of the participant, differing between and within diagnostic groups. Combining across disorder groups incurs disorder-specific differences, such as the lower baseline severity of the SP group and higher comorbidity in the DA group herein. These differences reflect the varying nature of the disorders and recruitment to treatment – for example, the high comorbidity of the DA group is likely to result from secondary consequences of avoiding dental treatment, such as a phobia of vomiting or social anxiety about visiting the dentist. Although we have sought to control for this heterogeneity statistically, it limits the conclusions of this investigation. Furthermore, many social and environmental influences on treatment response have been proposed, and the covariates controlled for within this analysis cannot correct for all possible confounds. Nevertheless, investigating biological correlates of therapy requires a pragmatic approach. Cohorts of individuals receiving psychological therapies, particularly those outside of clinical trials, are prone to heterogeneity and attrition. For any biological predictor to contribute valuably to therapeutic decision-making, it must be robust to these limitations.

Although no genome-wide gene expression studies have investigated response to CBT, single-gene studies have suggested a role for differential *FKBP5* expression in response to CBT for PTSD (Levy-Gigi et al. 2013; Yehuda et al. 2013). One probe in this study, ZdI45Se3VG7s869FKo, captures expression of FKBP5, but was not associated with either outcome (baseline to post-treatment: $P = 0.0533$, $q = 0.999$; baseline to follow-up: $P = 0.607$, $q = 0.997$). However, the low power of the analysis (and differences between this cohort and those examined previously), limit strong conclusion.

Gene expression differs between different tissues and organs; expression observed in peripheral blood may not reflect that in the brain. Previous studies suggest moderate correlation between gene expression in different tissues, varying by individual genes (Sullivan et al. 2006). The emergence of reference panels such as the GTEx Portal has made in silico assessment of blood–brain expression correlations at the individual gene level viable (Consortium 2015). As such, peripheral blood gene expression can provide relevant insights into gene expression in the brain, and this will improve as further brain expression samples are added to the reference. From a pragmatic standpoint, gene expression markers of treatment response will only be useful if they can be obtained from peripheral tissues – while the effect of gene expression in brain tissues is of biological interest, it cannot be of practical utility in this case.

Assessing the severity of anxiety disorders can be performed using different rating scales, with varying characteristics. No consensus regarding the best means of measuring response to CBT exists (Loerinc et al. 2015). In this study, the CGI-S was used as a measure of clinical concern across treatment groups, allowing a single measure to be used to assess general functioning. However, this measure bears a number of limitations. It is a subjective measure of clinical judgement that may fail to capture the participant's anxiety as appropriately as a self-report measure. Treatment response is likely to involve multiple components, including reduction in fear and increase in functioning, that a single measure may not capture. One potential solution is to combine

a number of scales using different assessors and assessing different aspects of treatment response. However, this increases the complexity of the analysis and the potential for spurious results. In addition, it would be difficult to interpret in a useful manner.

Anxiety disorders are widespread and disabling, and CBT is a first-line treatment for these conditions. CBT involves a considerable investment from the recipient, and a significant minority of those receiving it do not respond adequately. Stable pre-treatment predictors of outcome are required. To date, genetic variants and gene expression levels have not provided these predictors, individually or in combination. However, this does not argue against the continued study of the biology underlying CBT response. The pattern of findings to date is consistent with the highly polygenic model that has been proposed to influence behavioural traits (Chabris et al. 2015). Although individual genetic variants seem extremely unlikely to be valuable predictors, prediction might be achieved through the combined effect of many genetic variants, at multiple levels of analysis.

Response to CBT is likely to be influenced by genes and by the environment, and continued research to define reliable environmental and clinical predictors of response is vital – genetics can only be clinically useful in the context of known environmental and clinical risk factors (Hudson et al. 2015). Studies of genetic variation, gene expression and epigenetics should either adopt a hypothesis-neutral approach (exploring variation genome-wide), or be informed by robust associations in related traits (rather than assumed biological relevance). The effects of individual transcript differences are likely to be small. For these insights to be discovered, cohorts of thousands of individuals must be treated in as homogenous a manner as possible from recruitment to the analysis of the resulting data. This is not straightforward (especially given the heterogeneity inherent to CBT) but the example of the many international consortia driving advances in complex trait genetics demonstrates such investigations can yield valuable insights.

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Summary statistics from all analyses are available on request from the authors.

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G. Breen is a consultant in pre-clinical genetics for Eli Lilly. All other authors declare no financial interests.

ORCID

Jonathan R.I. Coleman http://orcid.org/0000-0002-6759-0944
Kathryn J. Lester http://orcid.org/0000-0002-0853-2664
Susanna Roberts http://orcid.org/0000-0002-2616-2644
Robert Keers http://orcid.org/0000-0001-7954-7583

Simone De Jong http://orcid.org/0000-0002-8002-7195
Héléna Gaspar http://orcid.org/0000-0002-5540-2707
Silvia Schneider http://orcid.org/0000-0003-0698-8411
Jürgen Margraf http://orcid.org/0000-0001-5207-7016
Gerome Breen http://orcid.org/0000-0003-2053-1792
Thalia C. Eley http://orcid.org/0000-0001-6458-0700

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