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Gene expression biomarkers of response to citalopram treatment in major depressive disorder

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There is significant variability in antidepressant treatment outcome, with ~ 30–40% of patients with major depressive disorder (MDD) not presenting with adequate response even following several trials. To identify potential biomarkers of response, we investigated peripheral gene expression patterns of response to antidepressant treatment in MDD. We did this using Affymetrix HG-U133 Plus2 microarrays in blood samples, from untreated individuals with MDD ($N = 63$) ascertained at a community outpatient clinic, pre and post 8-week treatment with citalopram, and used a regression model to assess the impact of gene expression differences on antidepressant response. We carried out technical validation of significant probesets by quantitative reverse transcriptase PCR and conducted central nervous system follow-up of the most significant result in post-mortem brain samples from 15 subjects who died during a current MDD episode and 11 sudden-death controls. A total of 32 probesets were differentially expressed according to response to citalopram treatment following false discovery rate correction. *Interferon regulatory factor 7* (*IRF7*) was the most significant differentially expressed gene and its expression was upregulated by citalopram treatment in individuals who responded to treatment. We found these results to be concordant with our observation of decreased expression of *IRF7* in the prefrontal cortex of MDDs with negative toxicological evidence for antidepressant treatment at the time of death. These findings point to *IRF7* as a gene of interest in studies investigating genomic factors associated with antidepressant response.
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Introduction

Major depressive disorder (MDD) is a debilitating and chronic condition that affects between 5 and 10% of the general population.[1] The treatment of MDD includes a variety of biopsychosocial approaches, but in frontline medical practice, it is primarily based on pharmacological interventions, commonly with an initial prescription of a serotonin selective reuptake inhibitor antidepressant.[2–5] Although antidepressants are generally effective, and in spite of a growing number of different types and classes, there is significant variability in antidepressant treatment outcome. Almost 60% of patients do not recover following a single antidepressant trial,[6] and 30 to 40% of patients do not present with an adequate response following several trials.[2,4,5] The failure to respond has important individual, economic and social consequences for both patients and their families.

Although it would be ideal to better target available treatments to individual patients, we know little about predictors of response. Among factors associated with response to antidepressant treatment in MDD, depression severity, comorbid anxiety disorders, longer duration and frequency of previous episodes and an older age of onset have all been identified as predictors of poor response.[7] However, given low sensitivity and specificity, none of these factors are useful when planning treatment options for MDD

patients.[7] Response to pharmacological treatment has also been demonstrated to segregate in families. Only a handful of studies, however, have investigated familial patterns of response to antidepressants,[8–10] and subsequent studies have investigated the association of genetic variants with treatment response. As the downstream effects of different predictors of antidepressant response—be they genetic and/ or environmental—should ultimately be mediated by changes in gene function, investigating predictors and underlying biological factors associated with response by means of gene expression studies may provide valuable information and help identify biomarkers of antidepressant response. This approach has been favorably used in the investigation of response to treatment for several medical conditions, such as breast cancer,[11–13] colon cancer[14] and cardiovascular disease.[15–17]

In the context of neuropsychiatric disorders, peripheral blood gene expression studies of drug response have value beyond a proxy model of central nervous system (CNS) phenomena, given that both peripheral and central processes are involved in the individual response to treatment. Surprisingly, to date, most studies investigating gene expression changes to antidepressant treatment have been performed in patients with Alzheimer's disease,[18–20] whereas most studies

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of antidepressant response in depression have been carried out in rodent models. There is limited, although promising, work probing gene expression changes associated with antidepressant response in patients with MDD.[21–23] Here, we report on a study assessing changes in genome-wide expression as a function of 8-week citalopram treatment response in a sample of patients with current MDD who were untreated at the study onset. To our knowledge, this is the first genome-wide expression study investigating patterns of response to antidepressant treatment in MDD patients.

Materials and methods

MDD sample. Our subjects were untreated males and females ($N = 63$) with a diagnosis of MDD without psychotic features, according to the SCID-1 (Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders-fourth edition (DSM-IV) Axis I Disorders) ascertained at a community outpatient clinic at the Douglas Mental Health University Institute (see Supplementary Table 1 for clinical details). Subjects were excluded from the study if they had comorbidity with other major psychiatric disorders, including a previous manic or hypomanic episode (diagnosis of bipolar disorder) or a diagnosis of schizophrenia. However, subjects were not excluded if they presented with anxious traits, as anxiety is highly comorbid with MDD. They were also excluded if they presented comorbidity with substance disorders or if they had positive tests for illicit drugs, both pre- and post-citalopram treatment. Subjects could not have had previously used citalopram and could not have been treated for the current episode of major depression if they had a recurrent form of MDD (46.8% recurrent MDD; Figure 1).

Citalopram treatment. Patients were treated with citalopram for a total of 8 weeks, starting with an initial dose of 10 mg per day, which was subsequently titrated up to a maximum of 60 mg per day. Assessments of depression severity were carried out three times during the trial (pretreatment, 1 month after and post-treatment commencement) using the 21-item Hamilton Rating Scale for Depression (HAMD-21).

Blood samples and RNA processing. Blood samples were collected in six PAXgene blood RNA tubes (PreAnalytix, Hombrechtikon, Switzerland) and two EDTA vacutainer tubes

(BD, Mississauga, Ontario, Canada) from cases before the start of the trial and again at trial completion. PAXgene tubes were frozen using a sequential freezing process. This involved storing tubes at room temperature for 3 h, transferring to 4 °C overnight, −20 °C for 6–8 h and then finally to −80 °C. Four tubes were used for RNA extraction and microarray processing and the remaining two tubes were kept at −80 °C for subsequent RNA extraction and microarray validation studies. The EDTA tubes were used to obtain plasma for citalopram concentration measures. All subjects included in the study provided informed consent and the project was approved by the internal review board for the Douglas Mental Health University Institute.

The RNA used in this study had a minimum A260/A280 ratio of > 1.9. Integrity and possible degradation of the RNA samples was determined using a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and samples had an average RNA integrity number of 8.4 and a 28S/18S ratio of > 1.6.

High-performance liquid chromatography. High-performance liquid chromatography analysis was carried out to determine subject compliance at the end of the trial. Citalopram concentrations were determined from plasma removed from week 8 blood tubes following centrifugation. Plasma samples were transferred to cryogenic tubes for storage at −80 °C until time for use.

For drug extraction, 1 ml of plasma was first spiked with 150 ng desipramine, which acted as an internal standard, and then basified with 100 µl NaOH. To this, 10 ml of 1% butanol:isoamyl alcohol was added to bring the drug into the aqueous phase. The drug was then back-extracted using 200 µl HCl, and subsequently 50 µl was injected into the high-performance liquid chromatography machine. The samples were run through a reverse-phase C-18 column and peaks were detected with an ultraviolet detector. Citalopram concentration measures were derived using a standard curve constructed from plasma spiked with known concentrations of citalopram and processed as mentioned above. To take into account intraday variability, new standard curves were made before each high-performance liquid chromatography run.

Microarrays. We used the Affymetrix GeneChip Human Genome U133 Plus 2.0 array (Affymetrix, Santa Clara, CA, USA), consisting of 54 000 probesets, allowing for the analysis of 47 000 transcripts, representative of 38 500
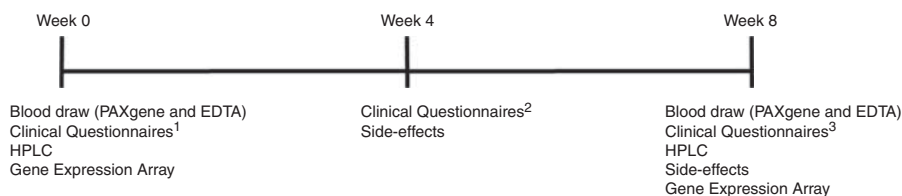
Figure 1 Timeline and steps taken within the 8-week citalopram trial. [1]SCID-I; SCID-II; sociodemographic questionnaire; HAMD-21 and Hamilton Anxiety questionnaire. [2]HAMD-21 and Hamilton Anxiety questionnaire. [3]HAMD-21 and Hamilton Anxiety questionnaire. Abbreviations: HAMD-21, 21-item Hamilton Rating Scale for Depression; HPLC, high-performance liquid chromatography; SCID-I, Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders-fourth edition (DSM-IV) Axis I Disorders; SCID-II, Structured Clinical Interview for DSM-IV Axis II Personality Disorders.

human genes. Sample preparation and processing, hybridization to the array and normalization were performed as suggested by the manufacturer and described in the Affymetrix GeneChip Expression Analysis Manual (Affymetrix). The GeneChip IVT Express and the GeneChip Hybridization, Wash and Stain kits from Affymetrix were used for first and second complementary DNA synthesis, IVT/labeling and purification of RNA, fragmentation and purification. GeneChip analysis was performed as described below.

Data analysis. Normalization of gene expression arrays was executed using Robust Multi-Array as implemented in Partek Genomics Suite (Partek, St Louis, MO, USA; Version 6.3 (03.10.08)), with initial probeset filtering based on Microarray Suite 5 (MAS5) present/absent calls, whereby only those probesets present in at least 75% of the total sample were retained for further analysis. This filtering procedure is based on the methods described by McClintick and Edenberg[24] whereby the use of present-call filtering functions in minimizing the impact of large differential signals explained by a small proportion of the sample. Following this procedure, the number of probesets was reduced to 19 277. We also removed probesets associated with the Y chromosome to minimize the likelihood of false positive results arising from the inclusion of probesets from this chromosome. Several indicators of RNA quality were used to prefilter samples before analysis; these included β-actin $5'/3'$ ratio ($\geqslant 0.3$), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) $5'/3'$ ratio ($\geqslant 0.5$) and RawQ. Outlier detection for arrays was also carried out using principal component analysis.

Following initial filtering, the remaining analyses were carried out using the statistical software R (www.r-project.org). For the identification of the number of probesets to be used in our analyses to identify a set of differentially expressed genes according to response to citalopram treatment, we used a two-step statistical approach. First, we preselected a set of 1160 genes showing the highest fold change in $\log_2$ expression between pre- and post-treatment. In our sample, this corresponded to a fold change $\geqslant 1.08$ or, alternatively, $\leqslant 0.92$. Subsequently, a regression model was carried out for each gene using the difference between pre- and post-HAMD-21 scores as the dependent variable and the difference in $\log_2$ expression as the independent variable. We accounted for the multiplicity of tests by adjusting the P-values such that the false discovery rate (FDR) was $<10\%$.

Gene expression validation. The GenomeLab GeXP Genetic Analysis System (Beckman Coulter, Mississauga, Ontario, Canada) was used to validate significant gene expression findings. This was carried out through Genome Quebec at the RNomics Laboratory at the University of Sherbrooke (Sherbrooke, Quebec, Canada). The GeXP system enabled the multiplexing of 30 genes within a single sample well through the use of both gene-specific and universal primers for amplification of complementary DNA fragments of varying length. Using capillary electrophoresis, at the end of the PCR, fragments migrate by size and thus separate into gene-specific bands, which can be visualized as peaks in a chromatogram. The area under the curve is

equivalent to the concentration of mRNA in the original sample. The concentration measures are scaled to a control RNA sample, which was included on each plate and then normalized to GAPDH. The sample used for this consisted of pre- and post-RNA samples for all subjects included in the analyses and a control RNA sample from peripheral blood.

Follow-up gene expression studies in the prefrontal cortex. A TaqMan gene expression assay for *interferon regulatory factor 7* (*IRF7*) was used to investigate the expression of this gene in an all-male sample of 15 subjects who died during a depressive episode (average age $= 36.8 \pm 11.2$ years) and a group of 11 sudden-death, psychiatrically normal controls (average age $= 34.3 \pm 11.5$ years) in the prefrontal cortex. The groups were similar in age ($P = 0.42$), brain pH ($P = 0.481$) or post-mortem interval ($P = 0.854$). Toxicological investigation of post-mortem tissue showed no evidence of antidepressants or other psychotropic treatments. RNA was extracted from brain tissue using the RNeasy Lipid Tissue Kit from Qiagen (Toronto, Ontario, Canada). RNA concentration was assessed using a Nanodrop (Thermo Scientific, Wilmington, DE, USA) and RNA quality using the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and brain samples had an average RNA integrity number of 7.2. Complementary DNA was produced using Moloney murine leukemia virus reverse-transcriptase and Oligo-dT primers and subsequently diluted to a concentration of 2.5 ng μl^{-1} for use in quantitative reverse transcriptase-PCR. Quantitative reverse transcriptase- was performed in 384-well plates on the 7900HT Real-Time PCR system from Applied Biosystems (Carlsbad, CA, USA) under fast conditions using PerfeCTa qPCR Fastmix (Quanta Biosciences, Gaithersburg, MD, USA). Target and endogenous control (β-actin) were run in the same well and plates were analyzed using the ΔCt method in RQ Manager (Applied Biosystems) with the calibrator being a sample with low variability between replicates.

Results

A total of 63 subjects with MDD were treated with citalopram for 8 weeks and their gene expression profiles were obtained pre- and post-treatment. Demographic and baseline clinical variables had no significant effect on treatment outcome (Supplementary Table 2). We observed no instances of suicidal behavior or significant increase in ideation during the trial, necessitating trial discontinuation. As well, past suicidal behavior had no effect on the results observed in the citalopram sample. Side effects were measured after 4 and 8 weeks of treatment, and there were no significant differences between responders and nonresponders in side-effect profiles at week 4 (P-value $= 0.98$) or at week 8 (P-value $= 0.08$). All subjects had detectable levels of serum citalopram at the end of 8 weeks, and we observed a significant correlation between citalopram dose and plasma concentration ($r = 0.392$, P-value $= 0.002$).

Differential gene expression according to treatment irrespective of treatment response. At the outset, probesets were filtered based on their presence in 75% of

the total sample, resulting in a reduction of the total number of probesets to 19 277. The first step in our analyses was to select a subset of probesets showing change in expression according to treatment irrespective of response. Three criteria were used for this purpose: (1) significance, (2) fold change and (3) effect size. The lists of probesets pertaining to each criterion are summarized in Supplementary Tables 3–5. The Venn diagram (Supplementary Figure 1) illustrates the number of probesets unique and common to each of the aforementioned criteria. The 417 common probesets were then used in the analysis of treatment response, as outlined below.

Gene expression changes associated with response to antidepressant treatment. For the assessment of response, we did not utilize the more traditional procedure of dichotomization of subjects into responder and nonresponder groups based on a HAMD cutoff, but opted instead to analyze response quantitatively. This is because of HAMD scores being continuous measures and the fact that depressive/ response states are not necessarily categorical following 8 weeks of treatment. Furthermore, from a statistical point of view, dichotomization of continuous variables is not recommended as it can result in an unnecessary loss of information, as well as lead to reduced statistical power and an increased probability of type II error.[25]

In order to determine gene expression changes associated with treatment response, we conducted regression analyses using the log fold change of the 417 differentially expressed probesets, which are differentially expressed according to treatment, to predict the change in HAMD scores between pre and post-treatment. We found that changes in the expression of 69 probesets significantly predicted the change in HAMD scores following treatment (P-value <0.05). Of these, 32 probesets remained statistically significant after FDR correction (FDR $\leqslant 0.10$; Table 1). Using DAVID[26] to probe over-represented ontologies, these probesets were found to code for genes involved in immune response, transcription factors, apoptosis and other cellular processes (Table 2). Among the 32 probesets, probeset 208436_s_at, which maps to the gene coding for *IRF7*, a transcription factor regulating the cytokine interferon-α, was the only one that was significantly associated with the change in depression severity using an FDR of 0.05.

Technical validation of microarray findings. The validation results are shown in Table 3. Expression values in the validation assays were consistent and in the same direction with those observed in the array; however, only eight of these genes displayed significant correlations. *IRF7* expression values observed using these techniques were similar to those seen using the microarrays. To exclude the possibility that the differences observed in IRF7 were because of inflammatory processes associated with possible infections, we investigated whether they were associated with changes in white cell counts. However, no significant differences were observed between pre- and post-treatment measures ($P = 0.12$).

Expression of *IRF7* in prefrontal cortex. To gain insight into the possible CNS validity of our peripheral results, we investigated *IRF7* expression in prefrontal cortex, a brain region thought to play an important role in the neurobiology of major depression.[27] To this end, we studied the levels of *IRF7* by means of quantitative reverse transcriptase-PCR using a TaqMan assay in 15 subjects who died in the context of an episode of MDD and 11 sudden-death controls with comparable age, post-mortem interval, RNA integrity number and pH, and no current or lifetime history of MDD. We found that *IRF7* was significantly downregulated in MDD subjects when compared with controls (fold change $= -1.74$, P-value $= 0.01$).

Discussion

A large proportion of patients taking antidepressants display unfavorable or minimal response and, as a consequence, they typically are exposed to multiple antidepressant trials and endure a protracted illness course. The potential individual and societal advantages resulting from the effective identification of biomarkers of antidepressant response are numerous and ranging from economic benefits to reduced medical and social burden. In this study we carried out an analysis of peripheral gene expression with the aim of determining potential biomarkers for response to citalopram. We have identified a set of 32 probesets whose differential expression is significantly associated with the change observed in depression severity following treatment. As well, we have found additional evidence for the role of immune dysregulation in both the periphery and the brain.

IRF7 is part of a nine-member transcription factor family responsible for the transcriptional regulation of interferon-mediated signaling pathways.[28] IRF7 has been shown to complex with IRF1, IRF3 and histone transacetylases to regulate transcription of the proinflammatory cytokine, interferon-α. In our study, we observed an upregulation of IRF7 following treatment with citalopram. IRF7 upregulation could be the result of citalopram stimulating fluctuations in interferon levels,[29] or through IRF7 promoter methylation.[30] Initiation of the inflammatory immune response gives rise to changes in neuromodulatory effects mimicking the outcomes elicited by stressful life events.[31] The effects of immune-derived stress on the CNS and the periphery, and the subsequent effects on depressed and anxious mood, are not a point of contention in MDD research;[32–34] however, the exact mechanistic nature of this process and the specific effects of immunological agents are not completely elucidated. In psychiatric disorders, with particular emphasis on depression, there is evidence for a dysregulation of the balance of immunomodulatory cytokines in consequence to an increased preponderance of proin-flammatory cytokines (for example, interleukin-1β, interleukin-6, tumor necrosis factor-α and interferon-α-α) due to stress.[35–38] As well, the administration of pro-inflammatory cytokines in both animals[39] and euthymic subjects[40,41] triggers the onset of depressive symptomatology, such as depressed mood, fatigue, anhedonia and anorexia.[42–44] Furthermore, alterations in the proinflammatory/anti-inflammatory cytokine balance has been demonstrated as being an explanatory factor for the way the brain adapts to stress.[45] The administration of interferon-α has been demonstrated to generate depressive-like symptoms,[46,47] although its exact mechanism of action

Table 1 The 32 probesets found to be significantly associated with the change in HAMD score and meeting 0.10 FDR criteria

Probeset	Gene symbol	Gene title	Cytoband	P-value (uncorrected)	FDR	Fold change	Direction
208436_s_at	IRF7	Interferon regulatory factor 7	11p15.5	0.0002	0.0316	1.12	UP
228230_at	PRIC285	Peroxisomal proliferator-activated receptor A interacting complex 285	20q13.33	0.0021	0.0644	1.10	UP
218429_s_at	C19orf66	Chromosome 19 open reading frame 66	19p13.2	0.0024	0.0653	1.11	UP
209417_s_at	IFI35	Interferon-induced protein 35	17q21	0.0025	0.0656	1.10	UP
239266_at	—	—	—	0.0027	0.0660	−1.07	DOWN
53720_at	C19orf66	Chromosome 19 open reading frame 66	19p13.2	0.0037	0.0676	1.11	UP
203258_at	DRAP1	DR1-associated protein 1 (negative cofactor 2 α)	11q13.3	0.004	0.0680	1.09	UP
212647_at	RRAS	Related RAS viral (r-ras) oncogene homolog	19q13.3-qter	0.004	0.0680	1.11	UP
229803_s_at	—	—	—	0.0042	0.0682	−1.08	DOWN
227299_at	CCNI	Cyclin I	4q21.1	0.0044	0.0684	1.10	UP
210740_s_at	ITPK1	Inositol 1,3,4-triphosphate 5/6 kinase	14q31	0.005	0.0688	1.08	UP
206284_x_at	CLTB	Clathrin, light chain (Lcb)	4q2-q3l5q35	0.0054	0.0691	1.11	UP
1555491_a_at	C19orf66	Chromosome 19 open reading frame 66	19p13.2	0.0057	0.0692	1.09	UP
229350_x_at	PARP10	Poly (ADP-ribose) polymerase family, member 10	8q24.3	0.0066	0.0735	1.08	UP
215749_s_at	GORASP1	Golgi reassembly stacking protein 1, 65kDa	3p22-p21.33	0.0078	0.0781	1.08	UP
205875_s_at	TREX1	Three prime repair exonuclease 1	3p21.31	0.0097	0.0838	1.10	UP
1562836_at	—	—	—	0.0109	0.0867	−1.12	DOWN
1558937_s_at	—	—	—	0.0122	0.0893	−1.13	DOWN
202779_s_at	UBE2S	Similar to ubiquitin-conjugating enzyme E2 Subiquitin-conjugating enzyme E2S	19q13.43	0.0128	0.0904	1.13	UP
225058_at	GPR108	G protein-coupled receptor 108	19p13.3	0.0132	0.0911	1.10	UP
220998_s_at	UNC93B1	Unc-93 homolog B1 (C. elegans)	11q13	0.0147	0.0933	1.09	UP
235292_at	FLJ32255	Hypothetical protein LOC643977	5p12	0.0148	0.0935	1.10	UP
202687_s_at	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	3q26	0.0157	0.0947	1.13	UP
207713_s_at	RBCK1	RanBP-type and C3HC4-type zinc finger containing 1	20p13	0.0164	0.0955	1.09	UP
223152_at	PPP1R12C	Protein phosphatase 1, regulatory (inhibitor) subunit 12C	19q13.42	0.0169	0.0961	1.12	UP
211250_s_at	SH3BP2	SH3-domain binding protein 2	4p16.3	0.0172	0.0964	1.09	UP
213621_s_at	GUK1	Guanylate kinase 1	1q32-q41	0.0178	0.0971	1.15	UP
218961_s_at	PNKP	Polynucleotide kinase 3´-phosphatase	19q13.3-q13.4	0.0187	0.0980	1.08	UP
212983_at	HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	11p15.5	0.0193	0.0985	1.12	UP
222660_s_at	RNF38	Ring finger protein 38	9p13	0.0197	0.0989	−1.08	DOWN
225294_s_at	TRAPPC1	Trafficking protein particle complex 1	17p13.1	0.0202	0.0993	1.11	UP
204858_s_at	TYMP	Thymidine phosphorylase	22q13l22q13.33	0.0207	0.0997	1.13	UP

Abbreviations: DOWN, downregulated; FDR, false discovery rate; HAMD, Hamilton Rating Scale for Depression; UP, upregulated.
Information of differential gene expression between pre- and post-treatment values is represented through a fold change (post/pre) and the directionality of expression following treatment (UP and DOWN).

and pathological consequences still remain predominantly underexplored.[48]

Recently, Spijker et al.[49] published peripheral gene expression findings following stimulation with lipopolysaccharide, a lipoglycan shown to produce depressive-like behaviors in humans.[50] In this study, the authors found a difference in stimulated gene expression in MDD versus controls, leading to the identification of a predominantly immune-related biomarker with enough sensitivity and specificity to differentiate between cases and controls.

Traditionally, studies have used peripheral cells, such as lymphocytes or platelets, from affected patients to investigate markers of illness in vitro. These studies have several limitations. For instance, cell models, although informative, do not represent normal cell environment and lack the complexity of the host organism. The investigation of peripheral cells in vivo, rather than in vitro, addresses some

of the limitations of previous studies, and as such, a few gene expression studies investigating peripheral samples have been recently conducted in neuropsychiatric conditions such as autism,[51–55] schizophrenia[56,57] and Alzheimer's disease.[19,20] However, one of the central questions of these studies, particularly when they are interested in the investigation of markers of illness, is whether or not their findings are representative of gene changes taking place in the brain. Peripheral studies of drug response, on the other hand, have value not only as a proxy model of CNS phenomena, but as an indicator of peripheral processes involved in individual response to treatment.[58–62] Examples of peripheral factors involved in drug response include pharmacokinetic, pharmacodynamic, metabolizing and endocrinal factors, all of which may influence peripheral mRNA expression.

That said, it remains important to assess the CNS validity of mRNA expression differences seen in peripheral tissues.

Table 2 Gene ontology analysis of probesets significantly associated with citalopram response

Category	Term	Count	%	P-value
Biological process	Nucleobase, nucleoside and nucleotide metabolic process	4	13.33%	0.0057
Molecular function	Hydrolase activity, acting on acid anhydrides	5	16.67%	0.0109
Molecular function	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	5	16.67%	0.0133
Molecular function	Pyrophosphatase activity	5	16.67%	0.0146
Molecular function	Nuclease activity	3	10.00%	0.0278
Cellular component	Golgi apparatus	4	13.33%	0.0302
Molecular function	Ribonucleotide binding	7	23.33%	0.0341
Cellular component	Golgi apparatus	4	13.33%	0.0399
Molecular function	Purine nucleotide binding	7	23.33%	0.0409
Biological process	Nucleotide metabolic process	3	10.00%	0.0434
Molecular function	Purine ribonucleotide binding	7	23.33%	0.0460
Biological process	DNA replication	3	10.00%	0.0474

Count refers to the number of probesets (out of 32) that belong to the ontological category and term, the percentage (%) to which this corresponds and the P-value of the association.

Table 3 Validation of microarray findings using GeXP or qPCR

Gene	Probeset	Uncorrected P-value Microarray	FDR	Fold change (pre/post)	Fold change (pre/post) GeXP or real time	Pearson's correlation	
						r	P-value
IRF7	208436_s_at	0.0002	0.032	1.12	1.09	0.631	<0.0001
PRIC285	228230_at	0.002	0.064	1.10	1.15	−0.115	0.234
C19orf66	218429_s_at	0.002	0.065	1.11	1.17	0.286	0.003
IFI35	209417_s_at	0.003	0.066	1.10	1.03	0.477	<0.0001
C19orf66	53720_at	0.004	0.068	1.11	1.17	0.248	0.01
CCN1	227299_at	0.004	0.068	1.10	1.06	−0.063	0.255
DRAP1	203258_at	0.004	0.068	1.09	1.06	0.141	0.066
CLTB	206284_x_at	0.005	0.069	1.11	1.02	0.009	0.461
ITPK1	210740_s_at	0.005	0.069	1.08	1.06	0.036	0.352
C19orf66	1555491_a_at	0.006	0.069	1.09	1.17	0.265	0.006
TREX1	205875_s_at	0.009	0.084	1.10	1.09	0.281	0.001
GPR108	225058_at	0.013	0.091	1.10	1.02	−0.076	0.208
FLJ32255	235292_at	0.015	0.093	1.10	1.00	0.085	0.184
UNC93B1	220998_s_at	0.015	0.093	1.09	1.10	0.157	0.046
TNFSF10	202687_s_at	0.016	0.095	1.13	1.03	0.056	0.276
PPP1R12C	223152_at	0.017	0.096	1.12	1.02	−0.017	0.428
SH3BP2	211250_at	0.017	0.096	1.09	1.01	−0.015	0.438
GUK1	213621_s_at	0.018	0.097	1.15	1.12	0.332	<0.0001
HRAS	212983_at	0.019	0.099	1.12	1.07	0.072	0.225
RNF38	222660_s_at	0.019	0.099	−1.08	1.01	0.116	0.108
TRAPPC1	225294_s_at	0.02	0.099	1.11	1.03	−0.101	0.141

Abbreviations: *CCN1, cyclin 1; CLTB, clathrin, light chain (Lcb); C19orf66, chromosome 19 open reading frame 66; DRAP1, DR1-associated protein 1 (negative cofactor 2 α)*; FDR, false discovery rate; *FLJ32255, hypothetical protein LOC643977; GUK1, guanylate kinase 1; GPR108, G protein-coupled receptor 108; HRAS, v-Ha-ras Harvey rat sarcoma viral oncogene homolog; IFI35, interferon-induced protein 35; IRF7, interferon regulatory factor 7; ITPK1, inositol 1,3,4-triphosphate 5/6 kinase; PARP10, poly (ADP-ribose) polymerase family, member 10; PPP1R12C, protein phosphatase 1, regulatory (inhibitor) subunit 12C; PRIC285, peroxisomal proliferator-activated receptor A interacting complex 285; RNF38, ring finger protein 38; SH3BP2, SH3-domain binding protein 2; TNFSF10, tumor necrosis factor (ligand) superfamily, member 10; TRAPPC1, trafficking protein particle complex 1; TREX1, three prime repair exonuclease 1; UNC93B1, Unc-93 homolog B1 (C. elegans)*.
The first three columns contain data from the microarray, whereas the fourth column presents fold change results from quantitative validation and the last two columns give Pearson's correlation (*r*) and P-value of the correlation between the microarray results and quantitative validation.

When investigating the pattern of prefrontal cortex gene expression of IRF7, we found its expression to be significantly downregulated in MDD compared with controls in frontal cortex. A limitation of post-mortem investigations is that information on antidepressant medication history of individual subjects is restricted. However, availability of toxicological data partially reduces the impact of this limitation. Although our post-mortem results do not represent by themselves a validation of our peripheral findings, they certainly support the hypothesis that IRF7 may play a role in brain processes associated with depression. We are limited in our design by the lack of a placebo or control group for comparison. However, it must be said that the aim of the present study was to determine biomarkers of response and not to perform an assessment of drug efficacy. Although assessing molecular processes associated with placebo effects is also a relevant question, withholding potentially beneficial treatment from actively depressed individuals is nowadays considered unethical in many countries, including the one where this study was conducted. It was nevertheless reassuring that we observed similarities with other studies having used citalopram regarding noticeable side effects during the course of treatment[63] and in the range of citalopram concentrations quantified in plasma.[64–67] In all, our findings do provide good indications of plausible treatment targets and likely biomarkers for depression and citalopram response.

Conflict of interest

The authors declare no conflict of interest.

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