Pharmacogenomic Predictors of Citalopram Treatment Outcome in Major Depressive Disorder

Firoza Mamdani¹, Marcelo Berlim¹, Marie-Martine Beaulieu¹, and Gustavo Turecki^{*,1}

¹McGill Group for Suicide Studies, Depressive Disorders Program, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada

Abstract

Objectives—A significant proportion of patients with major depressive disorder (MDD) do not improve following treatment with first-line antidepressants, and currently, there are no objective indicators of predictors of antidepressant response. The aim of this study was to investigate pre-treatment peripheral gene expression differences between future remitters and non-responders to citalopram treatment and identify potential pharmacogenomic predictors of response.

Methods—We conducted a gene expression study using Affymetrix HG-U133 Plus2 microarrays in peripheral blood samples from untreated individuals with MDD (N = 77), ascertained at a community outpatient clinic, prior to an 8-week treatment with citalopram. Gene expression differences were assessed between remitters and non-responders to treatment. Technical validation of significant probesets was carried out by qRT-PCR.

Results—A total of 434 probesets displayed significant correlation to change in score and 33 probesets were differentially expressed between eventual remitters and non-responders. Probesets for SMAD 7 (SMA- and MAD-related protein 7) and SIGLECP3 (sialic acid-binding immunoglobulin-like lectin, pseudogene 3) were the most significant differentially expressed genes following FDR correction, and both were down-regulated in individuals who responded to treatment.

Conclusions—These findings point to SMAD7 and SIGLECP3 as candidate predictive biomarkers of antidepressant response.

Keywords

Depressive disorder; major; peripheral gene expression; citalopram; pharmacogenomics; biomarkers; immune response genes

Introduction

Major depressive disorder (MDD) affects approximately 10–15% of the general population and is one of the leading causes of disability-adjusted life years (Murray and Lopez 1998).

*Corresponding Author: Gustavo Turecki MD, PhD, McGill Group for Suicide Studies, Douglas Mental Health University Institute, 6875 LaSalle Blvd., Verdun, Quebec, Canada, H4H 1R3.

Conflicts of interest

None to declare

While there are several different treatment options available for MDD, the most common are based on the use of antidepressants (AD), and among these, selective serotonin reuptake inhibitors (SSRI) are the most commonly prescribed. However, a significantly large proportion of patients (~ 50%) do not respond to first-line AD treatment (Trivedi et al. 2006). Considering the large number of individuals affected by MDD, this represents an excessive amount of individuals not reaping any or only partial benefit from the treatment they are receiving. The failure of AD to provide adequate levels of response to large number of patients incurs considerable socio-economic and emotional burdens on both patients and their families. Currently, there are no objective measures to help clinicians identify patients who are more or less likely to respond to individual AD treatments. As such, the decision of what is the best first line antidepressant to a given patient is primarily subjective, based to a large degree on the clinician's subjective perception. The identification of predictors of individual response to specific AD would help better target specific ADs to individual patients.

There is evidence that response to pharmacological treatment segregates in families. For instance, relatives of bipolar disorder probands that respond to lithium treatment are themselves, when affected by bipolar disorder, more likely to respond to lithium (Alda et al. 1994; Grof et al. 1994; Mendlewicz et al. 1972; Mendlewicz et al. 1973). Although data are not extensive, there have been a handful of studies providing evidence that AD response may also be familial. For instance, depressed first-degree relatives of MDD probands responsive to treatment with fluvoxamine are likely to respond to fluvoxamine treatment (Akiskal 1982; Franchini et al. 1998; PARE et al. 1962; Serretti et al. 1998).

In addition to familial history of treatment response, several other factors have been shown to predict response to antidepressant treatment in MDD patients. For instance, clinical correlates such as patterns of comorbidity, illness severity, age of onset and course of the disorder, including longer duration and frequency of prior episodes (Kemp et al. 2008). However, these factors are not useful when trying to predict treatment outcome of MDD patients because they are of low sensitivity and specificity (Kemp et al. 2008).

Several 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 of response by means of gene expression studies may provide valuable information and help identify possible biomarkers of antidepressant response. This approach has been successfully used in several studies investigating predictors of response of medications used to treat several medical conditions (Chang et al. 2003; Zembutsu et al. 2009; Nagasaki and Miki 2008; Schetter et al. 2008; Kittleson et al. 2005; Marziliano et al. 2009; Matkovich et al. 2009). Peripheral blood gene-expression studies have shown to provide a good proxy for what may take place in the CNS of psychiatric patients through comparative studies (Glatt et al. 2005; Rollins et al. 2010; Gladkevich et al. 2004; Mill et al. 2002; Nicholson et al. 2004; Sharp et al. 2006) and can prove valuable because peripheral factors are also involved in an individual's response to treatment. There has been progress in the search for gene expression biomarkers of response, although to our knowledge not at a genome-wide level (Cattaneo et al. 2012; Malki et al. 2012). In this study, we sought to

identify possible biomarkers predicting remission to citalopram, we conducted a study in a sample comprising 77 untreated MDD outpatients analyzing differences in baseline peripheral gene expression between remitters and non -responders to an 8-week citalopram treatment trial.

Methods

Major Depressive Disorder Sample

Our subjects (N = 77) were untreated males (N = 35) and females (N = 42) patients prospectively recruited among outpatients attending psychiatric and community clinics associated with the Douglas Mental Health Institute, Montreal, QC and with the necessary approval from the hospital ethics and internal review board. All patients included in this study met criteria for current major depressive disorder (MDD), as diagnosed by means of the Structured Clinical Interview for DSM-IV Axis I diagnoses (SCID-1) using DSM -IV criteria and with a Hamilton Rating Scale for Depression (HAM-D-21) score on initial assessment of at least 20. Clinical and demographic details of the subjects are outlined in Table 1. Subjects were excluded from the study if they had experienced previous manic or hypomanic episodes or if they had psychotic features or current/past psychotic disorders, had previously been treated with citalopram, had comorbid substance disorders or if they tested positive for illicit drugs during urinalysis screens pre and/or post -treatment. In addition, patients included in this study could not have had any active medical illness in addition to MDD and could not take any other pharmacological treatment.

Citalopram Treatment and Remission Status

Patients were treated with citalopram for a total of 8 weeks, starting with an initial dose of 10mg die, which was subsequently titrated up to a maximum of 60 mg die. Assessments of depression severity were carried out at three times during the trial (prior to treatment initiation, after 4 weeks of treatment, and at the end of the trial) using the HAM-D-21. Remission status was based on HAM-D-21 cutoffs at the end of the trial – where subjects having scores at 8 weeks of 9 or below were classified as remitters.

Blood Samples

Blood samples were collected in 6 PAXgene™ blood RNA tubes (PreAnalytix, New Jersey, USA) and 2 EDTA vacutainer tubes (BD, New Jersey, USA) from cases prior to the start of the trial. A similar number of tubes were collected and processed at the end of the 8-week trial from which an EDTA tube was used for HPLC as described below. PAXgene™ tubes were frozen using a sequential freezing process. This involved storing tubes at room temperature for 3 hours, transferring to 4°C overnight, -20°C for 6–8 hours and then finally to -80°C. Four tubes were used for RNA extraction and microarray processing and the remaining 2 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 and for DNA extraction. All subjects included in the study provided informed consent and the project was approved by the internal review board of the Douglas Mental Health University Institute.

High Performance Liquid Chromatography (HPLC)

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

For drug extraction 1ml of plasma was first spiked with 150 ng desipramine, which acted as an internal standard, and then basified with 100 μl NaOH. To this 10ml 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, subsequently 50 μl was injected into the HPLC machine. The samples were run through a reverse-phase C-18 column and peaks were detected with a UV 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 intra-day variability new standard curves were made prior to each HPLC run.

Microarrays

RNA used in this study had a minimum A260/A280 ratio of more than 1.9. Integrity and possible degradation of the RNA samples was determined using the 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and samples had an average RNA integrity number (RIN) of 8.4 and a 28S/18S ratio of more than 1.6. We used the Affymetrix GeneChip Human Genome U133 Plus 2.0 array, consisting of 54 000 probesets allowing for the analysis of 47 000 transcripts, representative of 38 500 human genes.

Data Analysis

Gene-expression analysis was performed using Robust Multi-Array (RMA) as implemented in Partek Genomics Suite (Partek Inc, St. Louis, MO, USA; Version 6.5), with probeset filtering based on Microarray Suite 5 (MAS5) present/absent calls. Probesets were filtered based on being called present (P) in at least 50% of the total pre-treatment sample. Several indicators of RNA quality were used to pre-filter samples prior to analysis; these included β -actin 5'/3' ratio (> 0.3), GAPDH 5'/3' ratio (> 0.5) and RawQ. Outlier detection for arrays was also carried out using principal component analysis (PCA).

Following initial filtering, to minimize the potential of obtaining false positives, gene expression measures for the 22297 probesets passing the 50% presence criteria were initially assessed for correlation to the residuals of the regressed change in HAMD-21 score between pre and post-treatment values. The regressed change was assessed by predicting HAMD-21 scores at the end of 8 weeks of treatment using the baseline scores. This was carried out in SPSS (version 14) and the output residuals, which are representative of goodness of fit of the model, were used in further analyses as a measure of remission. Use of regressed change between pre-and post-treatment avoids increased false -positives, loss of information and reduced power (Streiner 2002). The probesets having a Pearson correlation (r) of greater than or equal to 0.2 and a p -value < 0.05 were retained. These probesets were then used in an ANCOVA model within Partek Genomics Suite (v6.5). The ANCOVA carried out had remission status as the main effect, with gender as a covariate and scan date included as a

random effect in the model. If any factors were found to impart a lot of variation, their effects were removed using the batch removal function implemented in Partek Genomics Suite and the analyses were re-run on the corrected data. Furthermore, power analysis was carried out using G*Power and it was determined that our sample has 85% power to detect effect sizes of 0.9 at a 0.01 significance level.

Gene ontology analysis, to identify over-represented functional classes of genes, was carried out using DAVID (Dennis, Jr. et al. 2003; Huang et al. 2009). Pathway analysis was carried out using IPA (Ingenuity Pathway Analysis, Redwood City, CA, USA).

Gene Expression Validation of Microarray Findings

TaqMan gene expression assays for SMAD7 and SIGLECP3 were used to validate significant gene expression findings. cDNA was produced using M-MLV reverse transcriptase and Oligo-dT primers and subsequently diluted to a concentration of 2.5ng/μl for use in qRT-PCR. Quantitative RT-PCR was performed in 384-well plates on the 7900HT Real-Time PCR system from Applied Biosystems under fast conditions using PerfeCTa qPCR Fastmix (Quanta Biosciences, Gaithersburg, MD). Target and endogenous control (β-actin) were run in the same well and plates were analyzed using the Ct method in RQ Manager (Applied Biosystems) with the calibrator being a sample with low variability between replicates.

Results

Of the 77 patients included in this study, 46 had HAMD-21 scores lower than 9 (5.20 ± 2.76) after 8 weeks of treatment and were considered citalopram -treatment remitters. The remaining patients were considered non-responders (18.03 ± 4.99). When comparing remitters and non-responders, no differences were observed in distribution of age, gender or HAMD -21 score at study entry. All subjects enrolled in the trial were ascertained as being compliant due to the presence of detectable levels of citalopram in their individual plasma samples using HPLC.

The total number of probesets included in the analysis was 22 297, which is the number resulting after removing probesets not present in at least 50% of the subjects in at least one of the groups. While this procedure potentially prevents us from detecting treatment -related gene-expression differences that may be present in a minority of individuals, it helps reduce false -positive findings resulting from spurious or non -treatment related variation in gene-expression (Mamdani et al. 2011; Sequeira et al. 2006; Sequeira et al. 2009). We assessed the correlation between baseline gene expression levels and the regressed change in HAMD-21 scores according to citalopram treatment. In order to minimize regression to the mean effects, a well-known source of erroneous inferences, we used the residuals of the regressed change between pre -and post-citalopram HAMD-21 scores as a measure of remission. A total of 434 probesets displayed significant correlation to change in score ($P < 0.05$ and $r > 0.2$, Supplemental Table 1).

Gene ontology analysis of the 434 probesets significantly correlated to change in score identified several over-represented ontologies (Table 2). The most significant categories

were related to the cell's protein metabolic processes, protein binding and other actions involving the plasma membrane. These probesets (N = 434) are associated to a global change in score following treatment, and are not indicative of antidepressant remission state. To identify probesets associated with remission, we carried out a post-hoc ANCOVA controlling for gender and other confounders such as batch effects. This analysis indicated that 88 probesets significantly differentiated between remitters and non-responders (P 0.05), while 42 probesets were equally significant and had fold changes of ± 1.2 between (remitters vs. non responders) groups. When these two groupings were compared there remained 33 probesets meeting both criteria thresholds (P 0.05 and fold changes ± 1.2) (Table 3).

Of the 33 probesets differentially expressed between remitters and non-responders, SMAD7 and SIGLECP3, both of which were down-regulated in remitters, were the only ones that remained significant after FDR correction for multiple testing. SMAD 7 (SMA-and MAD - related protein 7) is a regulator of transforming growth factor -beta signaling, while SIGLECP3 (sialic acid-binding immunoglobulin-like lectin, pseudogene 3) is a lectin-like adhesion molecule.

Technical validation of probesets for SMAD7 and SIGLECP3 was performed using TaqMan assays. Both genes displayed significant correlation with microarray data (SMAD7: $r = 0.439$, P - value = 0.007; SIGLECP3: $r = 0.467$, P - value = 0.011) and were also differentially expressed between remitters and non-responders in similar direction and fold - change magnitude as in the microarrays (SIGLECP3: fold change = -1.420 , P - value = 0.001; SMAD7: fold change = -1.140 , P - value = 0.073). Figure 1, graphically displays the differences seen between remitters and non-responders for gene expression of SMAD7 and SIGLECP3, in both the microarray and by qPCR. In addition, to gain insight into the probable CNS validity of our peripheral findings we investigated the post-mortem brain gene expression of the probesets for SMAD7 and SIGLECP3 in the cortex. For this purpose we examined expression levels of the probesets corresponding to our genes of interest from Affymetrix microarrays, previously processed in the lab, in a sample of 6 subjects who died during a depressive episode and a sample of 6 sudden-death controls, having equivalent age, post-mortem interval and pH. We observed low levels of SIGLECP3 in both groups, while SMAD7 was downregulated in MDD subjects.

Ingenuity Pathway Analysis was performed to identify, if present, the pathways to which the 33 significant, remission-related probesets belonged. One of the top networks was that of inflammatory response, which comprised 10 of the 33 significant probesets. As well, when exploring the relationship between the significant probesets, SMAD7 was identified as being a central node in the produced network (Figure 2).

Discussion

Response to antidepressant treatment is clinically difficult to predict. Studies such as the STAR*D (Rush et al. 2003) have investigated predictors of various treatment modalities. Recently, Belzeaux and colleagues (Belzeaux et al. 2010) have shown changes using real-time PCR in peripheral gene expression of depression-related candidate genes following 8

weeks of any antidepressant treatment. However, without the use of a unique treatment, Belzeaux et al (2010) (Belzeaux et al. 2010) could not identify treatment-specific predictors or gene expression alterations. In our study, we have identified plausible biomarkers for future response to citalopram treatment.

Through analyses of predictors to citalopram treatment, we identified two genes: SMAD7 and SIGLECP3, which remained significant following FDR correction for multiple testing and had significant differential expression between response groups. SMAD7 is responsible for the recruitment of ubiquitinases to induce the degradation of Transforming Growth Factor- β (TGF β) receptor and thus negatively regulates the TGF β signalling pathway (Wicks et al. 2006). TGF β is considered to be a Th3-type cytokine and plays a major role in guarding a balance between Th1 and Th2 cytokines (or pro and anti-inflammatory) (Myint et al. 2005). In psychiatric disorders, particularly in depression, there is evidence for a dysregulation of the balance of immunomodulatory cytokines in consequence to an increased preponderance of Th1 cytokines due to stress (Maes 1995; Seidel et al. 1996). Furthermore, alterations in the Th1/Th2 cytokine balance has been demonstrated as being an explanatory factor for the way the brain adapts to stress (Anisman and Merali 2003).

Antidepressant treatment has been shown to have an immunomodulatory function whereby it acts to increase anti-inflammatory cytokine levels (Kubera et al. 2000; Kubera et al. 2001; Maes 2001) and helps to re-establish a balance between Th1 and Th2 cytokines. A study by Kubera et al (Kubera et al. 2000) in mice demonstrated that alteration in cytokine levels is contingent upon the SSRI administered and the length of treatment administration. As well, there is evidence for up-regulation in the levels of TGF β (Myint et al. 2005) following 6 to 8 weeks of antidepressant treatment. From what is known of the regulatory effects of TGF β , the lower baseline SMAD7 gene expression levels we observed before citalopram treatment, among future responders, suggests that less endogenous SMAD7 is available to form complexes with SMURF (SMAD Ubiquitination Regulatory Factor) to induce TGF β degradation, (Asano et al. 2004) and thus blunts negative regulation of TGF β signalling. So in our study, remitters appear to have an advantage in that TGF β levels are likely to be at a higher, and possibly advantageous, level resulting in a more rapid increase of TGF β following treatment. This hypothesis is consistent with recent findings by our group of increased methylation in the promoter of TGF β 2 in the hippocampus of suicides as compared to psychiatrically normal sudden-death controls (Labonté B et al., unpublished results) since suicide is an unfortunate consequence of both MDD and treatment non-response (Isacson et al. 2010).

SIGLECs (sialic acid-binding immunoglobulin-like lectins) are lectins involved in interactions between individual cells and take part in cellular regulation within the immune system, with most immune cells, at a minimum, expressing one SIGLEC (Crocker et al. 2007). Even with their extensive presence throughout the body, the exact mechanisms by which SIGLECs function and the identity of their binding partners remains incompletely characterized (Crocker et al. 2007). In the context of neurological disorders, Siglec-11, has been found to be involved in plaque formation in Alzheimer's disease (Salminen and Kaarniranta 2009), however, to our knowledge, there have been no previous studies of SIGLECs and MDD. SIGLECs are in the same family as myelin associated glycoprotein,

which can induce the inhibition of neurite growth (Tang et al. 1997). Recently, Siglec-9 (Carlin et al. 2009), Siglec-10 (Chen et al. 2009) and Siglec-11 (Wang and Neumann 2010) have been shown to dampen response of innate immunity through binding to other sialylated compounds. Wang and Neumann (2010) (Wang and Neumann 2010) demonstrated this on microglia, where microglial cells expressing a splice variant of Siglec-11 had reduced induction of pro-inflammatory cytokines following lipopolysaccharide (LPS) stimulation and were found to have diminished detrimental effects on neuronal growth. The authors' also underlined the need for polysialylation on neurons, in this case through PSA-NCAM (polysialylated neural cell adhesion molecule), for Siglec-11 expressing microglia to have minimal cytotoxic effects on neurons. The apparent reduction of the related SIGLECP3 gene, which we observed in future citalopram remitters, could be understood as a possible adaptive advantage in remitters to reduce the possible detrimental effects of immunological response on neuronal growth. However, with the results presented, concrete conclusions cannot be made, although it can be postulated that SIGLECP3 could function as a peripherally-based surrogate of SIGLEC function in the CNS.

Although, not passing FDR correction, several probesets of interest demonstrated large fold-change differences between response groups, among these were NCAM1 and FGFBP2, which have previously been reported as being associated with MDD.

We are limited in our design by the lack of a placebo or control group for comparison. Having such a group would have allowed us to separate gene expression changes that are related to the actual effect of citalopram from changes due to a placebo effect. The ideal scenario would be to carry out a larger prospective trial including both antidepressant and placebo arms, as well as an independent validation sample. Forthcoming investigations would benefit from a larger CNS sample, encompassing several brain areas, for a thorough assessment of blood-brain comparability. As well, with regards to compliance, HPLC analyses should take into consideration citalopram metabolites to allow for an account of drug metabolism. In the end even though this study is not without limitations, our results provide evidence that is consistent with previous literature, supporting a role for immune dysregulation in MDD and the association of immune genes with antidepressant response. In addition, this study has pointed out new mechanisms which should be further explored when investigating factors associated with antidepressant treatment response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Gene expression differences for SMAD7 and SIGLECP3 between future remitters and non-responders to citalopram treatment

Figure 1. Plot of gene expression differences between citalopram remitters and non-responders by microarray and qPCR

Plot of differences seen between future remitters and non -responders to citalopram treatment for gene expression of SMAD7 and SIGLECP3, in both the microarray and by qPCR. The gene expression measures illustrated for qPCR are based on RQ values obtained using TaqMan assays, while those from the microarray represent probeset intensities, resulting in differences in the scales used. RES = remitter; NRES = non-responder; ** = p-value 6×10^{-5} ; * = p-value 1×10^{-3} .

Figure 2. Schematic representation of a gene network based on genes significantly associated to response

Gene network including the 33 significantly differentially expressed probesets. Genes in pink originate from the list inputted into IPA, with SMAD7 being a central player, indicated by the bright red. Other informative genes are shown in white.

Table 1

Clinical and demographic information of the sample included in the study

Subjects Included in Study (N=77)	
Age	38.93
Males	35 (45%)
Females	42 (55%)
Baseline HAMD	29.26
Max Cit Dose (mg)	37.50
Remitters	46
Non-Responders	31
Depressive subtype	
Melancholic	50 (65%)
Atypical	20 (26.0%)
MDD-NOS	7 (9.0%)
Recurrent MDD	37 (48.05%)
Baseline HAMA	21.09
Suicidal Ideation	43 (55.84%)

HAMD: Hamilton Depression Rating Scale; **MDD-NOS:**Major depressive disorder – not otherwise specified; **HAMA:**Hamilton Anxiety Rating Scale

Gene ontologies over -represented by probesets significantly correlated to change in HAMD-21 score following citalopram treatment

Table 2

Category	Term	Ontology	Count	%	PValue
BP	GO:0044267	cellular protein metabolic process	55	13.99	0.04
CC	GO:0005829	cytosol	44	11.20	0.0007
CC	GO:0005737	cytoplasm	169	43.00	0.0020
CC	GO:0044444	cytoplasmic part	119	30.28	0.0036
CC	GO:0043231	intracellular membrane-bounded organelle	177	45.04	0.01
CC	GO:0043229	intracellular organelle	195	49.62	0.01
MF	GO:0005515	protein binding	171	43.51	0.04

BP:biological process; CC:cellular component; MF:molecular function

Table 3

List of significantly differentially expressed probesets between future remitters (RES) and non-responders (NRES) to citalopram treatment

ProbesetID	Gene Symbol	Gene Title	p-value	Fold-Change (RES vs. NRES)	Fold-Change (RES vs. NRES)
204790_at	SMAD7*	SMAD family member 7	1.42E-05	-1.36	RES down vs NRES
232686_at	SIGLECP3*	sialic acid binding Ig-like lectin, pseudogene 3	5.99E-05	-1.37	RES down vs NRES
213996_at	YPEL1	yippee-like 1 (Drosophila)	0.002	-1.26	RES down vs NRES
207314_x_at	KIR3DL2	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail	0.002	-1.6	RES down vs NRES
207313_x_at	KIR3DL2	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail	0.003	-1.37	RES down vs NRES
214617_at	PRF1	perforin 1 (pore forming protein)	0.003	-1.34	RES down vs NRES
219304_s_at	PDGFD	platelet derived growth factor D	0.003	-1.36	RES down vs NRES
213822_s_at	UBE3B	ubiquitin protein ligase E3B	0.004	-1.27	RES down vs NRES
203989_x_at	F2R	coagulation factor II (thrombin) receptor	0.004	-1.26	RES down vs NRES
210164_at	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	0.004	-1.38	RES down vs NRES
211242_x_at	KIR2DL4	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4	0.004	-1.26	RES down vs NRES
223836_at	FGFBP2	fibroblast growth factor binding protein 2	0.005	-1.51	RES down vs NRES
202073_at	OPTN	optineurin	0.006	-1.28	RES down vs NRES
209160_at	AKR1C3	aldo-keto reductase family 1, member C3	0.006	-1.48	RES down vs NRES
1566608_at	---	---	0.008	-1.27	RES down vs NRES
1553177_at	SH2D1B	SH2 domain containing 1B	0.008	-1.39	RES down vs NRES
216907_x_at	KIR3DL2	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail	0.008	-1.42	RES down vs NRES
220646_s_at	KLRF1	killer cell lectin-like receptor subfamily F, member 1	0.008	-1.42	RES down vs NRES
207509_s_at	LAIK2	leukocyte-associated immunoglobulin-like receptor 2	0.001	-1.62	RES down vs NRES
211597_s_at	HOPX	HOP homeobox	0.01	-1.38	RES down vs NRES
232568_at	MGC24103	hypothetical MGC24103	0.012	-1.23	RES down vs NRES
211687_x_at	KIR3DL1	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail	0.014	-1.34	RES down vs NRES
212843_at	NCAM1	neural cell adhesion molecule 1	0.015	-1.32	RES down vs NRES
213533_at	D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence	0.016	-1.23	RES down vs NRES
211245_x_at	KIR2DL4	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail	0.018	-1.21	RES down vs NRES
242751_at	---	---	0.019	-1.22	RES down vs NRES

ProbesetID	Gene Symbol	Gene Title	p-value	Fold-Change (RES vs. NRES)	Fold-Change (RES vs. NRES)
218638_s_at	SPON2	spondin 2, extracellular matrix protein	0.024	-1.31	RES down vs NRES
209569_x_at	D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence	0.03	-1.39	RES down vs NRES
209570_s_at	D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence	0.032	-1.31	RES down vs NRES
239956_at	---	---	0.035	-1.22	RES down vs NRES
222816_s_at	ZCCHC2	zinc finger, CCHC domain containing 2	0.036	1.27	RES up vs NRES
219529_at	CLIC3	chloride intracellular channel 3	0.04	-1.28	RES down vs NRES
204103_at	CCL4	chemokine (C-C motif) ligand 4	0.043	-1.22	RES down vs NRES

* Probeset passed FDR step-up correction