Title:

Reduced expression of apolipoprotein E receptor type 2 in peripheral blood lymphocytes from patients with major depressive disorder

Authors:

Katsuaki Suzuki[a], Yasuhide Iwata[b], Hideo Matsuzaki[c], Ayyappan Anitha[a], Shiro Suda[a], Keiko Iwata[a], Chie Shinmura[b], Yosuke Kameno[b], Kenji J. Tsuchiya[a], Kazuhiko Nakamura[b], Nori Takei[a] and Norio Mori[a,b].

Affiliations:

a Research Center for Child Mental Development, Hamamatsu University School of Medicine, Hamamatsu, Japan.

b Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu, Japan.

c Research Center for Child Mental Development, Osaka University Graduate School of Medicine, Suita, Japan.

Address correspondence to:

Katsuaki Suzuki, M.D., Ph.D.

Research Center for Child Mental Development,

Hamamatsu University School of Medicine,

1-20-1 Handayama, Hamamatsu 431-3192, Japan.

Phone: +81 53-435-2295; Fax: +81 53-435-3621.

Email: k-suzuki@hama-med.ac.jp

Abstract

We measured the mRNA levels of apolipoprotein E receptor type 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR) in peripheral blood lymphocytes from 43 patients with major depressive disorder (27 drug-free patients and 16 medicated patients) and 43 age-matched healthy controls using a quantitative real-time RT-PCR method. The correlations between mRNA levels of both receptors and clinical variables in patients were also examined. The expression of ApoER2 mRNA, but not VLDLR, was significantly lower in patients as compared to controls, irrespective of the medication status. There was no statistically significant correlation between the reduction of ApoER2 mRNA levels and any of the clinical variables measured in patients. Results from this preliminary study suggest that the expression of ApoER2 may serve as a trait marker for major depressive disorder.

Keywords

Major depressive disorder, biomarker, low-density lipoprotein receptor family, ApoER2, real-time RT-PCR

Abbreviations

ApoE, apolipoprotein E;
ApoER2, apolipoprotein E receptor type 2;
BPRS, Brief Psychiatric Rating Scale;
GAPDH, glyceraldehyde-3-phosphate dehydrogenase;
HAM-D, Hamilton Rating Scale for Depression;
LDL, low-density lipoprotein;
MDD, major depressive disorder;
RT-PCR, reverse transcriptase-polymerase chain reaction;
SD, standard deviation;
VLDLR, very low-density lipoprotein receptor

Introduction

Major depressive disorder (MDD) is one of the most common psychiatric disorders worldwide [Ebmeier et al., 2006; Ustün et al., 2004]. Evidence implicates the involvement of altered immune response and inflammation in the development of MDD [Irwin and Miller, 2007]. Patients with MDD have been reported to show signs of activated innate immune responses as manifested by increased inflammatory cytokines, including tumor necrosis factor-alpha, interleukin (IL)-1 and IL-6, in the peripheral blood and cerebrospinal fluid [Khairova et al., 2009; Kim et al., 2007; Müller and Ackenheil, 1998; Zorilla et al., 2001]. The potential role played by lymphocytes in the adaptive immune response has also been implicated in MDD [Miller, 2010]. For instance, T cell responses to mitogens have been shown to be decreased in depressed individuals [Kronfol et al., 1986; Schleifer et al., 1984], and the skin response to commonly encountered antigens as a measure of T cell-mediated immune function is also decreased in patients with MDD [Hickie et al., 1993]. Furthermore, the number of B cells in patients with MDD is reported to be decreased as compared to the number in controls [Pavón et al., 2006; Schleifer et al., 1984], although conflicting results have also been reported [Hernandez et al., 2010; Ravindran et al., 1998; Robertson et al., 2005]. A decrease in the responsiveness, or possibly the number, of peripheral blood lymphocytes in MDD patients is believed to be related to increased apoptosis [Eilat et al., 1999; Szuster-Ciesielska et al., 2008], although the detailed mechanism of this relationship remains to be elucidated.

Apolipoprotein E (ApoE) is a multifunctional component of plasma lipoproteins [Nimpf and Schneider, 2000]. In addition to its functions in lipoprotein metabolism via the low-density lipoprotein (LDL) receptor family, apoE is known to act as an immunomodulator. ApoE is produced by macrophages [Basu et al., 1983; Kockx et al., 2008], which activate T cells by antigen presentation [Mistry et al., 1995; van den Elzen et al., 2005]. ApoE has also been implicated in the activation of natural killer T cells by acting as a molecular chaperone for bacterial antigens, delivering them to antigen-presenting cells via LDL receptors [Allan et al., 2009]. These findings indicate that apoE and its receptors on lymphocytes are implicated in the adaptive immune responses via intercellular signaling systems. With regard to the LDL receptor family that binds to apoE, apolipoprotein E receptor type 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR) are implicated in the pathophysiology of MDD. These receptors bind to reelin, an extracellular matrix glycoprotein that plays crucial roles in brain development

as well as in synaptic plasticity in the adult brain [Herz and Chen, 2006]. Blood levels of the 180kD isoform of reelin were shown to be reduced in patients with MDD [Fatemi et al., 2001].

Considering the altered immune responses in MDD and the roles played by apoE and its receptors in the immune system, we hypothesized that the expression of ApoER2 and VLDLR in peripheral blood lymphocytes may be reduced in patients with MDD. To test this, we examined the mRNA expression of ApoER2 and VLDLR in lymphocytes from patients with MDD and matched healthy subjects by quantitative real-time RT-PCR analysis. In addition, we sought to identify relationships between the alteration in mRNA levels of either receptor, if any, and clinical variables in the MDD patients.

Materials and Methods

Subjects

A total of 86 subjects participated in this study. Forty-three people with MDD (mean [SD] age 39.7 [9.1] years) according to the DSM-IV-TR criteria and 43 age- and gender-matched healthy controls (mean [SD] age 38.8 [6.8] years) were recruited. Twenty-seven patients were either drug-naïve (n=22) or free from drug treatment for more than 2 months (n=5) at the sampling (drug-free). Remaining 16 patients were medicated by any psychotropic drug (medicated). The clinical symptoms of patients were evaluated using the Hamilton Rating Scale for Depression (HAM-D) and the Brief Psychiatric Rating Scale (BPRS), whose Japanese versions in both scores have been validated [Kitamura et al., 1987; Kitamura et al, 1985]. Lifetime doses of antidepressants, mood stabilizers and antipsychotics were calculated as imipramine-, lithium- or chlorpromazine-equivalent doses in medicated patients. All of the participants provided written informed consent for participation in this study. The study was approved by the ethics committee of Hamamatsu University School of Medicine.

Measurement of ApoER2 and VLDLR mRNA

The expression of mRNA of both ApoER2 and VLDLR was evaluated by means of quantitative real-time RT-PCR methods, the details of which have been described elsewhere [Suzuki et al., 2008]. In brief, fasting blood samples were collected between 11:00 AM and noon by venipuncture. Lymphocytes were isolated from blood samples by means of the Ficoll-Paque

gradient method (purity 80%) within 2 hours after sampling. Total RNA was extracted from lymphocytes and the final RNA preparations were resuspended in diethylpyrocarbonate-treated water, quantified and stored at -80 °C. Complementary DNA was prepared by incubating DNase-treated total RNA (1.0 μg) with M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA) in the presence of random primers. Quantitative real-time RT-PCR analysis was performed using an ABI PRISM 7700 Sequence Detector (PE Applied Biosystems, Foster City, CA). The housekeeping gene GAPDH was used as an endogenous control. TaqMan probes and primers were constructed according to known sequences for the ApoER2 (Genbank D50678) and VLDLR (Genbank NM_003383) genes as shown in Table 1. The primer set and probe for GAPDH were made by PE Applied Biosystems (Table 1). The ApoER2 and VLDLR mRNA expression levels are presented as the mRNA copy number per μg of total RNA, which was calibrated with the level of GAPDH mRNA expression; the levels are described in the Figures as powers of 10 (e.g., 10,000 copies are shown as 10^4).

Statistical analysis

We used SPSS version 17.0 (SPSS Inc., Tokyo) for statistical analyses. All the analyses were performed on actual data, although presentations in the figures used a log scale. We performed the Shapiro-Wilk normality test and found that ApoER2 mRNA levels in patients did not distribute normally. Therefore, we chose nonparametric tests as follows. For the comparison of ApoER2 and VLDLR mRNA levels between two groups, we used the Wilcoxon signed-rank test. For multiple comparison of ApoER2 mRNA levels in drug-free or medicated patients and their matched controls, the Kruskal-Wallis test followed by *post hoc* Dunn's multiple comparison test were used. To test relationship between mRNA levels and clinical variables, Spearman's rank correlation and regression analyses were carried out. A P value less than 0.05 was considered statistically significant. The data are presented as mean ± SD with range in Tables.

Results

The characteristics of all the participants are summarized in Table 2. Since the difference in age between controls and patients with MDD was not statistically significant (Z = -1.22, P = 0.22), the age matching was considered to be successful. Out of 16 medicated patients, 13

responded to treatment and entered remission, which was reflected by the medicated patients' lower scores on the HAM-D and BPRS compared to the drug-free patients. The expression levels of the mRNA of ApoER2 and VLDLR in control subjects were 69779.1 ± 23414.9 and 16903.9 ± 8263.9 copies/μg of total RNA (Fig. 1). The averaged expression of ApoER2 mRNA in all the patients with MDD was 43123.2 ± 27645.1 copies/μg, which was significantly lower than the corresponding value in controls (Z = -3.83, P < 0.001; Fig 1A). In contrast, the expression of VLDLR mRNA in all the patients (15346.2 ± 8479.6 copies/μg) did not differ from that in controls (Z = -0.93, P = 0.35; Fig 1B). There was no statistically significant correlation between ApoER2 and VLDLR mRNA expressions (r = 0.17, P = 0.27). We further examined the reduction in ApoER2 mRNA expression in patients with respect to their medication status. Kruskal-Wallis test revealed significant differences (H = 22.25, P < 0.001) among 4 groups, i.e., drug-free and medicated patients and matched controls for each of patient subgroups. The *post hoc* Dunn's test revealed significantly lower levels of ApoER2 mRNA in both drug-free (P = 0.02) and medicated (P < 0.001) patients compared to their corresponding healthy controls (Fig. 2). The difference in ApoER2 mRNA levels between drug-free and medicated MDD patients was not statistically significant (P = 0.12).

We then examined the correlation between ApoER2 mRNA levels and the clinical variables in patients with MDD. There was no statistically significant correlation between ApoER2 mRNA levels and the severity of symptoms as assessed by HAM-D (r = 0.21, P = 0.17) or by BPRS (r = 0.13, P = 0.38). ApoER2 mRNA levels did not correlate with the duration of illness (r = 0.02, P = 0.92). There was no correlation between ApoER2 mRNA levels and either the total medicated period (r = -0.20, P = 0.19) or the life-time dose of antidepressants (imipramine equivalent, r = -0.05, P = 0.84), mood stabilizers (lithium equivalent, r = -0.40, P = 0.12), or antipsychotics (chlorpromazine equivalent, r = -0.38, P = 0.15) in the medicated patients. To test the relationship between ApoER2 mRNA and clinical symptoms in patients with MDD, we further carried out regression analyses. After controlling for the duration of illness, total medicated period, and medication used, we confirmed that there was no significant relationship between ApoER2 mRNA levels and HAM-D (R^2 = 0.21, β = 0.68, P = 0.19) or BPRS (β = -0.48, P = 0.37) scores.

Discussion

The present study demonstrated that the mRNA levels of ApoER2, but not VLDLR, in peripheral blood lymphocytes from patients with MDD were significantly lower than those of age- and gender-matched healthy control individuals, and that reductions in ApoER2 mRNA levels were significant in both drug-free and medicated patients. These results suggest that the expression of ApoER2 is altered at the transcriptional level in lymphocytes from patients with MDD. The reduction of ApoER2 mRNA levels did not correlate with the severity of depressive symptoms or with other clinical variables, including the duration of illness, the total medicated period, or the medication dose. Thus, it is likely that the reduction in ApoER2 mRNA levels in the peripheral blood lymphocytes was associated with the diagnosis of MDD. Although the functional relevance of lower levels of ApoER2 mRNA in MDD patients compared to healthy controls remains unclear from this preliminary study, it appears that the level of ApoER2 mRNA in the peripheral blood lymphocytes may serve as a biological *trait* marker of MDD. The reason for the distinct expression pattern between ApoER2 and VLDLR in MDD patients is unknown. Although the two receptors are closely related to each other in function as well as in structure [Herz and Chen, 2006], their binding properties to lipoproteins are different. ApoER2 is capable of binding very low-density lipoproteins that are deficient to apoE [Tacken et al., 2000], whereas VLDLR requires apoE for lipoprotein binding [Takahashi et al., 2004]. Such a difference between the two receptors might be related to our observation, although further studies will be necessary.

The mechanisms by which ApoER2 expression is reduced in lymphocytes are unknown. To the best of our knowledge, this is the first demonstration of ApoER2 expression in peripheral blood lymphocytes from patients with MDD. One possible explanation is that the reduction in ApoER2 mRNA expression may represent an altered function of lymphocytes in the lipoprotein-mediated immune responses in patients with MDD. This interpretation of the results is not in conflict with the findings of functional changes in lymphocytes, in addition to abnormalities in the innate immune response, which were observed in both the non-medicated and medicated patients [Irwin and Miller, 2007; Khairova et al., 2009; Kim et al., 2007; Miller, 2010; Müller and Ackenheil, 1998; Zorilla et al., 2001]. At this time, it is unclear whether or not the decreased transcriptional expression of ApoER2 observed here was associated with the expression of the receptor protein. It is also unknown which populations of lymphocytes were responsible for the reduction in ApoER2 expression in MDD patients, although T cells and

natural killer T cells are the candidates, because these cell populations are known to play a role in apoE-mediated responses [Allan et al., 2009; Mistry et al., 1995; van den Elzen et al., 2005]. Further studies are required.

In addition to its roles in the immune system, apoE plays roles in cellular uptake of lipoproteins, in neuronal maintenance and repair, and in regulating synaptic transmission in the brain [Herz and Chen, 2006]. ApoE has three major isoforms encoded by three alleles (ε2, ε3, ε4) from two single nucleotide polymorphisms. Evidence suggests that the ε4 allele is the major risk factor for early- and late-onset Alzheimer's disease, while the ε2 allele is protective [Strittmatter et al., 1993; Kim et al., 2009]. A recent meta-analysis of case-control studies, considering a range of candidate MDD susceptibility genes, has shown that the APOE ε2 allele was associated with lower risk for MDD as compared to the ε3 allele, and that the effect of ε4 allele was similar to the ε3 allele [López-León et al., 2008]. This suggests that APOE may be one of susceptibility genes for MDD. Given that ApoER2 serves as a receptor for apoE in the brain, one might expect that apoE could be a means by which ApoER2 mRNA expression was reduced in patients with MDD. It is unclear from the current study whether the levels of ApoER2 mRNA in peripheral lymphocytes reflect those in the brain. Further studies are required to test the effect of *APOE* genotype on the ApoER2 mRNA levels in MDD.

There are some limitations to our study. The small sample size renders the data presented here preliminary, and a larger study with more subjects with MDD will be necessary. Furthermore, the lack of another psychiatric group might cast doubt as to whether or not ApoER2 is specific to MDD. However, in our previous study [Suzuki et al., 2008] we measured the levels of ApoER2 mRNA in lymphocytes from patients with schizophrenia by RT-PCR. Since the mRNA levels of both ApoER2 and VLDLR in control subjects were similar between our previous and current studies, the results of the two studies can be compared. ApoER2 levels in drug-naïve patients with schizophrenia were not significantly different from those in the controls [Suzuki et al., 2008], supporting our hypothesis that ApoER2 may be a marker for MDD.

The need for characterizing measurable biological markers in MDD remains urgent so that individuals at risk for the illness can be identified. Our findings suggest that peripheral ApoER2 mRNA levels may serve as a peripheral biological marker of MDD, and that the apoE-ApoER2 signaling might play a role in the pathophysiology of the disorder.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research (A) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to N.M.); this funding had no further role in the study design; in the collection, analysis or interpretation of the data; in the writing of the report; or in the decision to submit the paper for publication.

References

Allan LL, Hoefl K, Zheng DJ, Chung BK, Kozak FK, Tan R et al. Apolipoprotein-mediated lipid antigen presentation in B cells provides a pathway for innate help by NKT cells. Blood 2009; 114:2411-6.

Basu SK, Goldstein JL, Brown MS. Independent pathways for secretion of cholesterol and apolipoprotein E by macrophages. Science 1983; 219:871-3.

Ebmeier KP, Donaghey C, Steele JD. Recent developments and current controversies in depression. Lancet 2006; 367:153-67.

Eilat E, Mendlovic S, Doron A, Zakuth V, Spirer Z. Increased apoptosis in patients with major depression: a preliminary study. J Immunol 1999; 163:533-4.

Fatemi SH, Kroll JL, Stary JM. Altered levels of reelin and its isoforms in schizophrenia and mood disorders. Neuroreport 2001; 12:3209-15.

Hernandez ME, Martinez-Fong D, Perez-Tapia M, Estrada-Garcia I, Estrada-Parra S, Pavón L. Evaluation of the effect of selective serotonin-reuptake inhibitors on lymphocyte subsets in patients with a major depressive disorder. Eur Neuropsychopharmacol 2010; 20:88-95.

Herz J, Chen Y. Reelin, lipoprotein receptors and synaptic plasticity. Nat Rev Neurosci 2006; 7:850-9.

Hickie I, Hickie C, Lloyd A, Silove D, Wakefield D. Impaired in vivo immune responses in patients with melancholia. Br J Psychiatry 1993; 162:651-7.

Irwin MR, Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. Brain Behav Immun 2007; 21:374-83.

Khairova RA, Machado-Vieira R, Du J, Manji HK. A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. Int J Neuropsychopharmacol 2009; 12:561-78.

Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. Neuron 2009; 63:287-303.

Kim YK, Na KS, Shin KH, Jung HY, Choi SH, Kim JB. Cytokine imbalance in the pathophysiology of major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2007; 31:1044-53.

Kitamura T, Machizawa S, Maruyama S, Nakagawa Y, Morita M, Sato T et al. Test-retest reliability of Oxford University version of Brief Psychiatric Rating Scale (BPRS). Seishin

Eisei Kenkyu 1985; 32:1-15 [Japanese].

Kitamura T, Suga R, Morita M, Ito j. Reliability of operational diagnostic criteria and its problems – III. Test-retest reliability of the Research Diagnostic Criteria (RDC), Hamilton's Rating Scale for Depression and the Scale for the Assessment of Negative Symptoms. Seishin Igaku 1987; 29:579-585 [Japanese].

Kockx M, Jessup W, Kritharides L. Regulation of endogenous apolipoprotein E secretion by macrophages. Arterioscler Thromb Vasc Biol 2008; 28:1060-7.

Kronfol Z, House JD, Silva Jr. J, Greden J, Carroll BJ. Depression, urinary free cortisol excretion and lymphocyte function. Br J Psychiatry 1986; 148:70-3.

López-León S, Janssens AC, González-Zuloeta Ladd AM, Del-Favero J, Claes SJ, Oostra BA, et al. Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry 2008; 13:772-85.

Miller AH. Depression and immunity: a role for T cells? Brain Behav Immun 2010; 24:1-8.

Mistry MJ, Clay MA, Kelly ME, Steiner MA, Harmony JA. Apolipoprotein E restricts interleukin-dependent T lymphocyte proliferation at the G1A/G1B boundary. Cell Immunol 1995; 160:14-23.

Müller N, Ackenheil M. Psychoneuroimmunology and the cytokine action in the CNS: implications for psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 1998; 22:1-33.

Nimpf J, Schneider WJ. From cholesterol transport to signal transduction: low density lipoprotein receptor, very low density lipoprotein receptor, and apolipoprotein E receptor-2. Biochim Biophys Acta 2000; 1529:287-98.

Pavón L, Sandoval-López G, Hernández ME, Loría F, Estrada I, Pérez M et al. Th2 cytokine response in Major Depressive Disorder patients before treatment. J Neuroimmunol 2006; 172:156-65.

Ravindran AV, Griffiths J, Merali Z, Anisman H. Circulating lymphocyte subsets in major depression and dysthymia with typical or atypical features. Psychosom Med 1998; 60:283-9.

Robertson MJ, Schacterle RS, Mackin GA, Wilson SN, Bloomingdale KL, Ritz J et al. Lymphocyte subset differences in patients with chronic fatigue syndrome, multiple sclerosis and major depression. Clin Exp Immunol 2005; 141:326-32.

Schleifer SJ, Keller SE, Meyerson AT, Raskin MJ, Davis KL, Stein M. Lymphocyte function in

major depressive disorder. Arch Gen Psychiatry 1984; 41:484-6.

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977-81.

Suzuki K, Nakamura K, Iwata Y, Sekine Y, Kawai M, Sugihara G et al. Decreased expression of reelin receptor VLDLR in peripheral lymphocytes of drug-naive schizophrenic patients. Schizophr Res. 2008; 98:148-56.

Szuster-Ciesielska A, Slotwinska M, Stachura A, Marmurowska-Michalowska H, Dubas-Slemp H, Bojarska-Junak A et al. Accelerated apoptosis of blood leukocytes and oxidative stress in blood of patients with major depression. Prog Neuropsychopharmacol Biol Psychiatry 2008; 32:686-94.

Takahashi S, Sakai J, Fujino T, Hattori H, Zenimaru Y, Suzuki J et al. The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. J Atheroscler Thromb 2004; 11:200-8.

Tacken PJ, Beer FD, Vark LC, Havekes LM, Hofker MH, van Dijk KW. Very-low-density lipoprotein binding to the apolipoprotein E receptor 2 is enhanced by lipoprotein lipase, and does not require apolipoprotein E. Biochem J 2000; 347:357-61.

Ustün TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ. Global burden of depressive disorders in the year 2000. Br J Psychiatry 2004; 184:386-92.

van den Elzen P, Garg S, León L, Brigl M, Leadbetter EA, Gumperz JE et al. Apolipoprotein-mediated pathways of lipid antigen presentation. Nature 2005; 437:906-10.

Zorilla EP, Luborsky L, McKay JR, Roesnthal R, Houldin A, Tax A, et al. The relationship of depression and stressors to immunological assays: A meta-analytic review. Brain Behav Immun 2001; 15:199 -226.

Figure legends

Figure 1. Scatter plot of ApoER2 and VLDLR mRNA expression in all the patients with major depressive disorder and healthy controls. (A) The levels of ApoER2 mRNA in patients were significantly lower than those in healthy controls. (B) The levels of VLDLR mRNA in patients were comparable to those in healthy controls. Bars represent the means. *$P < 0.001$ (Wilcoxon signed-rank test).

Figure 2. Scatter plot of ApoER2 mRNA levels in drug-free or medicated patients with major depressive disorder and in matched healthy controls. ApoER2 mRNA levels in patients were significantly lower than corresponding values in matched controls. *$P < 0.05$ and **$P < 0.01$ (Kruskal-Wallis test followed by *post hoc* Dunn's multiple comparison test).

Figure 1

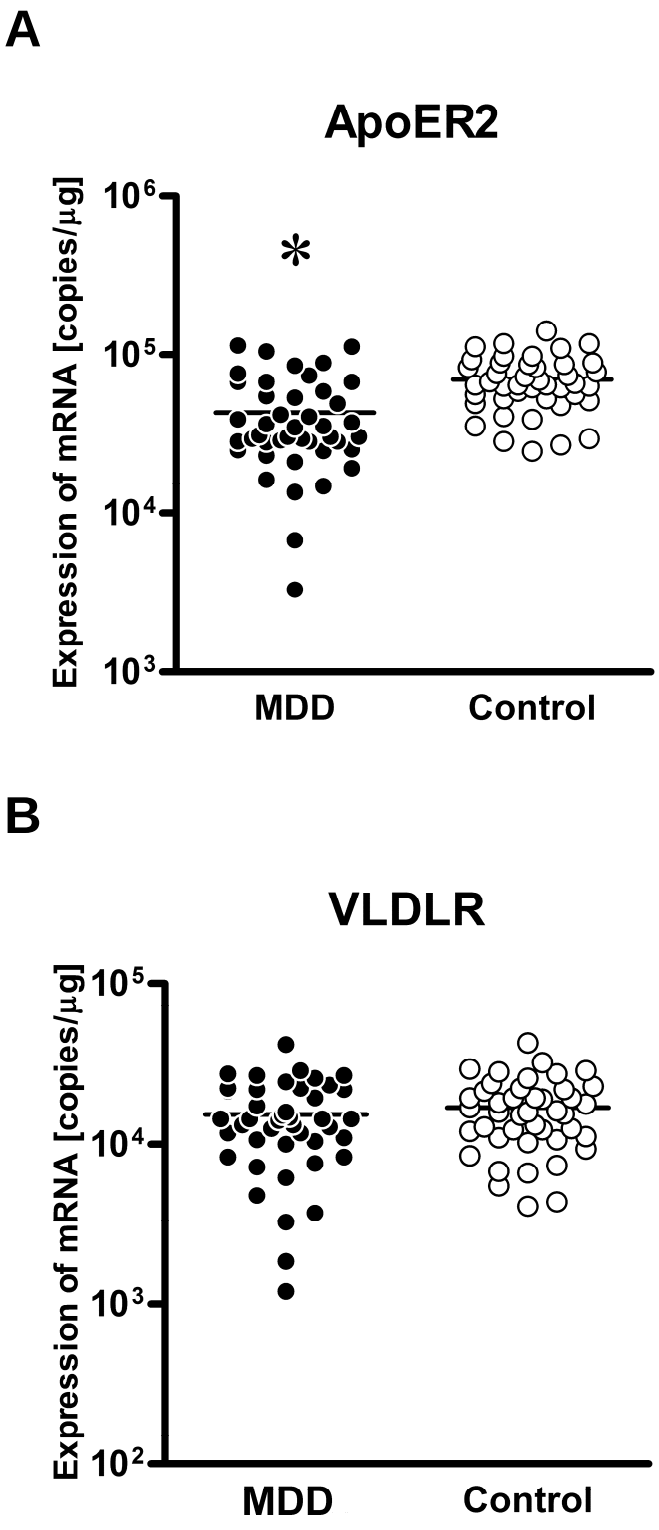
A
ApoER2
Expression of mRNA [copies/µg]
10^6
10^5
10^4
10^3
*
MDD
Control
B
VLDLR
Expression of mRNA [copies/µg]
10^5
10^4
10^3
10^2
MDD
Control

Figure 2

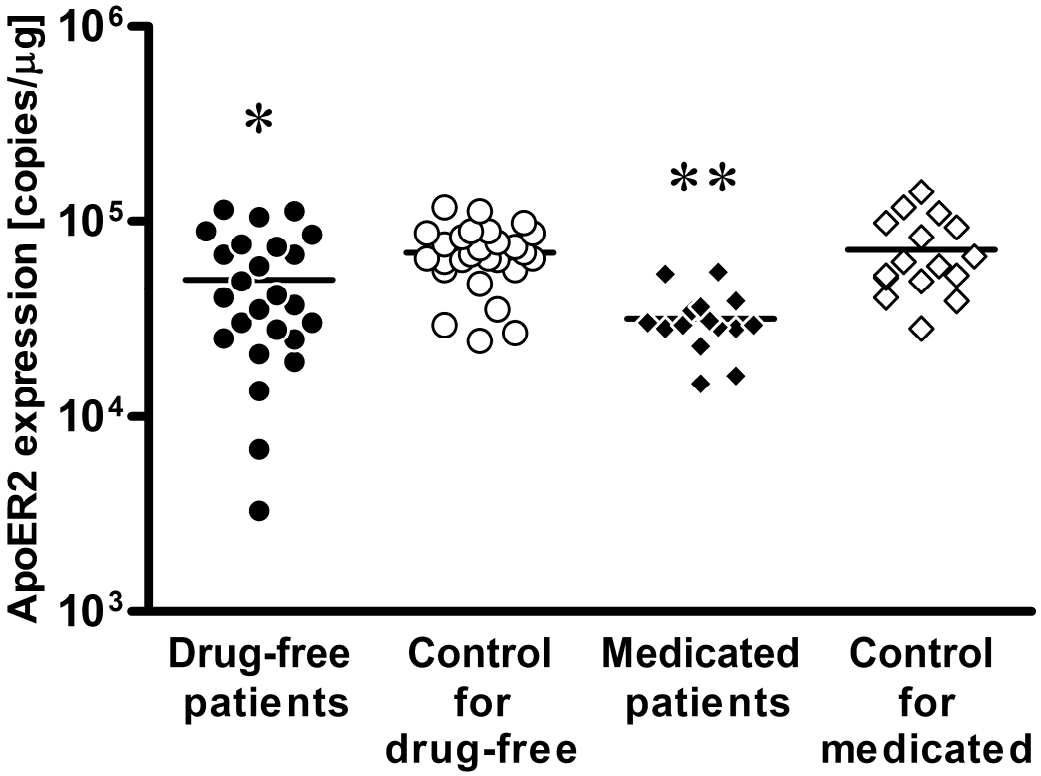
ApoER2 expression [copies/μg]
10^6
10^5
10^4
10^3
*
**
Drug-free patients
Control for drug-free
Medicated patients
Control for medicated

Table 1. TaqMan primers and probes.

Gene		Sequence
ApoER2	forward	5'-TGTGAGTGCTACCCTGGCTACGA-3'
	reverse	5'-GCCTTGTCCATGTAGGCGCTATAG-3'
	probe	5'-TCACCAACCGGTACGAGGTGCGGAGG-3'
VLDLR	forward	5'-CTGCAGGGACTGGAGTGATGAG-3'
	reverse	5'-GCAGATTCCTGGATTTTGGCA-3'
	probe	5'-CGAGTGTGACTGTGCAGCTGGGTTTGA-3'
GAPDH	forward	5’-GAAGGTGAAGGTCGGAGTC-3’
	reverse	5’-GAAGATGGTGATGGGATTC-3’
	probe	5’-CAAGCTTCCCGTTCTCAGCC-3’

Abbreviations: ApoER2, apolipoprotein E receptor type 2; VLDLR, very low-density lipoprotein receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Characteristics and test scores of study participants.

	Controls	Patients		
		total	drug-free	medicated
N (male/female)	43 (31/12)	43 (31/12)	27 (18/9)	16 (13/3)
Age, year	38.8 ± 6.8 (28-55)	39.7 ± 9.1 (26-55)	38.5 ± 9.8 (26-55)	41.7 ± 7.6 (27-55)
Duration of illness, month	N/A	55.0 ± 72.1 (1-268)	22.7 ± 31.0 (1-110)	109.4 ± 88.6 (6-268)
Medicated period, month	N/A	28.3 ± 54.6 (0-240)	3.0 ± 9.8 (0-48)	70.9 ± 71.4 (6-240)
HAM-D total score	N/A	17.3 ± 8.3 (0-31)	20.4 ± 7.1 (0-31)	11.9 ± 7.7 (0-26)
BPRS total score	N/A	9.2 ± 4.9 (0-18)	11.1 ± 4.2 (0-18)	6.0 ± 4.5 (0-15)
Lifetime dose of antidepressants, g	N/A	15.4 ± 28.6 (0-113)	5.7 ± 17.7 (0-75)	31.9 ± 35.9 (5-113)
Lifetime dose of mood stabilizers, g	N/A	7.7 ± 29.9 (0-185)	0.0 ± 0.0 (0-0)	20.8 ± 47.0 (0-185)
Lifetime dose of antipsychotics, g	N/A	0.6 ± 3.0 (0-19)	0.0 ± 0.0 (0-0)	1.6 ± 4.8 (0-19)

Data are expressed as mean ± standard deviation (SD) with range in parentheses. Abbreviations: HAM-D, Hamilton Rating Scale for Depression; BPRS, Brief Psychiatric Rating Scale; N/A, not applicable.