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P2RX7 polymorphisms Gln460Arg and His155Tyr are not associated with major depressive disorder or remission after SSRI or ECT

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ABSTRACT

Purinergic receptor P2X, ligand-gated ion channel, 7 (*P2RX7*) gene polymorphism, has been suggested to be associated with major depressive disorder (MDD). The association between *P2RX7* gene polymorphism and remission after serotonin selective reuptake inhibitors (SSRI) or electroconvulsive therapy (ECT) has not previously been studied. The aims of the present study were to test for an association between *P2RX7* polymorphisms Gln460Arg (rs2230912) and His155Tyr (rs208294) and MDD in two patient populations compared to controls. The first patient sample consisted of 119 subjects with treatment-resistant major depressive disorder, who were treated with ECT and the second of 99 depressive outpatients treated with SSRI. Genotype frequencies were also compared between remitters (Montgomery and Åsberg Depression Rating Scale (MADRS) < 8) and non-remitters (defined as MADRS ≥ 8) to SSRI or ECT treatment. There were no differences in allele or genotype frequencies of either rs2230912 or rs208294 between patient groups and controls. Neither rs2230912 nor rs208294 was associated with MDD or remission after SSRI or ECT. The results suggest that *P2RX7* gene polymorphisms Gln460Arg (rs2230912) and His155Tyr (rs208294) are not associated with MDD or remission after SSRI or ECT.

Depression is a heterogeneous disorder which arises from complex interactions between genetic, developmental and environmental factors. Recently, the role of inflammatory factors in the depression pathogenesis has been studied. The excessive secretion of proinflammatory cytokines, such as interleukin-1β, interferon-α and tumor necrosis factor-α from activated macrophages has been suggested to play a role in the pathogenesis of depression [19].

The purinergic P2X7 receptor is an ATP-gated non-selective cation channel activated by high concentrations of ATP (>100 μM) [27]. The P2X7 receptors mediate the influx of Ca^{2+} and Na^{+} ions and also the release of proinflammatory cytokines. P2X7 receptors are supposed to be expressed on cells from hematopoietic lineages, such as erythrocytes, lymphocytes, neutrophils, eosinophils, mast cells, monocytes and macrophages as well as in brain glial cells (microglia, astrocytes and Muller cells) [27,36,5,18,32,30]. They have also been found to be expressed in epithelial and endothelial cells as well as in bone cells (osteoclasts, osteoblasts) [27,36]. Their expression in neurons is still controversial [1]. They can mediate and/or enhance neurotransmitter release (e.g. glutamate) [40,9], and may affect neuronal plasticity and neuronal cell death [31]. The P2X7 receptors mediate the release of interleukin-1β, which is a key mediator in chronic inflammation, neurodegeneration and chronic pain [31]. Therefore, altered P2X7 receptor expression of function may lead to abnormal inflammatory or immune response. P2X7 receptor-deficient mice have substantially attenuated inflammatory responses [7]. The P2X7 receptors have been claimed to represent a link between the nervous and immune system and to play a role in the pathogenesis of different psychiatric and neurodegenerative diseases, such as major depressive disorder (MDD), anxiety, bipolar disorder and Alzheimer's disease [3,10,23,31,28].

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Table 1
Distribution of Gln460Arg (rs2230912) and His155Tyr (rs208294) in ECT- and SSRI-treated patients and controls.

Gln460Arg[¤]	A/A	A/G	G/G	A-allele	G-allele
ECT patients (N = 119)	91 (76.5%)	26 (21.8%)	2 (1.7%)	208 (87.4%)	30 (12.6%)
SSRI patients (N = 99)	76 (76.8%)	21 (21.2%)	2 (2.0%)	173 (87.4%)	25 (12.6%)
Total patients (N = 218)	167 (76.6%)	47 (21.6%)	4 (1.8%)	381 (87.4%)	55 (12.6%)
Controls (N = 391)	277 (70.8%)	103 (26.3%)	11 (2.8%)	657 (84.0%)	125 (16.0%)
His155Tyr[¤¤]	**C/C**	**C/T**	**T/T**	**C-allele**	**T-allele**
ECT patients (N = 119)	50 (42.0%)	51 (42.9%)	18 (15.1%)	151 (63.4%)	87 (36.6%)
SSRI patients (N = 98)	42 (42.9%)	38 (38.8%)	18 (18.4%)	122 (62.2%)	74 (37.8%)
Total patients (N = 217)	92 (42.4%)	89 (41.0%)	36 (16.6%)	273 (62.9%)	161 (37.1%)
Controls (N = 382)	158 (41.4%)	162 (42.4%)	62 (16.2%)	478 (62.6%)	286 (37.4%)

[¤] $p = 0.642$ (Pearson χ^2 test between ECT-treated, SSRI-treated patients and controls).
[¤¤] $p = 0.954$ (Pearson χ^2 test between ECT-treated, SSRI-treated patients and controls).

P2RX7 gene located on chromosome 12q24.31 encodes the P2X7 receptor. Of *P2RX7* single nucleotide polymorphism (SNP), rs2230912 has been reported to be associated with MDD [21] and its severity [15,26]. It is located in exon 13 of *P2RX7* and results in a change of the amino acid glutamine to arginine at 460 position (Gln460Arg) [8].

However, two recent, well-conducted studies have failed to show the linkage between rs2230912 polymorphism and MDD [11,12]. Another *P2RX7* SNP, rs208294 (His155Tyr or 489C > T) may also be associated with MDD [29]. It leads to the His 155 into Tyr change in the extracellular domain of the receptor and it has been suggested to act as a gain-of-function polymorphism of the P2RX7 [6].

Dysfunction of the hypothalamic–pituitary–adrenal axis (HPA) is well established in depression [34]. Some proinflammatory cytokines are potent activators of the HPA axis. Antidepressants have been shown to reduce the release of proinflammatory cytokines from activated macrophages [22,33] and thus play a role in the inhibitory feedback of the HPA axis, which, in turn, leads to reduced release of glucocorticoids from the adrenal glands [19]. In addition, many antidepressants can increase the release of endogenous cytokine antagonists such as interleukin-1 receptor antagonist [37] and interleukin-10 [22]. Antidepressants have also been suggested to modulate the expression of *P2RX7* in the central nervous system [16]. To the best of our knowledge the association between *P2RX7* polymorphism and remission to neither serotonin selective reuptake inhibitors (SSRI) nor electroconvulsive therapy (ECT) has been previously studied. The aims of the present work were to study the association between *P2RX7* polymorphisms rs2230912 and rs208294 and MDD in two clinically different MDD patients groups compared to controls, and also an association between these polymorphisms and proportion of patients in remission after SSRI or ECT.

The ECT population consisted of 119 patients with a diagnosis MDD according to DSM-IV criteria. They were all referred to and treated with ECT because of treatment-resistant depression. Patients with neurological disorders, dementia, schizophrenia, bipolar disorder and alcohol or other substance abuse were excluded. The severity of their depression was assessed on the Montgomery–Åsberg Depression Rating Scale (MADRS) [25] at baseline and after the series of ECT treatments. Primary outcome measure of the treatment was remission, defined as <8 points on MADRS. Blood DNA samples were taken. The ECT treatment procedure [2] and a more detailed description of the patients [38] are given elsewhere.

A total of 99 outpatients with DSM-IV MDD were recruited. The eligibility criterion for the study was a baseline score of 20 or more on MADRS. Severe somatic diseases, medication affecting the mood, other axis I psychiatric disorder, severe personality disorders or alcohol-substance abuse led to exclusion from the study. At the baseline visit the severity of MDD was assessed by MADRS and SSRI (citalopram, fluoxetine or paroxetine) was initiated; the choice of medication was made by an experienced psychiatrist. According to the study protocol patients met a psychiatrist at 3 and 6 weeks. At the final visit the outcome was assessed on MADRS; those with MADRS scores <8 were considered remitters. Of 99 patients 86 (87%) completed the study. A more detailed description of these patients is available elsewhere [38,17].

The control population consisted of 395 healthy Finnish blood donors. They completed a health questionnaire including information on their mental health, and were also interviewed by a nurse about their medications and possible chronic illnesses. All patients and controls were of Caucasian origin (Finnish).

For DNA extraction, 9.0 ml EDTA–whole blood was taken from the participants and stored in a freezer at −20 °C. Genomic DNA was extracted from peripheral blood leukocytes using QIAamp®DNA Blood Minikit and automated biorobot M48 extraction (Qiagen, Hilden, Germany). Genotyping of the P2RX7 was performed using Taqman®SNP Genotyping Assays (for rs2230912 the assay C__15853715_20, and for rs208294 C___3019032_1_) and ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

Pearson's chi-square ($\chi 2$) test was used to compare numbers of genotypes and allele frequencies between the study groups. Analysis of covariance (ANCOVA) was used in treatment response comparisons between different *P2RX7* genotypes for both SNPs studied. In the models, MADRS score change was used as dependent variable, *P2RX7* polymorphisms and gender as factors, and age as covariate. The limit of statistical significance was set at 0.05. Data analysis was carried out using SPSS/Win software (Version 16.0, SPSS Inc., Chicago, IL, USA). Power analysis calculation was carried out with PS Power and Sample Size Calculations Software (version 3.0, http://biostat.mc.vanderbilt.edu/PowerSampleSize). The power analyses revealed a 12% difference between patient and control groups in rs208294 and a 9% difference in rs2230912 as statistically significant ($p < 0.05$) with a power of ≥ 0.8.

All 119 ECT treated, 99 SSRI treated and 391 control patients were successfully analyzed for rs2230912 and 119, 98 and 382 for rs208294, respectively. The patients in the ECT group were older (57.7 ± 14.0 yrs) than in the SSRI (40.7 ± 14.0 yrs) or in the control groups (44.4 ± 11.1 yrs). The mean MADRS scores at baseline were 32.5 ± 8.2 in the ECT group and 27.0 ± 5.7 in the SSRI group, and after the treatment the MADRS scores were 11.3 ± 8.8 and 12.0 ± 8.2, respectively. Detailed clinical and demographic data are presented elsewhere [18]. Of 119 ECT treated patients, 45 were remitters and 74 non-remitters and of 86 SSRI treated, 30 were remitters and 56 non-remitters.

The two *P2RX7* polymorphisms studied, rs2230912 and rs208294, were not associated with MDD (Table 1) or with remission rates after ECT or SSRI (Tables 2 and 3). Percentages of