Analysis of biomarkers in major depression

PhD thesis

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1. Introduction

Mood disorders are among the most common psychiatric disorders. Usually they have an early onset and a long-lasting time course. Therefore, besides personal suffering, these disorders impose a great burden on all nations through direct costs (such as hospitalization and pharmacological treatments) and indirect costs (e.g., inability to work, and the loss of productive forces due to early death rates). According to the World Health Organization's estimates depression will be the second main reason for reduced work capacity and early death by 2020, and will demand great expenses on health care budgets. Unfortunately, the currently available treatment options for major depression are only partially effective, since many patients do not or only moderately respond to drug therapies. The lack of cooperation (which can be due to drug side effects) is also a frequent problem. Searching for the underlying mechanisms in mood disorders has been a hot topic in the medical field over the last decades. There are several methodological approaches in this research; one of them is aiming to unravel the complex genetic background of mood disorders. The findings of these psychiatric genetic studies can help to identify those vulnerable individuals who would develop depressive symptoms as a response to stressful situations. Also, these genetic studies might identify novel molecular targets with a potential for innovative antidepressant drug design.

The aim of my PhD research was to investigate the effectiveness of two potential biomarkers (which have been shown previously to play a role in the pathomechanism of mood disorders) in our patient population treated during a major depressive episode (MDE). The peripheral level of a protein biomarker, the plasma Vascular Endothelial Growth Factor (VEGF) was analyzed in antidepressant drug response. In the hope of finding an early diagnostic biomarker for mood disorders, a potential genetic risk factor was also investigated in the Hungarian patient population. Based on earlier results, we chose the P2RX7 (purinergic receptor P2X, ligand-gated ion channel, 7) as our candidate gene.

My thesis presents genetic association analyses of two P2RX7 single nucleotide polymorphisms (SNPs) in unipolar major depression (MDD), bipolar disorder (BPD), and depressive symptom severity. One of the investigated P2RX7 polymorphisms (Gln460Arg, rs2230912) was already associated with both MDD and BPD in earlier studies. In addition, we tested a new potential microRNA binding site polymorphism, rs1653625 from the 3' end of the P2RX7 gene.

2. Aims

The aim of our study was to analyze two possible biomarkers (VEGF protein level and P2RX7 gene polymorphisms) which could affect depression symptom severity and/or could potentially predict treatment success. The patients diagnosed with either MDD or BPD had an active depressive episode; whereas the controls were recruited from the general population.

2.1 Peripheral VEGF protein level as a potential predictor for treatment response

Based on previous findings, the VEGF might have an important role in the molecular pathomechanism of mood disorders, and it might also affect treatment outcome.
The present study tested if plasma VEGF levels before and after the 28 day treatment period were in association with antidepressant drug response on a sample of patients treated during MDE.

2.2 P2RX7 polymorphisms as possible genetic risk factors for depression

In this genetic association study we tested if P2RX7 gene polymorphisms (rs2230912, rs1653625) were in association with the occurrence of MDD or BPD. First a case-control analysis was carried out. Then a dimensional model was also tested, since it can be more sensitive in identifying small genetic effects. In this dimensional analysis we used symptom severity as the dependent variable. Depression and anxiety symptoms were measured with the Hospital Anxiety and Depression Scale (HADS) and the Montgomery–Åsberg Depression Rating Scale (MADRS).

3. Methods

Data was collected from more than 400 patients diagnosed with mood disorder and from almost 560 healthy control participants. However, the inclusion criteria for the two types of analyses (i.e., the VEGF and the P2RX7 study) were different in terms of age, clinical status (possible chronic diseases), and valid phenotype and genotype data.

Patients with current MDE were recruited at the Department of Clinical and Theoretical Mental Health, Kútvölgyi Clinical Center, Semmelweis University, or at the Psychiatric Ward, Nyírő Gyula Hospital (Budapest, Hungary). For the control sample, healthy young adults were recruited at the Eötvös Loránd University. Participants filled out a set of

questionnaires, which included the Hospital Anxiety and Depression Scale (HADS), the Montgomery–Åsberg Depression Rating Scale (MADRS) was collected only from patients with MDE. The study was approved by the Scientific and Research Ethics Committee of the Medical Research Council. (ETT-TUKEB reference number: 4514-0/2010-1018EKU(294/PI/10.)

Study of the VEGF biomarker was carried out on 34 inpatients (MDD=21 and BPD=13, 9 men and 25 women, mean age 44.6±12.6 year). Patients were excluded if their MADRS score was less than 20 at the time of the first visit (V_1). Furthermore, major cardiovascular events, malignant and/or hematopoietic diseases and rheumatoid arthritis were also in the exclusion criteria. Levels of plasma VEGF were measured by dr. Judit Dobos in the National Institute of Oncology (Department of Molecular Immunology and Toxicology). Levels of plasma VEGF and the severity of depressive symptoms were monitored at two dates: at baseline and after a four-week treatment period. For the analyses of plasma VEGF levels paired sample t-tests were used.

For testing the peripheral VEGF protein level as a potential predictor for treatment response, we divided our sample into a responder and a non-responder group (responders were defined as those whose MADRS total score at the second visit (V_2) was reduced by at least 50% compared to their MADRS total score at baseline (first visit, V_1). Conversely, patients with less than a 50% improvement in MADRS total scores during the period between V_1 and V_2 were regarded as non-responders. Then, VEGF levels at baseline and after treatment were tested in both groups. Also, in a dimensional approach VEGF and MADRS scores were analyzed in a regression model.

Genetic association analyses were carried out in two waves: in the first study 171 patients were included (MDD=107 and BPD=64; 25.1% male and 74.9% female; mean age 48.5±11.0 years). In this sample only the exon polymorphism (rs22300912) was genotyped using allele-specific TaqMan probes on a real-time PCR machine. Afterwards additional patients were recruited, and the second set of analyses included 315 patients (MDD=195 and BPD=120; 24.8% male and 75.2% female; mean age of 47.3±11.5 years) and 373 controls (30.0% male and 70.0% female; mean age 28.3±12.4 years). On this expanded dataset the microRNA binding site polymorphism of P2RX7 (rs1653625) was also genotyped with a restriction fragment length polymorphism (RFLP) method developed at the Molecular Genetic Laboratory, Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University. Since the mean age of the clinical sample was

higher than in the control sample, we selected an age-matched control subsample (N=163; mean age was 37.8±12.9 years) from the control sample.

In the case-control genetic analyses chi-square tests were used to test the genotype frequency differences in the MDD and BPD vs control samples. In the dimensional analyses we compared the mean depression symptom scores of the different P2RX7 genotypes in the patient group with analysis of variance (ANOVA) to test if any genotype would affect the severity of symptoms. Two-way analysis of variance was used to test the possible interactional effect of the groups (clinical or control) and P2RX7 genotypes.

4. Results

4.1 Changes in the peripheral levels of VEGF before and after treatment

As expected, the responder group showed a bigger difference in the MADRS scores after treatment as compared to the non-responders. In the responder group the MADRS mean score was 75% less in the control visit, as compared to the baseline ($p<0.0001$), whereas the non-responder group showed only an average 25% change ($p<0.001$). The peripheral levels of VEGF did not show any significant difference before and after the 28 day treatment either in the total sample, nor in the responder or in the non-responder groups.

4.2 Baseline VEGF plasma level as a possible predictor of treatment response

The non-responder group showed higher plasma VEGF level at baseline as compared to the responders' baseline VEGF level ($p=0.055$). This trend was observable after treatment as well ($p=0.097$). Further tests of the dimensional approach showed that the baseline VEGF level is a predictor of MADRS symptom severity score after antidepressant treatment ($p=0.02$). These results suggest, that the peripheral level of VEGF might be a good biomarker of treatment response in patients with MDE. However, further independent replications are needed.

4.3 P2RX7 genotypes as possible risk factors of depression (case-control model)

No significant genotype or allele difference was observed in the clinical subsamples (MDD, BPD) vs. control group in the first sample or in the expended sample at any of the investigated P2RX7 polymorphisms (rs2230912 and rs1653625).

4.4 G allele of P2RX7 rs2230912 as a possible biomarker for depression symptom severity (dimensional approach)

Significant difference of the mean depression and anxiety scores was observed between the different genotype groups at the P2RX7 exon polymorphism (rs2230912) in both the first and the expanded sample. MDD and BPD patients with the G allele of P2RX7 rs2230912 showed significantly higher mean score on the HADS depression (p=0.0004) and anxiety scale (p=0.012) compared to the AA genotype. At the same time, no significant association was observed between this exon polymorphism and anxiety or depression scores in the control sample. These results were replicated on the expended sample. Furthermore, in the BPD group the rs2230912 G allele was associated with higher MADRS mean score (p=0.028).

4.5 MicroRNA binding site polymorphism of P2RX7 (rs1653625) as possible biomarker for symptom severity (dimensional approach)

BPD patients carrying the C allele of the rs1653625 showed lower mean depression symptom severity scores on both depression scales as compared to the AA genotype group (for HADS: p=0.017, for MADRS: p=0.019).

5. Conclusions

First, we tested the possible associations between VEGF plasma levels and antidepressant drug therapy with different approaches. We did not find any significant difference in the total clinical sample comparing the baseline VEGF level and VEGF level after the 28 day treatment. However, responders and non-responders showed a different VEGF levels at baseline (p=0.055) and after treatment as well (p=0.097). Dimensional analysis identified a significant association between plasma VEGF at baseline and symptom severity measured by MADRS (p=0.02): the low peripheral VEGF level at baseline predicted low MADRS scores after treatment. These results suggest that plasma VEGF level might be a biomarker for successful antidepressant treatment in patients with major depressive episode if effectiveness of therapy is measured by quantitative methods. These results are in line with previous studies suggesting that VEGF might have an important role in the molecular changes occurring during antidepressant treatment.

The genetic analyses of the P2RX7 polymorphisms (rs2230912 and rs1653625) tested the possible associations with MDD or BPD diagnosis, as well as with depression and anxiety symptom severity. In the case-control analyses no significant difference was observed in the P2RX7 allele or genotype frequencies at either polymorphisms between patient (MDD, BPD) and the control samples. In the dimensional analyses we tested if P2RX7 polymorphisms were in association with symptom severity. Our first results showed that MDD and BPD patients with the rs2230912 G allele had higher mean scores on the HADS depression (p=0.0004) and anxiety scales (p=0.012) compared to the AA genotype. Analyses of the expanded dataset confirmed and further clarified this association: BPD patients carrying the rs2230912 G allele showed not only higher mean scores on the HADS depression scale (p=0.003), but on MADRS as well (p=0.028). These results support previous studies showing association between the P2RX7 exon polymorphism and major depression, and also support the idea that dimensional approaches are more sensitive to detect small genetic effects as compared to case-control analyses. According to our best knowledge, our study was the first to report significant association between the P2RX7 microRNA binding site polymorphism (rs1653625) and depression symptom severity measured by both HADS and MADRS.

6. Publications of the PhD candidate

6.1 Publications in the topic of the dissertation

Halmai Z, Dome P, Vereczkei A, Rahman OA, Szekely A, Gonda X, Faludi G, Sasvari-Szekely M, Nemoda Z. (2013) Associations between depression severity and purinergic receptor P2RX7 gene polymorphisms. J Affect Disord 150(1):104-9. (IF.: 3.705)

Halmai Z, Dome P, Dobos J, Gonda X, Szekely A, Sasvari-Szekely M, Faludi G, Lazary J. (2013) Peripheral vascular endothelial growth factor level is associated with antidepressant treatment response: Results of a preliminary study. J Affect Disord, 144(3):269-73. (IF.: 3.705)

Hejjas K, Szekely A, Domotor E, **Halmai Z**, Balogh G, Schilling B, Sarosi A, Faludi G, Sasvari-Szekely M, Nemoda Z. (2009) Association between depression and the Gln460Arg polymorphism of P2RX7 gene: A dimensional approach. Am J Med Genet B Neuropsychiatr Genet 150B:295–9. (IF.: 3.932)

6.2 Other Publications

Kotyuk E, Nemeth N, **Halmai Z**, Faludi G, Sasvari-Szekely M, Szekely A. (2013) A hangulati dimenziók és a glia-eredetű növekedési faktort kódoló gén polimorfizmusainak összefüggése depresszióval diagnosztizált mintán. Neuropsychopharmacol Hung, 15(2):63-72.

Dome P, **Halmai Z**, Dobos J, Lazary J, Gonda X, Kenessey I, Sallai T, Makkos Z, Faludi G. (2012) Investigation of circulating endothelial progenitor cells and angiogenic and inflammatory cytokines during recovery from an episode of major depression. J Affect Disord, 136(3):1159-63. (IF.: 3.295)

Szekely A, Kovacs-Nagy R, Bányai É, Gősi-Greguss A, Varga K, **Halmai Z**, Ronai Z, Sasvari-Szekely M. (2010) Association between hypnotizability and the catechol-o-methyltransferase (COMT) polymorphism. Int J Clin Exp Hypn, 58(3): 301-15. (IF.: 1.842)

Halmai Z, Dömötör E, Balogh G, Sárosi A, Faludi G, Székely A. (2008) Egy új hangulati kérdőív validálása egészséges mintán. Neuropsychopharmacol Hung, 10(3): 151-157.