Biological mechanisms in the background of ruminative response style

PhD thesis abstract

Nóra Eszlári

Semmelweis University

Doctoral School of Mental Health Sciences

Supervisor: Gabriella Juhász, MD, PhD

Official reviewers: Judit Gervai, CSc
Róbert Urbán, DSc

Head of the Final Examination Committee: Gábor Faludi, MD, DSc

Members of the Final Examination Committee: Edit Buzás, MD, DSc
Zsolt Demetrovics, DSc
Adrien Pigniczkiné Rigó, PhD

Budapest

2018

1. Introduction

Rumination, or in other words depressive rumination or ruminative response style, is a trait-like, passive, repetitive and perseverative mode of thinking in response to distress and depressed mood. As one of the several, partly independent negative cognitive styles, it is a risk factor for major depression. It also denotes a considerable cognitive endophenotype for this disorder, since it has been demonstrated that common genetics of rumination and depression accounts for 45-68% of their phenotypic correlation. Replicated candidate gene studies have suggested the role of *FKBP5*, *5-HTTLPR*, *CREB1* and *BDNF* gene variants in rumination.

Although markers of the folate metabolism, particularly high homocysteine and low folate levels have been linked to both depression and inflexible cognition, candidate gene studies for rumination have not yet addressed any gene of the folate pathway.

Another gap in our present knowledge regarding rumination is to understand the role of serotonergic candidate genes, such as *HTR2A* encoding the 5-HT_{2A} receptor. In addition, it would be important to investigate the moderating effect of childhood adversity in these genetic associations, not only with rumination, but also with its two subtypes (subscales), the more maladaptive

brooding and the less maladaptive reflection. While brooding embodies a gloomy and passive criticism, comparisons of the self with unachieved standards, reflection denotes a neutral and purposeful self-analysis with the aim of finding a solution for the problem. From these two subtypes, only brooding but not reflection has been found to mediate the depressogenic effect of childhood adversity in previous studies.

2. Objectives

The aim of my PhD work was to identify genetic risk factors that raise the level of rumination, and, presumably via this endophenotype, contribute to the emergence of depression. We carried out genetic association studies in a sample of two independent population cohorts (from Budapest and Manchester) to answer the questions below. In our first study we investigated the associations with two genes of the folate metabolism, and in the second study we tested the role of *HTR2A* and childhood adversity.

A) Genetics of folate metabolism in the background of rumination

2.A.1. Is rs1801133 polymorphism of the *MTHFR* gene related to rumination?

2.A.2. Is rs11754661 polymorphism of the *MTHFD1L* gene related to rumination?

2.A.3. In case of a significant polymorphism-rumination association, is it mediated by depression?

2.A.4. In case of a significant polymorphism-rumination association, does it mediate the polymorphism-depression association?

2.A.5. In case of a significant polymorphism-rumination association, can it be replicated in the separate Budapest and Manchester subsamples?

2.A.6. Can the findings regarding the mediation of the polymorphism effect be replicated in the separate Budapest and Manchester subsamples?

B) Serotonin receptor gene *HTR2A* and childhood adversity in the background of rumination

2.B.1. Is rs3125 polymorphism (a microRNA binding site) of the *HTR2A* gene related to rumination?

2.B.2. Is rs6311 polymorphism (a methylation site) of the *HTR2A* gene related to rumination?

2.B.3. In case of a significant polymorphism-rumination association, is it dependent on rumination subscale, and / or childhood adversity level?

2.B.4. In case of a significant polymorphism-rumination association, is it mediated by depression?

2.B.5. In case of a significant polymorphism-rumination association, does it mediate the polymorphism-depression association?

2.B.6. In case of a significant polymorphism-rumination association, can it be replicated in the separate Budapest and Manchester subsamples?

2.B.7. Can the findings regarding the mediation of the polymorphism effect be replicated in the separate Budapest and Manchester subsamples?

2.B.8. If we cannot find any significant association of rs6311 with depression, can it be detected in a complex model, taking recent stress and six other depression-related polymorphisms into consideration?

3. Methods

European white adults aged 18-60 years were recruited from the general population of Budapest, Hungary and Manchester, United Kingdom, to fill out the NewMood questionnaire pack and provide DNA by a genetic saliva sampling kit. For the investigation of the folate pathway genes, 2204 participants had available data on *MTHFR* rs1801133, and 2120 on *MTHFD1L*

rs11754661. For the *HTR2A* investigation, data from 1501 participants were analysed. For the complex model on the role of *HTR2A* rs6311 in depression, 1682 participants provided data.

Rumination was measured by the 10-item Ruminative Responses Scale, comprising also brooding and reflection as subscales. Depression was measured both as lifetime depression based on self-report, and as a current depression score based on the Brief Symptom Inventory (BSI). We also used the BSI to measure current anxiety. Childhood adversity score was derived from the Childhood Trauma Questionnaire. Recent stress was measured by the List of Threatening Experiences.

Plink v1.07 was used to build linear regression models to test additive, dominant and recessive genetic associations with rumination or a subscale as the outcome, and the polymorphism, population, gender and age as predictors. In the models testing the gene-by-environment (GxE) interaction effect of rs3125 or rs6311 in the *HTR2A* gene and childhood adversity, main effects of these two variables were also included as predictors. In case of a subscale as the outcome, the other subscale was an additional predictor. Bonferroni correction or q-values of false discovery rate were applied to correct for multiple testing, and Quanto v1.2 was used to calculate the statistical power of the analyses.

For significant polymorphism-rumination associations, the mediation effect of depression on rumination or the mediation effect of rumination on depression were tested. Thus the above described regression model was run on rumination with the two depression variables as additional predictors. Moreover, the same model was run on lifetime and current depression, as outcome variable, with rumination as an additional predictor. To test replicability of findings within Budapest and Manchester, the models were run in the two subsamples without population as predictor.

Additional calculations were performed with SPSS and R.

We performed a Bayesian multi-level analysis of relevance to investigate the role of *HTR2A* rs6311 in depression in a complex model.

4. Results

A) Genetics of folate metabolism in the background of rumination

Although *MTHFR* rs1801133 had no association with rumination, the A allele of *MTHFD1L* rs11754661 was associated with higher rumination.

While the significant *MTHFD1L*-rumination association was not only due to depression, it entirely mediated the *MTHFD1L*-depression associations.

The *MTHFD1L*-rumination association could be replicated in the separate Budapest and Manchester subsamples. In contrast, this genetic effect on the depression phenotypes could not be replicated, because *MTHFD1L* was associated with neither depression phenotype in Manchester. Nevertheless, in Budapest, as in the combined sample, the *MTHFD1L*-rumination association was not exclusively due to depression, but it entirely explained the *MTHFD1L*-depression associations.

B) Serotonin receptor gene *HTR2A* and childhood adversity in the background of rumination

HTR2A rs3125 was associated only with brooding, and only in interaction with childhood adversity.

Similarly to the *MTHFD1L* results, the *HTR2A* rs3125 x childhood adversity interaction on brooding was not exclusively due to depression, however, brooding totally mediated the same GxE interaction effect on the depression phenotypes.

The *HTR2A* rs3125 x childhood adversity interaction on brooding could be replicated in the separate Budapest and Manchester subsamples. In contrast, this GxE effect was

significant on neither depression phenotype in neither subsample.

HTR2A rs6311 was associated only with rumination, and, similarly to the rs3125 effect, only in interaction with childhood adversity. However, this *HTR2A* rs6311 x childhood adversity interaction on rumination could neither be replicated in Budapest, only in Manchester, nor proved this GxE interaction significant on either depression phenotype. Moreover, rs6311 was not relevant with respect to a complex depression-anxiety phenotype, taking recent stress and six other depression-related polymorphisms into consideration.

5. Conclusions

1.) Our study demonstrated that the widely but inconclusively investigated *MTHFR* rs1801133 polymorphism is not associated with rumination in an adult general population sample.

2.) Our study was the first to reveal that the A allele of *MTHFD1L* rs11754661, already established as a risk factor for Alzheimer's disease in previous genome-wide association studies, is associated with higher rumination score in an adult general population sample.

3.) The fact that the association of *MTHFD1L* rs11754661 with rumination was replicable within the Budapest and Manchester subsamples suggests that *MTHFD1L* represents part of the rumination endophenotype that is generalizable across European populations.

4.) The association of *MTHFD1L* rs11754661 with rumination was not exclusively explained by depression, but fully mediated the same genetic association with depression, suggesting that the association of this genetic variant with rumination goes beyond this one disorder, implicating *MTHFD1L* as a contributor in the rumination endophenotype possessing transdiagnostic relevance.

5.) In spite of the replicable genetic association with rumination, *MTHFD1L* rs11754661 was associated with depression only in Budapest but not in Manchester, which discrepancy in replicability may be a clue that an endophenotype has a biological background more homogeneous and less sensitive to cultural and societal impacts than the disorder itself.

6.) Our results were the first to demonstrate that *HTR2A* rs3125 exerts an effect only on the maladaptive brooding subtype of rumination, and that this effect is a function of childhood adversity exposure, which finding entails the compelling importance of involving gene-by-environment

interaction models in the endophenotype concept, especially in case of polymorphisms transmitting epigenetic impacts, such as alterations of microRNA binding.

7.) Methylation site *HTR2A* rs6311 was associated only with rumination, and, similarly to the effect of microRNA binding site rs3125, this association was also a function of childhood adversity exposure, but, in contrast to the rs3125 effect, it could be replicated only in Manchester but not in Budapest, implicating the possible role of the epigenetic mechanism itself in the robustness of these *HTR2A* x childhood adversity interaction findings.

8.) As the association with *MTHFD1L* rs11754661, the *HTR2A* rs3125 x childhood adversity interaction also contributed to rumination endophenotype in a generalizable manner across European populations.

9.) As in case of the *MTHFD1L*-rumination association, the *HTR2A* rs3125 x childhood adversity interaction on brooding was not exclusively mediated by depression, but fully accounted for the same interaction results on depression, suggesting that this effect also goes beyond this one disorder, implicating this GxE interaction as a risk factor for rumination endophenotype possessing transdiagnostic relevance.

10.) As the associations with *MTHFD1L*, the *HTR2A* rs3125 x childhood adversity interaction results also corroborate the biologically homogeneous endophenotype nature of rumination, in that this GxE effect is replicable on brooding but unreplicable on depression.

6. Publications

6.1. Original publications related to the thesis

- **Eszlari N**, Kovacs D, Petschner P, Pap D, Gonda X, Elliott R, Anderson IM, Deakin JF, Bagdy G, Juhasz G. (2016) Distinct effects of folate pathway genes MTHFR and MTHFD1L on ruminative response style: a potential risk mechanism for depression. *Transl Psychiatry*. 1:19. **IF: 4.730**
- Gonda X, Hullam G, Antal P, **Eszlari N**, Petschner P, Hökfelt TG, Anderson IM, Deakin JFW, Juhasz G, Bagdy G. (2018) Significance of risk polymorphisms for depression depends on stress exposure. *Sci Rep*. 8:018-22221. **IF: 4.259**

6.2. Original publications not related to the thesis

- Juhasz G, **Eszlari N**, Pap D, Gonda X (2012) Cultural differences in the development and characteristics of depression. *Neuropsychopharmacol Hung*. 14:259-265.

- Juhasz G, Hullam G, **Eszlari N**, Gonda X, Antal P, Anderson IM, Hokfelt TG, Deakin JF, Bagdy G. (2014) Brain galanin system genes interact with life stresses in depression-related phenotypes. *Proc Natl Acad Sci U S A.* 111:24. **IF: 9.674**
- Kovacs D, Gonda X, Petschner P, Edes A, **Eszlari N**, Bagdy G, Juhasz G. (2014) Antidepressant treatment response is modulated by genetic and environmental factors and their interactions. *Ann Gen Psychiatry*. 13:13-17.
- Juhasz G, Gonda X, Hullam G, **Eszlari N**, Kovacs D, Lazary J, Pap D, Petschner P, Elliott R, Deakin JF, Anderson IM, Antal P, Lesch KP, Bagdy G. (2015) Variability in the effect of 5-HTTLPR on depression in a large european population: The role of age, symptom profile, type and intensity of life stressors. *PLoS ONE*. 10. **IF: 3.057**
- Gonda X, **Eszlari N**, Anderson IM, Deakin JF, Bagdy G, Juhasz G (2016) Association of ATP6V1B2 rs1106634 with lifetime risk of depression and hippocampal neurocognitive deficits: possible novel mechanisms in the etiopathology of depression. *Transl Psychiatry*. 6:221. **IF: 4.730**
- Gonda X, **Eszlari N**, Kovacs D, Anderson IM, Deakin JF, Juhasz G, Bagdy G. (2016) Financial difficulties but not other types of recent negative life events show strong interactions with 5-HTTLPR genotype in the development of depressive symptoms. *Transl Psychiatry*. 3:57. **IF: 4.730**

- Lazary J, **Eszlari N**, Juhasz G, Bagdy G (2016) Genetically reduced FAAH activity may be a risk for the development of anxiety and depression in persons with repetitive childhood trauma. *Eur Neuropsychopharmacol*. 26:1020-1028. **IF: 4.239**
- Kokonyei G, Szabo E, Kocsel N, Edes A, **Eszlari N**, Pap D, Magyar M, Kovacs D, Zsombok T, Elliott R, Anderson IM, Deakin JFW, Bagdy G, Juhasz G. (2016) Rumination in migraine: Mediating effects of brooding and reflection between migraine and psychological distress. *Psychol Health*. 31:1481-1497. **IF: 2.225**
- Kovacs D, **Eszlari N**, Petschner P, Pap D, Vas S, Kovacs P, Gonda X, Bagdy G, Juhasz G. (2016) Interleukin-6 promoter polymorphism interacts with pain and life stress influencing depression phenotypes. *J Neural Transm*. 123:541-548. **IF: 2.392**
- Kovacs D, **Eszlari N**, Petschner P, Pap D, Vas S, Kovacs P, Gonda X, Bagdy G, Juhasz G. (2016) Effects of IL1B single nucleotide polymorphisms on depressive and anxiety symptoms are determined by severity and type of life stress. *Brain Behav Immun*. 56:96-104. **IF: 5.964**
- Kovacs D, Kovacs P, **Eszlari N**, Gonda X, Juhasz G (2016) Psychological side effects of immune therapies: symptoms and pathomechanism. *Curr Opin Pharmacol*. 29:97-103.

- Gonda X, Sarginson J, **Eszlari N**, Petschner P, Toth ZG, Baksa D, Hullam G, Anderson IM, Deakin JFW, Juhasz G, Bagdy G. (2017) A new stress sensor and risk factor for suicide: the T allele of the functional genetic variant in the GABRA6 gene. *Sci Rep*. 7:017-12776. **IF: 4.259**
- Juhasz G, Csepany E, Magyar M, Edes AE, **Eszlari N**, Hullam G, Antal P, Kokonyei G, Anderson IM, Deakin JF, Bagdy G. (2017) Variants in the CNR1 gene predispose to headache with nausea in the presence of life stress. *Genes Brain Behav*. 16:384-393. **IF: 3.743**
- Lazary J, **Eszlari N**, Juhasz G, Bagdy G (2017) Az endokannabinoid rendszer genetikai asszociációs vizsgálatai szorongással összefüggésben [Genetic association analyses of the endocannabinoid system on anxious phenotype]. *Neuropsychopharmacol Hung*. 19:177-182.
- Petschner P, Gonda X, Baksa D, **Eszlari N**, Trivaks M, Juhasz G, Bagdy G. (2018) Genes Linking Mitochondrial Function, Cognitive Impairment and Depression are Associated with Endophenotypes Serving Precision Medicine. *Neuroscience*. 370:207-217.