Genetic and environmental risk factors, informative subtypes and putative endophenotypes in major depressive disorder

Ph.D. Thesis

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Introduction

It is well documented that major depression is most likely resulted from complex interactions of genetic, epigenetic, environmental and developmental factors, nevertheless the exact mechanisms underlying the disease are still largely unknown. There is fairly consistent evidence that childhood onset depression has familial determinants. To achieve the goal of developing more effective treatment for major depressive disorder (MDD), a greater understanding of the neurobiology of psychopathology and the identification of the risk and resilience factors are needed.

Putative genetic liability to depression- Oxytocin and prolactin genes

Molecular genetic studies of depression-related phenotypes in pediatric samples are relatively few in number, with many focused on serotonin system genes. Genetic association studies of variants in the serotonin transporter (SLC6A4) and serotonin receptor (e.g. 5HT1A, 1B, 2A) genes have found weak, inconsistent associations with depression. This suggests that genes outside of the serotonergic system, may also be important in the etiology of depressive disorders such as childhood-onset mood disorder. Oxytocin (OXT) and prolactin (PRL) are neuropeptide hormones that have been shown to be associated with antidepressant activity, to interact with the serotonin system and to be involved in the stress response and human biomarker studies provide a line of evidence implicating oxytocin in depressive symptoms Prolactin secretion is also dependent on the serotonergic system. Other results support an OXT-PRL feedback loop in the hypothalamus.

BDNF gene

BDNF Val66Met is a single nucleotide polymorphism (SNP) of nucleotide 196 in exon 5, resulting in a Val/Met amino acid change in codon 66, affecting the pro-BDNF sequence, with no functional effect on the mature BDNF, but causing changes in its cellular transport and secretion. The encoded protein is a neurotrophin, playing a role in hippocampal dendritic morphology and synaptic function. It has been implicated in depression, evidenced by decreased hippocampal volume and hippocampal and serum BDNF levels associated with the disease.

Inflammatory cytokines

It is known that the genetic variants of inflammatory cytokines can play a role in the appearance of depression. In previous studies, increased interferon-gamma (IFNγ) level was found in a sample of adolescents with major depression, additionally, the increase in the level of interferon-gamma correlated with the interferon-gamma +874 T/A single nucleotide polymorphism.

Factors which may influence the link between genetic liability and depression

In this work a number of factors are considered that could contribute to and/or moderate the liability to depression. The limited success of genetic studies might arise from current classification schemas including DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition), since they are based on clusters of syndromes in a heterogeneous patient group. MDD as defined in DSM-IV is a heterogeneous disorder with regard to etiology, symptoms and level of functioning and response to treatment. One of the clinical subtypes of MDD that showed distinct clinical and biochemical features was melancholic depression. Several biological markers have been suggested to identify this putative endophenotype. Preliminary evidence suggests that genetic factors may discriminate melancholic and non-melancholic depression.

Somatic type of depression

Another putatively interesting phenotype is the somatic type of depression (STOD). Inflammatory cytokines induce behavioral symptoms, known as sickness behavior (SB), that are similar to symptoms seen in depression (fatigue, altered sleep patterns, psychomotor retardation, social withdrawal, appetite loss, anhedonia and impaired cognitive function). INF-γ is primarily secreted by T-lymphocytes, it usually provokes fatigue, malaise, headache, lack of appetite, weight loss, weakness, lethargy and decreased concentration, which can all be symptoms of depression. This similarity led to the theory that the imbalance of inflammatory cytokines can contribute to the development of depression.

Role of stressful life events

Indeed, the role of stressful life events has been proven in depression and several studies showed the effect of gene by environment (G×E) interaction on mood disorders. Many environmental factors have been identified, including early and recent stressful life events and parental depression, which may interact with the genetic background. Interaction between BDNF Val66Met polymorphism and environmental stress on depression was found even when separating pure environmental factors from the environmental factors under partial genetic control and adopting a prospective longitudinal design.

Aims and hypotheses

1) We tested whether single nucleotide polymorphisms at the loci for *OXT, PRL* and their receptors are associated with childhood-onset mood disorders (COMD).

   Based on our preliminary studies regarding the *BDNF* Val66Met polymorphism in depressed children and their siblings, to improve our knowledge about the potential genetic contributors to the melancholic type of depression, we examined whether an effect of the *BDNF* Val66Met polymorphism, and interaction of the *BDNF* Val66Met polymorphism with stressful life events could differentiate juvenile melancholic and non-melancholic patients.

2) Specifically, we hypothesized that:

   a. Melancholic and non-melancholic depressed probands are characterized by different *BDNF* Val66Met genotype and allele distributions.
   b. Past exposure to stressful life events interacts with the *BDNF* Val66Met polymorphism to predict a melancholic depression phenotype.

   Furthermore, we examined the possible association between interferon-gamma +874 T/A single nucleotide polymorphism and the development of a symptomatically homogenous type of childhood onset depression.

3) We hypothesized that:

Depressed probands showing and those not showing the investigated group of somatic symptoms are characterized by different *IFN*-γ +874 T/A gene and allele distributions.

These studies are innovatively integrating recent work on the molecular genetics of MDD.

Methods

Participants, enrollment and assessment procedures in the Childhood Onset Major Depression study

In my present work I report on three different but connected studies using depressed probands and their unaffected siblings on a large sample, which is representative to the Hungarian outpatient and clinic-referred population of depressed youths as the recruitment sites supported more than approximately 85% of the newly referred cases during the study period.

Diagnostic tools and questionnaires

General Information Sheet for Children and Adolescents (GIS)

The GIS is a fully structured interview containing pre-coded item response choices. In the GIS the parent is interviewed about the child's socio-demographic/family background, developmental, educational, and health history, and major life events.

Interview Schedule for Children and Adolescents – Diagnostic Version (ISCA-D)

Major depression was diagnosed using the ISCA-D semi-structured psychiatric interview, assessing both lifetime and current disorders in children and youths. Furthermore, it includes most DSM-IV Axis-I diagnoses and even some DSM-III (Diagnostic and Statistical Manual of Mental Disorders, Third Edition) symptoms. The clinicians rating is based on both the parent informants and the child's/youth's answer, by deriving a final rating for each symptom.

Children depression Inventory-short version (CDI-Short Form)

As a part of the initial assessment, the 10-item CDI Short Form was developed. The CDI in its original form is a 27-item self-rated questionnaire for children and adolescents. The Short Form is a multi-rater assessment of depressive symptoms in youth aged 7 to 17 years correlating with the full inventory (r = 0,89), administered and scored using paper-and-pencil format in the present study, providing streamline evaluation of depressive symptoms.

Statistical analyses

To study oxytocin and prolactin gene variants, several analyses were performed using Haploview v 3.32 (Barrett et al., 2005), including Hardy-Weinberg equilibrium and Mendelian inheritance, and the transmission disequilibrium test (TDT). A corrected significance threshold was calculated for each gene using Nyholt's (2004) spectral decomposition (SNPSpD) method. Correction by SNPSpD was followed by correction for the number of genes tested, to account for multiple comparisons. Haplotype blocks were defined using the Gabriel et al. (2002) criteria. All haplotypes with a frequency greater than 0.05 were also tested for association with COMD.

To compare melancholic depressed participants to the nonmelancholic depressed ones and to compare melancholic depressed participants to their unaffected siblings (with no detectable Axis I DSM-IV disorder), we applied the following statistical methods: Group characteristics were investigated using an independent-samples t-test and the $\chi 2$ -test. Fisher's exact test was used to compare allele frequencies and the $\chi 2$ - test of statistical significance set at P less than 0.05 was used to compare genotype frequencies among the nonmelancholic and melancholic subgroups, furthermore among melancholic probands and their unaffected siblings. Logistic regression models were used to examine the total weighted and grouped weighted life event scores and *BDNF* Val66Met genotypes. We used these models in the total melancholic group. Main effects and possible interactions were tested using the likelihood ratio test (stepwise regression). In the first model (Model 1), the main effects of the total weighted life event score and Val66Met genotypes were tested. Second, we included the interaction term of total weighted life event scores and Val66Met genotypes. In the second model (Model 2), the genotype and the main effects of grouped life events scores were tested, later adding the interaction terms between grouped life event scores and Val66Met genotypes. The main effects of SLEs were analyzed

continuously as continuous score yields more information than dichotomous variables, yielding more sensitive results. Effect size was counted for the genotype-by-melancholia interaction and power analysis was carried out using GPower (Faul et al., 2007).

To compare probands with and without the somatic type of depression, SPSS software was used, chi-square test was applied to compare genotype frequencies and Fisher's exact test was applied to compare allele frequencies among the investigated groups.

Results

Investigating the *OXT, OXTR, PRL*, and *PRLR* genes

A total of 678 families were genotyped for 16 single nucleotide polymorphisms across the *OXT, OXTR, PRL*, and *PRLR* genes. Two of the three SNPs of the *OXT* gene, showed significant results (rs2740210 and rs4813627). After using SNPSpD, both were nominally significant, but they were not significant after further Bonferroni correction by the number of genes tested. No significant associations were found for any SNPs in the *OXTR, PRL*, or *PRLR* genes. Only one haplotype block, spanning rs1205960 and rs849886 in the *PRL* gene, was identified. None of the haplotypes were significant. We performed two additional exploratory analyses to examine parent-of-origin effects (POEs) and proband sex effects for the three SNPs downstream of the *OXT* gene, followed by a onetailed Fisher's Exact text, based on the hypothesis that oestrogen influences on OXT expression would be more relevant in transmissions to and from females. Three *OXT* SNPs revealed biased maternal transmissions of rs2740210 and rs4813627, while paternal transmissions were not significant; most of the paternal transmissions were in the same direction as the maternal transmissions, with no parent-of-origin effect by Fisher's Exact Test. Similarly, all three *OXT* SNPs showed biased transmission to daughters, but proband sex effects were not significant after correction for multiple tests for rs2740210 or rs2770378.

The preliminary studies investigating the *BDNF* Val66Met polymorphism

In the preliminary studies investigating the role of *BDNF* Val66Met polymorphism in the melancholic phenotype, *BDNF* Val66Val genotype had a significantly higher frequency in the depressed group than in the controls. We did not find significant difference in the frequency of

BDNF alleles between the melancholic and the non-melancholic group. From the investigated somato-vegetative symptoms (poor concentration, memory or attention, insomnia, hypersomnia, weight loss or weight gain, psychomotor retardation or agitation) hypersomnia showed association with the frequency of Val66Met genotype. Regarding the categorized life event groups the occurrence of psychiatric disease of family members showed association with *BDNF* Val allele.

Examining *BDNF* Val66Met genotype and allele distributions in melancholic and non-melancholic depressed participants and interaction with SLEs

Testing the first hypothesis of this study, the allele frequency was not significantly different across the two diagnostic groups. There was a trend among the nonmelancholic depressed youth of showing a slightly higher rate of the Met-containing genotype, although not statistically significant. Examining our second hypothesis, in the total melancholic group with the Model 1 of the logistic regression, described in the Statistical analysis section, taking Met containing genotypes as the reference category, we did not find either significant main effect or significant interaction effect of the total SLEs and the *BDNF* polymorphism on the melancholy outcome. When we applied Model 2 (again the Met-containing genotype was considered the reference category) to examine life event groups separately, neither of the events contributed significantly toward the model.

Investigating the association between interferon gamma +874 T/A polymorphism and the somatic type of depression

In the SB group, the frequencies of the A allele containing genotypes were higher, than in the comparison group, without statistically significant difference. The comparison of allele frequencies also did not reveal statistically significant differences. These results did not reveal association between *IFN-γ* +874 polymorphism and SB. However we observed a tendency, being the genotypes with the A allele more frequent in the SB group.

Conclusion

We genotyped SNPs across the *OXT* and *PRL* genes and their receptors. Our results show a trend towards association of two *OXT* SNPs, rs2740210 and rs4813627, with COMD, not significant after correction for multiple testing. Supplementary analyses initially suggested proband sex effects for *OXT* SNPs rs2740210 and rs2770378, but were not significant after multiple testing correction. To our knowledge, this is the first genetic association study of *OXT* and *PRL* variants in a pediatric mood disorder sample. We report evidence that was initially suggestive of possible association between two *OXT* SNPs (rs2740210 and rs4813627) and COMD. The results survived spectral decomposition, but were not significant after correcting for the number of genes tested. *OXTR, PRL* and *PRLR* SNPs were not associated with COMD. In secondary analyses, the relative significances of association for two *OXT* SNPs were increased with parental and proband sex effect analyses. A trend towards proband sex effects to daughters failed to reach statistical significance after correcting for multiple tests. Considering the *OXT* SNPs are located in a putative regulatory region, our exploratory analyses by parent and proband sex are of interest as they may be relevant to epigenetic effects and can serve as basis for further studies.

Since the results of our preliminary studies showed that the *BDNF* Val66Met polymorphism might play a role in the onset of melancholic type of depression, we decided to perform the analyses on a larger database and with improved criteria for melancholic depression.

Consequently, we studied the contribution of the BDNF polymorphism and its interaction with SLE toward early onset melancholic depression. Our analysis could not be used to discriminate between the melancholic and the nonmelancholic groups.

The frequency of melancholia in our sample was in the range of the previously reported prevalences of 20% to almost 50% in juvenile samples; the ratio of females was significantly higher in the melancholic than in the nonmelancholic group (t=0.262, P(two-tailed=0.01)), suggesting that females are more prone to developing the early-onset melancholic phenotype. Our result may represent an interesting suggestion for future research as the presence of the subtype might influence treatment response and complications.

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