Hormones, monoamines and peripartum affective symptoms
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Chapter 8
Depression following severely complicated pregnancies:
Accumulating effects of genotype and environment

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Abstract

**Background:** Life events have been related to the onset of depressive symptoms in vulnerable people. This vulnerability has been explained by genetic variability in the serotonin transporter (5-HTT) gene. We prospectively investigated the relation between depression and life-events in the presence of variants of the 5-HTT gene in subjects who experienced a relatively homogeneous life-event: i.e. women with severely complicated pregnancies.

**Methods:** Eighty patients with the HELLP syndrome, pre-eclampsia or preterm prelabour rupture of membranes completed BDI-II and PSS-SR questionnaires during pregnancy and six weeks postpartum to assess symptoms of depression and post-traumatic stress disorder. Data on history of depression were collected. The 5-HTT transporter genotypes were determined.

**Results:** Incidence of postpartum depression was 21% in 5-HTT ll carriers, which was significantly higher than 5% in sl (p= 0.05) and 0% in ss carriers (p=0.03). PTSD was not found to be associated with variation in the 5-HTT gene. In a regression analyses on BDI scores, 5-HTT genotype ($\beta$=.158) and history of depression ($\beta$=.280) were found to be independent risk factors for postpartum depression.

**Conclusion:** Both 5-HTT genotype and a history of depression explained some of the variance in depressive symptoms in women who suffered from severely complicated pregnancies. The involvement of the 5-HTT l allele is probably related to decreased tryptophan and serotonin availability in the peripartum. These results indicate that the genetics of depression can be understood when the accumulating effects of history of an individual, life events and changing physiology are all taken into account, emphasizing the complexity of the pathophysiological processes of depression.
Introduction

Depressive episodes are often preceded by stressful life-events (1), which are therefore thought to causally contribute to the onset of depression. However, since the majority of the population has been exposed to potentially stressful events and only a minority of the exposed individuals develop psychopathology (1) other individual characteristics are thought to contribute to the development of psychopathology. It is likely that the vulnerability for depression induced by life-events can at least partly be explained by genetic variability (2).

Until now, the hunt for candidate genes explaining the genetic vulnerability for life-event induced depression yielded only one reproducible result, i.e. a functional length-polymorphism in the promoter region of the serotonin transporter (5-HTT) gene. The 5-HTT is a protein that plays a key role in the regulation of extracellular serotonin levels. The short allele (s) and the long allele with an A to G substitution (l) in the 5-HTT promoter region are associated with a decreased expression and functionality of the 5-HTT (3, 4). Heterozygous carriers of the s allele, with a history of childhood abuse, were found to have higher rates of depression and suicidality following several life events (5). This result has been replicated (15 times) as well as rejected (2 times) in other studies (6). This inconsistency may be related to the method of assessment of past stressful events (6).

In this study, we will evaluate the effects of genotype and environment in participants who have experienced a relatively similar life event, i.e. women with severely complicated pregnancies. The conditions studied here are pre-eclampsia (PE) (pregnancy related hypertension, proteinuria), the HELLP syndrome, (a severe form of pre-eclampsia) and PPROM (Preterm Prelabour Rupture of Membranes). During HELLP and PE the fetal condition is endangered due to placental dysfunction as well as the risk of preterm birth, and in patients with proven PPROM the fetal condition is endangered by intra-uterine infections and pre-term birth.

We propose that these pregnancy complications are a suitable candidate for genotype by environment research, since they fulfill all criteria for an environmental pathogen as defined by Moffitt et al. (7): a) there is a marked variability in the psychological response to these pregnancy complications (8); b) the complications have an effect by themselves as normal pregnancies have a lower incidence of psychopathology (8) and they are outside the individuals control, thus ruling out gene-environment correlations; c) the complications are proximal pathogens, of which the effects can be followed up,
with a fixed time between the stressor and evaluation of psychopathology; d) these complications are age and sex specific by their nature.

In uncomplicated pregnancies depressive symptoms have been associated with the 5-HTT l allele (9, 10). In these studies it has been proposed that carriers of the 5-HTT l allele, i.e. the high expression variants (11), are more vulnerable for the decrease in cerebral tryptophan availability and the concomitant decrease in serotonergic metabolism as occurs in the peripartum period (12), an idea which is further supported by the findings of a tryptophan depletion study, indicating that ll carriers with a history of depression developed depressive symptoms following tryptophan depletion (13).

Stressful life events are not only associated with depression, but also with Post Traumatic Stress Disorder (PTSD), a disease within the anxiety spectrum, characterized by re-experiencing of the stressful condition, avoidance of reminders to that condition and a persistent hyper-aroused state (14). In general co-morbidity between depression and PTSD is 50% (15). The genetics of PTSD are an understudied area. The sparse literature regarding this subject also indicates a role for the 5-HTT s allele in the onset of PTSD (16, 17).

We designed a prospective longitudinal study in which we evaluated the relationships between 5-HTT polymorphisms and depression in women with complicated pregnancies. In addition, we also measured PTSD symptoms. Based on the literature we formulated two hypotheses. According to studies investigating the interaction between 5-HTT, life events and depression, carriers of the s allele would be more vulnerable for depression and PTSD following severely complicated pregnancies. However, in line with the literature on the association of 5-HTT and peri-partum depression, there is also a role for the 5-HTT l allele.

**Materials and Methods**

**Participants**
This study is part of a larger study which was designed to identify the incidence of and risk factors for depression and PTSD following HELLP, PE and PPROM. Results of this study are described elsewhere (Doornbos et al, 2008 in prep). Pregnant women admitted to the obstetric department of the University Medical Center Groningen between February 2005 and February 2008 were asked for participation when they fulfilled the criteria for PE and HELLP as defined according to the criteria of the
International Society for the Study of Hypertension in Pregnancy; or PPROM according to the ACOG practice bulletin on PPROM (18). All women had singleton pregnancies and were native Dutch speakers. Exclusion criteria for all groups were pre-existing medical conditions (hypertension, cardiovascular or renal diseases, SLE, Diabetes Mellitus); and a history of intra-uterine fetal death. All women included in this study were also asked to provide material (either blood or buccal swabs) for the isolation of DNA. All women gave written informed consent. Approval was obtained from the medical ethical committee of the University Medical Centre Groningen, (nr: METc 2004.122).

Measures
Participants were tested during pregnancy (pre-test) and six weeks postpartum (follow-up). Patients were tested as soon as possible after admission. During the pre-test, participants completed a brief self-report measure of general demographic information. Information regarding psychiatric history was obtained in an interview, containing questions on: 1) The life-time presence of episodes of depressed mood or anhedonia, i.e. ‘In the past, did you ever experience one or more periods: (a) in which you felt depressed or down for most of the day?; (b) or in which you lost interest in activities you usually enjoy?’; 2) Questions regarding previous post traumatic stress complaints i.e. (a) ‘Have you ever witnessed or experienced a traumatic situation, (such as experiencing or witnessing a life-threatening situation, physical or sexual abuse, a disaster or serious accident’ and (b) ‘Has this experience affected you afterwards? (for example with nightmares, or intrusive thoughts)’; and 3) the psychological treatment history. Based on these questions, 2 variables were constructed: 1) indication for a previous depressive episode (0 = absent; 1 = present); 2) indication for previous post traumatic stress symptoms (0 = absent; 1 = present). Data regarding current and past obstetric status were collected from the medical record.

During both test-sessions the PTSD Symptom Scale self report questionnaire (PSS-SR) (19) and the Beck Depression Inventory, second edition (BDI-II) (20), were completed. The PSS-SR is a questionnaire containing 17 items corresponding to the 17 PTSD symptoms described in the DSM-IV. These items are rated using 4 point scales asking for the frequency with which each symptom occurred over the past month (0= never; 1 = once per week, 2 = 2 -4 times per week, 3 = at least 5 times per week). The total PSS-SR score ranges from 0 – 51. The re-test reliability has been calculated .74 (19). In the present sample the internal consistency was good (for the pre-test $\alpha = .86$ and for the follow up $\alpha = .94$).
The PSS-SR that was administered during the pre-test investigated past month PTSD symptoms related to any stressful event experienced before. The follow-up PSS-SR investigated PTSD symptoms in the past month that were specifically related to pregnancy and parturition. In addition, during follow-up assessment participants rated the extent to which they had felt fear, helplessness, or horror during the most shocking pregnancy-related event on three 100 mm Visual Analogue Scales (VASs).

Pregnancy-related PTSD was considered to be present when participants: 1) scored 80 or more on one of the VAS for horror, fear or helplessness (subjective stress, DSM-IV A2 criterion); 2) reported at least 1 re-experiencing, 3 avoidance and 2 hyper-arousal symptoms on the PSS-SR (DSM IV, B, C, and D criterion) and symptoms were considered present if an item was rated 2 (‘2 – 4 times per week’) or more; 3) obtained a total PSS-SR score of 18 or higher (severity, DSM IV F criterion). It should be noted that the time criterion of 4 weeks (DSM IV E) was met because follow-up assessment was at six weeks postpartum.

The BDI-II (20) is a self-report measure of depressive symptoms over the past two weeks. It consists of 21 items containing four statements that reflect increasing symptom severity (scoring 0-3 per item). The total score ranges from 0 to 63. The BDI-II is found to have good psychometrical properties (20-22). The internal consistencies of the BDI-II in the current sample was good (pre-test $\alpha = .88$, and the follow up $\alpha = .91$). Depression was considered to be present if BDI scores were 21 or more, as this is the criterion for a moderate depression according to the BDI manual (20).

5-HTT genotyping
From the participants who agreed to participate in the genetic analyses, DNA was isolated from 10 ml EDTA anticoagulated blood or from buccal swabs. EDTA anticoagulated blood was centrifuged at 800g for 10 minutes a 4°C, after which the buffy coat was collected and stored at -20°C until DNA isolation. Buccal swabs were stored at -20°C until DNA isolation. DNA was isolated from the buffy coats using an automated DNA isolation system (X-tractor, Westburg, Leusden, The Netherlands) and the Sigma DNA isolation kit (Sigma, Zwijndrecht, The Netherlands). DNA from the buccal swabs was isolated using the Qiamp Mini blood Kit (QIAGEN Benelux B.V., Venlo, The Netherlands). 5-HTT Genotypes were determined using the HTTp2a and HTTp2B primer set to amplify 406 (S) and 450 (L) bp fragments with the polymerase chain reaction (PCR) (23). The L$_A$, L$_G$, and S alleles were determined by incubation of the PCR product with the restriction enzyme Msp I (New England Biolabs, Westburg, Leusden, The Netherlands) for at least 3 hours at 37°C. Msp I cuts the GGCC sequence,
resulting in fragments of 329, 62, and 59 (L_A), 174, 155, 62 and 59 bp (L_C), and 285, 62 and 59 bp (S) respectively. The resulting restriction fragments were separated using a 2% agarose gel and visualized using GelStar (SYBR-green; Cambrex Bio Science, Rockland, ME).

**Statistical analysis**
Statistical analyses were performed with SPSS 14.0. Alpha was set at 0.05. Group comparisons involved three groups based on the 5-HTT genotype: ss, sl and ll; L_G was considered as an s allele. For the dichotomous data, Chi^2 analyses were used. Exploration of the continuous data revealed that the BDI and PSS scores were not normally distributed. Therefore, for group comparisons appropriate non-parametrical tests were employed. In order to evaluate the relative contribution of genetic factors as compared to other risk factors depression a hierarchical multiple regression (HMR) analyses were performed on the continuous BDI-II scores. When appropriate, non-normally distributed variables were square-root transformed to meet assumptions of normality.

<table>
<thead>
<tr>
<th>Results</th>
<th>Prevalence and severity of PTSD and Depression</th>
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<tbody>
<tr>
<td></td>
<td>Six women (7.5%) fulfilled the criteria for depression and 9 women (11.3%) fulfilled criteria of PTSD. The prevalence of depression and PTSD and the BDI-II and PSS-SR</td>
</tr>
</tbody>
</table>
symptoms scores did not differ between women with HELLP, PE and PPROM. The prevalence of depression and PTSD according to 5-HTT polymorphisms is shown in table 2. There was a significant effect of genotype on the prevalence of depression (Pearson $\chi^2=7.058$, df=2, $p=.024$). Further analyses revealed that the prevalence of depression differed significantly in ll subjects as compared to ss ($\chi^2=6.692$, df=1, $p=.03$), and that the prevalence difference between ll and sl was borderline significant ($\chi^2=3.774$, df=1, $p=.052$). The prevalence of PTSD (table 2) and the BDI and PSS-SR sum scores (data not shown) did not differ between the three genotypes.

**Interaction 5-HTT genotype with history of depression**

These results implicate an association of the l allele with depression. As this association is previously reported to interact with a history of depression (see introduction), we further investigated this interaction. For this, 5-HTT genotypes were dichotomized (ss & sl vs. ll), to obtain sufficient power. Figure 1 shows that subjects with a history of depression showed higher BDI scores, and that this observation seemed more pronounced in ll carriers as compared with ss & sl carriers.

<table>
<thead>
<tr>
<th>LL (n=19)</th>
<th>SL (n=41)</th>
<th>SS (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDI &gt;20*</td>
<td>4 (21%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>PTSD</td>
<td>2 (11%)</td>
<td>5 (12%)</td>
</tr>
</tbody>
</table>

* Significant effect in the $\chi^2$ test $p=0.029$

**Table 2.** Diagnosis of depressive symptoms (BDI> 20) and PTSD at 6 weeks postpartum according to the 5-HTT genotypes.

BDI = Beck Depression inventory; PTSD = Post Traumatic Stress Disorder 5-HTT: serotonin transporter

These combined effects of 5-HTT genotype and history of depression were found to be highly significant (Pearson $\chi^2=14.412$, df=3, $p=.002$). Posthoc analyses indicated a significant effect of history of depression but not of genotype, as the ll carriers with a history of depression did not differ from s carriers with a history of depression. However, this might be due to the small sample size of the group of ll carriers with a history of depression (n=6). To evaluate if the effects of 5-HTT and history of depression were caused by an additive effect or by an interaction of genotype with environment, we performed a hierarchical linear regression analyses. In the first step we entered 5-HTT genotype (0= s carries, 1 = ll carriers), history of depression (0= no history, 1 = history of depression), BDI scores during pregnancy and the death of an infant in the postpartum period (0 = living infant, 1 = infant died), in the second step we added a genotype x history of depression interaction variable (ll genotype AND history of depression = 1; s genotype OR no history of depression = 0). The first step of the model is shown in table 3. Adding the interaction variable to the model for BDI increased the $R^2$ with $0.002 (p=0.621)$ with a standardized $\beta$ of $-0.054 (p=.621)$. As this was not significant, we removed this variable from the model.
Discussion

Results from this prospective study indicate that in a sample of women with severely complicated pregnancies the 5-HTT l allele (borderline significant) and history of depression were independently associated with depressive symptoms. PTSD was not associated with variation in the 5-HTT gene.

Depressive symptoms in the postpartum have been reported before in 2 studies (9, 10) and has been explained as a higher sensitivity of 5-HTT l allele carriers for the decrease in cerebral tryptophan availability and concomitant serotonin synthesis in the peripartum. The combined effect of low tryptophan, the 5-n is also found in a tryptophan depletion study, who were previously depressed, developed depressive symptoms following tryptophan depletion (13). Therefore, the relation seems to be specifically associated with a decreased serotonergic metabolism.

Apparent the two variants of the serotonin transporter are both related to depression; however the pathophysiological pathways leading to depression may differ. The s allele is related to depression in interaction with early life stress, indicating that the higher vulnerability for depression in this sample is due to an impaired developmental process (5, 26). The neurotrophic properties of serotonin might mediate this interaction. On the other hand, vulnerability to depression in l allele carriers might be related to a higher sensitivity for fluctuations in serotonin metabolism. Adding to that, both the current and previous studies (13), suggest that this sensitivity for fluctuations in serotonin levels is further increased when previous depressive episodes are taken into account, indicating that an underlying vulnerability for mood disturbances becomes manifest, due to a decrease in serotonergic tone.

Depression but not PTSD was found to be associated to the ll genotype, despite a correlation of 0.70 between depression and post traumatic stress symptoms. In contrast, all other risk factors were related to both depression and PTSD. Moreover, the relation between depression and the interaction ‘l allele * history of depression’ remained significant after correcting for PTSD symptoms. Based on this, we suggest that the 5-HTT genotype might be one of the factors involved in the differentiation of the response to a traumatic event within the anxiety-depressive spectrum. It must be noted however, that this finding cannot be extrapolated to psychopathology induced by other stressors.
as these observations were made in a postpartum cohort, and seem to be related to a pregnancy related change in tryptophan availability and serotonin metabolism. This finding is consequential for future research, as it indicates that genetic variation is not only associated with vulnerability for psychopathology, but also influences the pathophysiological outcome within the spectrum of affective disorders.

These results have to be interpreted in the light of several strengths and weaknesses of this study. Strong points comprehend the study design, with a fairly homogeneous cohort and including a relatively uniform stressor (a severe pregnancy complication). Adding to that, an exceptional feature of this study is the fixed time between the stressor and evaluation of PTSD and depression, which is unique for a genetic study of trauma-related psychopathology. Limitations of this study include the relatively small sample size (which is related to the low incidence of the stressor in the population) and the evaluation of the history of depression by self report. A final limitation is the absence of a control group with uneventful pregnancies, resulting in an inability to evaluate the specificity of this finding for complicated pregnancies.

In conclusion, we found the accumulated effects of the 5-HTT genotype and a history of depression explain the occurrence of depression following a complicated pregnancy. The complexity of this interaction is, in our opinion, illustrative for the pathophysiology of depression, and emphasizes that the accumulation of these risk factors should be taken into account both in causal models of depression as well as in treatment and prevention of depression.

Table 3. Regression analyses of postpartum BDI scores with genotype * history of depression interaction variable.

| Model statistics: (F = 19.161, p < 0.001), R² = 0.505, Adjusted R² = 0.479.
| BDI scores were square root transformed before addition. |

<table>
<thead>
<tr>
<th>Variable</th>
<th>B (SE)</th>
<th>Sign.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.48 (0.37)</td>
<td>0.155</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Death of infant</td>
<td>0.44 (0.11)</td>
<td>0.12</td>
<td>0.176</td>
</tr>
<tr>
<td>BDI during pregnancy*</td>
<td>0.61 (0.09)</td>
<td>0.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of depression</td>
<td>0.66 (0.20)</td>
<td>0.28</td>
<td>0.001</td>
</tr>
<tr>
<td>5HTT: I vs. 5HTT: L carriers</td>
<td>0.41 (0.21)</td>
<td>0.16</td>
<td>0.056</td>
</tr>
</tbody>
</table>

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Reference list


