Hormones, monoamines and peripartum affective symptoms
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Chapter 5

The course of peripartum depressive symptoms is associated with gene polymorphisms of MAOA, 5-HTT and COMT


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Abstract

Background: Polymorphisms of monoamine-related genes have been associated with depression following life-events. The peripartum is a physiologically and psychologically challenging period, characterized by fluctuations in depressive symptoms, therefore facilitating prospective investigations in this gene x environment interaction.

Methods: Using data of 89 women who filled in four depression questionnaires (EPDS) during the peripartum, we found a significant interaction between the course of depressive symptoms and polymorphisms in 5-HTTLPR (p=0.019); MAOA (p=0.044) and COMT (p=0.026), and MAOA*COMT (p<0.001). Particularly women carrying the combination of MAOA-L and COMT met/met alleles, had increased EPDS scores at week 36 of pregnancy and 6 weeks postpartum, but not during early pregnancy or 12 weeks postpartum.

Limitations: relatively small sample size; no information regarding (family) psychiatric history was collected.

Conclusion: These results support previous findings that these polymorphisms modify the stress-response and the development of depression. The present study suggests their role in peripartum depression, emphasizing that particular combinations of these polymorphisms may exhibit strong effects in specific periods.
Introduction

Depressive disorders are complex diseases that do not follow Mendelian inheritance law (1). Their genetic basis is anchored in ‘disease susceptibility genes’ that precipitate depression by environmental triggers, such as mild or severe life events (2). Particular polymorphisms of monoamine-related genes have been associated with depression following life events (2). The peripartum is an example of a stressful period, but the intensity of the experienced stress varies per person. Pregnancy and parturition confer not only emotional life events, but also exert (neuro)physiological challenges with, amongst others, changes in the functioning of several hormones (3) and cerebral monoamines (4). The predictable time course of the pregnancy-related events facilitates prospective investigations.

Polymorphisms in the promoter of their serotonin transporter (5-HTTLPR), monoamine-oxydase type A (MAOA) and catechol-O-methyl-transferase (COMT) genes are known to affect the expression or functionality of their products, i.e. the proteins involved in the clearance of serotonin (5-HTTLPR and MAOA) and of dopamine and noradrenalin (COMT and MAOA). Low activity variants of these polymorphisms have been related to processes implicated in the development of depression (2, 5, 6).

Considering the peripartum as a stressful period evoking emotional reactions, we hypothesize that emerging depressive symptoms might depend on variations monoamine-related gene-polymorphisms. Accordingly we investigated whether the course of depressive symptoms in the peripartum is associated with the presence or interactions of any of the polymorphic genes of 5-HTTLPR, MOAA and COMT. The depressive symptoms were measured in healthy women with uncomplicated pregnancies and essentially non-traumatic deliveries.

Materials and Methods

Data were used from a trial that investigated the effect of supplemental docosahexaenoic-acid with or without arachidonic-acid (both 220 mg/day) on affective functioning in the peripartum period. The study population was composed of healthy women with uncomplicated pregnancies who gave birth to healthy term infants (ref saskia). Previous analyses showed that affective functioning in the study groups was independent of the intervention (7). We therefore considered the entire cohort as representative for a healthy group of pregnant women. The protocol was approved by the Dutch Central Committee on Research Involving Human Subjects. The trial is
registered in the ISRCTN register nr. ISRCTN58176213. All participants gave written informed consent.

**Questionnaires**
Depressive symptoms were assessed with a Dutch version of the Edinburgh Postpartum Depression Scale (EPDS) (8) at weeks 16 and 36 of pregnancy and 6 and 12 weeks postpartum.

**Genotyping**

**DNA isolation**
DNA was isolated from EDTA anticoagulated blood using an automated DNA isolation system (X-tractor, Westburg, Leusden, The Netherlands) and the Sigma DNA isolation kit (Sigma, Zwijndrecht, The Netherlands).

**COMT genotyping**
Genotyping of the COMT val158met polymorphism (1947 G/A; GenBank Z26491; dbSNP: rs4680) was performed with the allelic discrimination technique on an Applied Biosystems 7500 real-time polymerase chain reaction (PCR) system (Applied Biosystems, Nieuwkerk a/d Ijssel, The Netherlands) according to the supplied protocol. We used primers COMT-GAF (5’-CGAGATCAACCGACTGT-3’) and COMT-GAR (5’-CAGGCATGCACACCTTGTC-3’), minor groove-binding probes VIC-5’-TTTCGCTGGCGTGAAG-3’-NFQ (G) and FAM-5’-TCGCTGGCATGAAG-3’-NFQ (A). The reaction was carried out in TaqMan universal PCR master mix (Applied Biosystems).

**MAO-A genotyping**
PCR for MAO-A genotyping was essentially performed as described by (9) and colleges, using an unlabelled forward primer (5’-ACA GCC TGA CCG TGG AGA AG-3’) and a VIC-labelled reverse primer (5’-GAA CGG ACG CTC CAT TCG GA-3’) (9). The resulting PCR fragments were separated using an ABI 3130 analyzer (Applied Biosystems). Fragment size was estimated using a size marker (Applied Biosystems) and ABI Prism® GeneMapper™, version 3.0 software (Applied Biosystems).

**5-HTTLPR genotyping**
5-HTTLPR genotypes were determined using the HTTt2a and HTTt2B primer set to amplify 406 (S) and 450 (L) bp fragments with the polymerase chain reaction (PCR) (10). 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 analyses
Statistical analyses were performed using SPSS 14.0. The significance level was set at p<0.05. Each polymorphism was classified into two groups, according to activity of the gene-product: 5-HTTLPR SS vs. SL & LL. (L_G was considered as S); COMT met/met vs. met/val & val/val; and MAOA high activity vs. low activity variants (MAOA-H MAOA-L), as described in (11).

The relation between the gene polymorphisms and demographic and obstetric variables was investigated with an independent t-test or $\chi^2$ test. The associations between the gene polymorphisms and the course of EPDS scores were analyzed with one run of repeated measures ANOVA, with EPDS scores (weeks 16 and 36 of pregnancy and weeks 6 and 12 postpartum) as within-subject factors, and the three gene polymorphisms as between-subject factors. Treatment groups were added as co-variant. The Greenhouse-Geisser correction was applied because sphericity was violated. If significant interactions were present, post hoc testing was performed comparing the differences between the various gene polymorphisms.

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<th>MAOA</th>
<th>5-HTTLPR</th>
<th>COMT</th>
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<tbody>
<tr>
<td>Homozygote high activity</td>
<td>30 ss</td>
<td>18 met/met</td>
</tr>
<tr>
<td>Heterozygote high activity</td>
<td>50 sl</td>
<td>41 val/met</td>
</tr>
<tr>
<td>Homozygote low activity</td>
<td>9 ll</td>
<td>30 val/val</td>
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Table 1. Frequencies of polymorphism for the MAOA, 5-HTTLPR, COMT genes.

Results

The polymorphisms of each of the three genes were in Hardy-Weinberg equilibrium. The distribution of the polymorphisms is shown in table 1. Eighty nine women filled in 4 EPDS questionnaires. Demographic and obstetric variables did not differ between carriers of each of the three polymorphisms. All women were Caucasian, the mean age was 31.7 ± 4.6. There were 48 first pregnancies. The mean gestation duration was 39.7 ± 1.3 weeks and average birth weight was 3591 ± 493 g. Six children were born with a caeserian section and 6 had to be transfered to a neonatal ward.
The associations between the polymorphisms on the courses of EPDS scores are shown in figure 1. Analyses showed a significant interaction between the course of depressive symptoms and polymorphisms for MAO-A (p=0.044); 5-HTTLPR (p=0.019) and COMT (p=0.026). Post hoc testing revealed a significantly increased EPDS score at 36 weeks of pregnancy for MAOA-L (p=0.018) and COMT met/met (p=0.022); and at 6 weeks postpartum for MAOA-L (p=0.006) and COMT met/met (p=0.027) and a trend for the 5-HTT SL&LL group (p=0.07). Adding treatment group as co-variant did not influence the outcome of the analyses.

The analyses also indicated a highly significant MAO-A* COMT interaction in the course of depressive symptoms (p<0.001) as shown in figure 2. Post hoc analyses indicated an increased EPDS score at week 36 of pregnancy (p=0.003) and at 6 weeks postpartum (p=0.006) in women carrying MAOA-L + COMT met/met. Nine women were MAOA-L carriers of whom 6 also carried a COMT met/met variant.

**Discussion**

We found an interaction between monoamine gene-polymorphisms and the course of peripartum depressive symptoms. Carriers of MAOA-L, COMT met/met, and 5-HTT L exhibited increased depression scores in the peripartum. Most pronounced was the interaction of the MAO-A x COMT with the course of depressive symptoms. We show that MAOA in combination with COMT appears to regulate not only the stress response in laboratory experiments (11), but also seems to influence mood during normal, mild, stressful events, such as experienced in the peripartum period as well.

This is the first study investigating gene-environment interactions on peripartum depressive symptoms using longitudinal data. There is only one study on 5-HTT and peripartum depression. Using a cross-sectional design, this study shows that carriers of the 5-HTT s allele with a history of abuse, had increased levels of mid-pregnancy depression (15-27 weeks) (12). As we did not investigate history of abuse, we were unable to confirm this result.

Strengths of the present study are the homogeneity of the study sample, both in gender and age (2), and the longitudinal design, including a well defined (physiological) stressful period. Using this quasi experimental design we circumvented problems of post-stressor studies lacking data on pre-morbid functioning, and problems of longitudinal epidemiological studies, constituted of heterogeneous populations and varying stressful events as well as the time between the event and the onset of
depression. A relative weakness is that the cohort was part of a supplementation study. We did not find any influence of the supplements on depressive symptoms at any time point (7), nor did including the intervention as a co-variant influence the outcome of the study. The sample size is modest, but sufficient power was obtained by the quasi experimental, repeated measure design; consequently some of the gene*environment interactions were highly significant. Another limitation is the lack of information regarding the (family) history of depression. We would also emphasize here that the present study is not so much concerned with depression, but rather with the development of depressive symptoms in the peripartum period. Our results add to a complex picture of apparently conflicting studies relating both types of MAO-A polymorphisms to depression in females (6), but the MAO-A-I has also been associated with increased depressive symptoms (13), increased fMRI amygdala activity in sad condition (14) and disturbed HPA-axis activity (11). Together with the present study these findings may emphasize that the involvement of the various polymorphisms may depend on the type of the stress-challenge or life event.

Studies have, until now, consistently indicated that the COMT met allele influences prefrontal cortex and limbic activity in response to aversive or emotionally-negative stimuli (5, 15, 16) and to the endocrine response to stress (17). It is therefore conceivable that the stress of the advancing parturition and the early postnatal care for the infant has notably increased depression scores in those carrying COMT met/met polymorphisms to induce elevated sympathetic tone associated anxious and depressive feelings. This interaction might have become enhanced by coexistence of the MAOA-L allele, since the increased EPDS scores were especially noticed in those carrying both the combination of COMT met/met and MAOA-L alleles. Due to the low activities of both MAOA and COMT in these women they have less capacity to neutralize the stress-evoked release of catecholamines, which might be experienced as a high sympathetic tone associated anxious and depressive feelings. The relation between the 5-HTTLPT L allele and psychopathology has mainly been reported in tryptophan depletion studies, suggesting that the low tryptophan status of the peripartum period might have induced depressive symptoms in this group (18). The transient character of the here reported effects signifies that these polymorphisms might be associated with the onset, but not with the pathological persistence of depressive symptoms. This might also explain the discrepancy between the gene-association studies reporting inconsistent or no results regarding these polymorphisms in depression (19), and endocrine and fMRI challenges, which report more consistent associations (11, 16, 18), as the latter focus on transient responses.
Our findings support the GxE concept for depression. We suggest that GxE interactions become especially noticeable from longitudinal study designs. Although the notion of a genetic vulnerability for peripartum depression has been proposed before we are the first to show genetic vulnerability to essentially non-pathological depressive symptoms. Interestingly, most of the here studied polymorphisms are found to modify depressive symptoms in other conditions. This might explain why the major risk factors for peripartum depression are a personal history of depression and neuroticism (20, 21). The here observed gene-associated vulnerability for peripartum depressive symptoms, together with our previous observations (4) might offer the possibility to identify subjects that may become depressed in the course of pregnancy or postpartum.
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