Chapter 6

The course of peripartum depressive symptoms is not associated with polymorphisms in mono-aminergic genes

**Abstract**

**Objective:** The higher incidence of depression during the peri-partum suggests that pregnancy induces depression in genetically vulnerable women. This vulnerable subgroup can be identified by the course of its depressive symptoms. Since mono-aminergic metabolism changes during the peri-partum, its genetic-vulnerability to depression might be explained by functional polymorphisms in mono-aminergic genes. We investigated whether subgroups identified with Latent Class Analyses (LCA) were related to functional polymorphisms in three candidate genes involved in mono-aminergic metabolism.

**Methods:** LCA was applied to data from EPDS questionnaires filled in by 101 women during pregnancy (16 and 36 weeks) and postpartum (6 and 12 weeks). LCA scores were related to functional polymorphisms in the promoter regions of the serotonin transporter and MAOA genes and one in the COMT gene.

**Results:** LCA identified three classes characterized by: 1) postpartum depression (N=8; 8%), 2) no depressive symptoms (N=70; 69%), and 3) depressed during pregnancy (N=23; 23%). None of the investigated alleles were related to these classes.

**Conclusions:** Based on the course of the depressive symptom this study identified two small subgroups within a large cohort of healthy pregnant women. The subgroups proved unrelated to mono-aminergic candidate genes. These results emphasize that clinically identifiable subgroups do not necessarily share the same characteristics with subgroups based on genetic features.
Introduction

The incidence of affective disorders in the peripartum is increased compared to other periods in life (1, 2), suggesting that pregnancy might induce depression in some women. Conversely, most of the women exhibiting depressive symptoms during pregnancy were depressed in the period preceding pregnancy (3). Thus, only a small fraction of the pregnant women develop ‘new’ depressive symptoms during the peripartum, and might therefore have a specific vulnerability for depression precipitating during pregnancy. Since the depressive symptoms in this subgroup develop during pregnancy, this subgroup can be identified retrospectively by the course of their depressive symptoms.

Because only a subgroups of women is susceptible for peripartum depression, their vulnerability might originate from a genetically determined sensitivity related to the physiology of pregnancy (4). Changes in mono-aminergic metabolism have been implicated in both the onset and recovery of depression (5), and also in the onset of peripartum affective disorders (6). We therefore hypothesized that the genetic-vulnerability might be explained by functional polymorphisms in mono-aminergic genes. (6).

Polymorphisms in several genes involved in mono-aminergic metabolism, i.e. the serotonin transporter (5-HTT), mono-amine-oxidase type A (MAOA) and catechol-O-methyl-transferase (COMT) have been implicated in the pathophysiology of depression. The 5-HTT gene located at 17q11.1-q12, codes for a protein with a key role in the regulation of extracellular serotonin levels. A short allele (s) or a long allele with an A-to-G substitution (lG) in the 5-HTT promoter region affect the expression and functionality of the 5-HTT (7, 8), which will together be referred to as the 5-HTT-L (low activity variants). The long (l) allele in this promoter region, corresponds for a normal functioning variant of the 5-HTT, and is here referred to as the 5-HTT-H (High activity). The 5-HTT-L has been associated with depression, particularly in interaction with stressful life events (9, 10). The 5-HTT-H is found to modulate depressive symptoms induced by tryptophan depletion (11) and in the peripartum (12) & (Doornbos B. et al in prep). The gene coding for MAOA, mapped to the short arm of the X chromosome, translates into an enzyme involved in the degradation of serotonin, dopamine and noradrenalin. The promoter region of this gene contains a variable-number-tandem-repeat polymorphism (VNTR’s), consisting of a 30-bp repeated sequence, that occurs as 2, 3, 3.5, 4 or 5 repeats (R). The 3.5R or 4R are transcribed 10 times more efficiently compared to the other variants and are therefore referred to as MAOA-H (high activity) and the and 2R,3R, 5R as MAOA-L (low activity) (13). Finally, the COMT-gene, which is
mapped on chromosome 22, codes for an enzyme involved in the deactivation of dopamine and noradrenalin. A G-to-A substitution in codon 158, translating into a valine (val) to methionine (met) substitution, has been shown to account for a fourfold decrease in enzyme activity (14), and is here referred to as a COMT-L variant. The low active variants of MAOA (MAOA-L) and COMT (COMT-L), and their combination have been related to an altered stress-response and the pathophysiology of depression (15-18). We have previously shown their role in the onset of peri-partum depressive symptoms (Doornbos, in prep). Taken together, 5-HTT-L, MAOA-L and COMT-L (i.e. the low active variants) are positively associated with the occurrence of depressive symptoms, and the 5-HTT-H with depressive symptoms in the peripartum.

The present study tested the hypothesis that a subgroup of pregnant women with a pregnancy-related vulnerability for depressive symptoms, as identified by the course of their depressive symptoms, is associated with the above mentioned polymorphisms, i.e. the low activity variants of MAOA and COMT, and the long variant of the 5-HTT.

**Subjects and study design**

Data were used from a trial investigating the effect of supplementation of docosahexaenoic acid (DHA) with or without arachidonic acid (AA) (both 220 mg) or placebo on affective functioning in the peri-partum period. The detailed study design is described elsewhere (19). Previous analyses showed that affective functioning in the study groups was independent of the administered supplements (19). We therefore considered the cohort as representative for a group of healthy pregnant women. The protocol was approved by the Dutch Central Committee on Research Involving Human Subjects. All participants gave written informed consent. The trial is registered in the ISRCTN Register number ISRCTN176213.

<table>
<thead>
<tr>
<th>Age (y) (mean ± sd)</th>
<th>31.7 ± 4.5</th>
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<tbody>
<tr>
<td>Caucasian [n (%)]</td>
<td>9 (98)</td>
</tr>
<tr>
<td>Completed higher education [n %]</td>
<td>76 (75)</td>
</tr>
<tr>
<td>Married or living together [n %]</td>
<td>101 (100)</td>
</tr>
<tr>
<td>Smoking [n (%)]</td>
<td>4 (4)</td>
</tr>
<tr>
<td>First born [n (%)]</td>
<td>57 (56)</td>
</tr>
<tr>
<td>Gestation duration (wk) (mean ± sd)</td>
<td>39.7 ± 1.3</td>
</tr>
<tr>
<td>Birth weight (g) (mean ± sd)</td>
<td>3601 ± 481</td>
</tr>
<tr>
<td>Instrumental delivery</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>9 (8.9)</td>
</tr>
<tr>
<td>Infant transferred to neonatal ward</td>
<td>7 (6.9)</td>
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</tbody>
</table>

**Table 1. social and perinatal characteristics of the 101 study participants**
Assessments

Questionnaires
Depressive symptoms were assessed using a Dutch version of the Edinburgh Postpartum Depression Scale (EPDS) in week 16 and 36 of pregnancy and at 6 and 12 weeks postpartum. The EPDS consists of 10 items containing four statements that reflect depressive symptoms in increasing severity (scoring 0-3 per item). The sum scores ranges from 0-30. (20, 21) and a score of 13 or more indicates that women are at risk of having a depression (22). Postpartum blues were assessed with a Dutch version of the Blues Questionnaire (23). The Dutch version of the Blues Questionnaire is a 30-item scale in which each item is rated as being ‘more than usual’, ‘much more than usual’, ‘less than usual’, ‘much less than usual’ or ‘not different’. A score of ‘1’ was allocated to each item if the response was anything other than ‘no different’, this being scored as ‘0’. The sum score ranges from 0-30.

Genetic analyses
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). The analyses of 5-HTT, MAOA and COMT polymorphisms have been described elsewhere (Doornbos et al, in prep). Each genotype was classified into two groups, according to the activity of the gene-product as described in the introduction. One group was composed of the homozygous low activity variants, while the second group contained the pooled heterozygous and homozygous high activity variants.

Statistical analyses
Statistical analyses were performed with SPSS 14.0. The LCA was performed with Mplus statistical package (Muthén & Muthén, Los Angeles CA, USA). The significance level was set at p<0.05 (two sided), and was corrected for multiple testing with the Bonferroni correction.

When one of the four EPDS assessments was missing, we used the single multiple-imputation method (25) to replace the missing EPDS score. Single imputation can be
used to replace missing data when less than 10% of the data are missing, without biasing the results (25).

LCA was performed to the 4 EPDS assessments of the entire cohort as described before (26). In short, LCA is a statistical model-fitting method to identify different classes of subjects within a given data set. LCA assumes unobserved latent variables to explain the associations among observed scores and can be seen as a categorical equivalent of factor analysis, which assumes continuously distributed latent variables. Instead of giving a particular true solution, LCA produces several solutions with relative fit indices. LCA computes two sets of parameters. The first set is the latent class probabilities or class prevalences. The other set of parameters is called the conditional probabilities and estimates the probability of the observed variables, given that the individual is a member of that class. The conditional probabilities are analogous to the factor loadings in factor analysis. The Bayesian information criteria (BIC = log (L) - 0.05 × log (n) × k, where k is the number of parameters) (27) are generally used for the goodness of fit to determine the optimal number of groups. The smallest BIC value gives the best fit. The null model is a model for one single class, i.e., the whole cohort belonging to the same latent class. This model is rejected when models with two or more parameters result in better fit indices. Accordingly, we choose the model with lowest BIC value, if the subject numbers were sufficiently large (n>5) for the subsequently applied statistical analyses. It should be noted however that all participants received a latent factor score on all of the identified courses.

The outcome of the LCA was related to that of the genetic analyses as follows: all subjects received an LCA score for each of the classes. The sum of the different LCA scores for one individual is always 1; accordingly all participants contribute equally to the model. We compared these scores for the high and low activity variants of all three genes, using an independent t-test. As mentioned before we corrected for multiple testing using the Bonferroni correction.

For the evaluation of the effects of supplementation on the course of depression another parameter of the LCA model was used, i.e. the dominant class. This is the class for which an individual has the highest LCA score (thus ‘contributes most to’). Using this measure we compared the number of DHA, DHA+ AA and placebo treated persons for every dominant class with a $\chi^2$ test.

**Results**
Participant characteristics are described in table 1. The course of the depression scores over time is shown in the box plot in figure 1. The Friedman’s Analyses of Variance test showed a significant time effect (p<0.001). The post hoc test revealed that EPDS scores at 12 weeks post partum were significantly lower than EPDS scores at week 6 postpartum and week 36 of pregnancy (p<0.01). Mean blues scores at day 5 postpartum were 8.3 ± 6.1.

**Latent class analyses**

We replaced 12 EPDS scores of participants who missed one of the questionnaires assessed in the postpartum period. Thus 101 women were included in the latent class analyses.

For the 1-, 2-, 3-, and 4-class solution the BIC values decreased for every additional class as shown in table 2. However as the 4 class solution contained a class with only two subjects who were depressed throughout the peripartum, we accepted the 3 class solution. The classes were characterized as: 1) postpartum depression (N=8; 8%), 2) no depressive symptoms (N=70; 69%), and 3) depressed during pregnancy (N=23; 23%). The course of the depressive symptoms of the three latent classes is shown in figure 2.

The treatment groups were equally distributed among the three dominant classes. The LCA classes did not correlate with the demographic variables age, marital status, and professional or education level, neither with obstetrical variables, such as birth weight.

Mean LCA scores did not differ between parturition, such as caesarian section according to a Mann-Whitney test. LCA and ‘depressed during pregnancy class’ (Spearman’s rho: -0.404 p=0.002; 0.351 p=0.017). The LCA scores correlate with blues score.

**Polymorphisms and Latent classes**

Allele frequencies by latent class are shown in table 3. They proved to be in Hardy-Weinberg equilibrium. The mean independent t-test indicated that the LCA scores of the high and low variants of COMT, MAOA and 5-HTT did not differ significantly. The results are shown in table 4.

**Discussion**

![Figure 1. Boxplots of the EPDS scores during pregnancy and postpartum.](image)

#*differs significant from week 6 postpartum and week 36 of pregnancy (p<.01); ○ = outlier.
This study is the first to use latent class analyses on data of peripartum depression. The technique identified 2 small groups differing in the specific courses of depressive symptoms. We found that these subgroups were not associated with polymorphisms in the candidate genes MAOA, COMT and 5-HTT. Apparently, other non-genetic factors have a stronger influence on the course of peripartum depressive symptoms than these 3 candidate genes. These results emphasize that clinically identifiable subgroups do not necessarily share the same characteristics with subgroups based on a genetic analyses.

Strengths of this study are the longitudinal design, enabling the characterization of symptoms and specification of these subgroups. The sample might seem relatively small for power to detect an effect-size of 0.5 dependent t-test. In addition, the design of the sample homogeneity, the repeated measures, the equal distribution of the treatment groups among the three classes that were identified by LCA.

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Table 2. Bayesian Information Criteria (BIC) Values for the Latent Class Analyses Classes

<table>
<thead>
<tr>
<th>Class solution</th>
<th>BIC Value</th>
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<tbody>
<tr>
<td>1-Class</td>
<td>2326.984</td>
</tr>
<tr>
<td>2-Class</td>
<td>2244.518</td>
</tr>
<tr>
<td>3-Class</td>
<td>2217.523</td>
</tr>
<tr>
<td>4-Class</td>
<td>2192.385</td>
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</table>

Table 4. Latent class scores for the high and low activity variants of 5-HTT, MAOA and COMT

<table>
<thead>
<tr>
<th></th>
<th>High activity</th>
<th>Low activity</th>
<th>High activity</th>
<th>Low activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>.073 (.195)</td>
<td>.082 (.264)</td>
<td>.076 (.240)</td>
<td>.126 (.352)</td>
</tr>
<tr>
<td>Class 2</td>
<td>.602 (.466)</td>
<td>.667 (.457)</td>
<td>.673 (.450)</td>
<td>.406 (.450)</td>
</tr>
<tr>
<td>Class 3</td>
<td>.324 (.432)</td>
<td>.214 (.386)</td>
<td>.229 (.391)</td>
<td>.342 (.477)</td>
</tr>
<tr>
<td></td>
<td>.100 (.280)</td>
<td>.040 (.174)</td>
<td>.656 (.459)</td>
<td>.646 (.460)</td>
</tr>
<tr>
<td></td>
<td>.228 (.390)</td>
<td>.257 (.414)</td>
<td>.656 (.459)</td>
<td>.646 (.460)</td>
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The majority of the women (70%) were not depressed during pregnancy. The relative stability of mood of this group seems quite logical from an evolutionary point of view. If pregnancy would be depressogenic it would largely influence the quality and frequency of reproduction with pertinent consequences for the survival of the species. However from a conceptual point of view it is fascinating because depressogenic qualities have been attributed to the pregnancy-related changes in the endocrine systems (29) and monoamine metabolism (6). The very existence of the vast majority of women who remain unaffected in spite of sizeable endocrine fluctuations questions the causal role of many hormones and other physiological parameters in depression. The largest subgroup of 20% of the women is depressed during pregnancy and recover postpartum. This finding is in line with a large body of literature showing that the highest incidence of depressive symptoms is found at the end of pregnancy (28, 30-32). Women in this subgroup still have increased EPDS scores at 12 weeks postpartum; apparently recovery from depression is a slow process. Only 10% of the women developed de novo postpartum depressive symptoms. Interestingly these symptoms developed notably after 6 weeks postpartum. This observation has been reported previously (33), questioning the six week qualifier for postpartum onset as proposed by the DSM IV.

The LCA scores were not related to any variable of socioeconomic status, nor to obstetric variables. However, the study sample was healthy, with mostly uncomplicated deliveries, and quite homogenous with respect to socio-economic status, and may therefore not provide the opportunity to investigate influences of these factors on the development of depression.

The LCA scores of the ‘depression-during-pregnancy class’ and ‘no-depression class’ were significantly correlated with blues scores, whereas the postpartum depressed LCA class was not. These findings are in agreement with the literature indicating that blues correlate with depression scores during pregnancy (34), but seem to contradict studies reporting that blues score correlate with depression at six months postpartum (35, 36). This correlation however, could be explained by the finding that 50% of the women who were depressed during pregnancy, were also depressed 6 months later (3). The LCA analyses specifically identified those women who developed a new depression during the postpartum and thus were not depressed during pregnancy. Therefore the lack of correlation seems obvious, and may be regarded as a specific quality of this subgroup in which depression develops independent from the peripartum related events. This result underlines the complexity of the relations between depression and risk factors during pregnancy, as stated before by Pop et al: ‘during different assessments in the postpartum period different women are depressed, women who do not necessarily share the same characteristics’ (33).
None of the LCA classes was associated to polymorphisms in the three candidate genes. Previously, we have shown in the same cohort that 5-HTT high activity variants or MAOA and COMT low active variants confer a risk of a transient increase in peripartum depressive symptoms at 36 weeks of pregnancy and 6 weeks postpartum (Doornbos in prep.). The latter results are in line with another study that showed an association of the high activity variant of the 5-HTT with postpartum depression at 8 weeks postpartum (12). Thus the two methods for analyzing the effects of genes on the course of depression (one with a group-division based on genetics, and the other based on course of symptoms) give apparently discrepant results. However, both approaches provide different information; the comparison of two genotypes may elucidate characteristics of the gene, while linking genes to subgroups with different symptom courses may elucidate the genetic characteristics of a clinically relevant subgroup. We therefore consider the results of these studies complementary, rather than discrepant.

The results of this genetic analysis emphasize that other factors than genetics contribute significantly to a clinical reality as delineated by LCA, because the previously reported significant association of genetics with the course of depressive symptoms was not replicated by this approach. This is also seen in other area’s of psychiatry, in which genes seem to contribute significantly to endophenotypes of depression, like disturbances in the stress response (15, 24), or central disturbances in emotional processing (37, 38), but their contribution to depression, as indicated by association studies, appears to be rather small (39). At the clinical level a wide range of (interacting) risk factors together contribute to the onset and persistence of depression.

Despite the negative finding of this study we propose LCA as a useful method for genetic studies in psychiatry as: 1) it circumvents the use of debatable, power-wasting, cut off points or disputable DSM IV criteria, 2) it is a statistically powerful method and therefore useful in medium sized groups, and 3) it is especially useful for longitudinal studies during pregnancy because its course is predictable.

Conclusion

Based on the course of the depressive symptom this study identified two small subgroups within a large cohort of healthy pregnant women. The subgroups proved unrelated to mono-aminergic candidate genes, contradicting our previous findings. These results emphasize that clinically identifiable subgroups do not necessarily share the same characteristics with subgroups based on genetic features. However, due to the
moderate sample size of this study no definite answers on this relation could be given. Therefore the current approach should be replicated in a larger sample, with sufficient power to detect small effects. In the future LCA can be used to investigate to what extent other biological, psychological and socio-economic risk factors might influence the onset and course of depression. By that, risk factors for peripartum depression will be related to a major characteristic, i.e. its course.
Reference List


30. Gordon TE, Cardone IA, Kim JJ, Gordon SM, Silver RK. Universal perinatal depression...


