Case-control study of glucocorticoid receptor and corticotrophin-releasing hormone receptor gene variants and risk of perinatal depression

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Abstract

Background: Depression during pregnancy or after childbirth is the most frequent perinatal illness affecting women of reproductive age. It could result in unfavourable outcomes for both women and their newborns. The incidence of perinatal depression is higher for those with family history of depression and other mental illness, suggesting the contribution of genetic factors. There is postulation that disruption or fluctuation of reproductive hormones could play a part in women who are sensitive to such changes.

Methods: This is a case-control study comparing the frequencies of candidate gene variants in patients with perinatal depression with controls. Patients of Chinese descent (N = 725) were recruited from the outpatient clinics of the hospital between 2010 and 2013. Controls were patients who came for postnatal consultations at the obstetrics clinics and scored ≤ 7 on the Edinburgh Postnatal Depression Scale (EPDS) at the postnatal screening programme of the hospital. Cases with confirmed diagnosis of clinical (major) depression related to pregnancy/postpartum were recruited from the hospital's outpatient clinic. Genomic DNA was extracted from saliva samples and genotyped for the polymorphisms of interest. Differences between groups were assessed by chi-square analysis.

Results: CRHR1 rs242939 and rs1876828 were not polymorphic in the study population. There was no statistically significant association of perinatal depression for CRHR1 rs242941 and GR rs41423247 (BclI). When all subjects were grouped based on family history of mental illness, there was a statistically significant association of CRHR1 rs242941 with family history regardless of depression status (P = 0.043). There was also a statistically significant difference for GR rs41423247 and regularity of menstrual periods (P < 0.000). Although not statistically significant, women with perinatal depression showed a trend towards higher frequency of self-reported menstrual irregularity.

Conclusions: No evidence was found for the association of any of the genetic markers with perinatal depression in this study cohort. Instead, the possible genetic links were found in women with positive family history of mental illness and menstrual irregularity, suggesting these could be identifying risk markers for women.

Keywords: Genetic association, Family history, Menstrual period regularity, Perinatal depression, Pregnancy

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Background
Depression is a complex heterogeneous disorder that results from gene-environment interaction [1, 2]. Evidence for genetic contribution comes from twin and family studies, which suggests a heritability of 30 – 70 % and a 3-fold increased risk for first-degree relatives [3].

Depression affects all populations worldwide and is a major cause of disability and loss of productivity. The prevalence in women is higher than that of men. Women of childbearing age have the highest risk of developing depression, with close to 20 % suffering from postnatal depression or having depressive symptoms during pregnancy [4, 5]. The prevalence of antenatal depression in Singapore is between 12 % and 20 % and for postnatal depression it is 6.8 % [6, 7]. This rate is close to the range reported for western populations [8–10].

There is evidence that in some women, childbirth may be a specific trigger for depressive illness. Previous studies have proposed that the development of depression in some women could be triggered by striking changes in hormonal levels during pregnancy and following childbirth. These hormones include oestrogen, progesterone, corticotrophin-releasing hormone, cortisol and glucocorticoids [11–15]. In a genome-wide linkage study, the strongest signals that may be specific to postpartum mood symptoms were on 1q21.3-q32.1 and 9p24.3-p22.3, with modest association for Hemicentin gene SNPs on chromosome 1 [16]. The gene might have oestrogen receptor binding sites and was highly expressed in the hippocampus region. Drop in oestrogen levels after a hormone-stimulated pregnancy has been reported to alter hippocampal cell proliferation in rats [17]. Among transcripts which showed different expression patterns between women with postpartum depression and euthymic women, there was enrichment of those implicated in the oestrogen signalling pathway [18].

Disruption in ovarian function manifested as a change in menstrual cycle length has been linked to higher cardio-metabolic risk, higher scores for Center for Epidemiological Studies Depression Scale, and higher likelihood to have had a diagnosis of depression or used anti-depressant medication [19]. A link between psychiatric disorders and regularity of menstrual cycle has also been found in Caucasian women [20].

Past history of depression and family history of mental illness remain the two strongest predictors of perinatal depression [21]. Given the role of the hypothalamic-pituitary-adrenocortical (HPA) axis in the aetiology of depression and the changing levels of corticotrophin-releasing hormone (CRHR1) during pregnancy, it is likely that the predisposition to perinatal depression might be related to genetic polymorphisms in the hormone receptors. Indeed, several studies have shown that polymorphisms at the glucocorticoid receptor (GR), such as BclI and ER22/23EK were associated with dysfunction of the HPA axis and altered glucocorticoid sensitivity leading to depression [22–25]. Single nucleotide polymorphisms and haplotypes of CRHR1 were also found to be significantly over-represented in patients with major depression compared to controls [26–28].

Several studies have looked into specific single nucleotide polymorphisms (SNPs) of the glucocorticoid and corticotrophin-releasing hormone receptor genes in relation to the risk of developing depression during pregnancy and the postnatal period. The results range from none [29, 30] to very strong positive association with risk ratios of 2.9 – 5.48 [31].

In this study, we investigated the five SNPs in the study which showed high risk ratios [31] in a cohort of hospital patients that included controls who had been screened for postnatal depression and cases who met diagnostic criteria for perinatal depression. Besides providing replication for the positive association, our aim was also to obtain the genotypic frequencies of the five SNPs, namely BclI (rs41423247) and ER22/23EK (rs6189–6190) of GR; and rs242941, rs242939 and rs1876828 of CRHR1 in our Singaporean Chinese population.

Methods
Study participants
The study design was reviewed and approved by the SingHealth Centralised Institutional Review Board which oversees all research studies in the hospital. The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. Written informed consent of the participants was obtained after the procedures had been fully explained to them. The study sample has been described in a previous report [32]. In short, cases were recruited from outpatient psychiatric clinics and were all diagnosed by board-certified psychiatrists according to the criteria in Diagnostic Statistical Manual of Mental Disorders IV edition. Inclusion criteria for cases were being of Chinese ancestry and having a confirmed diagnosis of clinical (major) depression related to pregnancy/postpartum. However, those with comorbid predominant anxiety, psychotic disorders, or substance abuse were excluded from the analysis. Controls were recruited from patients who attended postnatal obstetrics clinics and were screened for absence of symptoms of postnatal depression using the Edinburgh Postnatal Depression Scale (EPDS). Only those with EPDS score ≤ 7 and self-reported as being Chinese were included. From a cohort of 725 subjects recruited between November 2010 and March 2013, 147 cases and 549 controls of self-reported Chinese ancestry were included in this study.

Data on demographics, personal and family history of mental illness, menstrual cycle regularity and mood changes were captured through in-person interviews based
on a standard set of questions and recorded on a data collection form at the time of recruitment.

**Molecular analysis**

DNA was extracted from saliva samples using either the Oragene DNA Kit (DNA Genotek Inc., Kanata, Canada) or the Norgen Saliva DNA collection kit (Norgen Biotek Corp., Thorold, Canada). DNA was quantitated using Nanodrop Spectrophotometer (Thermo Scientific, Wilmington, USA).

Genotyping of the three CRHR1 SNPs were done using Taqman assays: rs242941 - Assay ID: AHLJYBU; rs242939 - Assay ID: C_2544833_10, rs1876828 - Assay ID: 11935972_10. (Applied Biosystems, Foster City, CA, USA). Amplification was performed in a volume of 12 μl containing 25 ng genomic DNA, Taqman Universal Polymerase Chain Reaction Master Mix, 60 nM of each probe, and 270 nM of each primer. Cycling and hybridization conditions were set according to manufacturer’s instructions. The 50 cycles of denaturation and annealing/extension and post-polymerase chain reaction quantification of fluorescent intensity were performed using the Applied Biosystems StepOnePlus Real-Time PCR System. Alleles were called using the sequence detection software.

Genotyping of the GR BclI (rs41423247) was done by polymerase chain reaction followed by restriction with BclI and electrophoresis on 3 % agarose gel. Genotypes were scored manually. No genotyping was performed for GR ER22/23EK (rs6189 - rs242941 and regularity of menstrual periods (Table 3). Among the cases with perinatal depression, there was a higher proportion (22.5 %) who self-reported that their menstrual cycles were not regular, compared to 17.3 % among controls; although this was not statistically significant ($\chi^2 = 3.123, P = 0.210$).

**Discussion**

During pregnancy, the development of a transient organ of foetal origin, the placenta, causes major alterations in the hypothalamic-pituitary-adrenal (HPA) axis. It has been suggested that HPA axis dysregulation during this period of dramatic change in hormonal levels could cause the development of postpartum depression in vulnerable women [36]. Life stress was also found to be an important risk factor for depressive symptoms during pregnancy in a systematic review of 57 studies [37]. Genes related to HPA axis and stress reactivity are thus good candidate genes for perinatal depression.

A recent study by Engineer et al. found that the BclI single nucleotide polymorphism of the GR gene and the rs242939 SNP of the CRHR1 gene were associated with genetic risk to antenatal and postnatal depression [31]. We found no evidence of an association between the GR

| Gene | Genotypes | Controls (%) | Cases (%) | $\chi^2$ | P-value |
|------|-----------|--------------|-----------|----------|---------|
| CRHR1: rs242941 GG | 472 (86.0) | 127 (86.4) | | | |
| rs242941 GT | 71 (12.9) | 19 (12.9) | 0.199 | 0.905 |
| rs242941 TT | 6 (1.1) | 1 (0.7) | | | |
| GR: rs41423247 GG | 326 (60.0) | 88 (59.9) | | | |
| rs41423247 GC | 194 (35.7) | 50 (34.0) | 0.985 | 0.611 |
| rs41423247 CC | 23 (4.2) | 9 (6.1) | | | |

| Gene | Genotypes | No FH (%) | Positive FH (%) | $\chi^2$ | P-value |
|------|-----------|-----------|----------------|---------|---------|
| CRHR1: rs242941 GG | 536 (87.0) | 63 (78.8) | | | |
| rs242941 GT | 73 (11.9) | 17 (21.3) | 6.308 | 0.043 |
| rs242941 TT | 7 (1.1) | 0 (0.0) | | | |
| GR: rs41423247 GG | 368 (60.3) | 46 (57.5) | | | |
| rs41423247 GC | 217 (35.6) | 27 (33.8) | 3.461 | 0.177 |
| rs41423247 CC | 25 (4.1) | 7 (8.8) | | | |

| Gene | Genotypes | No FH (%) | Positive FH (%) | $\chi^2$ | P-value |
|------|-----------|-----------|----------------|---------|---------|
| CRHR1: rs242941 GG | 536 (87.0) | 63 (78.8) | | | |
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| rs41423247 GC | 217 (35.6) | 27 (33.8) | 3.461 | 0.177 |
| rs41423247 CC | 25 (4.1) | 7 (8.8) | | | |
There was no association between the specific SNPs and the diagnosis of perinatal depression in this cohort of Singaporean Chinese patients. However, based on the our findings on the associations with family history of mental illness and menstrual period regularity, the GR and CHR1 pathways may still be involved in the predisposition.
to mental illness, and menstrual period regularity may be a predisposing factor in susceptible women. There is a need for more studies in different populations to find the genetic factors associated with depression during pregnancy and following delivery. Identification of biomarkers facilitates screening of at-risk individuals for early intervention, choice of treatment, prediction of treatment response, and prognosis of outcome over a wide spectrum of symptoms associated with affective states, thereby optimizing clinical practice procedures.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ECT and HYC designed the study and obtained the funding. TEC, TL and HYC did the clinical assessment and oversaw the EPDS screening and the recruitment of controls. ECT and JLT did the literature search and wrote the first draft of the manuscript. HST did the subject recruitment and genotyping. All authors read and approved the manuscript.

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