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Association of premenstrual/menstrual symptoms with perinatal depression and a polymorphic repeat in the polyglutamine tract of the retinoic acid induced 1 gene

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ABSTRACT

**Background:** Depression during pregnancy or after childbirth is the most frequent perinatal illness affecting women. We investigated the length distribution of a trinucleotide repeat in *RAI1*, which has not been studied in perinatal depression or in the Chinese population.

**Methods:** Cases (n=139) with confirmed diagnosis of clinical (major) depression related to pregnancy/postpartum were recruited from the outpatient clinic. Controls were patients who came to the obstetrics clinics and scored <7 on the Edinburgh Postnatal Depression Scale (EPDS) (n=540). Saliva samples for DNA analysis, demographic information and self-reported frequency of occurrence of various premenstrual/menstrual symptoms were collected from all participants. Genomic DNA was extracted from saliva and relevant region sequenced to determine the number of CAG/CAA repeats that encodes the polyglutamine tract in the N terminal of the protein. Difference between groups was assessed by chi-square analysis for categorical variables and analysis of variance for quantitative scores.

**Results:** Compared to control subjects, patients with perinatal depression reported more frequent mood changes, cramps, nausea, vomiting, diarrhoea, and headache during premenstrual/menstrual periods (p=0.000). For the *RAI1* gene CAG/CAA repeat, there was a statistically significant difference in the genotypic distribution between cases and controls (p=0.031). There was also a statistically significant association between the 14-repeat allele and perinatal depression (p=0.016).

**Limitations:** Family history, previous mental illness, physical and psychological symptoms during the premenstrual/menstrual periods was self-reported. EPDS screening was done only once for controls.

**Conclusions:** The *RAI1* gene polyglutamine repeat has a different distribution in our population. The 14-repeat allele is associated with perinatal depression and more frequent experience of physical and psychological symptoms during menstrual period.

**Keywords:** mood symptoms, perinatal depression, polyglutamine repeats, *RAI1* gene, premenstrual/menstrual symptoms

1. **Introduction**

The recent Singapore Mental Health Study has shown that women have significantly higher risk of developing depression compared to men, and the rate of depression is higher in the childbearing age group (Chong et al., 2012). Depression during the antenatal and/or postnatal period affects about 12% of Singaporean women who undergo pregnancy and childbirth (Chee et al., 2005). This is in line with European studies which reported similar prevalence and even higher occurrence of close to 20% for symptoms of depression during pregnancy and the postpartum periods (Hubner-Liebermann et al., 2012). Although depression can occur at any age, it has been observed that the incidence in women closely mirrors the shift in oestrogen levels across a woman’s life cycle. In contrast, the incidence of
depression in men is constant for the years after puberty (Stahl, 2001). For both men and women, the incidence is higher for those with family history of depression and other mental illness, suggesting the contribution of genetic factors.

Previous studies have found premenstrual dysphoric disorder (PMDD), its related milder form premenstrual syndrome, and mood symptoms during past contraceptive use or third trimester to be risk factors for postpartum mood disorders (Bloch et al., 2005, 2006; Lee et al., 2007), which has been included as a new disorder under the 5th Edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013). However, there are limited studies on the association of premenstrual symptoms and the occurrence of reproductive cycle-related mood symptoms with regards to a psychiatric diagnosis (Buttner et al., 2013; Payne et al., 2007).

Most genetic studies on depression focus on neurotransmitters. The retinoic acid-induced 1 (RAI1) gene is one candidate gene for neuropsychiatric disorders as it is highly expressed in neuronal tissues and implicated in syndromes with neurobehavioural phenotypes. The gene is dosage sensitive: haploinsufficiency is shown to be the cause of the phenotype of Smith-Magenis syndrome (SMS, OMIM#182290) while duplication causes the milder Potocki-Lupski syndrome (PTLS, OMIM#610883). Presentations of these two syndromic disorders include developmental, physical, and neurobehavioural abnormalities, and circadian rhythm disruption (Elsea and Williams, 2011; Lee et al., 2012). The gene encodes a transcriptional regulator thought to be involved in the regulation of circadian rhythm, weight control and complex behavioural responses. Mouse model of PTLS showed behaviour representative of autistic features in humans (Molina et al., 2008; Walz et al., 2006). In a case-control study, the gene has also been linked to autism spectrum disorder (van der Zwaag et al., 2009).

The 120 kb RAI1 gene contains a polymorphic CAG/CAA repeat that encodes a polyglutamine tract at the N-terminal. There are also two polyserine tracts - one at the C-terminal and the other at the N-terminal. There is reported inverse association of the number of CAG/CAA repeats with the age of onset in spinocerebellar ataxia type 2 (SCA2), perhaps by interacting with the polyglutamine tract in the ataxin-2 protein (Hayes et al., 2000). This polymorphic repeat has also been linked to the severity of symptoms in schizophrenia and responsiveness to neuroleptic medication (Ivkovic et al., 2011; Joober et al., 1999). In this study, we investigated the length distribution of the trinucleotide repeat encoding the polyglutamine tract for this gene, which has not been studied in perinatal depression (which encompasses both antenatal and postnatal depression) or in the Chinese population.
2. Methods

2.1. Participants and sample characteristics

The study sample comprised 139 cases and 540 controls. All were women of Chinese
descent recruited from the outpatient clinic of KK Women’s and Children’s Hospital between
November 2010 and March 2013. Cases were patients with confirmed diagnosis of clinical
(major) depression related to pregnancy/postpartum who were recruited from the outpatient
psychiatric clinic. Women with comorbid predominant anxiety, psychotic disorders, or
substance abuse were excluded. Controls were obstetric patients who came for routine post-
natal consultations at the obstetrics clinics and scored <7 on the Edinburgh Postnatal
Depression Scale (EPDS). They were recruited via the post-natal screening programme
conducted by the Department of Psychological Medicine. All subjects provided written
informed consent to a protocol approved by the Institutional Review Board which oversees
research in the hospital.

Demographic information was collected from the medical records. Details on occupation,
housing and childcare arrangement, family history, and educational level were provided by
the patients. Patients were also asked to rate the occurrence of mood changes, cramps, nausea,
vomiting, diarrhoea and headache during the premenstrual/menstrual periods.

Saliva samples for genetic analysis were collected with either the Oragene DNA Kit
(DNA Genotek Inc., Canada) or Norgen Saliva DNA Collection kit (Norgen Biotek
Corporation, Canada).

2.2. Molecular analysis

Genomic DNA was extracted using the manufacturers’ protocols. DNA was checked for
quantity and purity with the Nanodrop Photospectrometer (Biofrontier Technology,
Wilmington, USA). To determine the number of CAG/CAA repeats, the relevant region was
amplified with primers 5’-CCACCTCCTCCACCTACTCC-3’ and 5’-
GCTGCCGTAGTGCTGATAC-3’. The amplicons were purified with ExoSAP and
sequenced using the BigDye® Direct Cycle Sequencing Kit (Applied Biosystems, Foster City,
USA) and electrophoresed on the Applied Biosystems Genetic Analyzer 3130.

2.3. Statistical analysis

Difference between groups was assessed by chi-square analysis for categorical variables
and analysis of variance (ANOVA) for quantitative scores. All analysis was performed using
IBM SPSS Statistics 19 (IBM Corporation, Armonk, NY, USA).
3. Results

The mean age was 33.81 ± 6.317 years for cases and 31.26 ± 4.682 years for controls (F=28.094, p=0.000). All controls were from the postnatal screening programme while some cases were patients who had their last delivery a few years ago. Mean age of menarche was 12.57 ± 1.484 years for cases and 12.97 ± 1.537 years for controls (F=7.636, p=0.006). All controls were married. Among cases, there were three who were not married at the time of recruitment – one single mother, one divorcee, and one widow (χ²=7.93, p=0.020). There were statistically significant associations between perinatal depression and past history of perinatal depression (χ²=74.943, p=0.000), personal history of mental illness (χ²=28.617, p=0.000), and self-reported family history of mental illness (χ²=125.148, p=0.000). There was also a statistically significant association with other existing illness (χ²=16.551, p=0.005) but only marginal association with pregnancy complications (χ²=10.839, p=0.055). Overall, cases with perinatal depression reported more frequent mood changes, cramps, nausea, vomiting, diarrhoea, and headache during their premenstrual/menstrual periods (Table 1).

For the RAI1 gene CAG/CAA repeat, amplification and sequencing failed in 12 subjects: three cases and nine controls. The distribution in cases ranged from 9 to 15 while the distribution for controls ranged from 10 to 21. The most common was the 13-repeat allele, followed by the 14-repeat allele. There was a statistically significant difference in distribution of the genotypes between cases and controls (χ²=8.908, p=0.031). Genotypes which were only found in cases were 9/13 and 14/14; genotypes which were unique to controls were 10/11, 10/13, 11/11, 11/14, 11/15, 12/14, 13/15, 13/17, 13/21 (Table 2). The 14-repeat allele also showed a statistically significant difference in the distribution between controls and cases (χ² = 8.320, p=0.016). Regardless of the case or control status, the 14-repeat allele was also significantly associated with self-reported headache (F=5.067, p=0.007) and marginally associated with mood changes (F=2.923, p=0.054) (Table 3). Headache was also significantly correlated with the occurrence of rare alleles (9, 10, 15, 17 and 21 repeats) (F=4.743, p=0.009).

4. Discussion

Our study found statistically significant differences between cases and controls in the mean frequency scores for all the premenstrual/menstrual symptoms investigated, with cases reporting more frequent occurrence across different domains. As the symptoms may be caused by sensitivity to changes in hormonal levels during a reproductive cycle, a similar mechanism may also be at play for the susceptibility to perinatal depression.
Our cases were older than control subjects because cases were from patients under psychiatric care who might have given birth years ago, while controls were all from the postnatal follow-up clinics and had just given birth about one to two months earlier. Age of menarche was also significantly lower in cases, indicating that longer exposure to fluctuation of reproductive hormones may be a risk factor in vulnerable women. Other factors found to be associated with the diagnosis were previous occurrence of perinatal depression, personal history of mental illness, and family history of mental illness. All were consistent with a genetic aetiology for perinatal depression.

The role of the RAI1 gene has not yet been explored in depression. It is one of the top eight genes identified for polyglutamine expansion disorders as it has a long CAG tract and relatively high Q-tract variance (Butland et al., 2007). In previous studies, the number of repeats was reported to range from 10 to 19 in the general population (Bi et al., 2006; Ivkovic et al., 2011), and 10 to 14 in a cohort of SMS patients (Vilboux et al., 2011). Relative frequencies of the various alleles also appear to be different in our population. The most common allele in multiple studies in Western populations was the 14-repeat allele while in this study it was the 13-repeat allele. We found the size range to be 9 to 21, with one control subject carrying a 17-repeat allele and another with a 21-repeat allele, the largest reported to date. We did not find any allele with 18, 19 or 20 repeats, and the 14-repeat allele was associated with a diagnosis of perinatal depression and more frequent experience of physical and psychological symptoms during menstrual periods.

Although there is no study on how the number of repeats correlate with protein level or activity, the N-terminal half which contains the polyglutamine tract is reported to contain transcription factor activity (Carmona-Mora et al., 2010). As there is evidence that retinoic acid receptor binding throughout the genome is highly coincident with oestrogen receptor α (ERα) binding, it may be important in the pathogenesis of perinatal depression by acting through crosstalk of retinoic acid and oestrogen signalling pathways (Hua et al., 2009).

In mouse studies, the RAI1 gene has been shown to be expressed mainly in neuronal brain structures during development and also in adult animals (Imai et al., 1995). Retinoic acid signalling in the mature brain may be important for neuronal maintenance, plasticity, repair and regeneration. Variability in the polyglutamine tract may modulate the activity of the mature protein as observed for other CAG-repeats containing genes. There is evidence that the protein is required for the maintenance of circadian rhythm, as haploinsufficiency of the gene causes the transcriptional dysregulation of the circadian clock (Williams et al., 2012).
The resulting abnormal sleep-wake cycle can contribute to sleep and mood problems, with sleep disturbance a key symptom of depression. This is supported by a wide body of evidence demonstrating the reciprocal connections between the serotonin and circadian master regulatory networks, each with its defined gene network of transcriptional regulators and signalling genes (Ciarleglio et al., 2011). Indeed, preliminary findings from a recent study have demonstrated individual differences in circadian phase shifts across the perinatal period correlate with postpartum depression (Sharkey et al., 2013).

There are a few limitations in this study. Family history, personal history of mental illness, and physical and psychological symptoms during the premenstrual/menstrual periods were all self-reported, and were not verified against any records. There might be recall bias of premenstrual/menstrual symptoms as the participants were recalling their last period, most of which would be at least 9 months ago. For controls, the EPDS screening was done at only one time-point during the postnatal period. Although we have tried to minimise confounding factors such as population stratification by including only subjects from one ethnic group, the results should be regarded as preliminary unless replicated in another population due to the potential for false-positive findings.

In conclusion, this study provides evidence that mood and physical symptoms during premenstrual/menstrual periods are related to the diagnosis of perinatal depression. We also found significant associations of the common 14-allele repeat with perinatal depression and risk factors such as mood changes during menstrual/premenstrual periods. These findings may be important in deciphering the pathophysiology of mood changes and pregnancy-related depression.

References


Table 1 Comparison of scores (mean ± standard deviation) between cases and controls for symptom occurrence during the premenstrual/menstrual periods

<table>
<thead>
<tr>
<th>*Symptom</th>
<th>Cases</th>
<th>Controls</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mood changes</td>
<td>2.19 ± 1.026</td>
<td>2.94 ± 1.015</td>
<td>61.246</td>
<td>0.000</td>
</tr>
<tr>
<td>Cramps</td>
<td>2.28 ± 1.043</td>
<td>2.71 ± 1.109</td>
<td>17.303</td>
<td>0.000</td>
</tr>
<tr>
<td>Nausea</td>
<td>3.48 ± 0.879</td>
<td>3.87 ± 0.470</td>
<td>49.874</td>
<td>0.000</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3.71 ± 0.664</td>
<td>3.92 ± 0.384</td>
<td>23.837</td>
<td>0.000</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>3.38 ± 1.003</td>
<td>3.74 ± 0.707</td>
<td>23.142</td>
<td>0.000</td>
</tr>
<tr>
<td>Headache</td>
<td>2.95 ± 1.112</td>
<td>3.63 ± 0.775</td>
<td>70.135</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Self-scored based on the following scale: 1 = always, 2 = sometimes, 3 = rarely, 4 = never
Table 2
Genotype distribution of the RAI1 repeat in cases and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n=532)</th>
<th>Cases (n=136)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>frequency</td>
</tr>
<tr>
<td>9,13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10,11</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>10,13</td>
<td>4</td>
<td>0.008</td>
</tr>
<tr>
<td>11,11</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>11,13</td>
<td>53</td>
<td>0.1</td>
</tr>
<tr>
<td>11,14</td>
<td>7</td>
<td>0.013</td>
</tr>
<tr>
<td>11,15</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>12,13</td>
<td>5</td>
<td>0.009</td>
</tr>
<tr>
<td>12,14</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>13,13</td>
<td>382</td>
<td>0.718</td>
</tr>
<tr>
<td>13,14</td>
<td>72</td>
<td>0.135</td>
</tr>
<tr>
<td>13,15</td>
<td>3</td>
<td>0.006</td>
</tr>
<tr>
<td>13,17</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>13,21</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>14,14</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3
Comparison of mean scores and standard deviation between groups carrying 0, 1 or 2 copies of the 14-repeat allele for symptom occurrence during the premenstrual/menstrual periods

<table>
<thead>
<tr>
<th>Symptom</th>
<th>0 (n = 559)</th>
<th>1 (n = 104)</th>
<th>2 (n = 2)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mood changes</td>
<td>2.79 ± 1.038</td>
<td>2.75 ± 1.164</td>
<td>1 ± 0.000</td>
<td>2.923</td>
<td>0.054</td>
</tr>
<tr>
<td>Cramps</td>
<td>2.65 ± 1.113</td>
<td>2.48 ± 1.072</td>
<td>2.00 ± 0.00</td>
<td>1.336</td>
<td>0.164</td>
</tr>
<tr>
<td>Nausea</td>
<td>3.78 ± 0.616</td>
<td>3.85 ± 0.498</td>
<td>3.00 ± 0.00</td>
<td>2.253</td>
<td>0.106</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3.86 ± 0.485</td>
<td>3.93 ± 0.349</td>
<td>4.00 ± 0.00</td>
<td>1.130</td>
<td>0.324</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>3.69 ± 0.760</td>
<td>3.61 ± 0.864</td>
<td>4.00 ± 0.00</td>
<td>0.635</td>
<td>0.530</td>
</tr>
<tr>
<td>Headache</td>
<td>3.50 ± 0.884</td>
<td>3.52 ± 0.924</td>
<td>1.5 ± 0.707</td>
<td>5.067</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Self-scored based on the following scale: 1 = always, 2 = sometimes, 3 = rarely, 4 = never

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Contributors
ECT and HYC designed the study and obtained the funding. ECT did the literature search and analyses and wrote the first draft of the manuscript. JN and HST did the subject recruitment and DNA sequencing. HYC, TEC, TL and CHC did the clinical assessment of patients. YCC oversaw the EPDS screening and the recruitment of controls. All authors read and approved the manuscript.
Conflict of interest

All authors declare no conflict of interest.

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