

# Evidence for oestrogen sensitivity in perinatal depression: pharmacological sex hormone manipulation study

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## Background

Enhanced sensitivity to oestrogen signalling may drive increased risk for depressive symptoms when exposed to peripartum sex-steroid hormone fluctuations.

## Aim

Testing if 116 pre-identified sex steroid-responsive transcripts that predicted perinatal depression (PND) translates to a pharmacological model of hormone-induced mood changes.

## Method

We generated longitudinal, genome-wide gene-expression and DNA-methylation data from 60 women exposed to a gonadotrophin-releasing hormone agonist (GnRHa) or placebo. We used linear mixed-effect models to assess differences between baseline and follow-up for gene expression and DNA methylation in the biphasic ovarian response to GnRHa.

## Results

Of the 116 PND-predictive transcripts, a significant (19%) overlap was observed with those differentially expressed post-GnRHa at both early and later follow-up, indicating sustained effects. Similarly, 49% of tested genes were differentially methylated post-GnRHa at the late follow-up. Within the GnRHa group, a large proportion of PND genes were significantly associated

(gene expression; DNA methylation) with changes in depressive symptoms (28%; 66%), oestradiol levels (49%; 66%) and neocortex serotonin transporter binding (8%; 45%) between baseline and follow-up.

## Conclusions

Our data bridge clinical PND biomarkers with a pharmacological model of sex hormone-induced mood changes and directly relate oestrogen-induced biological changes with depressive symptoms and associated serotonin-signalling changes. Our data highlight that individual variations in molecular sensitivity to oestrogen associate with susceptibility to hormone-induced mood changes and hold promise for candidate biomarkers.

## Declaration of interest

V.G.F. received honorarium for being a speaker for H. Lundbeck A/S. E.B.B. receives research funding from Böhringer Ingelheim to investigate FKBP5 as a potential drug target for depression.

## Keywords

Perinatal depression; biomarkers; oestrogen; gene expression; DNA methylation.

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The prevalence of major depressive disorder (MDD) among women is 1.5 to 3 times higher than in men.<sup>1</sup> In particular, women show heightened vulnerability for mood disorders in hormonal transition phases such as during puberty, pre- to postpartum<sup>2</sup> and perimenopause.<sup>3</sup> Perinatal depression (PND) is a common, debilitating disorder with a prevalence of 19%<sup>4</sup> that confers long-term detrimental effects on the health of both mother and child, which include adverse effects on mother–infant bonding and infant socio-cognitive development. Several biological markers, including sex-steroid hormonal fluctuations and alterations in the serotonergic system,<sup>5,6</sup> have been implicated in the pathophysiology of PND. In particular, oestrogen signalling affects the brain via diverse molecular mechanisms, including effects on hippocampal neurogenesis, neurotrophin signalling and stress hormone-axis function.<sup>7</sup> Oestrogen receptors are mainly nuclear receptors that serve as transcription factors by regulating the expression of target genes.<sup>8</sup> Upon activation, the oestrogen receptors bind to DNA-enhancer elements called oestrogen-response elements, which allow the oestrogen receptors to influence transcription of their target genes. Further, oestrogen can induce local changes in DNA methylation, effects which may be driven via the oestrogen receptors. Enhanced sensitivity to oestrogen signalling, rather than abnormal absolute levels of steroid hormones, may drive increased risk for depressive symptoms when exposed to sex-steroid hormone fluctuations across

peripartum.<sup>9,10</sup> We previously identified 116 genes whose expression levels in the highly sex steroid-stimulated state of the third trimester of pregnancy predicted later depression with postpartum onset. These predictive transcripts were significantly enriched for oestrogen-receptor targets and showed an increased dynamic change over pregnancy and the postpartum period in women with depression at postpartum onset, suggesting an enhanced sensitivity to oestrogen signalling in this group.<sup>11</sup> In another recent study, we used a gonadotropin-releasing hormone agonist (GnRHa) to induce a biphasic fluctuation in ovarian sex hormones as a pharmacological risk model for hormone-induced mood changes. We evaluated whether GnRHa treatment triggers depressive symptoms in a manner dependent on net changes in oestradiol levels and brain serotonin-transporter (SERT) binding.<sup>6</sup> Treatment with GnRHa induces a biphasic hormonal transition phase reflected by an initial stimulation of the hypothalamic–pituitary–gonadal axis; a desensitisation of the pituitary GnRH receptors; and subsequently a suppression of the ovarian sex-steroid production to menopausal level, which is reached within 10–14 days and sustained for 28 days post-intervention. Using this pharmacologically induced, biphasic, ovarian-hormone response by GnRHa via a randomised, double-blinded, placebo-controlled, within-subject study design, we have observed a significant association between SERT brain binding (measured with [<sup>11</sup>C]DASB Positron Emission Tomography (PET)) and scores from the 17-item Hamilton Rating Scale for Depression (HRSD-17) following GnRHa treatment.<sup>6</sup> This suggests that serotonergic signalling and oestradiol

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changes were key to mechanisms in hormone fluctuation-provoked depressive symptoms.

In this study, we have combined our findings from a previous study on biomarkers for depression with postpartum onset<sup>11</sup> and the GnRHa model;<sup>6</sup> there was no participant overlap between these two studies. This enabled us to determine whether transcriptional and epigenetic sensitivity to sex steroid-hormone manipulation of clinically derived genes map onto depressive symptoms and related SERT brain binding in a controlled pharmacological study. For this we assessed longitudinal gene-expression and DNA-methylation profiles of the 116 identified transcripts to predict PND in women undergoing GnRHa intervention or placebo.

## Methods and materials

### Participants

Healthy women were recruited via an internet advertisement and invited to a face-to-face screening by trained clinicians. Women with no history of neurological or psychiatric disorders (ICD-10 [1992] or DSM-IV [1994] Axis-I diagnostic criteria for obsessive-compulsive disorder, anxiety, major depression, bipolar disorder/mania or schizophrenia [Schedules for Clinical Assessment in Neuropsychiatry 2.1 interview]), no premenstrual dysphoric disorder (DSM-IV criteria), no alcohol misuse or illegal drug use, and a normal neurological and gynaecological examination were included in the study, as detailed in Frokjaer *et al.*<sup>6</sup> Participants scored within Danish norms on the Big Five Personality questionnaire (Revised NEO Personal Inventory) except for the 'openness' dimension where they scored higher, as expected for individuals volunteering for a PET study ( $127 \pm 18$  versus  $107 \pm 19$ , mean  $\pm$  s.d.,  $P = 0.0001$ ).<sup>6</sup>

A total of 63 healthy women participated in this randomised, placebo-controlled and double-blinded intervention study. Data sets from 60 participants were available for analyses. Of the remaining three participants, one did not receive intervention due to anovulation, one became pregnant and did not complete follow-up and one participant was discontinued in the programme due to technical error with brain imaging data. All participants had regular menstrual cycles and normal hormone status. Baseline assessments were performed in the mid-follicular phase when ovarian hormone levels are most stable and the time since the postovulatory oestradiol drop is maximised. Contingent upon ovulation in their natural cycle as confirmed by transvaginal ultrasound, participants received a subcutaneous injection of a GnRHa implant (ZOLADEX<sup>®</sup> containing 3.6 mg of goserelin; AstraZeneca, London, UK) ( $n = 30$ ) or saline ( $n = 30$ ) in the midluteal phase of their cycle, i.e. on day  $22.6 \pm 2.5$ . This timing adhered to the reproductive care guidelines for GnRHa use and allowed a matched timing of menstrual bleeding (placebo group) and withdrawal bleeding (GnRHa group), which enabled blinding. Follow-up (time point 2) was placed post-bleeding at a time point late enough to allow the GnRHa group to have entered their early ovarian suppression phase ( $16.2 \pm 2.6$  days after intervention). In a subgroup of the latter-enrolled 38 participants, an extra blood sample was also withdrawn in the initial stimulation phase of GnRHa (follow-up point 1 placed 3–5 days after GnRHa intervention) to capture peripheral features of the stimulatory phase of GnRHa treatment. Brain imaging, clinical rating with the HRSD-17 and collection of DNA was performed at baseline and at time point 2 exclusively (Fig. 1).

All participants gave written informed consent. The study was approved by the ethics committee (protocol identifier: H-2-2010-108), and registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) under the code NCT02661789.

### Experimental procedures

#### DNA and RNA collection

Samples were stored at  $-20^{\circ}\text{C}$ . RNA was isolated from peripheral blood collected in PAXgene tubes using the PAXgene Blood miRNA kit (PreAnalytiX, QIAGEN, Germany). RNA integrity and concentration were analysed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, US); the RNA integrity number (RIN) ranged from 6.6–9.4, with a mean (s.d.) RIN of 8.4 (0.4). The RNA samples were sent to the Max Planck Institute of Psychiatry to run on microarrays as previously described.<sup>11</sup> Illumina's GenomeStudio version 2011.1 with Methylation module 1.9.0 software (Illumina Inc, San Diego, CA, USA, [https://support.illumina.com/downloads/genomestudio\\_software\\_2011.html](https://support.illumina.com/downloads/genomestudio_software_2011.html)), with default Illumina settings, was used for the analysis.

#### Oestradiol concentrations

Serum oestradiol concentrations were determined as previously described.<sup>6</sup>

#### SERT imaging

Brain SERT binding was imaged by using [<sup>11</sup>C]DASB-PET based on 90-minute dynamic acquisition immediately after bolus injection. Further details on [<sup>11</sup>C]DASB imaging and quantification were previously described.<sup>6,12</sup>

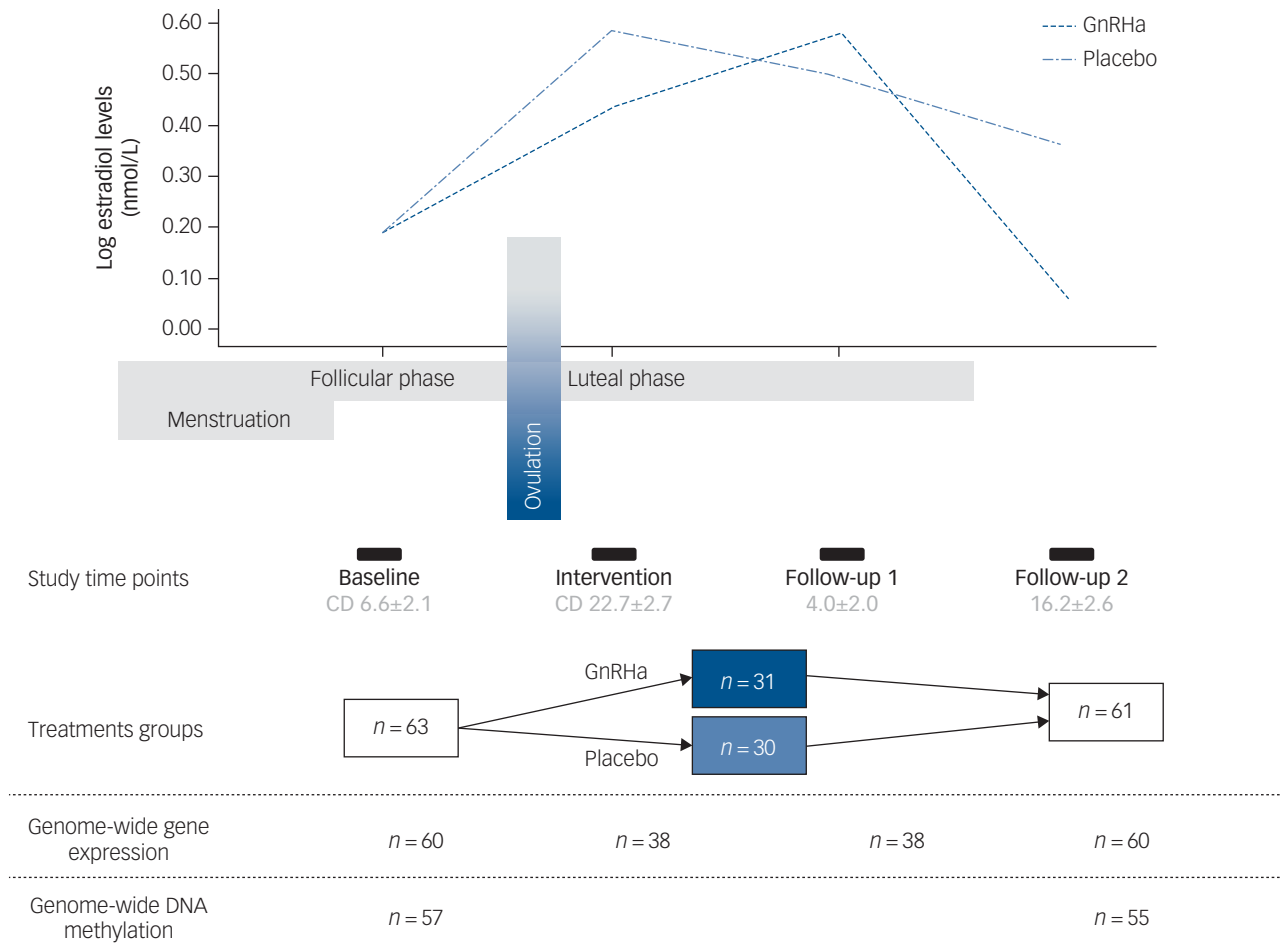
#### Statistical analyses

Raw data were exported using the Illumina Beadstudio program (Illumina Inc, San Diego, CA, USA, [https://support.illumina.com/downloads/genomestudio\\_software\\_2011.html](https://support.illumina.com/downloads/genomestudio_software_2011.html)) and loaded into R for downstream analysis (<http://www.R-project.org>). Samples with probe detection call rates less than 95% and those with an average intensity value of either under 50% of the experiment-wide sample mean or less than 2000 arbitrary units were excluded from further analysis.

Gene expression data were transformed and normalised using the variance stabilising normalisation.<sup>13</sup> Probes passing the filter criteria of Illumina probe detection  $P$ -value of less than 0.01 in at least one sample were used for subsequent analysis. We used the surrogate variable analysis (SVA) method to account for known and unknown factors such as batch effects by including them as covariates in the model, controlling for potential bias. After quality control and filtering, a total of 32 143 gene-expression probes were used for analysis.

For DNA methylation, the data were background and control normalised using the minfi package.<sup>14</sup> Subset-quantile Within-Array Normalisation was used to remove technical differences between Infinium-I and -II probes via the minfi package. The methylation status for each probe was recorded as a  $\beta$ -value ranging between 0 and 1. Probes where more than 50% of the samples had a detection  $P$ -value greater than 0.05, probes on the Y chromosome, and those with single-nucleotide polymorphisms within 50 bp from the query site were removed. This resulted in 482 227 CpG (5'-cysteine-phosphate-guanine-3') probes that were used for subsequent analysis.

Data were analysed using an established analysis pipeline comprising custom statistical programs and scripts<sup>15</sup> written in R and Linux. Surrogate variable analyses revealed seven significant SVA vectors which were used as covariates in the model to correct for technical artifacts and hidden confounders. Linear mixed-effects models were used to investigate differential gene expression and DNA methylation and to test their association with phenotypes as repeated measures, after correcting for the SVAs. Analysis-of-variance models were used to extract the  $P$ -values. As described previously,<sup>6</sup> oestradiol concentrations were log-transformed for



**Fig. 1** Study design depicting the different time points and samples available. The follow-up time point 1 depicts the early stimulatory phase of the gonadotrophin-releasing hormone agonist (GnRH $\alpha$ ) intervention, whereas time point 2 depicts the subsequent suppression phase. The line plot of the log oestradiol levels in the women is shown across the study at the different time points. The bars indicate time points, i.e. baseline assessment placed in follicular phase at cycle day (CD) 6.6  $\pm$  2.1 (mean  $\pm$  s.d.), intervention in luteal phase at cycle day 22.7  $\pm$  2.7, early follow-up (follow-up 1) at 4.0  $\pm$  2.0 days post-intervention and late follow-up (follow-up 2) at 16.2  $\pm$  2.6 days post-intervention.

analysis. For candidate analysis, we used the nominal  $P$ -value. The hypergeometric test was used to test for the enrichment and was performed in R. This test helps assess if the observation is indeed statistically significant, i.e. beyond what is expected by chance.

## Results

### Demographics

The groups were well matched demographically, i.e. age, body mass index and education, as well as for subclinical psychopathology and follicle-stimulating hormone levels ( $P > 0.05$ ).<sup>6</sup> Supplementary Table 1 available at <https://doi.org/10.1192/bjp.2018.234> shows the clinical profile. Details on genome-wide gene-expression and DNA-methylation samples available at different time points are depicted in Fig. 1.

### Gene expression and DNA methylation of the 116-gene PND panel in the GnRH $\alpha$ model

Post-GnRH $\alpha$ -induced gene-expression and DNA-methylation changes within the 116 PND genes (intervention group  $\times$  time)

To mirror the results from the third trimester of pregnancy,<sup>11</sup> we specifically assessed differences in gene expression between baseline

and the early post-GnRH $\alpha$  intervention time point (follow-up 1), which provides a model proxy for a hormone-stimulated phase. For the 116 PND transcripts, we tested if associations between changes in gene-expression profiles across baseline to early follow-up post-GnRH $\alpha$  induction were moderated by the intervention (intervention  $\times$  time point interaction). We observed that 22 of the 116 transcripts (19%) showed significant changes in gene expression ( $P < 0.05$ ) between baseline and early post-GnRH $\alpha$  intervention (follow-up 1) across the GnRH $\alpha$  and placebo group, this was significant relative to what would be expected by chance (1.7-fold enrichment,  $P = 0.02$ ). Of these, 10 transcripts also showed persistent changes in gene expression from baseline until the later post-GnRH $\alpha$  time point (follow-up 2). Among these 10 transcripts there were 6 (see Supplementary Table 4 for details) showing a significant change in this cohort at second follow-up; they were also in the same direction as in the PND cohort by Mehta *et al.*,<sup>11</sup> indicating that the gene-expression changes post-GnRH $\alpha$  intervention were persistent and had likely 'carry-over' effects from the stimulatory phase into the subsequent suppression or hormonal-withdrawal phase.

Next, we tested whether the GnRH $\alpha$  treatment also elicited changes in DNA methylation in loci encoding these 116 transcripts. DNA methylation was available at baseline and the later, second follow-up post-GnRH $\alpha$  intervention; hence the DNA-methylation differences represent longer-lasting changes post-GnRH $\alpha$  rather

than immediate changes. From the 116 transcripts, 98 had at least one CpG within 1.5 kb of the gene locus on the 450k DNA-methylation array available for analysis after quality control and filtering. For the 2756 CpG sites within these 98 transcripts, analysis was performed to identify those CpGs significant for intervention group  $\times$  time, using  $P < 0.05$ . DNA-methylation levels for the CpG sites within these genes were regressed against the group status (intervention/placebo)  $\times$  time (baseline to post-GnRHa intervention follow-up 2) ( $n = 60$  women). From those tested, 48 transcripts (49%, 208 CpG sites) had at least one CpG with  $P < 0.05$  (2.6-fold enrichment,  $P = 1.6 \times 10^{-5}$ ; Supplementary Table 5). The difference in DNA methylation from baseline to the later follow-up ranged from  $-4$  to  $+8\%$ , with 196 (95%) CpGs showing an increase in DNA methylation (average 4% [1%]) and 11 CpGs showing a decrease in methylation (average 3% [1%]). Among the 22 transcripts that had significant gene-expression changes, the majority ( $n = 18$ ) also had significant changes in DNA methylation (Table 1). Figure 2 depicts examples of gene-expression and DNA-methylation changes.

PND genes associated with oestradiol levels within the GnRHa treatment group

Our previous work demonstrated that GnRHa-triggered depressive symptoms were significantly associated with magnitude of oestradiol decline among the GnRHa-treated women.<sup>6</sup> To test if the observed changes in gene expression or DNA methylation were driven by fluctuations in oestradiol in response to GnRHa, i.e. proxy for sensitivity to GnRHa, we assessed the association between gene-expression/DNA-methylation changes and changes in oestradiol levels. In terms of gene-expression changes from baseline to early follow-up, 57 of the 116 transcripts (49%) were significantly associated with changes in oestradiol levels at the later follow-up, and 16 transcripts (14%) were associated with early oestrogen changes at the early follow-up (Table 2 and Supplementary Table 3). In terms of DNA-methylation, changes in 76 loci (66%,

368 CpG sites, twofold enrichment,  $P = 1.5 \times 10^{-4}$ ) were significantly associated with the change in oestradiol levels between baseline to late follow-up (Supplementary Table 2).

PND genes associated with HRSD-17 within the GnRHa treatment group

We had previously demonstrated that GnRHa intervention precipitated depressive symptoms in some women,<sup>6</sup> hence we tested if gene-expression/DNA-methylation changes between baseline and the two post-GnRHa time points were associated with changes in HRSD-17 scores. For 33 of the 116 transcripts (28%, twofold enrichment,  $P = 4.4 \times 10^{-7}$ ; Table 2) gene-expression changes between baseline and early follow-up were nominally significantly associated with changes in HRSD-17 scores to the early follow-up. For 18 of 116 genes (16%, fold enrichment  $P > 0.05$ ), early gene-expression changes were significantly associated with mood symptoms at the late follow-up (Table 2). In addition, the DNA-methylation changes from baseline to late follow-up of 77 transcripts (66%, 319 CpG sites) were also associated with changes in HRSD-17 scores from baseline to late post-GnRHa (follow-up 2) (twofold enrichment,  $P = 2.5 \times 10^{-4}$ ; Supplementary Table 2).

PND genes associated with SERT binding in neocortex within the GnRHa treatment group

In this cohort GnRHa induced increases in depressive scores, which were significantly associated with increases in neocortical SERT binding within the GnRHa group only in contrast to placebo.<sup>6</sup> We therefore tested whether neocortical SERT binding would also associate with gene-expression and DNA-methylation changes in these genes post-GnRHa treatment. We found that of the 116 PND genes, gene expression of 9 transcripts (8%) and 16 transcripts (14%) were associated with changes in SERT binding in neocortex from baseline to early and late post-GnRHa follow-up, respectively (Table 2). Similarly, DNA methylation of 52 transcripts (45%, 156 CpG sites) were also associated with changes in SERT binding in neocortex from baseline to late post-GnRHa (follow-up 2) (twofold enrichment,  $P = 9.9 \times 10^{-6}$ ; Supplementary Table 2).

**Table 1** A list of the 22 transcripts that had significant gene expression changes

Probe identifier	Gene symbol	<i>P</i> -value time point $\times$ status	Changes in DNA methylation (number of CpGs)
		Early follow-up	
ILMN_2208802	NPPI	$3e^{-03}$	2
ILMN_1695157	CA4	$4e^{-03}$	14
ILMN_1691432	PRDM4	$7e^{-03}$	12
ILMN_3239548	LOC100132740	$1e^{-02}$	
ILMN_1712035	TMEM115	$1e^{-02}$	12
ILMN_1775761	TSR1	$1e^{-02}$	1
ILMN_2147105	LOC440348	$1e^{-02}$	
ILMN_1681984	GALNT10	$1e^{-02}$	28
ILMN_1721344	MOBK2A	$2e^{-02}$	18
ILMN_1769135	DPP7	$2e^{-02}$	9
ILMN_2313901	PAM	$2e^{-02}$	15
ILMN_1720838	DECR1	$3e^{-02}$	10
ILMN_1805377	ZP3	$3e^{-02}$	21
ILMN_1711786	NFE2	$3e^{-02}$	6
ILMN_1753500	ARHGAP12	$3e^{-02}$	32
ILMN_1693394	BCKDK	$4e^{-02}$	12
ILMN_1899760	<sup>a</sup>	$4e^{-02}$	
ILMN_2094106	HSD17B12	$5e^{-02}$	19
ILMN_2355463	CYFIP1	$5e^{-02}$	49
ILMN_1663068	MGC33556	$5e^{-02}$	
ILMN_3238633	SDHAF2	$5e^{-02}$	2
ILMN_1779706	TP53BP2	$5e^{-02}$	18

Of these, 18 also had significant changes in DNA methylation after treatment with a gonadotrophin-releasing hormone agonist across the intervention and placebo groups. a. No gene symbol available.

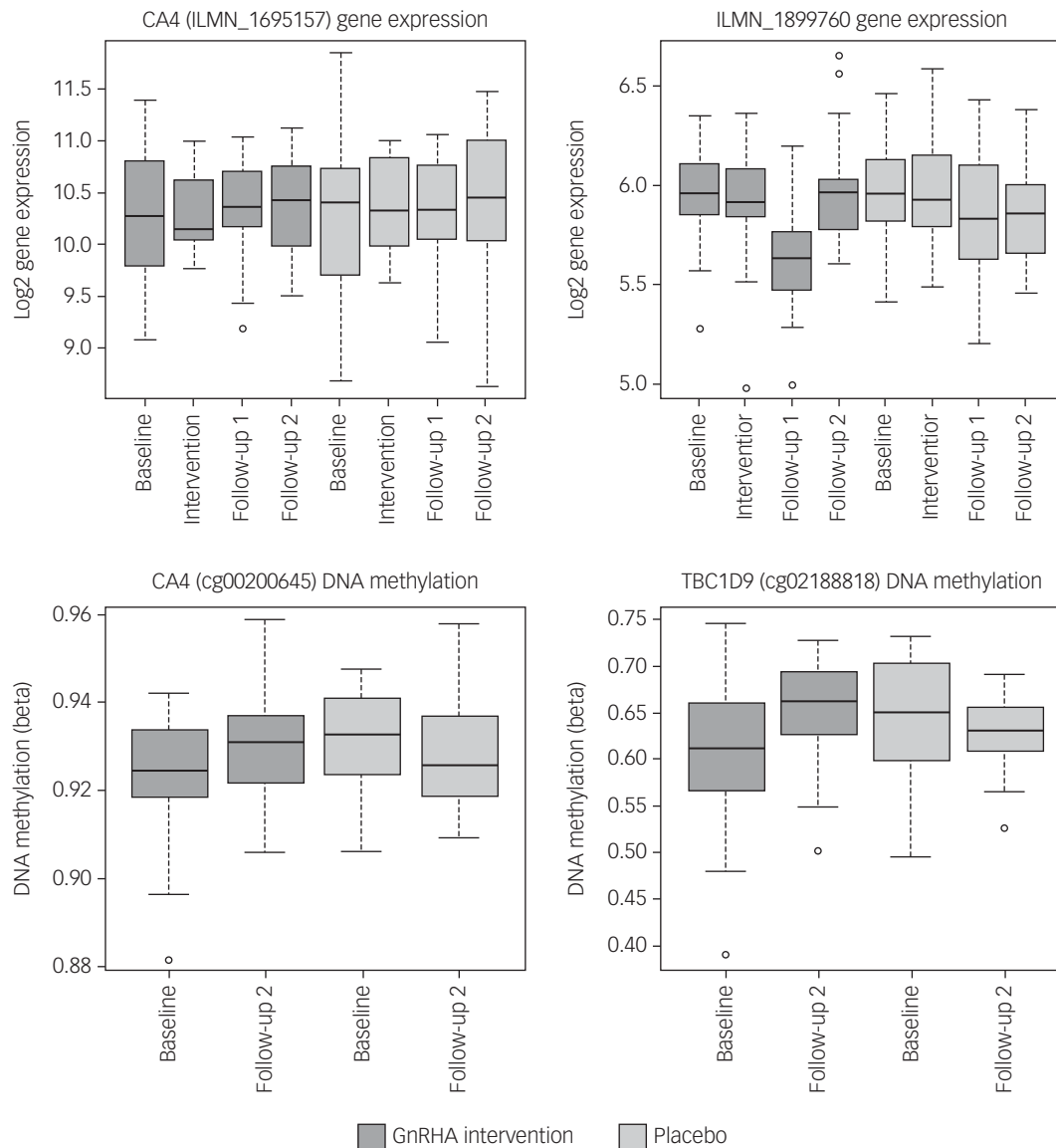
## Discussion

We provide the first direct human evidence of the role of oestrogen fluctuations and their biological consequences as a risk factor for development of hormone-induced depression.

We found (a) that the 116 PND genes were regulated over the time course of the GnRHa intervention on the gene-expression and DNA-methylation level, which correlated with GnRHa-induced changes in oestradiol; and (b) that their dynamic changes predicted changes in HRSD-17 score and SERT brain binding. Our data suggest that dynamic changes in gene expression and DNA methylation in peripheral blood of transcripts, which are regulated over pregnancy and are predictive of PND, also predict mood changes in a pharmacological model. Our results thus directly support a coupling of dynamic sensitivity to oestrogens on the molecular level and sensitivity to develop depressive symptoms across hormonal transitions.

Of the 116 PND-predictive transcripts, we found that a significant proportion of genes were also differentially expressed between baseline and early post-intervention (stimulation phase) across the GnRHa intervention relative to the placebo group. For a proportion of these transcripts, changes were still present at the later follow-up (16 days post-intervention), indicating more long-lasting gene-expression changes carried over from stimulation to the (early) hormonal suppression phase. The genes included NPPI, belonging to a family of a series of primate-specific nuclear pore complex-





**Fig. 2** Gene-expression and DNA-methylation changes post-GnRHa treatment. Top panel indicates gene-expression changes and bottom panel indicates DNA-methylation changes in 2 of the 116 PND genes. Left panel indicates both gene expression for CA4 ( $P = 0.04$ ) and the DNA methylation ( $P = 0.023$ ) that were significant. Right panel indicates other examples of an intergenic probe ( $P = 0.004$ ) that was significant at the level of gene expression (top) and DNA methylation for a CpG within the TBC1D9 gene (bottom) that was significant ( $P = 0.004$ ). ILMN\_1899760 was differentially expressed in the same direction as the previous PND study. CA4 and TBC1D9 showed both significant gene-expression and DNA-methylation changes.

interacting protein (NPIP) sequences with unknown function but which are well conserved from African apes to humans. Other genes included ZP3, whose protein is a structural component of the zona pellucida and functions in primary binding and induction of the sperm acrosome reaction. Mutations within the ZP3 gene have been reported to be involved in human fertility.<sup>16</sup> Furthermore, we observed that the extent of gene-expression changes in relationship to changes in oestradiol levels also associated with the occurrence of mood symptoms, suggesting that the transcripts identified to predict PND may more generally serve as dynamic biomarker for hormone-induced mood changes, even in a pharmacological model. This raises the question of whether this candidate biomarker can identify women who display oestrogen sensitivity, even outside the pregnancy-stimulated state. Future studies are warranted to test if short-term stimulation, e.g. of blood *ex vivo*, can unmask the biomarker. Among genes significantly associated with changes in depressive

symptoms and oestradiol were several genes related to the immune system such as *ILF3*, *IL10RB* and *IL18RAP*. Another gene was *LHPP* which encodes an enzyme known as phospholysine phosphohistidine inorganic pyrophosphate phosphatase. Polymorphisms in the *LHPP* gene together with functional interaction with serotonin receptor 1A (*HTR1A*) have been previously shown to be associated with MDD<sup>17</sup> and were associated with resting state brain activity in MDD.<sup>18</sup> Another interesting gene was the *ESRRA* gene encoding a nuclear receptor closely related to the oestrogen receptor. *ESRRA* is expressed in the central nervous system and is required for induction of mitochondrial biogenesis in the brain.<sup>19</sup> It may regulate multiple metabolic processes critical to neuronal function including mediating expression of brain-derived neurotrophic factor and other target genes, monoamine oxidase A and B, that regulate neurotransmitter metabolism and behaviour.

Oestrogen receptors serve as transcription factors by regulating expression of target genes<sup>8</sup> to mediate the physiological effects of

**Table 2** Gene expression changes associated with oestradiol, depressive symptoms and serotonin binding in neocortex within the 116 perinatal-depression transcripts

Probe identifier	Symbol	GnRHA HRSD <sup>a</sup>		GnRHA oestradiol <sup>b</sup>		GnRHA GlobNeoCort <sup>c</sup>	
		Early follow-up	Late follow-up	Early follow-up	Late follow-up	Early follow-up	Late follow-up
ILMN_1780937	MUS81	3.07e <sup>-05</sup>	2.41e <sup>-03</sup>	1.51e <sup>-03</sup>	7.64e <sup>-01</sup>	5.18e <sup>-01</sup>	6.54e <sup>-01</sup>
ILMN_2396148	HIP1R	4.69e <sup>-04</sup>	3.63e <sup>-01</sup>	1.21e <sup>-02</sup>	3.52e <sup>-01</sup>	6.93e <sup>-01</sup>	3.96e <sup>-01</sup>
ILMN_2387090	CGGBP1	1.11e <sup>-03</sup>	9.04e <sup>-01</sup>	2.46e <sup>-06</sup>	9.72e <sup>-01</sup>	9.22e <sup>-01</sup>	9.96e <sup>-01</sup>
ILMN_1698463	ILF3	2.31e <sup>-03</sup>	4.88e <sup>-02</sup>	3.24e <sup>-06</sup>	3.10e <sup>-01</sup>	1.36e <sup>-01</sup>	1.23e <sup>-01</sup>
ILMN_1740010	PCNX	2.41e <sup>-03</sup>	4.20e <sup>-01</sup>	1.22e <sup>-04</sup>	1.44e <sup>-02</sup>	5.09e <sup>-01</sup>	1.75e <sup>-02</sup>
ILMN_1699049	PMS2L3	2.71e <sup>-03</sup>	5.34e <sup>-01</sup>	3.05e <sup>-05</sup>	3.90e <sup>-01</sup>	9.50e <sup>-01</sup>	8.16e <sup>-01</sup>
ILMN_1712035	TMEM115	3.72e <sup>-03</sup>	4.92e <sup>-01</sup>	6.62e <sup>-06</sup>	2.31e <sup>-01</sup>	8.40e <sup>-01</sup>	3.41e <sup>-01</sup>
ILMN_2061732	YRDC	4.90e <sup>-03</sup>	1.54e <sup>-01</sup>	8.91e <sup>-06</sup>	1.34e <sup>-01</sup>	7.35e <sup>-01</sup>	4.22e <sup>-01</sup>
ILMN_1752199	LHPP	7.92e <sup>-03</sup>	9.49e <sup>-01</sup>	4.84e <sup>-05</sup>	5.51e <sup>-01</sup>	1.46e <sup>-01</sup>	2.91e <sup>-01</sup>
ILMN_1751615	COQ10B	8.56e <sup>-03</sup>	7.14e <sup>-01</sup>	8.91e <sup>-04</sup>	5.42e <sup>-01</sup>	6.58e <sup>-01</sup>	5.82e <sup>-02</sup>
ILMN_1663068	MGC33556	9.12e <sup>-03d</sup>	2.58e <sup>-02</sup>	1.20e <sup>-05</sup>	5.38e <sup>-01</sup>	2.83e <sup>-01</sup>	1.90e <sup>-01</sup>
ILMN_1683950	SNX11	1.19e <sup>-02</sup>	5.27e <sup>-02</sup>	1.69e <sup>-03d</sup>	6.14e <sup>-02</sup>	7.25e <sup>-01</sup>	8.72e <sup>-01</sup>
ILMN_1816713	<sup>e</sup>	1.25e <sup>-02</sup>	2.21e <sup>-01</sup>	1.11e <sup>-01</sup>	3.31e <sup>-01</sup>	5.20e <sup>-01</sup>	4.40e <sup>-01</sup>
ILMN_2358784	ASB3	1.31e <sup>-02d</sup>	9.05e <sup>-01</sup>	7.09e <sup>-06</sup>	2.22e <sup>-01</sup>	2.70e <sup>-01</sup>	8.45e <sup>-02</sup>
ILMN_1774272	ESRRA	1.35e <sup>-02</sup>	5.38e <sup>-01</sup>	2.92e <sup>-02d</sup>	6.63e <sup>-01</sup>	1.93e <sup>-01</sup>	3.09e <sup>-01</sup>
ILMN_2082314	TOM1	1.43e <sup>-02</sup>	1.12e <sup>-01</sup>	5.29e <sup>-03</sup>	1.03e <sup>-01</sup>	9.15e <sup>-01</sup>	2.64e <sup>-01</sup>
ILMN_2145280	DEF6	2.16e <sup>-02</sup>	3.02e <sup>-01</sup>	2.25e <sup>-04d</sup>	3.29e <sup>-01</sup>	2.00e <sup>-01</sup>	7.90e <sup>-01</sup>
ILMN_2378708	TAF6	2.49e <sup>-02</sup>	2.71e <sup>-01</sup>	6.95e <sup>-03d</sup>	8.78e <sup>-02</sup>	2.48e <sup>-01</sup>	8.62e <sup>-01</sup>
ILMN_1662848	TXNDC15	2.53e <sup>-02</sup>	7.44e <sup>-01</sup>	1.68e <sup>-04</sup>	2.25e <sup>-01</sup>	8.15e <sup>-01</sup>	8.61e <sup>-03d</sup>
ILMN_2230892	IL10RB	2.56e <sup>-02</sup>	2.05e <sup>-01</sup>	1.02e <sup>-04</sup>	1.18e <sup>-02d</sup>	7.17e <sup>-01</sup>	1.88e <sup>-01</sup>
ILMN_1749244	LYRM1	2.68e <sup>-02</sup>	1.78e <sup>-01</sup>	1.16e <sup>-02</sup>	1.77e <sup>-01</sup>	4.25e <sup>-01</sup>	7.41e <sup>-01</sup>
ILMN_1731596	AP3S2	2.73e <sup>-02</sup>	5.45e <sup>-01</sup>	6.69e <sup>-01</sup>	7.14e <sup>-02</sup>	1.58e <sup>-01</sup>	2.83e <sup>-01</sup>
ILMN_3251317	RER1	3.27e <sup>-02</sup>	6.37e <sup>-01</sup>	6.40e <sup>-04d</sup>	2.50e <sup>-01</sup>	8.82e <sup>-01</sup>	4.22e <sup>-01</sup>
ILMN_3232828	LOC728620	3.72e <sup>-02</sup>	7.49e <sup>-01</sup>	5.27e <sup>-05</sup>	2.65e <sup>-01</sup>	2.36e <sup>-01</sup>	1.95e <sup>-01</sup>
ILMN_1806999	B9D2	3.75e <sup>-02</sup>	9.72e <sup>-01</sup>	1.58e <sup>-05</sup>	1.86e <sup>-01</sup>	9.13e <sup>-01</sup>	5.37e <sup>-01</sup>
ILMN_1693394	BCKDK	3.85e <sup>-02</sup>	6.75e <sup>-01</sup>	2.08e <sup>-01</sup>	1.41e <sup>-02</sup>	3.04e <sup>-01</sup>	9.10e <sup>-01</sup>
ILMN_1724406	INO80E	4.03e <sup>-02d</sup>	3.01e <sup>-02</sup>	1.43e <sup>-04d</sup>	1.84e <sup>-01</sup>	5.09e <sup>-01</sup>	7.45e <sup>-01</sup>
ILMN_2208802	NPIP	4.27e <sup>-02d</sup>	7.07e <sup>-01</sup>	6.31e <sup>-08</sup>	1.74e <sup>-01</sup>	2.02e <sup>-01</sup>	6.30e <sup>-02</sup>
ILMN_1653266	DNAJB14	4.55e <sup>-02</sup>	5.99e <sup>-01</sup>	2.96e <sup>-02</sup>	3.44e <sup>-01</sup>	9.13e <sup>-01</sup>	8.02e <sup>-01</sup>
ILMN_1775761	TSR1	4.62e <sup>-02</sup>	5.59e <sup>-01</sup>	2.12e <sup>-03d</sup>	3.91e <sup>-01</sup>	2.43e <sup>-02</sup>	3.39e <sup>-02</sup>
ILMN_1721762	IL18RAP	4.75e <sup>-02</sup>	9.47e <sup>-01</sup>	1.28e <sup>-03</sup>	8.74e <sup>-02</sup>	9.69e <sup>-01</sup>	6.71e <sup>-01</sup>
ILMN_1720838	DECR1	4.86e <sup>-02d</sup>	1.94e <sup>-01</sup>	4.02e <sup>-05d</sup>	1.79e <sup>-01</sup>	5.87e <sup>-01</sup>	1.09e <sup>-01</sup>
ILMN_2143795	MGC4677	5.24e <sup>-02</sup>	3.92e <sup>-01</sup>	1.78e <sup>-01</sup>	4.12e <sup>-01</sup>	6.99e <sup>-03</sup>	4.37e <sup>-01</sup>
ILMN_1801246	IFITM1	5.49e <sup>-02</sup>	3.12e <sup>-01</sup>	1.83e <sup>-03</sup>	4.78e <sup>-01</sup>	9.18e <sup>-01</sup>	1.75e <sup>-01</sup>
ILMN_2344204	PRR13	6.29e <sup>-02</sup>	6.69e <sup>-02</sup>	2.61e <sup>-06</sup>	8.54e <sup>-03d</sup>	8.13e <sup>-01</sup>	2.68e <sup>-01</sup>
ILMN_3239548	LOC100132740	6.49e <sup>-02</sup>	2.09e <sup>-01</sup>	5.95e <sup>-02</sup>	1.44e <sup>-01</sup>	2.78e <sup>-01</sup>	6.09e <sup>-01</sup>
ILMN_1777765	C12orf10	6.99e <sup>-02</sup>	2.09e <sup>-01</sup>	1.10e <sup>-05</sup>	7.99e <sup>-01</sup>	7.61e <sup>-01</sup>	2.19e <sup>-01</sup>
ILMN_2230672	MRPL18	7.08e <sup>-02</sup>	6.69e <sup>-02</sup>	2.75e <sup>-01</sup>	8.72e <sup>-01</sup>	1.07e <sup>-01</sup>	2.91e <sup>-01</sup>
ILMN_1718303	PVRL2	7.32e <sup>-02</sup>	2.65e <sup>-04</sup>	6.09e <sup>-02</sup>	1.13e <sup>-03</sup>	5.41e <sup>-01</sup>	3.87e <sup>-02</sup>
ILMN_1744795	TBL1X	7.93e <sup>-02</sup>	1.03e <sup>-01</sup>	3.91e <sup>-02</sup>	7.52e <sup>-02</sup>	4.81e <sup>-01</sup>	8.46e <sup>-01</sup>
ILMN_1728844	PTPRN2	8.04e <sup>-02</sup>	4.56e <sup>-01</sup>	1.26e <sup>-01</sup>	9.32e <sup>-01</sup>	8.45e <sup>-01</sup>	2.72e <sup>-01</sup>
ILMN_1777881	TSPAN17	8.26e <sup>-02</sup>	7.97e <sup>-01</sup>	6.95e <sup>-05</sup>	8.02e <sup>-01</sup>	8.02e <sup>-01</sup>	7.10e <sup>-01</sup>
ILMN_1772329	LRRFIP2	8.41e <sup>-02</sup>	8.00e <sup>-01</sup>	2.30e <sup>-01</sup>	3.43e <sup>-01</sup>	7.91e <sup>-01</sup>	4.59e <sup>-01</sup>
ILMN_3248975	PPP4C	8.57e <sup>-02</sup>	7.58e <sup>-01</sup>	3.79e <sup>-01</sup>	8.64e <sup>-01</sup>	7.47e <sup>-01</sup>	6.65e <sup>-01</sup>
ILMN_1676010	SP1	8.66e <sup>-02</sup>	3.86e <sup>-02</sup>	3.98e <sup>-02d</sup>	1.27e <sup>-01</sup>	6.78e <sup>-01</sup>	7.51e <sup>-02</sup>
ILMN_1658883	ARAF	8.67e <sup>-02</sup>	8.65e <sup>-01</sup>	1.01e <sup>-03</sup>	4.23e <sup>-01</sup>	1.92e <sup>-01</sup>	3.71e <sup>-01</sup>
ILMN_1713636	S100A6	9.89e <sup>-02</sup>	3.83e <sup>-02</sup>	7.19e <sup>-03</sup>	1.11e <sup>-02</sup>	4.57e <sup>-01</sup>	9.61e <sup>-01</sup>
ILMN_2112357	CD300LF	1.05e <sup>-01</sup>	2.16e <sup>-01</sup>	3.68e <sup>-03d</sup>	2.06e <sup>-02d</sup>	3.64e <sup>-01</sup>	8.49e <sup>-01</sup>
ILMN_1701875	ZYX	1.08e <sup>-01</sup>	2.67e <sup>-01</sup>	1.76e <sup>-01</sup>	2.48e <sup>-02</sup>	3.99e <sup>-01</sup>	8.25e <sup>-01</sup>
ILMN_1683916	PEX13	1.13e <sup>-01</sup>	6.11e <sup>-01</sup>	3.47e <sup>-01</sup>	5.78e <sup>-01</sup>	8.57e <sup>-01</sup>	4.74e <sup>-01</sup>
ILMN_1691508	PLAUR	1.25e <sup>-01</sup>	3.90e <sup>-01</sup>	4.13e <sup>-06d</sup>	2.33e <sup>-01</sup>	5.86e <sup>-01</sup>	6.44e <sup>-01</sup>
ILMN_1668721	CCND3	1.30e <sup>-01</sup>	5.90e <sup>-02</sup>	2.46e <sup>-01</sup>	5.39e <sup>-01</sup>	4.93e <sup>-02</sup>	5.97e <sup>-01</sup>
ILMN_1676986	NPIP	1.31e <sup>-01</sup>	4.45e <sup>-01</sup>	8.40e <sup>-05</sup>	9.30e <sup>-01</sup>	5.55e <sup>-02</sup>	3.74e <sup>-02</sup>
ILMN_1768016	TNFRSF17	1.36e <sup>-01</sup>	1.84e <sup>-01</sup>	4.51e <sup>-02</sup>	3.38e <sup>-01</sup>	2.97e <sup>-02</sup>	1.99e <sup>-01</sup>
ILMN_1782377	LOC440354	1.37e <sup>-01</sup>	4.20e <sup>-01</sup>	1.75e <sup>-01</sup>	3.63e <sup>-01</sup>	8.63e <sup>-01</sup>	9.59e <sup>-01</sup>
ILMN_1728106	TNF	1.40e <sup>-01</sup>	8.40e <sup>-01</sup>	2.47e <sup>-02</sup>	8.01e <sup>-02</sup>	9.80e <sup>-01</sup>	6.45e <sup>-01</sup>
ILMN_1789535	DHDDS	1.42e <sup>-01</sup>	3.95e <sup>-01</sup>	6.21e <sup>-02</sup>	3.79e <sup>-01</sup>	4.42e <sup>-01</sup>	4.73e <sup>-01</sup>
ILMN_1755850	ZNF350	1.48e <sup>-01</sup>	5.68e <sup>-01</sup>	4.89e <sup>-01</sup>	6.76e <sup>-02</sup>	4.91e <sup>-01</sup>	3.08e <sup>-02</sup>
ILMN_3235168	MUL1	1.55e <sup>-01</sup>	7.46e <sup>-01</sup>	6.46e <sup>-01</sup>	9.36e <sup>-01</sup>	2.55e <sup>-01</sup>	7.76e <sup>-01</sup>
ILMN_1770977	TMEM134	1.58e <sup>-01</sup>	5.90e <sup>-01</sup>	2.92e <sup>-02d</sup>	9.52e <sup>-01</sup>	6.99e <sup>-02</sup>	1.53e <sup>-02</sup>
ILMN_1867321	<sup>e</sup>	1.66e <sup>-01</sup>	9.24e <sup>-01</sup>	2.73e <sup>-05</sup>	7.90e <sup>-01</sup>	2.97e <sup>-01</sup>	1.41e <sup>-01</sup>
ILMN_1772731	HAGH	1.90e <sup>-01</sup>	2.70e <sup>-02</sup>	5.56e <sup>-01</sup>	2.56e <sup>-02</sup>	5.39e <sup>-01</sup>	1.18e <sup>-01</sup>
ILMN_1673936	KHSRP	2.05e <sup>-01</sup>	8.25e <sup>-03</sup>	4.47e <sup>-01</sup>	5.76e <sup>-01</sup>	9.72e <sup>-03</sup>	2.24e <sup>-01</sup>
ILMN_1659843	DNAJC3	2.08e <sup>-01</sup>	3.05e <sup>-01</sup>	1.89e <sup>-01</sup>	4.35e <sup>-01</sup>	9.23e <sup>-01</sup>	9.37e <sup>-02</sup>
ILMN_1703891	TBC1D9	2.10e <sup>-01</sup>	4.27e <sup>-01</sup>	8.00e <sup>-01</sup>	4.67e <sup>-02</sup>	3.73e <sup>-01</sup>	6.82e <sup>-01</sup>
ILMN_1739508	LOC652493	2.11e <sup>-01</sup>	1.41e <sup>-02</sup>	1.79e <sup>-01</sup>	1.20e <sup>-01</sup>	6.86e <sup>-02</sup>	5.68e <sup>-02</sup>
ILMN_1695157	CA4	2.18e <sup>-01</sup>	1.21e <sup>-05</sup>	1.12e <sup>-02</sup>	4.68e <sup>-03</sup>	5.33e <sup>-01</sup>	4.45e <sup>-02</sup>
ILMN_1719661	SEPX1	2.20e <sup>-01</sup>	4.10e <sup>-01</sup>	4.41e <sup>-02</sup>	9.34e <sup>-01</sup>	1.90e <sup>-01</sup>	3.81e <sup>-01</sup>
ILMN_1671288	ODF2	2.25e <sup>-01</sup>	1.56e <sup>-01</sup>	5.87e <sup>-01</sup>	1.28e <sup>-01</sup>	9.55e <sup>-01</sup>	5.88e <sup>-01</sup>

(Continued)

Table 2 (Continued)

Probe identifier	Symbol	GnRHA HRSD <sup>a</sup>		GnRHA oestradiol <sup>b</sup>		GnRHA GlobNeoCort <sup>c</sup>	
		Early follow-up	Late follow-up	Early follow-up	Late follow-up	Early follow-up	Late follow-up
ILMN_1721344	MOBK2A	2.31e <sup>-01</sup>	6.30e <sup>-01</sup>	2.85e <sup>-01</sup>	7.12e <sup>-01</sup>	1.50e <sup>-01</sup>	2.39e <sup>-02</sup>
ILMN_1727984	F8A3	2.74e <sup>-01</sup>	7.01e <sup>-01</sup>	4.46e <sup>-01</sup>	6.12e <sup>-01</sup>	2.97e <sup>-01</sup>	9.07e <sup>-01</sup>
ILMN_1701603	ALPL	2.92e <sup>-01</sup>	2.58e <sup>-01</sup>	2.85e <sup>-01</sup>	1.00e <sup>+00</sup>	8.04e <sup>-02</sup>	7.94e <sup>-01</sup>
ILMN_1670801	MTR	3.00e <sup>-01</sup>	7.22e <sup>-01</sup>	4.35e <sup>-01</sup>	7.26e <sup>-01</sup>	1.93e <sup>-01</sup>	3.74e <sup>-01</sup>
ILMN_1795286	C6orf47	3.32e <sup>-01</sup>	9.29e <sup>-01</sup>	8.71e <sup>-01</sup>	8.81e <sup>-01</sup>	7.89e <sup>-01</sup>	6.35e <sup>-01</sup>
ILMN_1899760	<sup>e</sup>	3.45e <sup>-01</sup>	3.32e <sup>-01</sup>	1.80e <sup>-04</sup>	7.28e <sup>-01</sup>	3.75e <sup>-01</sup>	7.88e <sup>-01</sup>
ILMN_1758105	ZNF791	3.59e <sup>-01</sup>	3.48e <sup>-01</sup>	7.12e <sup>-01</sup>	3.53e <sup>-01</sup>	7.92e <sup>-02</sup>	6.50e <sup>-01</sup>
ILMN_1673933	LOC440341	4.10e <sup>-01</sup>	9.61e <sup>-01</sup>	6.97e <sup>-01</sup>	2.18e <sup>-01</sup>	6.11e <sup>-01</sup>	7.49e <sup>-01</sup>
ILMN_1708375	IRF1	4.22e <sup>-01</sup>	3.60e <sup>-01</sup>	3.85e <sup>-02d</sup>	3.45e <sup>-02</sup>	6.45e <sup>-01</sup>	5.13e <sup>-01</sup>
ILMN_1701331	UBE2M	4.27e <sup>-01</sup>	4.21e <sup>-03</sup>	2.35e <sup>-01</sup>	6.55e <sup>-02</sup>	8.08e <sup>-01</sup>	4.81e <sup>-02</sup>
ILMN_2098126	CCL5	4.28e <sup>-01</sup>	1.86e <sup>-03</sup>	3.32e <sup>-03</sup>	4.23e <sup>-02</sup>	1.60e <sup>-01</sup>	8.24e <sup>-02</sup>
ILMN_2279367	FCAR	4.38e <sup>-01</sup>	5.68e <sup>-01</sup>	2.94e <sup>-04</sup>	5.95e <sup>-01</sup>	3.08e <sup>-01</sup>	8.27e <sup>-01</sup>
ILMN_1795435	ZNF264	4.46e <sup>-01</sup>	7.49e <sup>-01</sup>	5.13e <sup>-01</sup>	8.06e <sup>-01</sup>	4.86e <sup>-01</sup>	4.07e <sup>-01</sup>
ILMN_1711786	NFE2	4.58e <sup>-01</sup>	2.24e <sup>-03</sup>	2.43e <sup>-03</sup>	7.74e <sup>-02</sup>	1.69e <sup>-01</sup>	1.23e <sup>-01</sup>
ILMN_2147105	LOC440348	5.25e <sup>-01</sup>	3.16e <sup>-01</sup>	3.29e <sup>-01</sup>	9.56e <sup>-01</sup>	1.45e <sup>-01</sup>	6.60e <sup>-02</sup>
ILMN_1738347	RNPEP	5.38e <sup>-01</sup>	2.73e <sup>-02</sup>	2.61e <sup>-01</sup>	3.16e <sup>-04</sup>	6.82e <sup>-01</sup>	7.59e <sup>-01</sup>
ILMN_3267670	LOC100130550	5.58e <sup>-01</sup>	1.93e <sup>-01</sup>	7.70e <sup>-02</sup>	1.97e <sup>-01</sup>	2.46e <sup>-01</sup>	1.29e <sup>-01</sup>
ILMN_2355463	CYFIP1	5.59e <sup>-01</sup>	3.16e <sup>-01</sup>	1.90e <sup>-01</sup>	6.66e <sup>-01</sup>	7.24e <sup>-01</sup>	5.62e <sup>-01</sup>
ILMN_1760174	MCCC1	5.67e <sup>-01</sup>	2.60e <sup>-01</sup>	9.48e <sup>-03d</sup>	1.85e <sup>-01</sup>	9.35e <sup>-01</sup>	5.96e <sup>-01</sup>
ILMN_2339779	ATP6V1E1	5.70e <sup>-01</sup>	8.16e <sup>-02</sup>	6.72e <sup>-01</sup>	2.43e <sup>-01</sup>	3.18e <sup>-01</sup>	1.43e <sup>-01</sup>
ILMN_1681984	GALNT10	6.13e <sup>-01</sup>	7.64e <sup>-01</sup>	9.30e <sup>-02</sup>	2.39e <sup>-01</sup>	3.46e <sup>-01</sup>	8.95e <sup>-01</sup>
ILMN_1893633	LOC439949	6.27e <sup>-01</sup>	5.88e <sup>-02</sup>	1.17e <sup>-01</sup>	2.74e <sup>-01</sup>	9.21e <sup>-01</sup>	4.01e <sup>-01</sup>
ILMN_1769135	DPP7	6.34e <sup>-01</sup>	9.87e <sup>-01</sup>	5.48e <sup>-01</sup>	1.19e <sup>-01</sup>	2.93e <sup>-02</sup>	2.02e <sup>-02</sup>
ILMN_3237241	FAM32A	6.50e <sup>-01</sup>	7.26e <sup>-01</sup>	3.47e <sup>-03</sup>	8.02e <sup>-01</sup>	3.14e <sup>-01</sup>	2.51e <sup>-03</sup>
ILMN_2150196	LRRC25	6.64e <sup>-01</sup>	1.00e <sup>+00</sup>	4.13e <sup>-01</sup>	3.77e <sup>-01</sup>	9.11e <sup>-01</sup>	2.59e <sup>-01</sup>
ILMN_3238633	SDHAF2	6.72e <sup>-01</sup>	1.30e <sup>-02</sup>	7.53e <sup>-01</sup>	1.98e <sup>-01</sup>	8.21e <sup>-01</sup>	5.32e <sup>-02</sup>
ILMN_1703337	LOC441763	6.74e <sup>-01</sup>	1.83e <sup>-01</sup>	1.88e <sup>-01</sup>	6.06e <sup>-03</sup>	4.82e <sup>-01</sup>	9.75e <sup>-01</sup>
ILMN_1776047	LOC646197	6.82e <sup>-01</sup>	4.07e <sup>-01</sup>	4.69e <sup>-04</sup>	7.63e <sup>-02</sup>	9.25e <sup>-01</sup>	5.77e <sup>-01</sup>
ILMN_1780546	OSM	7.18e <sup>-01</sup>	9.88e <sup>-02</sup>	1.43e <sup>-01</sup>	9.11e <sup>-02</sup>	6.34e <sup>-01</sup>	3.54e <sup>-01</sup>
ILMN_1691432	PRDM4	7.32e <sup>-01</sup>	2.56e <sup>-01</sup>	5.18e <sup>-01</sup>	7.26e <sup>-01</sup>	7.95e <sup>-01</sup>	1.17e <sup>-01</sup>
ILMN_2313901	PAM	7.61e <sup>-01</sup>	8.20e <sup>-02</sup>	7.33e <sup>-03</sup>	8.13e <sup>-02</sup>	9.88e <sup>-01</sup>	1.54e <sup>-01</sup>
ILMN_1753500	ARHGAP12	8.09e <sup>-01</sup>	2.14e <sup>-01</sup>	9.84e <sup>-02</sup>	7.70e <sup>-02</sup>	1.55e <sup>-02</sup>	4.06e <sup>-01</sup>
ILMN_1780334	KCNJ2	8.11e <sup>-01</sup>	5.34e <sup>-04</sup>	2.00e <sup>-01</sup>	8.88e <sup>-02</sup>	7.26e <sup>-03</sup>	2.96e <sup>-01</sup>
ILMN_1805377	ZP3	8.22e <sup>-01</sup>	6.91e <sup>-01</sup>	1.34e <sup>-01</sup>	7.51e <sup>-01</sup>	6.79e <sup>-02</sup>	6.14e <sup>-01</sup>
ILMN_1656621	CHMP2A	8.46e <sup>-01</sup>	2.40e <sup>-01</sup>	2.80e <sup>-02</sup>	1.34e <sup>-01</sup>	9.23e <sup>-01</sup>	5.52e <sup>-01</sup>
ILMN_1677446	TMEM189-UBE2V1	8.47e <sup>-01</sup>	6.32e <sup>-01</sup>	9.51e <sup>-01</sup>	8.72e <sup>-01</sup>	8.71e <sup>-01</sup>	4.63e <sup>-02</sup>
ILMN_2131493	VISA	8.55e <sup>-01</sup>	9.10e <sup>-01</sup>	4.63e <sup>-04</sup>	2.33e <sup>-01</sup>	3.78e <sup>-01</sup>	5.42e <sup>-02</sup>
ILMN_1657977	MSRB2	8.72e <sup>-01</sup>	7.88e <sup>-01</sup>	8.94e <sup>-01</sup>	8.83e <sup>-01</sup>	1.27e <sup>-01</sup>	1.27e <sup>-01</sup>
ILMN_1676005	KPNA1	8.74e <sup>-01</sup>	3.93e <sup>-01</sup>	9.71e <sup>-01</sup>	3.52e <sup>-01</sup>	3.61e <sup>-01</sup>	6.54e <sup>-01</sup>
ILMN_1784352	CCM2	9.28e <sup>-01</sup>	1.38e <sup>-01</sup>	7.52e <sup>-02</sup>	5.89e <sup>-01</sup>	2.67e <sup>-02</sup>	5.32e <sup>-01</sup>
ILMN_1779706	TP53BP2	9.32e <sup>-01</sup>	1.01e <sup>-01</sup>	2.51e <sup>-01</sup>	2.50e <sup>-02</sup>	5.76e <sup>-01</sup>	4.38e <sup>-02</sup>
ILMN_1665457	CLEC4C	9.47e <sup>-01</sup>	4.42e <sup>-01</sup>	1.19e <sup>-01</sup>	9.96e <sup>-01</sup>	1.98e <sup>-01</sup>	6.02e <sup>-01</sup>
ILMN_1675946	LOC401238	9.48e <sup>-01</sup>	9.26e <sup>-01</sup>	3.74e <sup>-01</sup>	2.70e <sup>-01</sup>	2.60e <sup>-01</sup>	6.22e <sup>-01</sup>
ILMN_1749612	LOC729101	9.49e <sup>-01</sup>	4.65e <sup>-01</sup>	1.09e <sup>-01</sup>	4.46e <sup>-01</sup>	6.14e <sup>-01</sup>	1.09e <sup>-01</sup>
ILMN_1754489	FBXL20	9.78e <sup>-01</sup>	7.40e <sup>-01</sup>	4.30e <sup>-01</sup>	6.15e <sup>-02</sup>	1.01e <sup>-01</sup>	3.63e <sup>-01</sup>
ILMN_2094106	HSD17B12	9.84e <sup>-01</sup>	5.59e <sup>-01</sup>	5.93e <sup>-01</sup>	1.33e <sup>-01</sup>	5.61e <sup>-01</sup>	4.03e <sup>-01</sup>
ILMN_1770290	CNN2	9.94e <sup>-01</sup>	5.80e <sup>-01</sup>	9.22e <sup>-01</sup>	5.29e <sup>-01</sup>	2.50e <sup>-01</sup>	9.81e <sup>-01</sup>

The results shown are from the treatment group. 'Early' indicates at early follow-up post-intervention with gonadotrophin-releasing hormone agonist; 'late' indicates the late post-intervention follow-up. GnRHA, gonadotrophin-releasing hormone agonist; HRSD, Hamilton Rating Scale for Depression; GlobCortNeo, global neocortex.

a. Indicates genes associated with changes in depressive symptoms.

b. Indicates genes associated with changes in oestradiol symptoms.

c. Indicates genes associated with changes in serotonin binding in the global neocortex.

d. Indicates gene-expression changes that are also associated in the placebo group.

e. No gene symbol available.

oestrogen. Oestrogen itself can also induce local changes in DNA methylation, which may lead to more lasting regulatory effects of changing sex-steroid levels. We found that changes in DNA methylation within the 116 target genes were even more pronounced than changes in gene expression, with a large proportion of genes differentially methylated from baseline to the post-GnRHa intervention time point. Both increases and decreases in DNA methylation were observed. Changes in DNA methylation over the intervention were associated with both changes in oestrogen levels and depressive symptoms. These results demonstrate that sex-steroid changes induce lasting changes in both gene expression and DNA methylation, and that DNA-methylation changes are more pervasive. Moreover, DNA-methylation changes that occur without concurrent gene-expression changes could be related to poised states of

these genes following GnRHa stimulation, reflecting an altered responsiveness to oestrogen once stimulated.

Inter-individual differences in dynamic changes of gene expression and DNA methylation that associated with a different susceptibility in hormone-induced mood changes could relate to genetic differences in oestrogen-sensitive enhancers of these target genes. In fact, we have previously shown that genetic variants that alter the transcriptional responsiveness to glucocorticoids in peripheral blood associate with an altered amygdala response in a threat task and an increased risk for depression as well as schizophrenia.<sup>20</sup> A different set of genetic variants located in oestrogen-responsive enhancers could be risk factors for oestrogen-induced mood changes. In addition, exposure to adverse life events, a known risk factor for the development of hormone-related mood disorders,<sup>21</sup>

has also been shown to associate with lasting changes in DNA methylation,<sup>22</sup> which could in turn shape future transcriptional and epigenetic responses.

One of the systems downstream of oestrogen activation is the serotonergic system and – intriguingly – human, rodent and non-human primate studies suggest that oestrogens might influence mood symptoms via effects on this system, as also recently demonstrated in humans.<sup>6</sup> Further support for this comes from molecular brain imaging studies that indicate a compromised monoamine brain signalling in the depressed state, including in postpartum depression,<sup>23</sup> and from the efficacy of selective serotonin reuptake inhibitors in PND,<sup>24</sup> a main class of antidepressants that selectively increase serotonin in the synapse. In line with our earlier findings,<sup>10</sup> we observed significant associations between gene-expression changes of the 116 PND genes (from baseline to early follow-up) with neocortical SERT binding, and several transcripts showed persistent associations with SERT binding even at the later follow-up. Similar, more pronounced, effects were observed for DNA-methylation changes. We speculate that during postpartum, sharply dropping oestrogen levels may further add to serotonergic deficiency and trigger other neurobiological changes, such as disturbed neurogenesis, that may increase risk for depression. Such effects, including oestrogen level-dependent changes in serotonergic signalling, are likely to be more accentuated in women with a heightened sensitivity to oestrogen as discussed previously.<sup>11</sup> These findings are interesting in light of a known crosstalk between sex hormones and neurotransmitter systems, thereby providing a potential mechanism for sex hormones to affect brain structures and functions that are central in the pathophysiology of depression.<sup>5</sup>

Given that our observations extended from PND to a pharmacological model, our set of biomarkers may also apply to other hormonal transition phases in women's lives, and thus could help characterise an important clinical subgroup of women at risk for hormone-induced depression. Future studies will be necessary to determine if these biomarkers can in fact identify such a subgroup and thus inform personalised prevention or treatment.

This study has several caveats. Although the longitudinal nature of the study is largely an advantage, a few samples had missing data, thereby reducing the power of the study. Replication of these findings in a larger cohort is warranted. Moreover, the gene-expression profiles were measured at baseline, intervention, early and late follow-up, whereas the DNA-methylation profiles were only measured at baseline and later follow-up, thereby restricting the interpretation of the DNA-methylation data and possibility to link changes in DNA methylation with changes in gene expression. Cell-count differences might confound some of our findings, but we have used the SVA method to account for known and unknown factors. Lastly, the GnRH $\alpha$  cohort displayed only subtle depressive symptoms, so one might speculate whether these clinical manifestations are relevant in a diagnostic context or whether they represent correlates of more non-pathological phenomena, such as postpartum blues or unspecific side effects. Nevertheless, the GnRH $\alpha$ -induced symptoms mapped onto the biomarker-associated states of a manifest depression with postpartum onset in the clinical cohort and, therefore, validate GnRH $\alpha$  exposure as a useful means of modelling mechanisms by which hormonal transition can trigger depressive episodes in certain sensitive women. Future studies in clinical populations must elucidate to what extent different diagnostic criteria for depressive episodes and onset specifiers across the peripartum can be discriminated by their gene-expression and epigenetic profiles. As for the function of the specific genes beyond being biomarkers for oestradiol sensitivity, the observed gene-expression signatures of GnRH $\alpha$  were derived from peripheral blood and therefore we interpret their potential direct effects in the central nervous system with caution.

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## Supplementary material

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## Psychiatry in history

### Ishaq Ibn Imran ... Constantine the African, *De Melancholia* ... *De Coitu* and Chaucer

Greg Wilkinson

Ishaq Ibn Imran, a physician in the city of Kairouan, in what is now Tunisia, produced a treatise on melancholy around AD 900. Omrani *et al*<sup>1</sup> say it is the oldest-known surviving manuscript dedicated entirely to melancholia that includes manic-depressive illness; it claims that melancholy could be due to hereditary and environmental influences and that 'Melancholy affects the soul through fear and sadness – the worst thing that can befall it. Sadness is defined by the loss of what one loves; fear is the expectation of misfortune'. They conclude that 'in many ways, our current views differ little from our medieval forebears'. Treatments comprise over half of the material.

Constantinus Africanus (AD 1015–1098) is one of the most influential medical scholars of the Middle Ages and a key figure in the revival of scientific medicine. In 1077, he arrived in Salerno, southeast of Naples, where he was linked with the *Schola Medica Salernitana*, the first European medical school. From Tunis, he brought manuscripts of the masters of Arabic medicine, which as a monk at Monte Cassino he translated and modified into an opus of Latin texts cited under his name. These circulated throughout Europe to the 17th century, laying the foundations for medical curricula. As an example of his reach, *De Melancholia* was copied at the priory of Kirkham, Yorkshire, in the mid-12th century. All that aside, Constantine is accused of plagiarism, and *De Melancholia* comprises parts of Ishaq Ibn Imran's treatise.

Constantine wrote *De Melancholia* because it was a malady prevalent 'in these regions': those intent on study and books of philosophy are especially liable to melancholy because of their scientific investigations, their tired memories and the failure of their minds, as are those who lose their beloved possessions, such as their children and dearest friends or some precious thing which cannot be restored. Many religious persons fall into this disease from their fear of God and contemplation of the Last Judgement: they become drunk, as it were, with excessive anxiety and vanity.

Treatments of contemporary charm include a magnetic mountain on the shore of the Indian Ocean that draws all the iron nails out of passing ships. Constantine recounts that Rufus of Ephesus says the magnet comforts those afflicted with melancholy and removes their fears and suspicions. Today, so it is claimed, transcranial magnetic stimulation uses magnetic fields to stimulate nerve cells in the brain to improve symptoms of depression.

Chaucer names Constantine in his *Prologue to The Canterbury Tales* (1387–1400) and in *The Merchant's Tale* as a source of aphrodisiacs:

'For he had many potions, drugs as fine  
As those that monk, accursed Constantine,  
Has numbered in his book *De Coitu*.  
He drank them all; not one did he eschew'

*De Coitu* is Constantine's translation of a treatise on sexual intercourse and fertility by Ibn al-Jazzar.

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