Distinctive gene expression profile in women with history of postpartum depression

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ABSTRACT

Postpartum depression (PPD) is a disease which incorporates a variety of depressive states differing in nature and severity. To assist in the understanding of the pathogenesis of the disease, we aimed to ascertain a molecular mechanism underlying PPD development. We applied microarray technology to characterize gene expression of euthymic women with a history of PPD and compared the results with healthy controls. Our study demonstrated that women who considered euthymic on a clinical level, in fact, had an altered molecular profile when compared to participants with no PPD history. We identified nine genes significantly distinguished expression in post-depressive women; they may serve as a diagnostic tool for the detection of a predisposition to PPD. Our findings contribute significantly to the understanding of PPD etiology and its pathogenesis, offer a plausible explanation for the risk of the PPD recurrence, and may also contribute to clinical treatment.

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1. Introduction

Postpartum depression (PPD) is a mood disorder emerging during the antenatal period and up to the end of the postnatal year [1]. PPD incorporates a variety of depressive and anxiety states varying in nature and severity. With respect to its clinical manifestations, postpartum depression is similar to depression during any other period in life. However, PPD is more complicated in terms of its potential effect on the health of the baby, the maternal-child bond, infant development, and familial interpersonal relationships [2–3].

Although the specific etiology of PPD remains unclear, several psychosocial and biological factors have been associated with the disease [4]. Psychosocial factors include a history of depression or anxiety during pregnancy, stressful life events or changes during pregnancy, inadequate social support, a history of a psychiatric disorder and, possibly, nicotine use [5–6].

There are also a number of possible physiological indicators of PPD that have been identified over the previous decade: decreased noradrenaline or serotonin activity in the brain [7], decreased Omega-3 fatty acids [8], 25(OH) Vitamin D [9], fluctuating oxytocin [10] and IL-1beta levels [11]. There is also strong evidence that abnormalities in the activity of the hypothalamic–pituitary-adrenal axis (HPA) play an important role in the etiology of major depressive disorders, as well as in postpartum depression [12–13]. The mechanism involved appears to be an altered response to stress and the inability to maintain its regulation [14]. In addition, a strong correlation between GABA receptor alteration and appearance of depressive symptoms in knock-out mice has been identified [15].

Of late, the contribution of genetic and epigenetic factors to the development of prenatal depression has been evaluated [16–17]. A recent study shows that estradiol- mediated DNA methylation change was associated with PPD risk [18].

Yet, with all the aforementioned data, we are still quite far from a complete understanding of PPD pathology. Why do some recuperate from birth with relative ease, while others develop psychiatric imbalance? Early identification of a predisposition to PPD and subsequent well-timed preventive psychological or/and medical treatment, could prevent disease development before it harms the mother, infant and family unit.

Microarray methodology enables the screening of thousands of genes at once; it allows for the identification of pathological modification both on the genetic and transcriptional levels. Peripheral mononuclear cells have been shown as an appropriate cellular model to investigate the molecular mechanism of psychiatric disorders [19–20]. Another study applied the microarray technology on mononuclear

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cells sampled from women immediately after labor and found a distinctive gene expression signature among nine women that developed PPD a short time after birth [21]. These data provide important evidence for molecular modifications in PPD patients.

However, the fact that the mononuclear cells were obtained so soon after delivery creates some disadvantages, as birth is associated with a wide range of chemical alterations triggered by stress and inflammation. This phenomenon can potentially distort the unique modifications in molecular pathways which are responsible for depression development. It is for this reason that we examined the molecular profiling of mononuclear cells obtained from women that had been diagnosed previously for PPD, but were considered euthymic at the time of the study.

The aims of this study were to clarify the pathogenesis of PPD and to develop a possible diagnostic tool for early recognition of the disease. Early identification of PPD predisposition may potentially prevent or offer future relief from this illness.

2. Material and methods

2.1. Recruitment

Subjects meeting the inclusion criteria for this study were identified at primary care and psychiatric clinics. Inclusion criteria were healthy women of reproductive age who had been previously diagnosed with PPD of at least mild severity, but had completely recovered from the disease (PD group). Women presenting with a diagnosis of any additional psychiatric disorder as well as women with positive family history of psychiatric disorders were excluded from the study. Subjects who were taking any medication were also excluded. The control group (Controls), recruited from local health clinics. consisted of healthy age-matched women, whom had given birth in the past and had no personal or family history of mental disorders. Eventually, analysis was conducted on six women in the PD group and 10 in the Control group.

In order to ensure that no women participating in the study had depression even of a mild nature, each participant completed the Zung Self-Rating Depression Scale [22], a 20 item self-administered questionnaire widely used as a screening tool, to identify symptoms associated with depression. Scores above 50, indicates that depression is absent. The questionnaire was translated into Hebrew and validated for those participants who weren't literate in English.

Written informed consent was obtained from the participants, in full compliance with the ethics committee requirement of the sponsoring institution.

2.2. Blood samples assessment and RNA extraction

Venous blood was collected and PBMC were freshly isolated using the Ficoll gradient method (Ficoll-Paque™ Plus; GE Healthcare), according to the manufacturer protocol. RNA was extracted from the isolated cells (RNeasy Mini Kit, Qiagen) and the integrity of the RNA samples was confirmed using Agilent Bioanalyzer 2100 system (Agilent Technologies). RNA samples were kept at -80oC for further microarray analysis.

2.3. The mRNA expression profiles of differentially expressed genes

To identify target genes that may be affected in PD participants, a comparative DNA microarray expression analysis was performed, using RNA extracted from their mononuclear blood cells. The results were compared with controls. Ten RNA samples obtained from the control women were pooled together. The RNA samples of 6 PD women were hybridized with the controls as follow: each one of the three PD RNA samples (1–3) was hybridized with the pooled RNA from the controls. The other 3 PD (4–6) RNA samples were pooled together and hybridized with the pooled RNA from the control samples likewise. The schematic illustration of the procedure is presented in Fig. 1. The use of a pooling design was decided after consulting with the
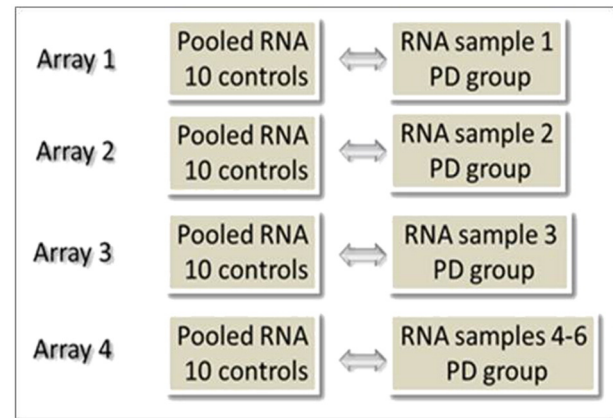
Fig. 1. Schematic description of the microarray analysis design. The RNA samples of 6 PD women hybridized with the controls; each one of the three PD RNA samples (1–3) was hybridized with the pooled RNA from the controls. 4–6 PD RNA samples were pooled together and hybridized with the pooled RNA from the total control samples.

biostatisticians of the Genomic Data Analysis Unit of the Hebrew University in Jerusalem. We specifically utilized the pooling design in order to reduce the effects of biological variation and to minimize differences due to subject-to-subject variation, making substantive features easier to find. This is a desirable choice, as our primary interest was not specific individuals but rather the general characteristics of the two distinct populations (PD versus controls).

In order to assure that the average expression level of a gene in the pool would not differ significantly from the average of the expression levels of the gene in the contributing samples, we made sure to take equal amounts of RNA from each sample.

Two hundred ng of total RNA for each sample was amplified and labeled with a fluorescent dyes (Cy3 or Cy5) using the Low RNA Input Linear Amplification & Labeling kit (Agilent Technology). The quality of the resulting labeled cRNA was measured using a Nanodrop ND-100 spectrophotometer. The Cy3 or Cy5 labeled cRNA was hybridized to the Agilent Whole Human Genome Oligo Microarray V2 (design 026652, Agilent Technologies, USA) for 17 hours at 65 °C in an Agilent DNA-Microarray Hybridization Oven. The arrays were later washed using the Gene Expression Wash Buffer Kit. The Microarray was scanned using the Agilent High-Resolution Microarray Scanner and the data was extracted from the resulting images using the Feature Extraction 10.7 software.

2.4. Array data analysis

Four microarray chips prepared from cDNA of PD samples compared with controls were color coded using cy3 and cy5 dyes. The data from the scanner were in the GPR format and we used the F635–B635 and F32–B532 column data. In each of the four microarrays, we performed flooring on the two columns setting to 20 values below that value (maximum values of those columns are around 65,000). We then performed a lowess normalization on the data using a 0.1 scale sliding window (plots (1/2) log2 of the product of the DataX and DataY intensities versus log2 of the intensity ratios). The corrected values from the four arrays created from the new intensity and ratio values (new_cy5 ¼ 2. ^ (intensity þ ratio/2); new_cy3 ¼ 2. ^ (- intensity-ratio/2) resulted in eight columns of data, four for the controls and four for the PD samples. We obtained p-values for all the genes using the t-test which we then used to create a volcano scatter plot showing significance versus gene expression ratio (fold change) of our data. To verify the statistical significance of our microarray results, we used the Stanford SAM package [23] that uses False Discovery Rates (FDRs) and a p-value, as described in