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Letter to the Editor
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Letter to the Editor

A molecular tool for menstrual cycle phase dating of endometrial samples in endometriosis transcriptome studies†

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Summary Sentence

Transcriptome profiling of 57 endometrial receptivity genes specifies the menstrual cycle phase of endometrial samples.

Key words: endometriosis, endometrium, menstrual cycle.

Dear Editor,

Here we report the usability of a panel of transcriptomic markers to determine the menstrual cycle phase of undated endometrial tissue samples for gene expression studies. Endometrial tissue transcriptomic studies are an important approach to find molecular characteristics and biomarkers of endometriosis and other endometrium-related diseases. However, endometrial gene expression is under strict hormonal control and the menstrual cycle phase-specific signature has to be considered in molecular studies of reproductive age women to avoid false-positive or -negative findings that may occur if studied individuals are from different menstrual cycle phases. The endometrial specimens' collection is generally well tolerated by patients: however, unnecessary procedures can be avoided if archival well-preserved RNA samples are available for research. Still, the use of archival samples may be complicated if there is no accompanying menstrual cycle information or only patients' self-reported menstrual cycle day is available, and no tissue has been left for histological evaluation and classification of samples. Although the self-reported menstrual cycle history has been extensively used in molecular studies, the length of the normal menstrual cycle varies between 24 and 35 days and thus self-reported menstrual history or calendar-based counting methods are insufficient to accurately determine menstrual cycle phase, or discriminate ovulatory cycles from anovulatory cycles [1]. Ponnampalam et al. [2] utilized high-throughput microarray technology and demonstrated that classification of the endometrial samples according to the global transcriptional profile is concordant with the histological evaluation. However, as global expression profiling is rather costly, we aimed to use a new cost-effective Targeted Allele Counting by sequencing (TAC-seq) methodology [3] to explore the capability of a panel of 57 well-described endometrial receptivity genes [4] to determine the exact molecular menstrual cycle phases of endometrial samples.

For that purpose, RNA was extracted from endometrial tissue samples collected from 45 women with and 33 women without endometriosis (suffering from pelvic pain or infertility) in menstrual (M, cycle days 1–5, n = 4), proliferative (P, cycle days 6–14, n = 17),

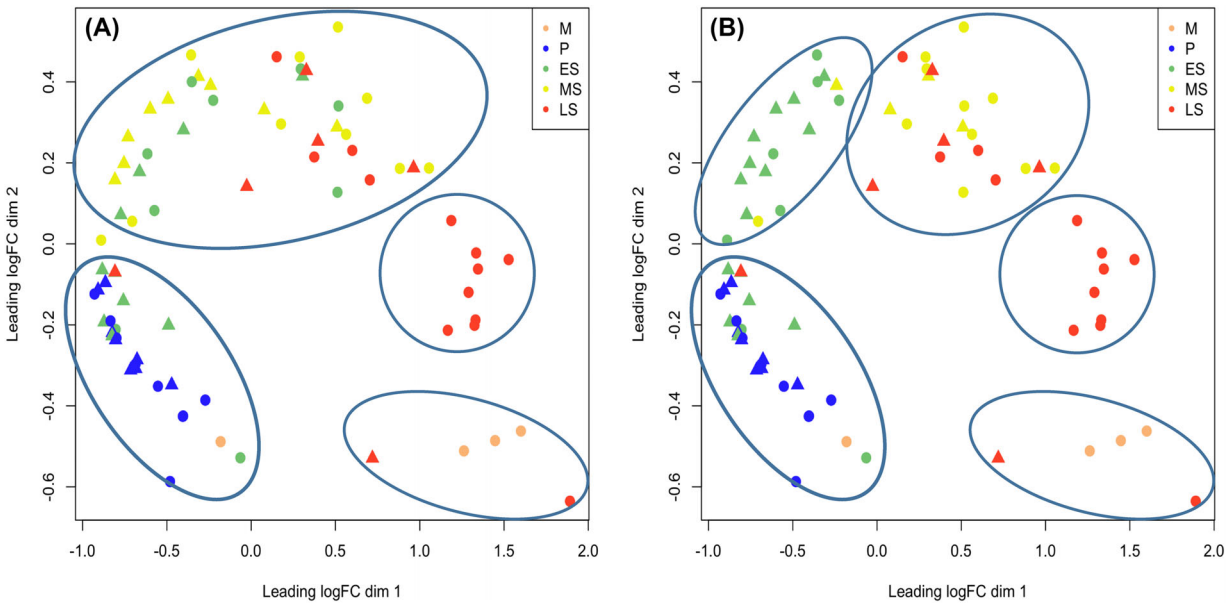

Figure 1. Multidimensional scaling plot of normalized RNA sequencing data of 57 endometrial receptivity genes in women with and without endometriosis. (A) Clustering analysis of RNA sequencing data. (B) Clustering after applying support vector machine classifier to ES and MS phase samples. P—proliferative, ES—early secretory, MS—mid-secretory, LS—late secretory, M—menstrual phase endometrial samples. Triangles represent women without endometriosis and circles mark women with endometriosis.

early-secretory (ES, cycle days 15–18, n = 19), mid-secretory (MS, cycle days 19–23, n = 19), and late-secretory (LS, cycle days 24–28, n = 19) phases according to the self-reported menstrual cycle days (Supplementary Table S1 and Supplementary Materials and Methods). The average age of women with and without endometriosis was 31.0 ± 4.7 and 32.0 ± 5.1 years, respectively, and they had not received any hormonal treatments for at least 3 months prior to the laparoscopy in Tartu University Hospital (Tartu, Estonia). The TAC-seq libraries were sequenced with NextSeq 500/550 v2.5 Kit (Illumina). Sequencing data analysis was performed as described previously [3], and each sample was normalized using geometric mean of gene expression levels of four housekeeper genes. The same sequencing protocol was applied to 54 paired endometrial samples from 27 healthy parous women, collected at the histologically and biochemically [predicted from the luteinizing hormone (LH) peak in urine] confirmed ES and MS cycle phases (described in [5]). The resulting data were used to create a machine learning support vector machine (SVM) model for discrimination of ES and MS phase samples.

Multidimensional scaling plot of normalized RNA sequencing data showed that expression pattern of the 57 endometrial receptivity genes divided the samples roughly into four distinct groups (Figure 1A). Also, no clear segregation was seen between women with and without endometriosis, which is concordant with a recent study by Garcia-Velasco et al. [6]. All endometrial samples from P phase clustered together and a subset of LS phase samples (n = 8) formed a distinct cluster; however, several samples from LS phase (n = 8) were more similar to MS samples and two LS samples grouped together with M phase samples. A similar phenomenon was described by Ponnampalam et al. [2], who suggested that the menstrual cycle is a continuum and the samples from the boarders of cycle phases may cluster to the adjacent phases. Interestingly, one LS sample showed similar gene expression pattern to P samples. We hypothesized that expression of the receptivity-related genes in anovulatory cycles remains similar to P phase throughout the cycle. Although the data about the endometrial receptivity-specific gene expression signature in women with anovulatory menstrual cycles is scarce, the level of glycodelin, which normally increases considerably and stays elevated during the secretory phase, has been shown to remain low throughout the anovulatory cycle [7]. The *PAEP* gene encoding glycodelin was also among the 57 genes analyzed in the current study and its low level in this LS sample was comparable to P samples, supporting our assumption about anovulatory cycle.

Furthermore, ES and MS samples formed one diffuse cluster (Figure 1A), indicating that self-reported menstrual cycle day does not allow reliable distinction between samples from these adjacent phases. Thereafter, the SVM model was successfully applied to segregate the studied self-reported ES and MS samples (Figure 1B) according to the receptivity gene expression pattern in endometrial tissues from women in biochemically confirmed ES and MS phases. After adjustment, 4 out of 19 ES phase samples were re-classified as MS samples and 9 MS samples were re-classified as ES, showing that molecular profiling helped to assign the endometrial samples from adjacent phases correctly even without precise chronological dating. The most widely used method to assign the endometrial samples collected at the second half of the cycle to ES or MS phase is determination of the LH peak from urine, which correlates significantly better with the histological dating than the calculations based on the onset of the next menstrual period [8]. However, as collection of tissue samples for research is for ethical reasons usually combined with clinical procedures that are scheduled long in advance, it is difficult if not impossible to obtain specimens at the particular LH day. Furthermore, the value of histological dating has been questioned as there are too many confounding factors influencing the interpretation of the results [9]. Therefore, new molecular tools, such as described in the current report, are useful to help specify the precise menstrual cycle phase of not only archived endometrial RNA samples but also of endometrial samples from uncertain cycle

phases in transcriptomic studies to facilitate the discovery of true disease-related markers.

Supplementary data

Supplementary data are available at *BIOLRE* online.
Supplementary Table S1. General characteristics of the study participants.

Conflict of interest

The authors have declared that no conflict of interest exists.

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