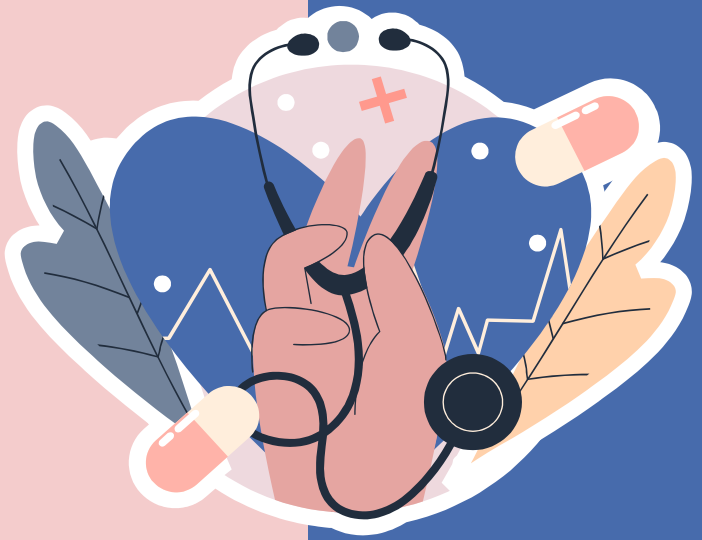
MicroRNA Sequencing of Saliva with Endometriosis

Anna Sim, Elise Kim, Manya Jain, Julian Saldana



Introduction

Title

A Bioinformatics Approach to MicroRNA-Sequencing Analysis Based on Human Saliva Samples of Patients with Endometriosis

Authors Sofiane Bendifallah[1, 2, 3], Yohann Dabi[1, 2, 3] , Stéphane Suisse[4], Ludmila Jornea[5], Delphine Bouteiller[6], Cyril Touboul[1, 2, 3], Anne Puchar[1], Emile Daraï[1, 2]

Importance

- Non-invasive
- easier to collect
- higher stability, reproducibility, and accessibility

Published

International Journal of Molecular Sciences on July 21, 2022

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Question Is there a bioinformatics approach for microRNA-sequencing analysis of saliva samples for miRNAome expression that can accurately diagnose endometriosis?

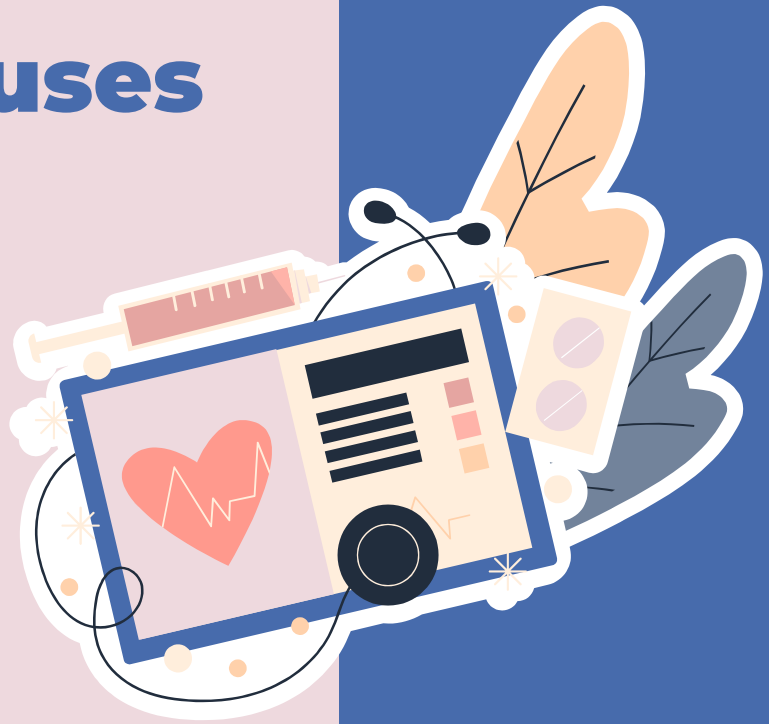
Hypothesis There is a bioinformatics approach for microRNA-sequencing using saliva samples that can accurately diagnose endometriosis.

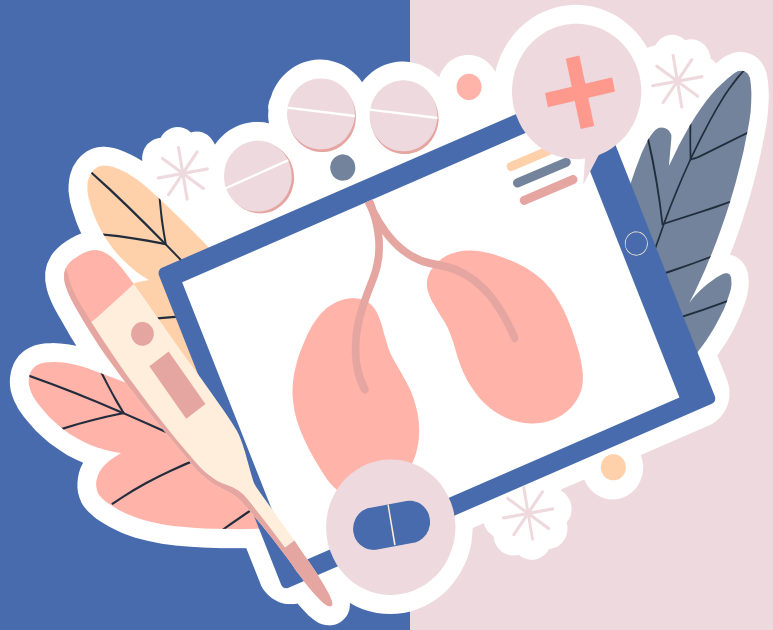
BACKGROUND

Endometriosis is a disease where tissue representing the lining of the uterus appears outside of the uterus. Can cause inflammation, scar tissue, etc.

microRNA and its uses

- miRNA is a region of highly conserved non-coding RNA
  - Regulate gene expression
- Reported regulation of oncogenes or tumor suppressor genes
- Used to help identify biomarkers in relation to cancer/disease





Why saliva samples?

It is a non-invasive approach to diagnosing endometriosis, if achievable. Forgoing blood samples and painful travel for patients to enter into a hospital and take a test for diagnosis.

Methods - Data Collection & Analysis

Sample Collection

Obtained 200 saliva samples using DNA Genetek's all-in-one at-home kit OME-505 (NCT04728152)

RNA Preparation

Isolated using miRNeasy kit and centrifuged

RNA Sequencing Libraries

Prepared with QIAseq miRNA Library Kit

Sequencing

Performed with the Novaseq6000 sequencer

Methods - Bioinformatics

Sequencing Preprocessing & Quality Control

- Trimmed using Cutadapt
- Aligned with Bowtie and MiRDeep2 package
- Assessed using FastQC software v0.11.7

Differential Expression Analysis of miRNA

- Quantified with miRDeep2
- Tests conducted in DESeq2
- Z-score normalization used for expressed miRNAs
- Corrected p-value < 0.05

Diagnostic Accuracy Analysis

- ROC analysis performed
- ROC AUC calculated
- Chi^2 test for their categorical variables

Supplementary: TABLE 2

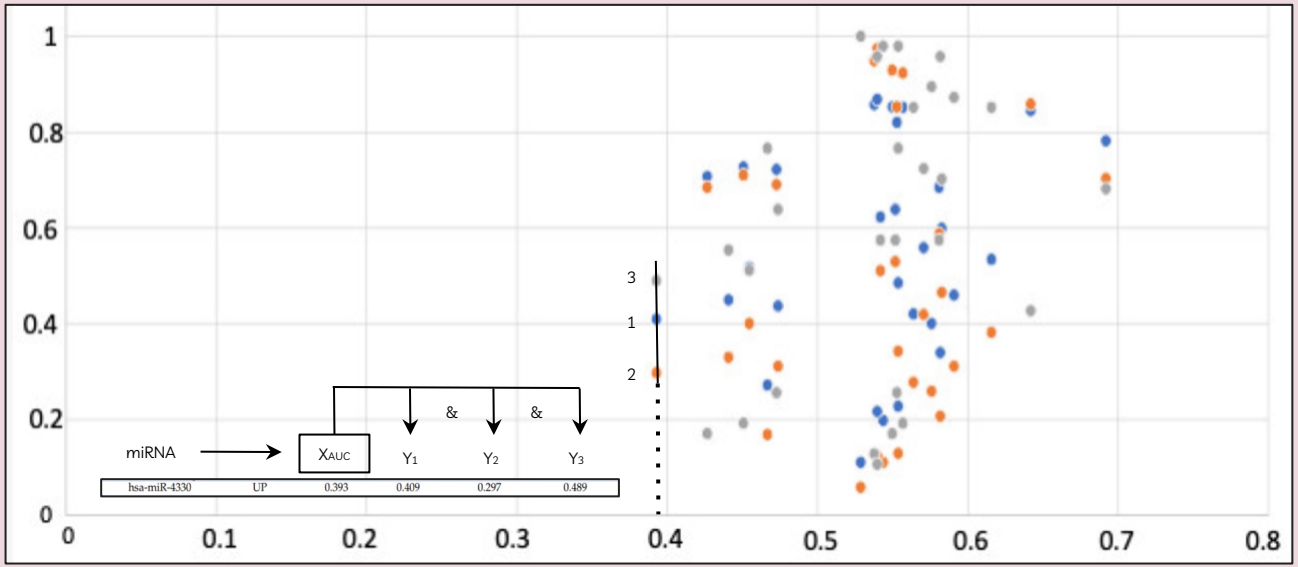
Diagnostic metrics for endometriosis for differentially expressed miRNAs in the saliva samples (n = 30)

miRNA	Regulation	AUC	F1-Score	Sensitivity	Specificity
hsa-let-7a-5p	UP	0.473	0.721	0.69	0.255
hsa-let-7i-5p	UP	0.451	0.726	0.71	0.191
hsa-miR-101-3p	UP	0.554	0.227	0.129	0.979
hsa-miR-103a-3p	UP	0.582	0.339	0.206	0.957
hsa-miR-142-3p	UP	0.529	0.11	0.058	1
hsa-miR-146a-5p	UP	0.538	0.857	0.948	0.128
hsa-miR-15a-5p	UP	0.571	0.558	0.419	0.723
hsa-miR-16-5p	UP	0.427	0.707	0.684	0.17
hsa-miR-199a-3p	UP	0.467	0.271	0.168	0.766
hsa-miR-203b-5p	UP	0.474	0.436	0.31	0.638
hsa-miR-205-5p	UP	0.544	0.197	0.11	0.979
hsa-miR-223-3p	UP	0.455	0.517	0.4	0.511
hsa-miR-23a-3p	UP	0.54	0.216	0.123	0.957
hsa-miR-23b-3p	UP	0.564	0.42	0.277	0.851
hsa-miR-24-3p	UP	0.542	0.622	0.51	0.574
hsa-miR-26a-5p	UP	0.54	0.868	0.974	0.106
hsa-miR-29a-3p	UP	0.552	0.638	0.529	0.574
hsa-miR-29c-3p	UP	0.441	0.449	0.329	0.553
hsa-miR-3191-3p	UP	0.55	0.852	0.929	0.17
hsa-miR-34c-5p	UP	0.642	0.844	0.858	0.426
hsa-miR-378a-3p	UP	0.554	0.484	0.342	0.766
hsa-miR-4330	UP	0.393	0.409	0.297	0.489
hsa-miR-4516	UP	0.581	0.684	0.587	0.574
hsa-miR-4677-3p	UP	0.692	0.781	0.703	0.681
hsa-miR-4754	UP	0.553	0.82	0.852	0.255
hsa-miR-4778-5p	UP	0.583	0.598	0.465	0.702
hsa-miR-523-5p	DOWN	0.576	0.4	0.258	0.894
hsa-miR-655-5p	UP	0.616	0.534	0.381	0.851
hsa-miR-6726-5p	UP	0.557	0.851	0.923	0.191
hsa-miR-6738-3p	DOWN	0.591	0.459	0.31	0.872

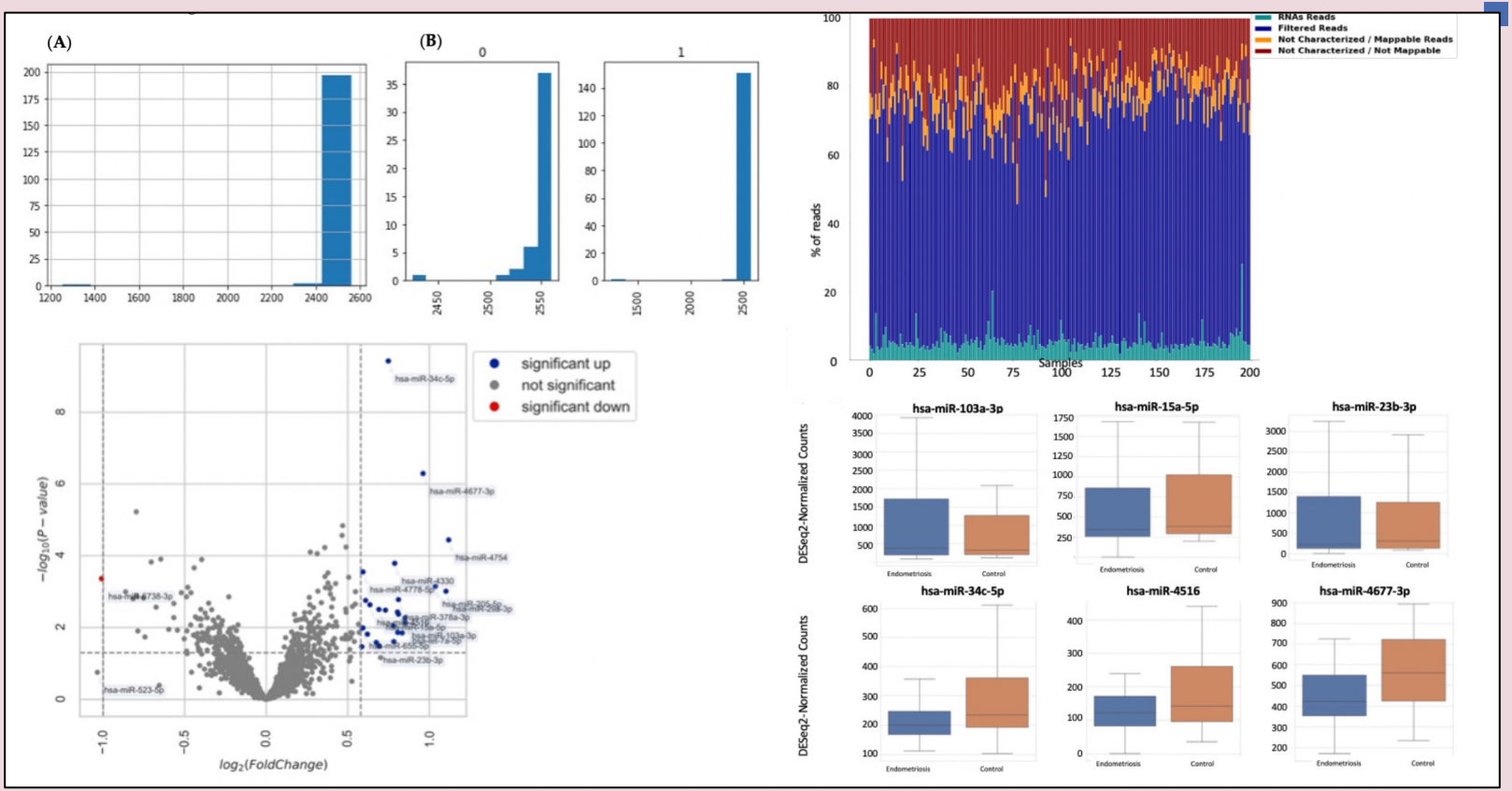
KEY FIGURE 5

Clustering of differentially expressed miRNAs in the saliva samples (n=30)

Y-axis: Values of
F1-Score
Sensitivity
Specificity



X-axis: AUC value


(A)
(B)
0
1
RNAs Reads
Filtered Reads
Not Characterized / Mappable Reads
Not Characterized / Not Mappable
% of reads
Samples
significant up
not significant
significant down
hsa-miR-34c-5p
hsa-miR-4677-3p
hsa-miR-4754
hsa-miR-4330
hsa-miR-4778-5p
hsa-miR-378a-3p
hsa-miR-103a-3p
hsa-miR-23b-3p
hsa-miR-523-5p
$-log_{10}(P-value)$
$log_2(FoldChange)$
hsa-miR-103a-3p
hsa-miR-15a-5p
hsa-miR-23b-3p
hsa-miR-34c-5p
hsa-miR-4516
hsa-miR-4677-3p
DESeq2-Normalized Counts
Endometriosis
Control

Authors' Conclusions vs Readers' Conclusion

The goal of the manuscript:

Develop a new bioinformatics approach

Present the results of the analysis:

30 miRNA sequences are up or down regulated

Analyze the validity and reliability of the new technique:

A biomarker of endometriosis has not been explored before

Draw conclusions:

Further studies and external validation are needed

The goal of the manuscript:

Understand new bioinformatics approach

Understand the results of the analysis:

3 miRNA had an AUC score higher than 0.6

Analyze the validity and reliability of the new technique:

The methodology and results can be questionable

Draw conclusions:

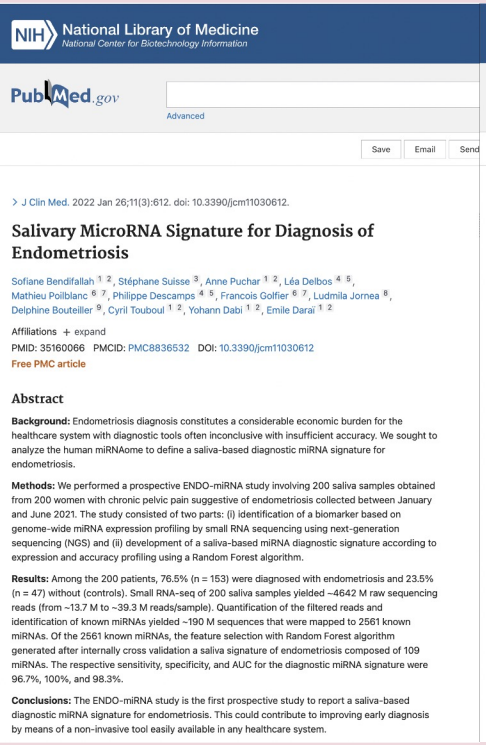
If proven to be valid and reliable, the method can revolutionize the modern diagnostic techniques

Limitations

- First report on bioinformatics approach using saliva to test diagnose of endometriosis
    - No scientifically proven salivary biomarkers for endometriosis have been studied before
        - miRNA is highly susceptible to degradation

          and can vary from tissue to tissue and even change during the menstrual cycle phase of the patients with endometriosis

Future Directions



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J Clin Med. 2022 Jan 26;11(3):612. doi: 10.3390/jcm11030612.

Salivary MicroRNA Signature for Diagnosis of Endometriosis

Sofiane Bendifallah [1 2], Stéphane Suisse [3], Anne Puchar [1 2], Léa Delbos [4 5], Mathieu Poilblanc [6 7], Philippe Descamps [4 5], Francois Golfier [6 7], Ludmila Jornea [8], Delphine Bouteiller [9], Cyril Touboul [1 2], Yohann Dabi [1 2], Emile Daraï [1 2]

Affiliations + expand

PMID: 35160066 PMCID: PMC8836532 DOI: 10.3390/jcm11030612

Free PMC article

Abstract

Background: Endometriosis diagnosis constitutes a considerable economic burden for the healthcare system with diagnostic tools often inconclusive with insufficient accuracy. We sought to analyze the human miRNAome to define a saliva-based diagnostic miRNA signature for endometriosis.

Methods: We performed a prospective ENDO-miRNA study involving 200 saliva samples obtained from 200 women with chronic pelvic pain suggestive of endometriosis collected between January and June 2021. The study consisted of two parts: (i) identification of a biomarker based on genome-wide miRNA expression profiling by small RNA sequencing using next-generation sequencing (NGS) and (ii) development of a saliva-based miRNA diagnostic signature according to expression and accuracy profiling using a Random Forest algorithm.

Results: Among the 200 patients, 76.5% (n = 153) were diagnosed with endometriosis and 23.5% (n = 47) without (controls). Small RNA-seq of 200 saliva samples yielded ~4642 M raw sequencing reads (from ~13.7 M to ~39.3 M reads/sample). Quantification of the filtered reads and identification of known miRNAs yielded ~190 M sequences that were mapped to 2561 known miRNAs. Of the 2561 known miRNAs, the feature selection with Random Forest algorithm generated after internally cross validation a saliva signature of endometriosis composed of 109 miRNAs. The respective sensitivity, specificity, and AUC for the diagnostic miRNA signature were 96.7%, 100%, and 98.3%.

Conclusions: The ENDO-miRNA study is the first prospective study to report a saliva-based diagnostic miRNA signature for endometriosis. This could contribute to improving early diagnosis by means of a non-invasive tool easily available in any healthcare system.

January 26, 2022

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Diagnostics (Basel). 2022 May 5;12(5):1150. doi: 10.3390/diagnostics12051150.

Endometriosis Associated-miRNome Analysis of Blood Samples: A Prospective Study

Sofiane Bendifallah [1 2], Yohann Dabi [1 2], Stéphane Suisse [3], Léa Delbos [4], Mathieu Poilblanc [5], Philippe Descamps [4], Francois Golfier [5], Ludmila Jornea [6], Delphine Bouteiller [7], Cyril Touboul [1 2], Anne Puchar [8], Emile Daraï [1 2]

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PMID: 35626305 PMCID: PMC9140062 DOI: 10.3390/diagnostics12051150

Free PMC article

Abstract

The aim of our study was to describe the bioinformatics approach to analyze miRNome with Next Generation Sequencing (NGS) of 200 plasma samples from patients with and without endometriosis. Patients were prospectively included in the ENDO-miRNA study that selected patients with pelvic pain suggestive of endometriosis. miRNA sequencing was performed using an Novaseq6000 sequencer (Illumina, San Diego, CA, USA). Small RNA-seq of 200 plasma samples yielded ~4228 M raw sequencing reads. A total of 2633 miRNAs were found differentially expressed. Among them, 8.6% (*n* = 229) were up- or downregulated. For these 229 miRNAs, the F1-score, sensitivity, specificity, and AUC ranged from 0-88.2%, 0-99.4%, 4.3-100%, and 41.5-68%, respectively. Utilizing the combined bioinformatic and NGS approach, a specific and broad panel of miRNAs was detected as being potentially suitable for building a blood signature of endometriosis.

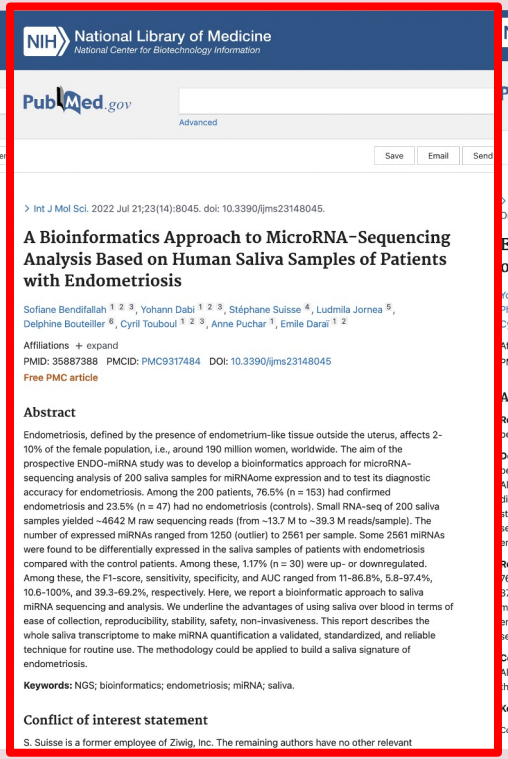
Keywords: NGS; bioinformatics; endometriosis; miRNA.

Conflict of interest statement

S. Suisse is a former employee of Ziwig, Inc. The remaining authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Figures

May 5, 2022



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Int J Mol Sci. 2022 Jul 21;23(14):8045. doi: 10.3390/ijms23148045.

A Bioinformatics Approach to MicroRNA-Sequencing Analysis Based on Human Saliva Samples of Patients with Endometriosis

Sofiane Bendifallah [1 2 3], Yohann Dabi [1 2 3], Stéphane Suisse [4], Ludmila Jornea [5], Delphine Bouteiller [6], Cyril Touboul [1 2 3], Anne Puchar [1], Emile Daraï [1 2]

Affiliations + expand

PMID: 35887388 PMCID: PMC9317484 DOI: 10.3390/ijms23148045

Free PMC article

Abstract

Endometriosis, defined by the presence of endometrium-like tissue outside the uterus, affects 2-10% of the female population, i.e., around 190 million women, worldwide. The aim of the prospective ENDO-miRNA study was to develop a bioinformatics approach for microRNA-sequencing analysis of 200 saliva samples for miRNAome expression and to test its diagnostic accuracy for endometriosis. Among the 200 patients, 76.5% (n = 153) had confirmed endometriosis and 23.5% (n = 47) had no endometriosis (controls). Small RNA-seq of 200 saliva samples yielded ~4642 M raw sequencing reads (from ~13.7 M to ~39.3 M reads/sample). The number of expressed miRNAs ranged from 1250 (outlier) to 2561 per sample. Some 2561 miRNAs were found to be differentially expressed in the saliva samples of patients with endometriosis compared with the control patients. Among these, 1.17% (n = 30) were up- or downregulated. Among these, the F1-score, sensitivity, specificity, and AUC ranged from 11-86.8%, 5.8-97.4%, 10.6-100%, and 39.3-69.2%, respectively. Here, we report a bioinformatic approach to saliva miRNA sequencing and analysis. We underline the advantages of using saliva over blood in terms of ease of collection, reproducibility, stability, safety, non-invasiveness. This report describes the whole saliva transcriptome to make miRNA quantification a validated, standardized, and reliable technique for routine use. The methodology could be applied to build a saliva signature of endometriosis.

Keywords: NGS; bioinformatics; endometriosis; miRNA; saliva.

Conflict of interest statement

S. Suisse is a former employee of Ziwig, Inc. The remaining authors have no other relevant

July 21, 2022

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Reprod Biomed Online. 2022 Sep 27;S1472-6483(22)00714-3. doi: 10.1016/j.rbmo.2022.09.019. Online ahead of print.

Endometriosis-associated infertility diagnosis based on saliva microRNA signatures

Yohann Dabi [1], Stéphane Suisse [2], Anne Puchar [3], Léa Delbos [4], Mathieu Poilblanc [5], Philippe Descamps [4], Julie Haury [3], Francois Golfier [5], Ludmila Jornea [6], Delphine Bouteiller [7], Cyril Touboul [1], Emile Daraï [8], Sofiane Bendifallah [9]

Affiliations + expand

PMID: 36411203 DOI: 10.1016/j.rbmo.2022.09.019

Abstract

Research question: Can a saliva-based miRNA signature for endometriosis-associated infertility be designed and validated by analysing the human miRNome?

Design: The prospective ENDOmiARN study (NCT04728152) included 200 saliva samples obtained between January 2021 and June 2021 from women with pelvic pain suggestive of endometriosis. All patients underwent either laparoscopy, magnetic resonance imaging, or both. Patients diagnosed with endometriosis were allocated to one of two groups according to their fertility status. Data analysis consisted of identifying a set of miRNA biomarkers using next-generation sequencing, and development of a saliva-based miRNA signature of infertility among patients with endometriosis based on a random forest model.

Results: Among the 153 patients diagnosed with endometriosis, 24% (n = 36) were infertile and 76% (n = 117) were fertile. Small RNA-sequencing of the 153 saliva samples yielded approximately 3712 M raw sequencing reads (from ~13.7 M to ~39.3 M reads/sample). Of the 2561 known miRNAs, the feature selection method generated a signature of 34 miRNAs linked to endometriosis-associated infertility. After validation, the most accurate signature model had a sensitivity, specificity and area under the curve of 100%.

Conclusion: A saliva-based miRNA signature for endometriosis-associated infertility is reported. Although the results still require external validation before using the signature in routine practice, this non-invasive tool is likely to have a major effect on care provided to women with endometriosis.

Keywords: Artificial intelligence; Endometriosis; Infertility; Machine learning; MiRNA signature.

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September 27, 2022

THANKS!

Do you have any questions?

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