



HAL
open science

New class of RNA biomarker for endometriosis diagnosis: The potential of salivary piRNA expression

Yohann Dabi, Stéphane Suisse, Yannick Marie, Léa Delbos, Mathieu Poilblanc, Philippe Descamps, Francois Golfier, Ludmila Jornea, Sylvie Forlani, Delphine Bouteiller, et al.

► **To cite this version:**

Yohann Dabi, Stéphane Suisse, Yannick Marie, Léa Delbos, Mathieu Poilblanc, et al.. New class of RNA biomarker for endometriosis diagnosis: The potential of salivary piRNA expression. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 2023, 291, pp.88-95. 10.1016/j.ejogrb.2023.10.015 . hal-04444857

HAL Id: hal-04444857

<https://hal.sorbonne-universite.fr/hal-04444857>

Submitted on 7 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**New class of RNA biomarker for endometriosis diagnosis :
the potential of salivary piRNA expression**

Yohann Dabi (MD) ^{1,2}, Stéphane Suisse ^{3,7}, Yannick Marie, Léa Delbos (MD) ^{4,5}, Mathieu Poilblanc (MD) ^{6,7}, Philippe Descamps (MD, PhD) ^{4,5}, Francois Golfier (MD, PhD) ^{6,7}, Ludmila Jornea (Msc) ⁸, Sylvie Forlani (MD) ⁸, Delphine Bouteiller (MD) ⁹, Cyril Touboul (MD, PhD) ^{1,2}, Anne Puchar (MD) ^{1,2}, Sofiane Bendifallah (MD, PhD) ^{1,2}, Emile Daraï (MD, PhD) ^{1,2}

1. Department of Obstetrics and Reproductive Medicine, Hôpital Tenon, 4 rue de la Chine, 75020 Paris.
2. Clinical Research Group (GRC) Paris 6: Centre Expert Endométriose (C3E), Sorbonne University (GRC6 C3E SU).
3. Ziwig, 19 rue Reboud, Lyon
4. Department of Obstetrics and Reproductive Medicine - CHU d'Angers.
5. Endometriosis Expert Center - Pays de la Loire
6. Department of Obstetrics and Reproductive Medicine, Lyon South University Hospital, Lyon Civil Hospices
7. Endometriosis Expert Center - Steering center of the EndAURA Network
8. Sorbonne Université, Paris Brain Institute - Institut du Cerveau - ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France.
9. Gentoyping and Sequencing core facility, iGenSeq, Institut du Cerveau et de la Moelle épinière, ICM, Hôpital Pitié-Salpêtrière, 47-83 Boulevard de l'Hôpital, 75013 Paris, FR.

Corresponding author :

Yohann DABI, MD

Tenon Hospital, 4 rue de la Chine, 75020 Paris, France

Mail : yohann.dabi@gmail.com

Tel : 01 56 01 70 00

Abstract

Objectives: In contrast to miRNA expression, little attention has been given to piwiRNA (piRNA) expression among endometriosis patients. The aim of the present study was to explore the human piRNAome and to investigate a potential piRNA saliva-based diagnostic signature for endometriosis.

Methods: Data from the prospective “ENDOMiRNA” study (ClinicalTrials.gov Identifier: NCT04728152) were used. Saliva samples from 200 patients were analyzed in order to evaluate human piRNA expression using the piRNA bank. Next Generation Sequencing (NGS), barcoding of unique molecular identifiers and both Artificial Intelligence (AI) and machine learning (ML) were used. For each piRNA, sensitivity, specificity, and ROC AUC values were calculated for the diagnosis of endometriosis.

Results: 201 piRNAs were identified, none had an AUC ≥ 0.70 , and only three piRNAs (piR-004153, piR001918, piR-020401) had an AUC between ≥ 0.6 and < 0.70 . Seven were differentially expressed: piR-004153, piR-001918, piR-020401, piR-012864, piR-017716, piR-020326 and piR-016904. The respective correlation and accuracy to diagnose endometriosis according to the F1-score, sensitivity, specificity, and AUC ranged from 0-0.862 %, 0-0.961%, 0.085-1, and 0.425-0.618. A correlation was observed between the patients' age (≥ 35 years) and piR-004153 ($p = 0.002$) and piR-017716 ($p = 0.030$). Among the 201 piRNAs, four were differentially expressed in patients with and without hormonal treatment: piR-004153 ($p = 0.015$), piR-020401 ($p = 0.001$), piR-012864 ($p = 0.036$) and piR-017716 ($p = 0.009$).

Conclusion: Our results support the link between piRNAs and endometriosis physiopathology and establish its utility as a potential diagnostic biomarker using saliva samples. Per se, piRNA expression should be analyzed along with the clinical status of a patient.

Keywords: endometriosis; non-coding RNA, piwiRNA; non-invasive biomarker;

Introduction

Endometriosis, defined by the presence of endometrial-like tissue outside the uterus, is thought to affect from 2-10% of women of reproductive age. However, its exact incidence – especially in adolescent and menopausal patients [1,2] and in asymptomatic patients [3] – has not been fully assessed. From a pathogenesis perspective, endometriosis is a multifactorial disease with genetic and epigenetic controls involving multiple pathways such as cell proliferation, cell differentiation, cell adhesion, apoptosis, angiogenesis, steroidogenesis, inflammatory and immune responses, oncogenic suppressors, as well as exposome factors such as persistent organic pollutants [4]. However, despite numerous investigations of these various pathways, the pathophysiology of endometriosis remains incompletely understood.

Recently, numerous studies have focused on the role of non-coding RNAs in the pathophysiology of endometriosis and their potential as diagnostic biomarkers [5–7]. In contrast to miRNAs, that have been extensively investigated in biofluids including blood and saliva [7–9], to our knowledge no study to date has investigated the expression of PIWI-interacting RNAs (piRNA) in patients with endometriosis. Similarly, no data are available about piRNAs as potential diagnostic biomarkers of endometriosis. piRNAs are single-stranded RNAs composed of 23–36 nucleotides [10], and are classified according to their origin and function: repeat-associated piRNAs, piRNAs derived from 3' untranslated mRNAs, and piRNAs originating from intergenic long non-coding RNAs (pachytene piRNAs) [11]. In contrast to other non-coding RNAs processed from a double-stranded RNAs, dicer-dependent, and bound by Argonaute proteins, piRNAs are transcribed from ssRNA transcripts, are dicer-independent, and bound to PIWI (P-element Induced Wimpy testis) proteins [12]. In the human, at least 30,000 piRNAs have been identified as being involved in transposon silencing, transcriptional gene silencing, post-transcriptional gene silencing, transgenerational epigenetic inheritance, exosomal communications, and mRNA decay [13–15]. piRNA

expression has been analyzed in a variety of human tissues and been shown to be involved in different physiological processes (sex determination and differentiation, germ cells) [16], in various benign diseases such as cardiovascular disorders and male infertility [17–20], and in gastric, breast, lung and colorectal cancer [21–26]. However, little data is available relative to piRNA expression in saliva and no data specific to piRNA expression among endometriosis patients is currently available.

Therefore, using data from the prospective “ENDO-miRNA” study [27], the aims of the present work were: (i) to describe the piRNAs differentially expressed in the saliva of patients with and without endometriosis; (ii) to estimate their value in terms of diagnostic accuracy; and (iii) to gain insights into the pathophysiology of endometriosis based on piRNA expression.

Methods

Study population

We used data from the prospective “ENDO-miRNA” study (ClinicalTrials.gov Identifier: NCT04728152). Data collection and analysis were carried out under the Research Protocol n° ID RCB: 2020-A03297-32. All patients gave written consent to participate to the study. The ENDO-miRNA study [27] included 200 saliva samples obtained from patients with chronic pelvic pain suggestive of endometriosis. In brief, as previously published, all patients underwent a laparoscopic procedure (either therapeutic or diagnostic laparoscopic) and/or MRI imaging detecting endometriosis by the presence of endometrioma and/or deep endometriosis with colorectal involvement. All the saliva samples were collected between January 2021 and June 2021. Analysis was performed blinded to the surgical and imaging findings. In case of endometriosis, the patients were stratified according to the revised American Society of Reproductive Medicine (rASRM) classification [28]. The main characteristics of the patients included in the ENDO-miRNA study were previously published [9] and are displayed in Supplementary Table 1 .

Saliva sample analysis

Saliva collection and processing has been previously described by our team [29]

RNA-sequencing libraries were prepared using the QIAseq miRNA Library Kit (Qiagen) according to the manufacturer’s instructions. Samples were indexed in batches of 96, with a targeted sequencing depth of 17 million reads per sample. Reads were first processed by trimming off the 3’ adapter and low-quality bases using cutadapt (cutadapt.readthedocs.io/en/stable/guide.html). Reads with no adapter sequence were tallied (no_adapter_reads). Following trimming, the insert sequences and UMI sequences were

identified. Reads with less than 16 bp insert sequences (`too_short_reads`) or less than 10 bp UMI sequences (`UMI_defective_reads`) were discarded. At each step, only unmapped sequences passed to the next step. Read counts for each RNA category (miRBase mature, miRBase hairpin, piRNA, tRNA, rRNA, mRNA and otherRNA) were calculated from the mapping results (`miRNA_Reads`, `hairpin_Reads`, `piRNA_Reads`, etc.). piRNABank was used for piRNA [30]. For each sample, all reads assigned to a particular miRNA or piRNA ID were counted, and associated UMIs were clustered to count unique molecules [31].

Bioinformatics

Sequencing reads were processed using the data processing pipeline. FastQ files were trimmed to remove adapter sequences using Cutadapt version v.1.18 and were aligned using Bowtie version 1.1.1 to the following transcriptome databases: the human reference genome available from NCBI (<https://www.ncbi.nlm.nih.gov/genome/guide/human/>), and piRNABank (piRNAs) using the piRDeep2 v0.1.0 package. The raw sequencing data quality was assessed using FastQC software v0.11.7 [32–36].

Differential expression analysis of the piRNAs was quantified by piRDeep2 [37]. Differential expression tests were then conducted in DESeq2 for piRNAs with read counts in ≥ 1 of the samples. DESeq2 integrates methodological advances with several novel features to facilitate a more quantitative analysis of comparative RNA-seq data using shrinkage estimators for dispersion and fold change [38] [39]. piRNAs were considered as differentially expressed if the absolute value of log₂-fold change was >1.5 (upregulated) and <0.5 (downregulated). The P value adjusted for multiple testing was $<.05$ [38].

To evaluate the accuracy of each piRNA, sensitivity, specificity, and ROC analysis have been performed, and the ROC AUC was calculated [40,41]

Additional statistical analysis was based on the Chi² test as appropriate for categorical variables as well as Wilcoxon-test. Values of P <.05 were considered to denote significant differences. Data were managed with an Excel database (Microsoft, Redmond, WA) and analyzed using R 2.15 software, available online (<http://cran.r-project.org/>).

Results

Global overview of piRNA transcriptome

Small RNA-seq of 200 saliva samples yielded ~4 642 M raw sequencing reads (from ~13.7 M to ~39.3 M reads/sample). Pre-filtering and filtering steps retained 70% (~3 205 M) of initial raw reads. Most of the filtered reads were of short read length. Quantification of the filtered reads and identification of known piRNAs yielded ~2,9 M sequences that were mapped to 2495 piRNAs from piRNABank. The number of expressed piRNAs ranged from 7 (outlier) to 247 per sample (169 = mean). The distribution of expressed piRNAs in the 200 saliva samples and the overall composition of processed reads is shown in Figure 1 – 3.

Comparison of piRNAs expressed in patients with and without endometriosis

The distribution of the piRNAs according to the AUC values is given in Supplementary Table 2. None had an AUC ≥ 0.70 , and only three piRNAs (piR-004153, piR-001918, piR-020401) had an AUC between ≥ 0.6 and < 0.70 .

Among the 201 piRNAs detected, none were up-regulated and 33 were down-regulated (Table 1). Four piRNAs were differentially expressed in patients with and without endometriosis: piR-004153, piR-012864, piR-017716, and piR-016904 (Figure 4). Only one piRNA had both an AUC > 0.60 and p-value using Wilcoxon-test < 0.05 . The Boxplots for the four piRNAs differentially expressed are given in Figure 5.

The respective relation and accuracy to diagnose endometriosis according to the F1-score, sensitivity, specificity, and AUC ranged from 0-0.862 %, 0-0.961%, 0.085-1, and 0.425-0.618.

Relation between epidemiological characteristics of the population and piRNA expression.

Relations between piRNAs expression and age, alcohol, smoking, BMI and infertility were evaluated using Mann-Whitney test. No relation was found between piRNAs and age < 30 years. Using a cut-off of 35 years, a relation was observed between piR-004153 ($p = 0.002$) and piR-017716 ($p = 0.030$), and the diagnostic of endometriosis. Among patients with endometriosis, no relation was found between piRNAs expression and infertility. No relation was observed between piRNAs expression and BMI (< or ≥ 30 kg/m²), alcohol consumption nor smoking.

Relation between piRNAs expression and dysmenorrhea, disease stage or hormonal treatment

No relation was observed between dysmenorrhea intensity using Visual Analogic Scale (VAS) or disease stage according to ASRM classification and piRNAs expression. Among patients with endometriosis, four piRNAs were differentially expressed in patients with and without hormonal treatment: piR-004153 ($p = 0.015$), piR-020401 ($p = 0.001$), piR-012864 ($p = 0.036$) and piR-017716 ($p = 0.009$). In both endometriosis and control groups, three other piRNAs were differentially expressed in patients using hormonal treatment: piR-011370 ($p=0.011$), piR-022296 ($p=0.005$) and piR-006701 ($p=0.029$),

Relation between pathophysiology of endometriosis and piRNA expression.

Among piRNAs of interest, only one piRNA, analyzed in blood (piR-004153), was previously reported in colorectal cancer [42]. No pathophysiological signaling pathway associated with piR-004153 expression was clearly identified. No saliva expression was previously reported for this piRNA. Among the three remaining piRNAs differentially

expressed, none was reported either in benign or malignant disorders. None of these piRNAs was previously reported in endometriosis.

Discussion

In the current study, we demonstrated that saliva piRNAs were differentially expressed in women with and without endometriosis. This finding is particularly relevant for identifying piRNAs as potential diagnostic biomarkers. Moreover, we demonstrated the relation between saliva piRNA expression and hormonal treatment and age. Finally, our analysis underlines the overall lack of data to designate the specific contribution of piRNAs in terms of pathophysiology.

In the present study, saliva piRNA expression was evaluated using NGS with Unique Molecular Identifier (UMI) barcoding that is the recommended approach to avoid amplification bias [31]. In the current study, using Poisson regression and the Wilcoxon test, we observed that none of the piRNAs detected had an AUC ≥ 0.70 , and that only three piRNAs (piR-004153, piR001918, piR-020401) had an AUC between ≥ 0.6 and < 0.70 for the diagnosis of endometriosis. Four saliva piRNAs appear particularly relevant for the diagnosis of endometriosis (piR-004153, piR-012864, piR-017716 and piR-016904). None of them were previously reported in endometriosis. Thus, further studies are required to confirm the implication of piRNAs in endometriosis and their potential contribution as a diagnostic biomarker.

The most relevant data of the present study is the relation between saliva piRNA expression and hormonal treatment – including the contraceptive pill, progestins, and GnRH analog – which is the first-line treatment for endometriosis [43,44]. In the present study, among the piRNAs of interest, four were differentially expressed in patients with and without hormonal treatment (piR-004153, $p=0.015$; piR-020401, $p=0.001$; piR-012864, $p=0.036$; and piR-017716, $p=0.009$), and three additional piRNAs were differentially expressed in patients using hormonal treatment (piR-011370, $p=0.011$; piR-022296, $p=0.005$; and piR-006701, $p=0.029$) regardless the endometriosis status. So far, none of these piRNAs have been

reported to be associated with hormonal treatment whatever the disease opening new avenues to elucidate the role of hormonal therapies and their mechanisms of action in endometriosis. Only piR-004153 has previously been evaluated, in the specific context of colorectal cancer, but without evaluating the associated signaling pathways. Despite the lack of data about the mechanistic role in the estrogenic piRNA response [45], previous reports have highlighted that piRNA expression is differentially regulated by estrogens [46]. Moreover, Zhang et al (2010) indicated that piRNA pathways are regulated by sex steroids [47]. Previous studies [48] have shown that the piR-31470/PIWIL4 complex could regulate the methylation level of glutathione S-transferase pi 1 (GSTP1) by recruiting multiple DNA methylation enzymes. This is relevant as a recent meta-analysis [49] demonstrated that only five of 28 polymorphisms were associated with endometriosis including GSTP1 rs1695. In transcriptional gene silencing (TGS), which mainly occurs with PIWI proteins with low catalytic activity, Cheng et al reported that the piRNA/PIWI complex mediates chromatin silencing by collaborating with histone-modifying enzymes or DNA methyltransferases hence suppressing mRNA transcription [50]. Finally, evidence of chromatin remodeling, de novo methylation, and direct transcriptional regulation shows the multifaceted roles of piRNAs [51–75] and the special role of piRNAs in epigenetic modification [76,77].

PiRNAs could contribute to a better understanding of the physiopathology of endometriosis, improvement in these patients management when infertile, as well as the development of new therapies [78]. Indeed, Expression of PIWI proteins in somatic stem cells [79] suggests that piRNA may also be involved in stem cell function regulation. However, the mechanism, major functions, and pathways regulated by the Piwi-piRNA complex remain poorly understood. Regarding fertility, the small RNA population of both the oocyte and cumulus cells during in vitro maturation (IVM) have been sequenced and the portfolio of endo-siRNA, miRNA, and piRNA demonstrated in pigs [80]. Moreover, since piRNAs

regulate protein-coding genes involved in the development of various diseases, they can be considered molecules useful as innovative epidrugs [81].

Another interesting finding is the variation in piRNA expression according to the epidemiological characteristics of the population. In the current study, piRNAs were differentially expressed according to the age of the patients. Although no difference was observed for patients under 30 years, differential expression was noted for piR-004153 ($p=0.002$) and piR-017716 ($p=0.030$) for patients over 35. So far, no data exist about the relation between piRNA expression and age in patients with endometriosis. However, Erwin and Blumenstiel have previously reported that piRNA biosynthesis increases with aging [82]. Moreover, in drosophila, Feltzin et al indicated a role of the exonuclease Nibbler on age-associated processes to modulate the length of small non-coding RNAs including piRNAs [83]. Wang et al described that normal ageing was associated with 3' shortening of piRNAs coupled with a decline in piRNA abundance [84]. Finally, Rounge et al found that ageing was the strongest factor associated with small-non-coding RNA expression, regardless of the class. However, none of their top five piRNAs that were differentially expressed in patients according to age were common with those found in our study [85]. This could be explained by differences in age between the studies: our population was composed of women between 18 and 43 years with few women over 40 years, while Rounge et al found a difference in piRNA expression for patients of 40-60 years. In contrast to age, we found no difference in piRNA expression according to BMI and smoking unlike the findings of a previous study [85] highlighting the need of further studies to assess not only the pathology involved but also the epidemiological characteristics of the population.

Some limits of the present study deserve to be underlined. First, in contrast to miRNA analysis in endometriosis [5–9], the crucial issue is the difference in the naming and numbering rules of piRNAs from one database to another [86] giving rise to different names

in various studies [86,87]. For example, piR-30025 and piRNA_30025 are listed in the piRNAdb and piRNAQuest databases, respectively, but have different chromosomal positions [88]. Moreover, it has been pointed out that most of the small RNAs in somatic tissues that are annotated as piRNAs and map on the same genomic location as other ncRNAs, are not functional piRNAs but only fragments of other ncRNAs [87,89]. In the current study, we decided to evaluate piRNA expression using the piRNABank database only, with the potential risk of restricting the number of piRNAs evaluated. Second, although the present prospective study is the first to evaluate saliva piRNA expression in women with and without endometriosis, it is important to note that the study focused on a specific population in terms of age with a potential bias linked to the exclusion of adolescents and patients over 43 years old as well as menopausal women. Third, the absence of a relation between piRNA expression and some characteristics such as fertility, obesity and smoking could be explained by the relatively small sample size.

In conclusion, despite some limits, our results from a large cohort of saliva samples are the first to suggest that piRNAs play a role in endometriosis. A diagnostic tool developed around saliva piRNAs would have the advantage of being non-invasive and simple to administer. Further studies are necessary to confirm our findings since no data are available on piRNA expression in women with endometriosis using NGS and UMI in saliva, blood, or serum.

Declarations

Ethics approval and consent to participate: The authors state that the data used are from the prospective ENDO-miRNA study (ClinicalTrials.gov Identifier: NCT04728152). Data collection and analysis were carried out under Research Protocol n° ID RCB: 2020-A03297-32. Informed consent was obtained from all subjects involved in the study.

Consent for publication: not applicable

Availability of data and materials: All relevant data are presented in the manuscript or in the supplementary files. Authors are available to provide any complementary information if asked.

Competing interests: 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.

Funding: Part of this work was funded by a grant from the Conseil Régional d'Ile de France (grant number EX024087) and from Ziwig, Inc

Author's contributions

Methodology and Design: SB, ED, PD, FG

Data collection: SB, YD, AP, CT, LD, MP

Biologic data collection: YD, LJ, SF, DB, YM

Analysis: SS, SB, ED, FG, PD, YM, SF

Data Interpretation: MP, SB, SS, LJ, YD, AP, CT et ED.

Manuscript writing: SS, YD, MP, SB, ED, LD.

All authors reviewed the manuscript for critical intellectual content.

Acknowledgment: All authors would like to sincerely thank F. Neilson (matrixconsultants.fr) for her English revision of the manuscript. Part of this work was carried out in the DNA and cell bank and the iGenSeq core facilities of ICM. We gratefully acknowledge their contribution for sample management and analysis.

References

- [1] Secosan C, Balulescu L, Brasoveanu S, Balint O, Pirtea P, Dorin G, et al. Endometriosis in Menopause-Renewed Attention on a Controversial Disease. *Diagn Basel Switz* 2020;10:E134. <https://doi.org/10.3390/diagnostics10030134>.
- [2] Haas D, Chvatal R, Reichert B, Renner S, Shebl O, Binder H, et al. Endometriosis: a premenopausal disease? Age pattern in 42,079 patients with endometriosis. *Arch Gynecol Obstet* 2012;286:667–70. <https://doi.org/10.1007/s00404-012-2361-z>.
- [3] Giudice LC, Kao LC. Endometriosis. *The Lancet* 2004;364:1789–99. [https://doi.org/10.1016/S0140-6736\(04\)17403-5](https://doi.org/10.1016/S0140-6736(04)17403-5).
- [4] Matta K, Vigneau E, Cariou V, Mouret D, Ploteau S, Le Bizec B, et al. Associations between persistent organic pollutants and endometriosis: A multipollutant assessment using machine learning algorithms. *Environ Pollut Barking Essex 1987* 2020;260:114066. <https://doi.org/10.1016/j.envpol.2020.114066>.
- [5] Moustafa S, Burn M, Mamillapalli R, Nematian S, Flores V, Taylor HS. Accurate diagnosis of endometriosis using serum microRNAs. *Am J Obstet Gynecol* 2020;223:557.e1-557.e11. <https://doi.org/10.1016/j.ajog.2020.02.050>.
- [6] Vanhie A, O D, Peterse D, Beckers A, Cuéllar A, Fassbender A, et al. Plasma miRNAs as biomarkers for endometriosis. *Hum Reprod Oxf Engl* 2019;34:1650–60. <https://doi.org/10.1093/humrep/dez116>.
- [7] Dabi Y, Suisse S, Jornea L, Bouteiller D, Touboul C, Puchar A, et al. Clues for Improving the Pathophysiology Knowledge for Endometriosis Using Serum Micro-RNA Expression. *Diagn Basel Switz* 2022;12:175. <https://doi.org/10.3390/diagnostics12010175>.
- [8] Bendifallah S, Dabi Y, Suisse S, Jornea L, Bouteiller D, Touboul C, et al. MicroRNome analysis generates a blood-based signature for endometriosis. *Sci Rep* 2022;12:4051. <https://doi.org/10.1038/s41598-022-07771-7>.
- [9] Bendifallah S, Suisse S, Puchar A, Delbos L, Poilblanc M, Descamps P, et al. Salivary MicroRNA Signature for Diagnosis of Endometriosis. *J Clin Med* 2022;11:612. <https://doi.org/10.3390/jcm11030612>.
- [10] Iwasaki YW, Siomi MC, Siomi H. PIWI-Interacting RNA: Its Biogenesis and Functions. *Annu Rev Biochem* 2015;84:405–33. <https://doi.org/10.1146/annurev-biochem-060614-034258>.
- [11] Aravin AA, Naumova NM, Tulin AV, Vagin VV, Rozovsky YM, Gvozdev VA. Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the *D. melanogaster* germline. *Curr Biol CB* 2001;11:1017–27. [https://doi.org/10.1016/s0960-9822\(01\)00299-8](https://doi.org/10.1016/s0960-9822(01)00299-8).
- [12] Vagin VV, Sigova A, Li C, Seitz H, Gvozdev V, Zamore PD. A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* 2006;313:320–4. <https://doi.org/10.1126/science.1129333>.
- [13] Amaral PP, Dinger ME, Mercer TR, Mattick JS. The eukaryotic genome as an RNA machine. *Science* 2008;319:1787–9. <https://doi.org/10.1126/science.1155472>.
- [14] Rayford KJ, Cooley A, Rumph JT, Arun A, Rachakonda G, Villalta F, et al. piRNAs as Modulators of Disease Pathogenesis. *Int J Mol Sci* 2021;22:2373. <https://doi.org/10.3390/ijms22052373>.
- [15] T V, Ps S, R T. Non-coding RNAs: Functions and applications in endocrine-related cancer. *Mol Cell Endocrinol* 2015;416. <https://doi.org/10.1016/j.mce.2015.08.026>.
- [16] Burgos M, Hurtado A, Jiménez R, Barrionuevo FJ. Non-Coding RNAs: lncRNAs, miRNAs, and piRNAs in Sexual Development. *Sex Dev Genet Mol Biol Evol Endocrinol Embryol Pathol Sex Determ Differ* 2021;15:335–50. <https://doi.org/10.1159/000519237>.
- [17] Das B, Roy J, Jain N, Mallick B. Tumor suppressive activity of PIWI-interacting RNA

- in human fibrosarcoma mediated through repression of RRM2. *Mol Carcinog* 2019;58:344–57. <https://doi.org/10.1002/mc.22932>.
- [18] Vychytilova-Faltejskova P, Stitkovcova K, Radova L, Sachlova M, Kosarova Z, Slaba K, et al. Circulating PIWI-Interacting RNAs piR-5937 and piR-28876 Are Promising Diagnostic Biomarkers of Colon Cancer. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol* 2018;27:1019–28. <https://doi.org/10.1158/1055-9965.EPI-18-0318>.
- [19] Iyer DN, Wan TM-H, Man JH-W, Sin RW-Y, Li X, Lo OS-H, et al. Small RNA Profiling of piRNAs in Colorectal Cancer Identifies Consistent Overexpression of piR-24000 That Correlates Clinically with an Aggressive Disease Phenotype. *Cancers* 2020;12:E188. <https://doi.org/10.3390/cancers12010188>.
- [20] Peng L, Song L, Liu C, Lv X, Li X, Jie J, et al. piR-55490 inhibits the growth of lung carcinoma by suppressing mTOR signaling. *Tumour Biol J Int Soc Oncodevelopmental Biol Med* 2016;37:2749–56. <https://doi.org/10.1007/s13277-015-4056-0>.
- [21] Chu H, Hui G, Yuan L, Shi D, Wang Y, Du M, et al. Identification of novel piRNAs in bladder cancer. *Cancer Lett* 2015;356:561–7. <https://doi.org/10.1016/j.canlet.2014.10.004>.
- [22] Chu H, Xia L, Qiu X, Gu D, Zhu L, Jin J, et al. Genetic variants in noncoding PIWI-interacting RNA and colorectal cancer risk. *Cancer* 2015;121:2044–52. <https://doi.org/10.1002/cncr.29314>.
- [23] Mai D, Ding P, Tan L, Zhang J, Pan Z, Bai R, et al. PIWI-interacting RNA-54265 is oncogenic and a potential therapeutic target in colorectal adenocarcinoma. *Theranostics* 2018;8:5213. <https://doi.org/10.7150/thno.28001>.
- [24] Mei Y, Wang Y, Kumari P, Shetty AC, Clark D, Gable T, et al. A piRNA-like small RNA interacts with and modulates p-ERM proteins in human somatic cells. *Nat Commun* 2015;6:7316. <https://doi.org/10.1038/ncomms8316>.
- [25] Xin J, Du M, Jiang X, Wu Y, Ben S, Zheng R, et al. Systematic evaluation of the effects of genetic variants on PIWI-interacting RNA expression across 33 cancer types. *Nucleic Acids Res* 2021;49:90–7. <https://doi.org/10.1093/nar/gkaa1190>.
- [26] Yin J, Jiang X-Y, Qi W, Ji C-G, Xie X-L, Zhang D-X, et al. piR-823 contributes to colorectal tumorigenesis by enhancing the transcriptional activity of HSF1. *Cancer Sci* 2017;108:1746–56. <https://doi.org/10.1111/cas.13300>.
- [27] Bendifallah S. Evaluation of miRNAs in Endometriosis. clinicaltrials.gov; 2021.
- [28] Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril* 1997;67:817–21. [https://doi.org/10.1016/s0015-0282\(97\)81391-x](https://doi.org/10.1016/s0015-0282(97)81391-x).
- [29] Bendifallah S, Dabi Y, Suisse S, Jornea L, Bouteiller D, Touboul C, et al. A Bioinformatics Approach to MicroRNA-Sequencing Analysis Based on Human Saliva Samples of Patients with Endometriosis. *Int J Mol Sci* 2022;23:8045. <https://doi.org/10.3390/ijms23148045>.
- [30] Sai Lakshmi S, Agrawal S. piRNABank: a web resource on classified and clustered Piwi-interacting RNAs. *Nucleic Acids Res* 2008;36:D173-177. <https://doi.org/10.1093/nar/gkm696>.
- [31] Islam S, Zeisel A, Joost S, La Manno G, Zajac P, Kasper M, et al. Quantitative single-cell RNA-seq with unique molecular identifiers. *Nat Methods* 2014;11:163–6. <https://doi.org/10.1038/nmeth.2772>.
- [32] Lopez-Rincon A, Mendoza-Maldonado L, Martinez-Archundia M, Schönhuth A, Kraneveld AD, Garssen J, et al. Machine Learning-Based Ensemble Recursive Feature Selection of Circulating miRNAs for Cancer Tumor Classification. *Cancers* 2020;12:E1785. <https://doi.org/10.3390/cancers12071785>.
- [33] Gyvyte U, Juzenas S, Salteniene V, Kupcinskas J, Poskiene L, Kucinskas L, et al. MiRNA profiling of gastrointestinal stromal tumors by next-generation sequencing.

- Oncotarget 2017;8:37225–38. <https://doi.org/10.18632/oncotarget.16664>.
- [34] Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 2009;10:R25. <https://doi.org/10.1186/gb-2009-10-3-r25>.
- [35] Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. *Nucleic Acids Res* 2008;36:D154-158. <https://doi.org/10.1093/nar/gkm952>.
- [36] Gao L, Zhang L. Construction and comprehensive analysis of a ceRNA network to reveal potential prognostic biomarkers for lung adenocarcinoma. *BMC Cancer* 2021;21:849. <https://doi.org/10.1186/s12885-021-08462-8>.
- [37] Li Q, Liu G, Bao Y, Wu Y, You Q. Evaluation and application of tools for the identification of known microRNAs in plants. *Appl Plant Sci* 2021;9:e11414. <https://doi.org/10.1002/aps3.11414>.
- [38] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15:550. <https://doi.org/10.1186/s13059-014-0550-8>.
- [39] Bargaje R, Hariharan M, Scaria V, Pillai B. Consensus miRNA expression profiles derived from interplatform normalization of microarray data. *RNA N Y N* 2010;16:16–25. <https://doi.org/10.1261/rna.1688110>.
- [40] Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996;15:361–87. [https://doi.org/10.1002/\(SICI\)1097-0258\(19960229\)15:4<361::AID-SIM168>3.0.CO;2-4](https://doi.org/10.1002/(SICI)1097-0258(19960229)15:4<361::AID-SIM168>3.0.CO;2-4).
- [41] Steyerberg EW, Eijkemans MJ, Harrell FE, Habbema JD. Prognostic modelling with logistic regression analysis: a comparison of selection and estimation methods in small data sets. *Stat Med* 2000;19:1059–79. [https://doi.org/10.1002/\(sici\)1097-0258\(20000430\)19:8<1059::aid-sim412>3.0.co;2-0](https://doi.org/10.1002/(sici)1097-0258(20000430)19:8<1059::aid-sim412>3.0.co;2-0).
- [42] Qu A, Wang W, Yang Y, Zhang X, Dong Y, Zheng G, et al. A serum piRNA signature as promising non-invasive diagnostic and prognostic biomarkers for colorectal cancer. *Cancer Manag Res* 2019;11:3703–20. <https://doi.org/10.2147/CMAR.S193266>.
- [43] Endometriosis guideline n.d. <https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Endometriosis-guideline.aspx> (accessed December 14, 2021).
- [44] ESHRE. Endometriosis guideline 2022.
- [45] Toth GP, Bencic DC, Martinson JW, Flick RW, Lattier DL, Kostich MS, et al. Development of omics biomarkers for estrogen exposure using mRNA, miRNA and piRNAs. *Aquat Toxicol Amst Neth* 2021;235:105807. <https://doi.org/10.1016/j.aquatox.2021.105807>.
- [46] Öner Ç, Turgut Coşan D, Çolak E. Estrogen and Androgen Hormone Levels Modulate the Expression of PIWI Interacting RNA in Prostate and Breast Cancer. *PloS One* 2016;11:e0159044. <https://doi.org/10.1371/journal.pone.0159044>.
- [47] Zhang D, Duarte-Guterman P, Langlois VS, Trudeau VL. Temporal expression and steroidal regulation of piRNA pathway genes (mael, piwi, vasa) during *Silurana* (*Xenopus*) *tropicalis* embryogenesis and early larval development. *Comp Biochem Physiol Toxicol Pharmacol CBP* 2010;152:202–6. <https://doi.org/10.1016/j.cbpc.2010.04.005>.
- [48] Zhang L, Meng X, Pan C, Qu F, Gan W, Xiang Z, et al. piR-31470 epigenetically suppresses the expression of glutathione S-transferase pi 1 in prostate cancer via DNA methylation. *Cell Signal* 2020;67:109501. <https://doi.org/10.1016/j.cellsig.2019.109501>.
- [49] Méar L, Herr M, Fauconnier A, Pineau C, Vialard F. Polymorphisms and endometriosis: a systematic review and meta-analyses. *Hum Reprod Update* 2020;26:73–102. <https://doi.org/10.1093/humupd/dmz034>.
- [50] Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, et al. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Transduct Target Ther*

- 2019;4:1–39. <https://doi.org/10.1038/s41392-019-0095-0>.
- [51] Fu A, Jacobs DI, Hoffman AE, Zheng T, Zhu Y. PIWI-interacting RNA 021285 is involved in breast tumorigenesis possibly by remodeling the cancer epigenome. *Carcinogenesis* 2015;36:1094–102. <https://doi.org/10.1093/carcin/bgv105>.
- [52] Rayford KJ, Cooley A, Arun A, Rachakonda G, Kleschenko Y, Villalta F, et al. Trypanosoma cruzi Modulates PIWI-Interacting RNA Expression in Primary Human Cardiac Myocytes during the Early Phase of Infection. *Int J Mol Sci* 2020;21:E9439. <https://doi.org/10.3390/ijms21249439>.
- [53] Ayarpadikannan S, Kim H-S. The impact of transposable elements in genome evolution and genetic instability and their implications in various diseases. *Genomics Inform* 2014;12:98–104. <https://doi.org/10.5808/GI.2014.12.3.98>.
- [54] Ng KW, Anderson C, Marshall EA, Minatel BC, Enfield KSS, Sapruff HL, et al. Piwi-interacting RNAs in cancer: emerging functions and clinical utility. *Mol Cancer* 2016;15:5. <https://doi.org/10.1186/s12943-016-0491-9>.
- [55] Chen Y, Pane A, Schüpbach T. Cutoff and aubergine mutations result in retrotransposon upregulation and checkpoint activation in *Drosophila*. *Curr Biol CB* 2007;17:637–42. <https://doi.org/10.1016/j.cub.2007.02.027>.
- [56] Sarot E, Payen-Groschêne G, Bucheton A, Péliisson A. Evidence for a piwi-dependent RNA silencing of the gypsy endogenous retrovirus by the *Drosophila melanogaster* flamenco gene. *Genetics* 2004;166:1313–21. <https://doi.org/10.1534/genetics.166.3.1313>.
- [57] Girard A, Hannon GJ. Conserved themes in small-RNA-mediated transposon control. *Trends Cell Biol* 2008;18:136–48. <https://doi.org/10.1016/j.tcb.2008.01.004>.
- [58] Biryukova I, Ye T. Endogenous siRNAs and piRNAs derived from transposable elements and genes in the malaria vector mosquito *Anopheles gambiae*. *BMC Genomics* 2015;16:278. <https://doi.org/10.1186/s12864-015-1436-1>.
- [59] Vagin VV, Klenov MS, Kalmykova AI, Stolyarenko AD, Kotelnikov RN, Gvozdev VA. The RNA interference proteins and vasa locus are involved in the silencing of retrotransposons in the female germline of *Drosophila melanogaster*. *RNA Biol* 2004;1:54–8.
- [60] Savitsky M, Kwon D, Georgiev P, Kalmykova A, Gvozdev V. Telomere elongation is under the control of the RNAi-based mechanism in the *Drosophila* germline. *Genes Dev* 2006;20:345–54. <https://doi.org/10.1101/gad.370206>.
- [61] Péliisson A, Sarot E, Payen-Groschêne G, Bucheton A. A novel repeat-associated small interfering RNA-mediated silencing pathway downregulates complementary sense gypsy transcripts in somatic cells of the *Drosophila* ovary. *J Virol* 2007;81:1951–60. <https://doi.org/10.1128/JVI.01980-06>.
- [62] Shpiz S, Kwon D, Uneva A, Kim M, Klenov M, Rozovsky Y, et al. Characterization of *Drosophila* telomeric retroelement TAHRE: transcription, transpositions, and RNAi-based regulation of expression. *Mol Biol Evol* 2007;24:2535–45. <https://doi.org/10.1093/molbev/msm205>.
- [63] Li C, Vagin VV, Lee S, Xu J, Ma S, Xi H, et al. Collapse of germline piRNAs in the absence of Argonaute3 reveals somatic piRNAs in flies. *Cell* 2009;137:509–21. <https://doi.org/10.1016/j.cell.2009.04.027>.
- [64] Carmell MA, Girard A, van de Kant HJG, Bourc’his D, Bestor TH, de Rooij DG, et al. MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline. *Dev Cell* 2007;12:503–14. <https://doi.org/10.1016/j.devcel.2007.03.001>.
- [65] Huang XA, Yin H, Sweeney S, Raha D, Snyder M, Lin H. A major epigenetic programming mechanism guided by piRNAs. *Dev Cell* 2013;24:502–16. <https://doi.org/10.1016/j.devcel.2013.01.023>.
- [66] Yin H, Lin H. An epigenetic activation role of Piwi and a Piwi-associated piRNA in *Drosophila melanogaster*. *Nature* 2007;450:304–8. <https://doi.org/10.1038/nature06263>.

- [67] Watanabe T, Cui X, Yuan Z, Qi H, Lin H. MIWI2 targets RNAs transcribed from piRNA-dependent regions to drive DNA methylation in mouse prospermatogonia. *EMBO J* 2018;37:e95329. <https://doi.org/10.15252/embj.201695329>.
- [68] Pal-Bhadra M, Leibovitch BA, Gandhi SG, Chikka MR, Rao M, Bhadra U, et al. Heterochromatic silencing and HP1 localization in *Drosophila* are dependent on the RNAi machinery. *Science* 2004;303:669–72. <https://doi.org/10.1126/science.1092653>.
- [69] Gomes AQ, Nolasco S, Soares H. Non-coding RNAs: multi-tasking molecules in the cell. *Int J Mol Sci* 2013;14:16010–39. <https://doi.org/10.3390/ijms140816010>.
- [70] Rajasethupathy P, Antonov I, Sheridan R, Frey S, Sander C, Tuschl T, et al. A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. *Cell* 2012;149:693–707. <https://doi.org/10.1016/j.cell.2012.02.057>.
- [71] Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Totoki Y, Toyoda A, Ikawa M, et al. DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. *Genes Dev* 2008;22:908–17. <https://doi.org/10.1101/gad.1640708>.
- [72] Fu A, Jacobs DI, Zhu Y. Epigenome-wide analysis of piRNAs in gene-specific DNA methylation. *RNA Biol* 2014;11:1301–12. <https://doi.org/10.1080/15476286.2014.996091>.
- [73] Zoch A, Auchynnikava T, Berrens RV, Kabayama Y, Schöpp T, Heep M, et al. SPOCD1 is an essential executor of piRNA-directed de novo DNA methylation. *Nature* 2020;584:635–9. <https://doi.org/10.1038/s41586-020-2557-5>.
- [74] Schöpp T, Zoch A, Berrens RV, Auchynnikava T, Kabayama Y, Vasiliauskaite L, et al. TEX15 is an essential executor of MIWI2-directed transposon DNA methylation and silencing. *Nat Commun* 2020;11:3739. <https://doi.org/10.1038/s41467-020-17372-5>.
- [75] Mohn F, Handler D, Brennecke J. Noncoding RNA. piRNA-guided slicing specifies transcripts for Zucchini-dependent, phased piRNA biogenesis. *Science* 2015;348:812–7. <https://doi.org/10.1126/science.aaa1039>.
- [76] Taft RJ, Pang KC, Mercer TR, Dinger M, Mattick JS. Non-coding RNAs: regulators of disease. *J Pathol* 2010;220:126–39. <https://doi.org/10.1002/path.2638>.
- [77] Guo M, Wu Y. Fighting an old war with a new weapon--silencing transposons by Piwi-interacting RNA. *IUBMB Life* 2013;65:739–47. <https://doi.org/10.1002/iub.1192>.
- [78] Hale BJ, Keating AF, Yang C-X, Ross JW. Small RNAs: Their Possible Roles in Reproductive Failure. *Adv Exp Med Biol* 2015;868:49–79. https://doi.org/10.1007/978-3-319-18881-2_3.
- [79] Sharma AK, Nelson MC, Brandt JE, Wessman M, Mahmud N, Weller KP, et al. Human CD34(+) stem cells express the hiwi gene, a human homologue of the *Drosophila* gene piwi. *Blood* 2001;97:426–34. <https://doi.org/10.1182/blood.v97.2.426>.
- [80] Yang C-X, Du Z-Q, Wright EC, Rothschild MF, Prather RS, Ross JW. Small RNA profile of the cumulus-oocyte complex and early embryos in the pig. *Biol Reprod* 2012;87:117. <https://doi.org/10.1095/biolreprod.111.096669>.
- [81] Taverna S, Masucci A, Cammarata G. PIWI-RNAs Small Noncoding RNAs with Smart Functions: Potential Theranostic Applications in Cancer. *Cancers* 2023;15:3912. <https://doi.org/10.3390/cancers15153912>.
- [82] Erwin AA, Blumenstiel JP. Aging in the *Drosophila* ovary: contrasting changes in the expression of the piRNA machinery and mitochondria but no global release of transposable elements. *BMC Genomics* 2019;20:305. <https://doi.org/10.1186/s12864-019-5668-3>.
- [83] Feltzin VL, Khaladkar M, Abe M, Parisi M, Hendriks G-J, Kim J, et al. The exonuclease Nibbler regulates age-associated traits and modulates piRNA length in *Drosophila*. *Aging Cell* 2015;14:443–52. <https://doi.org/10.1111/ace1.12323>.
- [84] Wang H, Ma Z, Niu K, Xiao Y, Wu X, Pan C, et al. Antagonistic roles of Nibbler and Hen1 in modulating piRNA 3' ends in *Drosophila*. *Dev Camb Engl* 2016;143:530–9.

<https://doi.org/10.1242/dev.128116>.

[85] Rounge TB, Umu SU, Keller A, Meese E, Ursin G, Tretli S, et al. Circulating small non-coding RNAs associated with age, sex, smoking, body mass and physical activity. *Sci Rep* 2018;8:17650. <https://doi.org/10.1038/s41598-018-35974-4>.

[86] Geles K, Palumbo D, Sellitto A, Giurato G, Cianflone E, Marino F, et al. WIND (Workflow for piRNAs aNd beyonD): a strategy for in-depth analysis of small RNA-seq data. *F1000Research* 2021;10:1. <https://doi.org/10.12688/f1000research.27868.3>.

[87] Kärkkäinen E, Heikkinen S, Tengström M, Kosma V-M, Mannermaa A, Hartikainen JM. The debatable presence of PIWI-interacting RNAs in invasive breast cancer. *Cancer Med* 2021;10:3593–603. <https://doi.org/10.1002/cam4.3915>.

[88] Zhang P, Si X, Skogerbø G, Wang J, Cui D, Li Y, et al. piRBase: a web resource assisting piRNA functional study. *Database J Biol Databases Curation* 2014;2014:bau110. <https://doi.org/10.1093/database/bau110>.

[89] Watanabe T, Takeda A, Tsukiyama T, Mise K, Okuno T, Sasaki H, et al. Identification and characterization of two novel classes of small RNAs in the mouse germline: retrotransposon-derived siRNAs in oocytes and germline small RNAs in testes. *Genes Dev* 2006;20:1732–43. <https://doi.org/10.1101/gad.1425706>.

Figures Caption

Figure 1: Overall composition of piRNAs in the processed reads

Figure 2: Distribution of expressed piRNAs in the 200 samples.

Figure 3: Distribution of expressed piRNAs in the samples per diagnosis (0 = controls, 1 = cases with endometriosis).

Figure 4: ROC curves analysis of the four piRNAs differentially expressed in patients with and without endometriosis.

Figure 5: Boxplot of the four piRNAs of interest for the diagnostic of endometriosis.

Down - regulated	Up-regulated
hsa_piR_004153/gb/DQ575660/Homo	-
hsa_piR_012864/gb/DQ587426/Homo	-
hsa_piR_021740/gb/DQ599789/Homo	-
hsa_piR_020582/gb/DQ598312/Homo	-
hsa_piR_006701/gb/DQ579162/Homo	-
hsa_piR_002980/gb/DQ574006/Homo	-
hsa_piR_004800/gb/DQ576604/Homo	-
hsa_piR_015149/gb/DQ590703/Homo	-
hsa_piR_019675/gb/DQ596992/Homo	-
hsa_piR_016659/gb/DQ592932/Homo	-
hsa_piR_000848/gb/DQ571067/Homo	-
hsa_piR_020455/gb/DQ598110/Homo	-
hsa_piR_004152/gb/DQ575658/Homo	-
hsa_piR_019912/gb/DQ597341/Homo	-
hsa_piR_014539/gb/DQ589909/Homo	-
hsa_piR_001104/gb/DQ571422/Homo	-
hsa_piR_015150/gb/DQ590704/Homo	-
hsa_piR_010024/gb/DQ583491/Homo	-
hsa_piR_011374/gb/DQ585363/Homo	-
hsa_piR_014879/gb/DQ590348/Homo	-
hsa_piR_016658/gb/DQ592931/Homo	-
hsa_piR_017295/gb/DQ593909/Homo	-
hsa_piR_017833/gb/DQ594619/Homo	-
hsa_piR_017936/gb/DQ594740/Homo	-
hsa_piR_014635/gb/DQ590029/Homo	-
hsa_piR_003672/gb/DQ574991/Homo	-
hsa_piR_018749/gb/DQ595765/Homo	-
hsa_piR_007540/gb/DQ580277/Homo	-
hsa_piR_020809/gb/DQ598639/Homo	-
hsa_piR_011186/gb/DQ585093/Homo	-
hsa_piR_004019/gb/DQ575471/Homo	-
hsa_piR_020668/gb/DQ598445/Homo	-
hsa_piR_015511/gb/DQ591196/Homo	-

Table 1 : piRNAs up and down-regulated in the population