# MEA Summary Review: Assessing diagnostic value of microRNAs from PMBC's and EV's in ME/CFS

By: Charlotte Stephens 11th February 2020

Dr Elisa Oltra's research team from the University of Valencia, Spain, has just published their study, funded by the MEA Ramsay Research Fund, which used samples from the UK ME Biobank.


They were analysing the levels of microRNA's in PBMC's and EV's (all terms explained below) in the blood of people with severe ME/CFS, compared to healthy controls, to see if they could identify a diagnostic biomarker.

They found decreased levels of Creatine Phosphokinase and differences in the number and size of Extracellular Vesicles in the ME/CFS patients compared to controls.

27 miRNA's were found to be significantly different. Further studies are needed in order to validate the possibility of these findings being used as diagnostic biomarkers for ME/CFS.

In this summary review, we hope to explain the terms used and shed additional light on these findings.

> "In this study, our team has evaluated, for the first time, ME/CFS miRNomes in peripheral blood mononuclear cells (PBMCs) and extracellular vesicles (EVs) from severely ill patients recruited at the monographic UK ME biobank to assess, using standard operating procedures (SOPs), blood fractions with optimal diagnostic power for a rapid translation of a miR-based diagnostic method into the clinic.
>
> "Our results show that routine creatine kinase (CK) blood values, plasma EVs physical characteristics (including counts, size and zeta-potential), and a limited number of differentially expressed PBMC and EV miRNAs appear significantly associated with severe ME/CFS ($p<0.05$).
>
> "Gene enrichment analysis points to epigenetic and neuroimmune dysregulated pathways, in agreement with previous reports. Population validation by a cost-effective approach limited to these few potentially discriminating variables is granted."

## Key Points

- Blood samples of 15 severely affected ME/CFS patients and 15 healthy controls were obtained from the UK ME Biobank and analysed for differences in miRNA expression in PBMC's and EV's.
- Reduced levels of Creatine Phosphokinase were found in the ME/CFS samples, which is in agreement with a 2019 study from the ME Biobank team.
- Higher quantities but smaller sizes of Extracellular vesicles (EV's) were found in ME/CFS patients compared to controls, which also agrees with findings from other studies.
- 17 miRNA's in PBMC's and 10 miRNA's in EV's were significantly different in ME/CFS patient samples compared to controls.
- 15 out of the 27 miRNA's found to be significantly different in ME/CFS show promise of being possible diagnostic biomarkers.
- Further, larger studies are needed in order to validate the possibility of these miRNA's being used as diagnostic biomarkers for ME/CFS.

# Explanation of key terms

## What are microRNA's?

microRNA's (abbreviated to miRNA) are short, non-coding RNA's (ribonucleic acids) that play a key role in regulating gene expression (turning certain genes off). Each different miRNA molecule (over 2000 different types have been identified) is predicted to regulate up to hundreds of target genes.

All bodily fluids typically contain free miRNAs, but also miRNAs packed into extracellular vesicles (EVs). Abnormal expression of miRNA's has been linked to many diseases and they are being pursued as clinical diagnostic markers and as therapeutic targets.

## What are PBMC's?

Peripheral blood mononuclear cells (PMBC) are a category of specialised immune cells, including T cells, B cells and Natural Killer cells. These can be isolated from blood samples to be studied.

## What are EV's?

Extracellular vesicles (EV's) are like little parcels of information that are released from cells all over the body and circulate in the blood, where they then attach to and deliver information or 'cargo' to other cells in the body. They can be carrying proteins, metabolites, lipids and RNA.

They are known to facilitate cellular communication in processes such as immune response and inflammation.

EV's can also be released by bacterial cells and are thought to play a role in host-pathogen interactions. EV's have been implicated to be involved in diseases such as cancer and Alzheimer's.

## What did the study involve?

Dr Oltra's research team evaluated blood samples from 15 severely affected ME/CFS patients and 15 healthy controls, taken from the UK ME Biobank.

They isolated PBMC's and EV's from these samples and evaluated the quantity, size and characteristics of the EV's in ME/CFS patients and compared them to the control samples.

Next, they identified miRNA's in the PBMC's and EV's that were either over- or under-expressed (higher or lower levels of them). Finally, they looked to see if these differences in expression could be used as diagnostic biomarkers.

# Findings

## Creatine Phosphokinase

Among the 30 clinical parameters evaluated at the UK ME Biobank in the blood samples studied, only creatine phosphokinase (CPK) levels showed statistically significant differences between the groups.

Levels of CPK were significantly reduced in the ME/CFS patients, which agrees with a recent report by Nacul et al. (2019).

- We published a blog on the previous study finding low levels of CK and what this might mean.

Nacul et al. had also commented:

"Although a bias coming from patient inactivity cannot be ruled out at this point, the fact that a multivariate model controlling for activity maintains its significance, in addition to its potential to explain PEM in ME/CFS warrants further exploration."

## Extracellular vesicles

In the Spanish study, ME/CFS samples had higher numbers of EV's compared to the controls. The isolated EV's were also smaller in size in the ME/CFS group compared to controls.

These findings agree with a recent study by Castro-Marrero et al. (2019). The meaning of these findings is currently unknown.

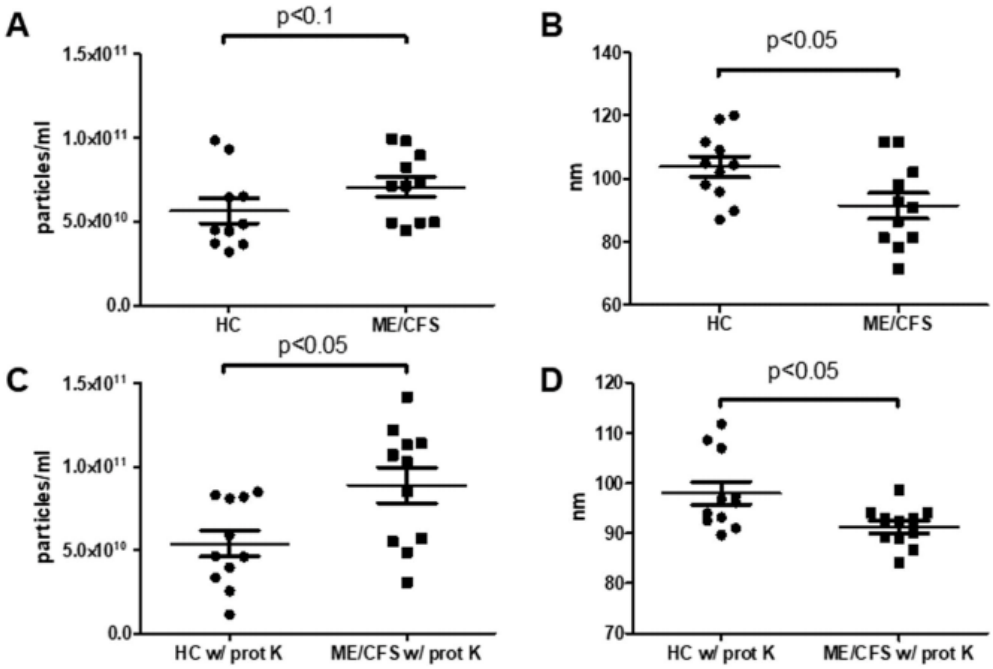


**Figure 1.** Shows the differences in the quantity (A and C) and size (B and D) of Extracellular Vesicles (EV's) in ME/CFS patients and controls.

## miRNA

Dr Oltra's team used differential expression analyses to identify miRNA's that were significantly different between ME/CFS patients and healthy controls PBMC's and EV's.

They found 17 miRNA's in PBMC's that were significantly different; 9 of them were overexpressed in ME/CFS and 8 of them under expressed. Interestingly, three of these had previously been reported as dysregulated by other authors in both ME/CFS and Fibromyalgia patients (Brenu et al. 2012; Cerda-Olmedo et al. 2015; Leinders et al. 2016).

10 miRNA's in the EV's were significantly different; 8 of them were overexpressed and 2 of them under-expressed in ME/CFS compared to healthy controls. Additionally, large differences in certain miRNA levels were noticed within the ME/CFS group, which may indicate the existence of patient subgroups.

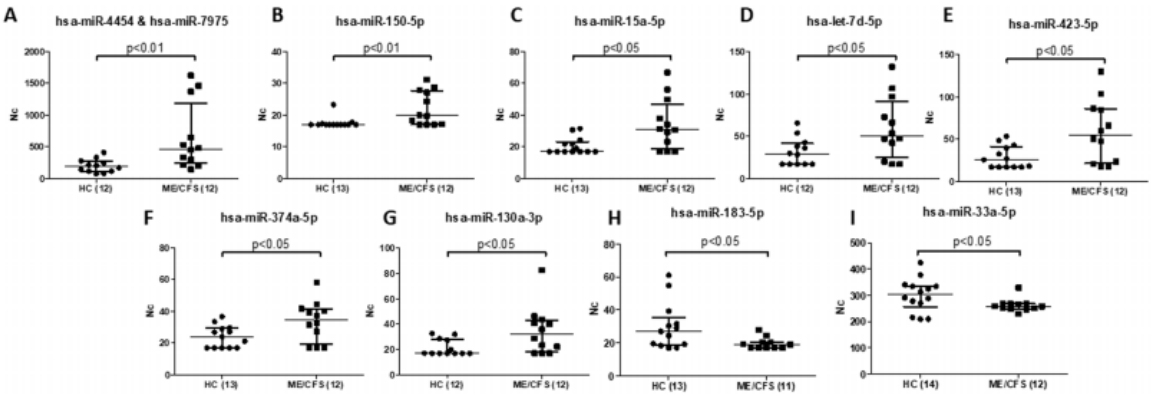


**Figure 2.** The miRNA's that were over expressed (A-G) and under-expressed (H and I) in Extracellular Vesicles (EV's) of ME/CFS patients compared to controls.

Using a database tool created by another group of researchers (Ludwig et al.,), the Spanish team tried to identify which specific tissues the miRNA's might have come from. They found that the ones differentially expressed in the EV's of ME/CFS patients were predominantly expressed in muscle and fascia, brain and nerves and hormonal glands (mostly thyroid). All of them correspond to tissues affected in ME/CFS.

The researchers noted that some of the miRNA's found to be different in ME/CFS can be influenced by anti-psychotic drugs and morphine, which may explain the observed overexpression. This highlights the importance of detailed medication history being obtained from study participants in order to rule this factor in or out.

Finally, the researchers looked at whether any of the miRNA's that were differentially expressed in ME/CFS patients could be used as diagnostic biomarkers. They found that 15 out of the 27 significantly different miRNA's they had detected may have an acceptable capacity to discriminate ME/CFS patients from healthy controls. Most of these were miRNA's from the PBMC's.

Two of the miRNA's with potential diagnostic value have been previously linked to inflammation by other researchers. However, at present we can only speculate on the potential significance of these findings.

# What does this mean/ what's next?

This study's findings of differences in miRNA expression between severe ME/CFS patients and healthy controls could help towards developing a diagnostic biomarker in the future, following further larger studies compared against different diseases, such as MS. It could also help to further our understanding of the biological pathways involved in ME/CFS disease pathology.

The researchers concluded:

> "This opens the exciting possibility for a directional low-cost screening method, including only a few miRNAs together with routine blood analytical parameters and some EV features, to evaluate larger ME/CFS cohorts towards population validation of the potential biomarkers of ME/CFS detected here.
>
> "Inclusion of diseased controls, such as multiple sclerosis (MS), systemic lupus erythematosus (SLE) or other diseases presenting overlapping symptoms will be of relevance in pursuance of ME/CFS specific biomarkers."

# References

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