Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVIII

Extracellular vesicles as a source of biomarkers for Fragile X Syndrome therapeutic assessment.

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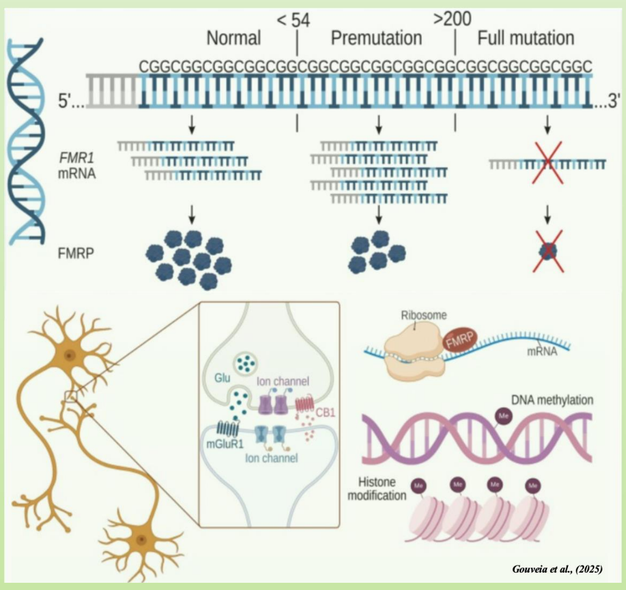
BACKGROUND

Fragile X syndrome (FXS) is a genetic disorder characterized by a range of cognitive, behavioural and physical deficits, including mild to moderate intellectual disability. The molecular cause is the extensive repetition and expansion of a CGG triplet in the 5' untranslated region[1], and consequential hypermethylation of the FMR1 gene results in the deficiency or absence of Fragile X Messenger Ribonucleoprotein 1 (FMRP)[2]. FMRP is an RNA-binding protein that plays a crucial role in synaptic plasticity and neuronal development.
No specific treatment is exploitable, and the preclinical assessment of drugs is difficult due to the limited availability of biomarkers. [3,4]



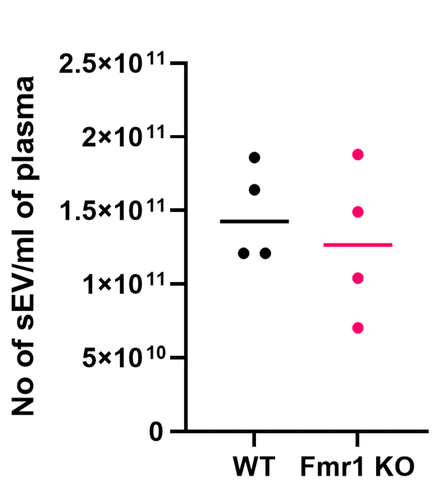
AIM

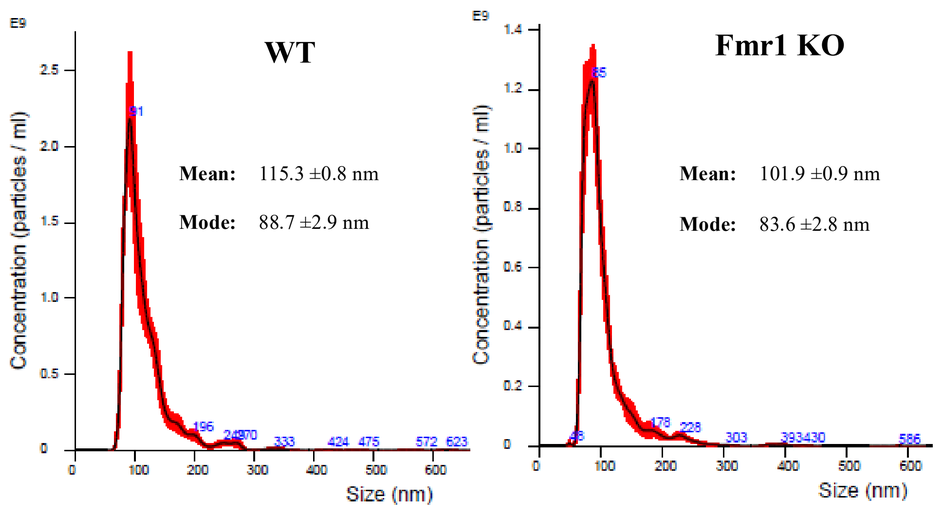
- To validate a novel approach based on the use of mouse model plasma-derived sEV for biomarker discovery in FXS.
- To identify new biomarker candidates suitable for preclinical evaluation of therapeutic interventions.[5]

METHODOLOGY

- **Sample collection**: Plasma of FXS mouse models (C57BL/6J).
- **sEV Isolation:** Size-Exclusion Chromatography (SEC).
- **Characterization:** NTA (Nanoparticle Tracking Analysis), SEM (Scanning Electron Microscopy) and Mass Spectometry (LC/MS) for sEV markers identification.
- **Proteomic and RNA analysis:** LC/MS ; WES capillary electrophoresis system. miRNA cargo inspected by Serum/Plasma panel (Qiagen); RT-qPCR.

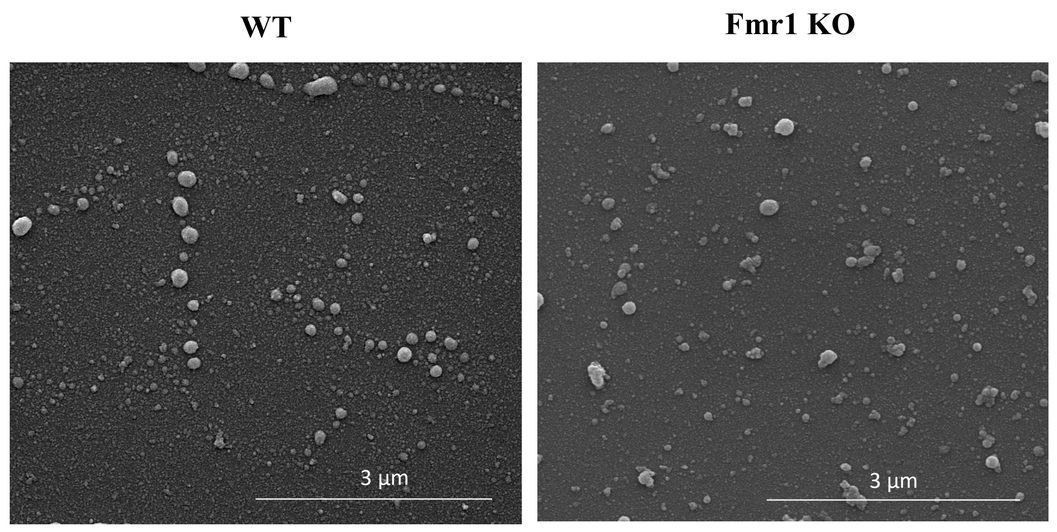
RESULTS





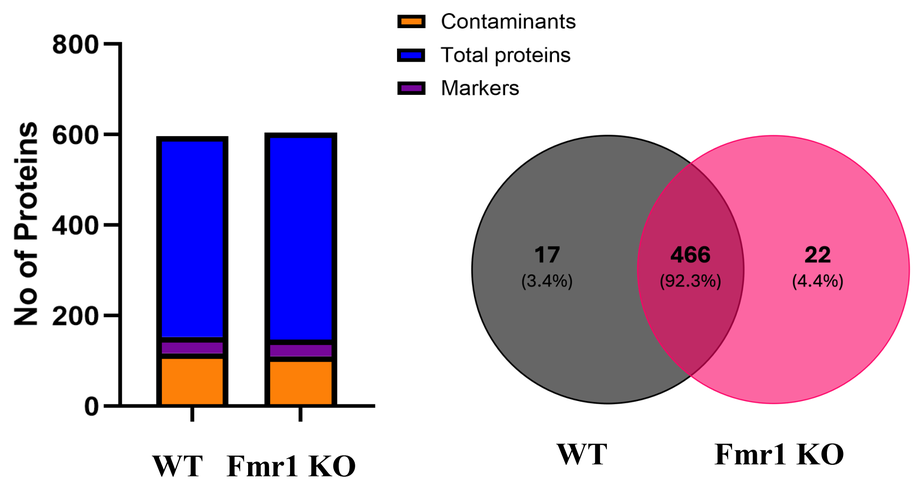
Particle number: Quantification of sEV isolated from WT and Fmr1 KO plasma indicates comparable yields.

Size distribution: NTA showed a similar size profile for WT and Fmr1 KO samples. Their average size, ranging from 101 to 115 nm, justifies their classification as small extracellular vesicles (sEV).



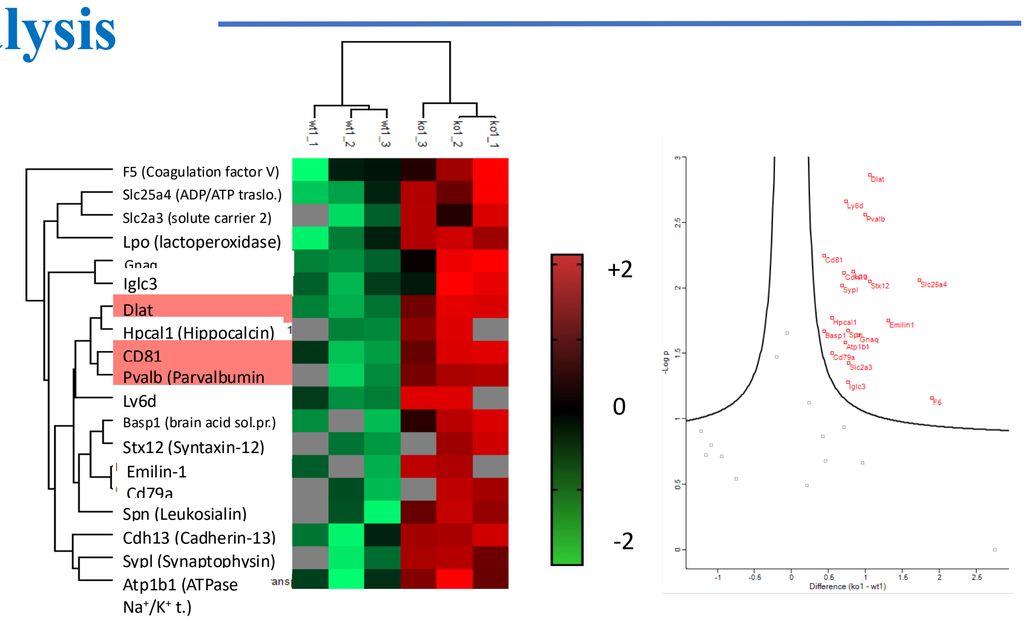
SEM images: Rapresentative SEM image of sEV isolated from WT (left) and Fmr1 KO (right) plasma sample.

Protein content analysis



Proteomics data: Bar blot represents the protein composition of sEV from WT and Fmr1 KO mice plasma analysed by LC/MS. In blue are represented total proteins, canonical EV markers are purple, and common plasma contaminants in orange. Venn diagram depicts the number of proteins identified in each sample. Furthermore, were confirmed the presence of EV protein markers in both samples, such as CD81, Rab7, Hspa8, Syntenin-1, ADAM10 and Cofilin-1.

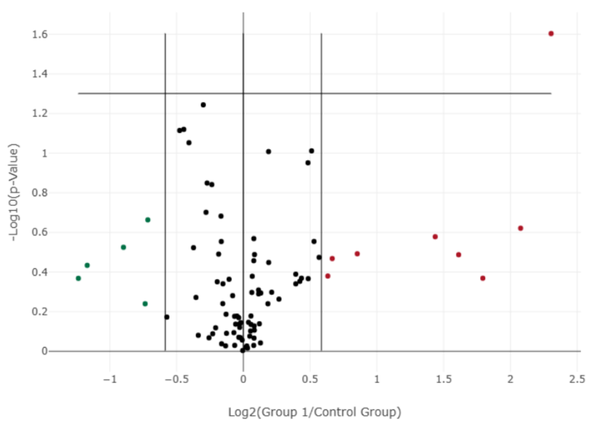
Proteins specifically expressed in WT and Fmr1 KO sEV	
WT	**Fmr1 KO**
COX2	Abcb6
Angptl 1/2	Ank1
Stx-11	Dnpep
Mb	Ehd4
Klkb1	Rtn4
Ttr	Slc1a5
Cyfip1	Slc2a1
	Lamtor1



Differential protein expression: Table highlights selected proteins specifically expressed in either WT or Fmr1 KO sEVs, selected for the ongoing validation using WES capillary system.
Heatmap and Volcano plot represent a comparative expression matrix of selected proteins identified by LC/MS in plasma-derived sEV from WT and Fmr1KO mice.

Biomarker identification

miRNA ID	Fold Regulation	p-Value
mmu-miR-215-5p	4.94	0.046319
hsa-miR-200c-3p	1.81	0.322022
hsa-miR-296-5p	3.47	0.244923
hsa-miR-9-5p	2.71	0.332714
mmu-miR-211-5p	4.22	0.149345
hsa-miR-96-5p	3.06	0.432328
mmu-miR-31-5p	1.59	0.340429
hsa-miR-184	1.55	0.417061



Protein assessment and miRNA prifiling:
Selected miRNA cargo was inspected by Serum/Plasma panel (Qiagen) using qRT-PCR.
Table and Volcano plot show obtained results (n=3, unpaired t-test) that will be further confirmed by qRT-PCR.

CONCLUSION

This study demonstrates the potential of sEVs as a source of novel measurable biomarkers for FXS. They could serve as an essential tool for the preclinical and clinical evaluation of therapies, as well as providing new insights into the cell signalling alterations underlying FXS.

REFERENCE

[1] Willemsen, R., et al. (2011)
[2] Bagni, C. et al, (2012) [4] Momen-Heravi, F., et al. (2018)
[3] Théry, C., et al. (2002) [5] Sharma, P., et al. (2019)

CONTACTS

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