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Quantitative NMR serum spectroscopy and tissue MALDI imaging decipher metabolomic and lipidomic heterogeneity in endometriosis

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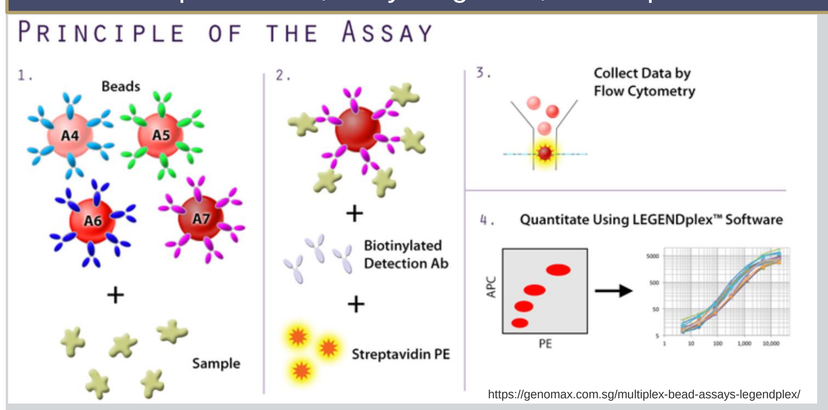
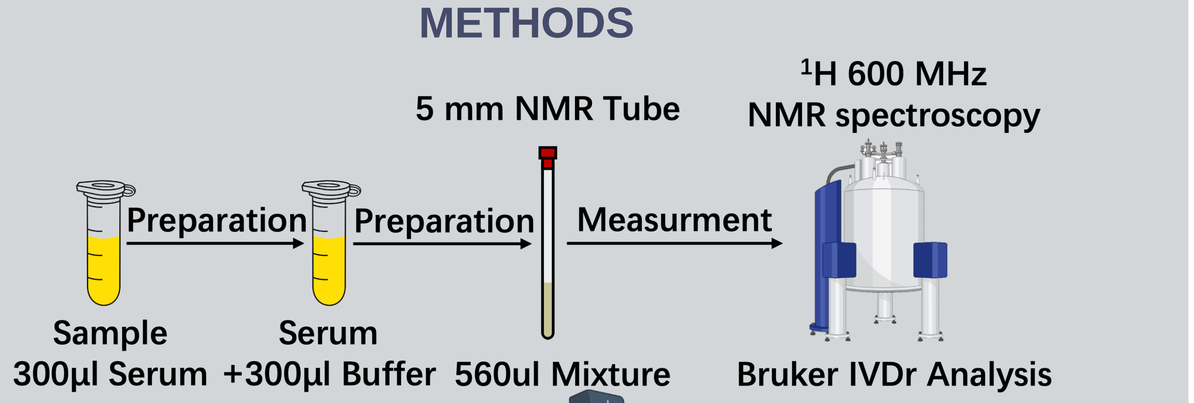
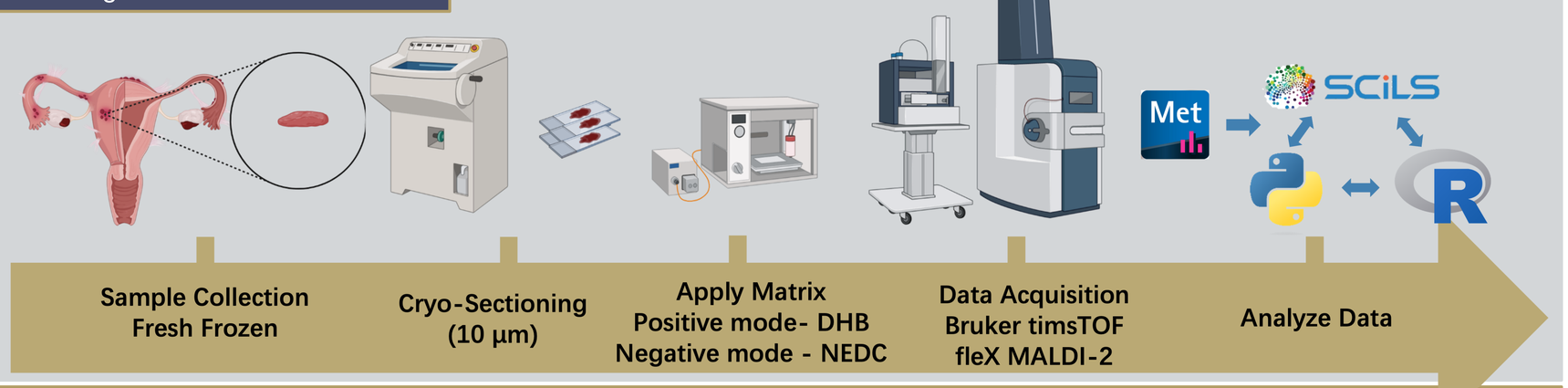
INTRODUCTION

- Endometriosis (EMT) affects about 190 million women of reproductive age worldwide with an economic burden comparable to or higher than other chronic diseases.
- Pelvic pain from endometriosis can only be temporarily managed via pharmacological intervention or surgery. It also causes infertility and a 50% increased risk for ovarian cancer.
- Currently, there are no accurate non-invasive diagnostic tests or biomarkers for endometriosis. Early diagnosis and timely intervention of endometriosis are urgent.
- We applied quantitative NMR spectroscopy to explore serum biomarkers for EMT but could not completely stratify all patient subgroups so we aimed for deeper phenotyping by MALDI.
- More research is needed to explore potential pathophysiological mechanisms and to ensure effective prevention, early diagnosis, and improved disease management.



METHODS





RESULTS

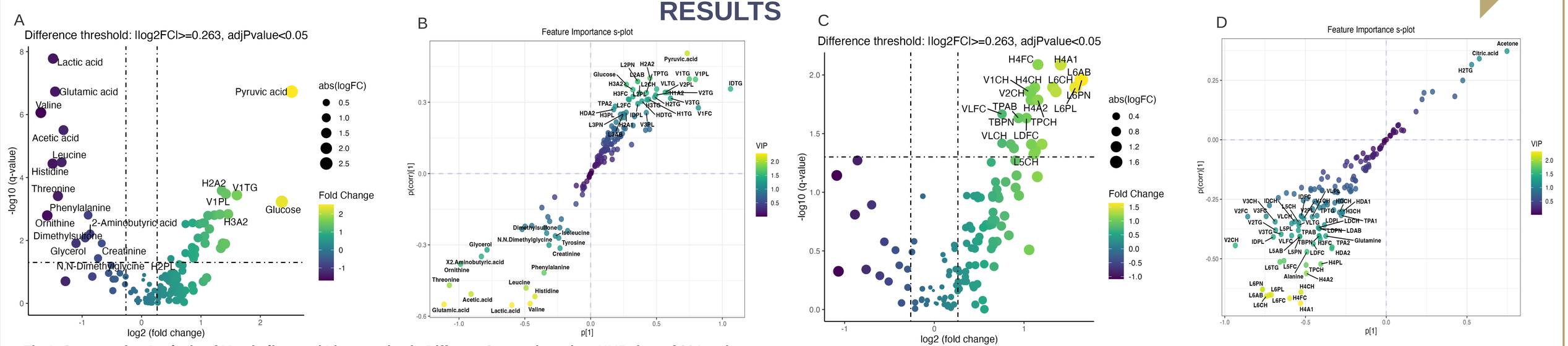


Fig 1. Comparative Analysis of Metabolites and Lipoproteins in Different Groups based on NMR data of 364 patients
A) Volcano Plot and B) S-Plot: Significant differences in metabolite levels were observed between the Endometriosis and Healthy groups. Pyruvic acid, glucose, and VLDL were significantly elevated in the Endometriosis group, whereas lactic acid, glutamic acid, valine, acetic acid, leucine, threonine, and phenylalanine were significantly reduced. Key metabolites distinguishing the Endometriosis group from the Healthy group are pyruvic acid and glucose, while acetic acid, threonine, and phenylalanine are prominent in the Healthy group. C) Volcano Plot and D) S-Plot: In the Borderline Ovarian Carcinoma (BOT) group compared to the Endometriosis group, there was a significant upregulation of LDL, HDL, and their subfractions, including L6AB, L6PN, H4FC, and H4A1 etc. The main distinguishing metabolites for the Borderline Ovarian Carcinoma group are acetone and citric acid, while HDL and LDL subfractions are more associated with the Endometriosis group.

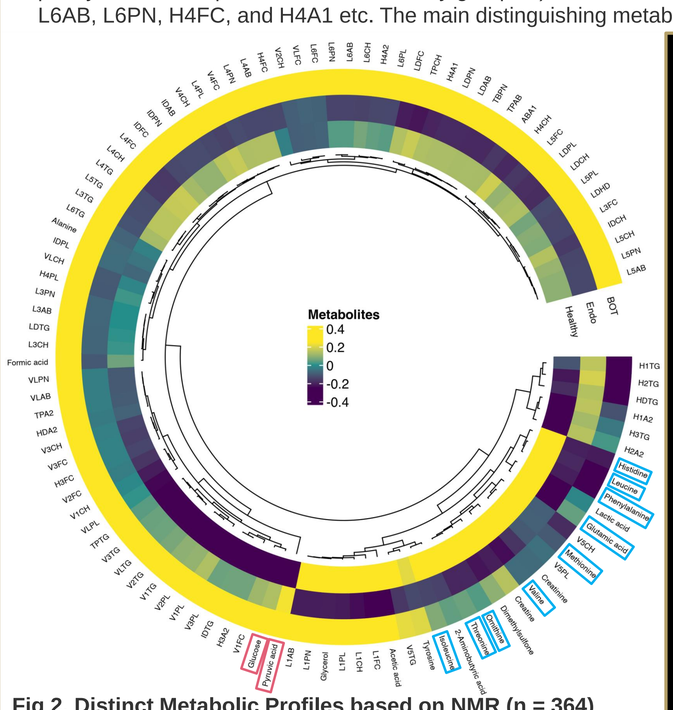


Fig 2. Distinct Metabolic Profiles based on NMR (n = 364)
The Endometriosis group exhibits elevated levels of metabolites such as glucose and pyruvic acid. Both the Endometriosis and BOT groups show increased lipid levels, including V3TG, H3TG, and TPTG, with BOT specifically upregulating unique lipid classes. Amino acids such as leucine, valine etc. are relatively downregulated in both the Endometriosis and BOT groups compared to healthy controls.

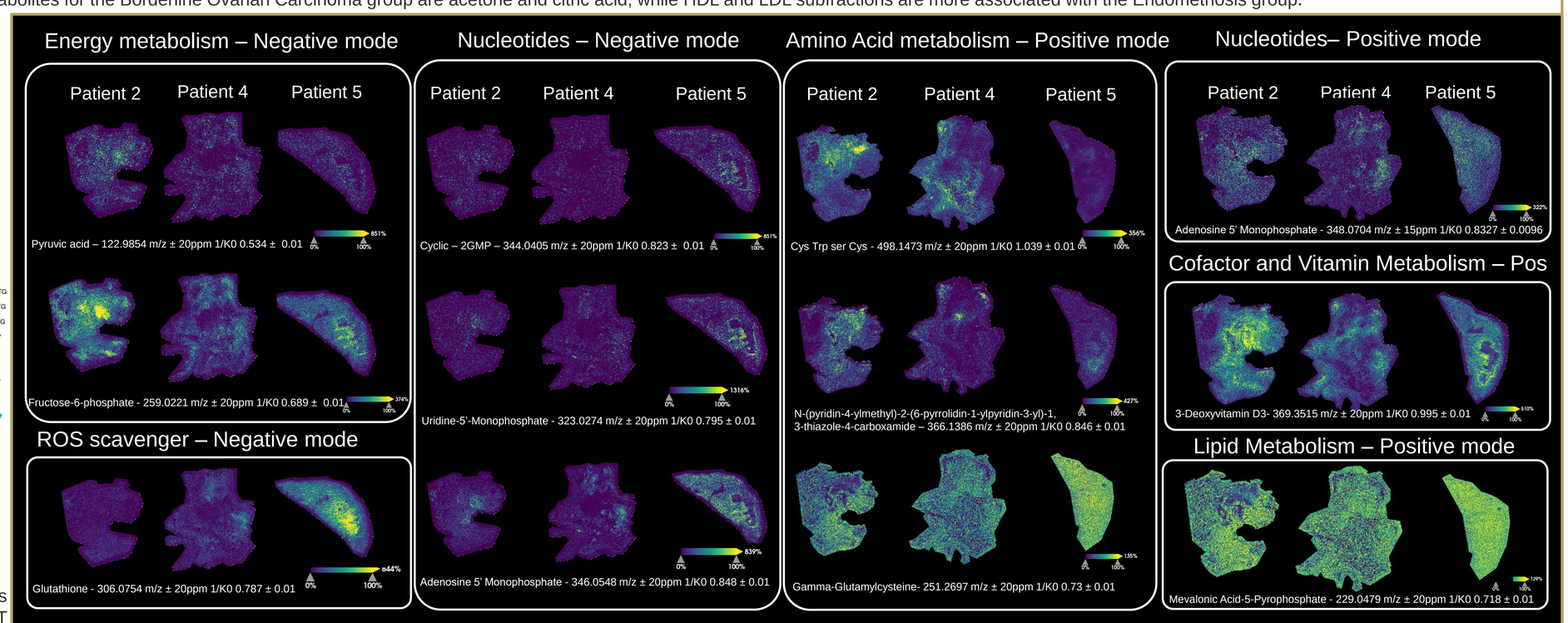


Fig 3. MALDI Imaging of Endometriosis Tissue
First explorative MALDI imaging (n = 3 patients with varying levels of lesion infiltration and distinct lesion locations) reveals distinct distributions of metabolites in endometriosis tissue. Energy metabolism-related metabolites such as pyruvic acid and fructose-6-phosphate are detected in negative MS mode. Nucleotide metabolism metabolites, including Cyclic-2GMP, Uridine-5'-Monophosphate (UMP), and Adenosine 5'-Monophosphate (AMP), are widely distributed. Amino acid metabolism metabolites like Cys-Trp-Ser-Cys, N-(pyridin-4-ylmethyl)-2-(6-pyrrolidin-1-ylpyridin-3-yl)-1,3-thiazole-4-carboxamide, and Gamma-Glutamylcysteine are observed in positive MS mode, showing varied intensities across different tissue locations.

CONCLUSION & OUTLOOK

B**ased on NMR serum data** of 364 patients, we hypothesize that **altered glycolysis and lipid metabolism** facilitate the growth and survival of ectopic endometrial tissue in endometriosis under previously reported hypoxic and inflammatory conditions, contributing to its characteristic clinically painful and inflammatory phenotype. BOT groups exhibit similar increases in glycolysis and lipid metabolism but display a more tumor-like profile with distinct upregulation of specific lipid species. The extent and specificity of these pathways suggest a higher degree of **metabolic reprogramming** associated with a pre-malignant state, characterized by enhanced energy production and biosynthesis to support cellular proliferation. These metabolic adaptations underscore key differences in the profiles of endometriosis, BOT, and OC as they evolve towards more aggressive malignancies. **First explorative MALDI imaging** provides insights into the spatial distribution of dysregulated metabolites **linked to energy, amino acid, and lipid metabolism**. Future research should include histological staining and comparative analysis of metabolic variations across different spatial locations within EMT, aiming to stratify endometriosis into distinct subtypes and elucidate underlying mechanisms.

ACKNOWLEDGEMENTS

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WSIC Werner Siemens Imaging Center

M3 MALIGNOME. METABOLOME. MICROBIOME.

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iFIT Visualizing and Targeting Cancer Stress

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