Assessing Urinary Proteins as Diagnostic Markers for Preeclampsia

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INTRODUCTION

- Preeclampsia (PE), a hypertensive pregnancy-specific disorder, is a leading cause of neonatal and maternal mortality affecting 5-8% of women globally.
- Currently, there is no reliable prognostic leading lack of diagnosis to be the primary cause of PE deaths.
- This project aimed to develop a reliable method of detecting PE by surveying urinary proteins associated with PE in PE and non-PE urine.
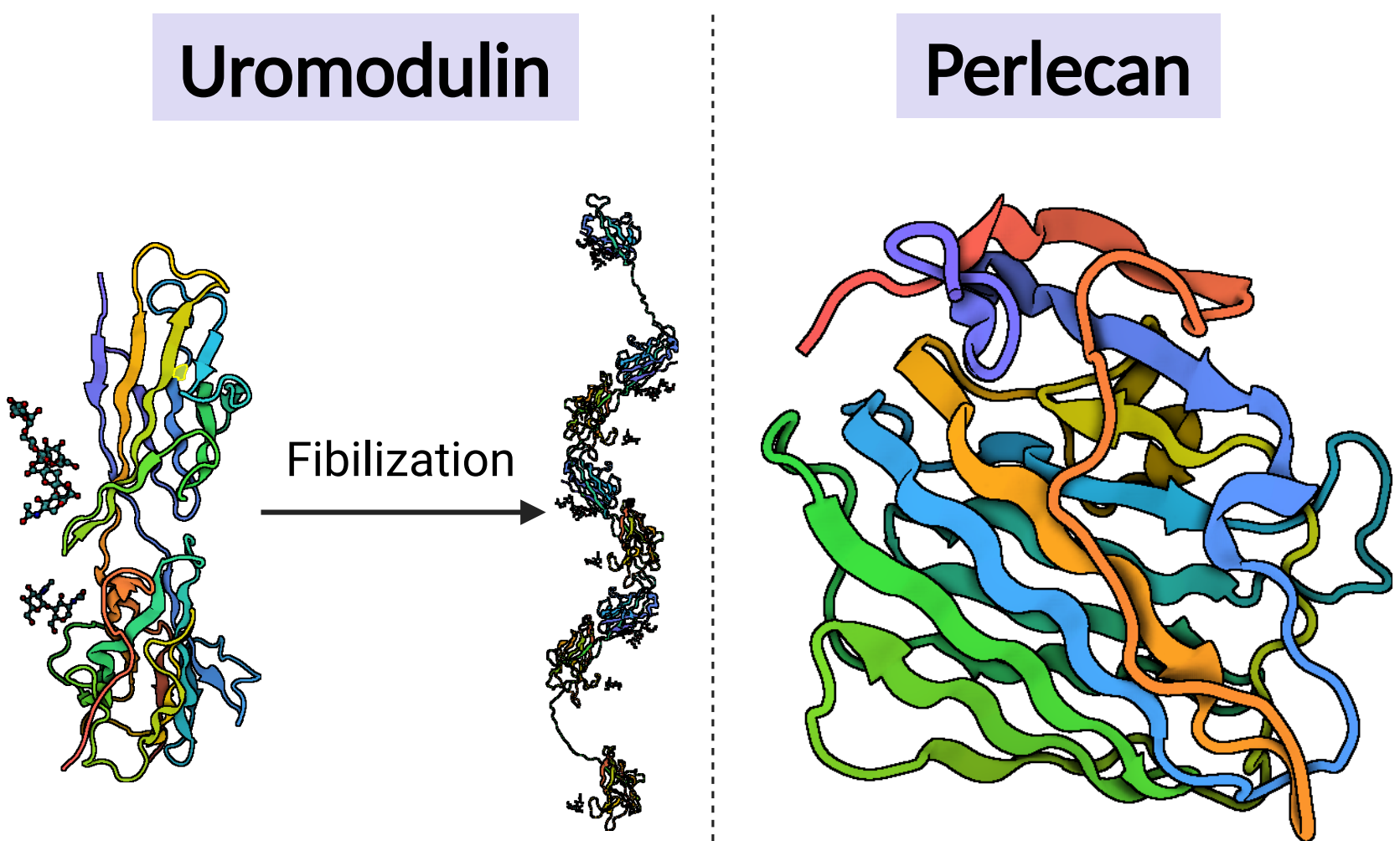
Uromodulin	Perlecan
Function: Fibrillary uromodulin traps bacteria in its net-like structure.	**Function:** Perlecan crosslinks components of the extracellular matrix
Diseased state: Fibrillary uromodulin traps the bacteria present in UTIs	**Diseased state:** Perlecan interacts with amyloid-β, which creates tangles that characterizes neurodegenerative diseases.
Relation to PE: UTIs increase one's risk of PE during pregnancy by 1.31 fold	**Relation to PE:** PE increases one's risk of developing dementia by 1.31-1.38 fold

Studies have found uromodulin and perlecan present in the urine of patients with PE at elevated levels compared to healthy urine.

Figure #1: Chart discussing the proteins of interest, uromodulin and perlecan

DOT BLOT

- Dot blots were used to quantify the amount of each protein present in the urine samples.
- Samples **were spun down to isolate the insoluble fraction and diluted by a factor of 1:1000.**
- This was spotted onto nitrocellulose membrane alongside a standard urine control
- The membranes were incubated with a **primary antibody** specific to the protein and a secondary florescent antibody
  - Uromodulin: The primary antibody bound to the bacterial binding domain, only quantifying fibrillary uromodulin.
  - Perlecan: The primary antibody bound to domain IV.
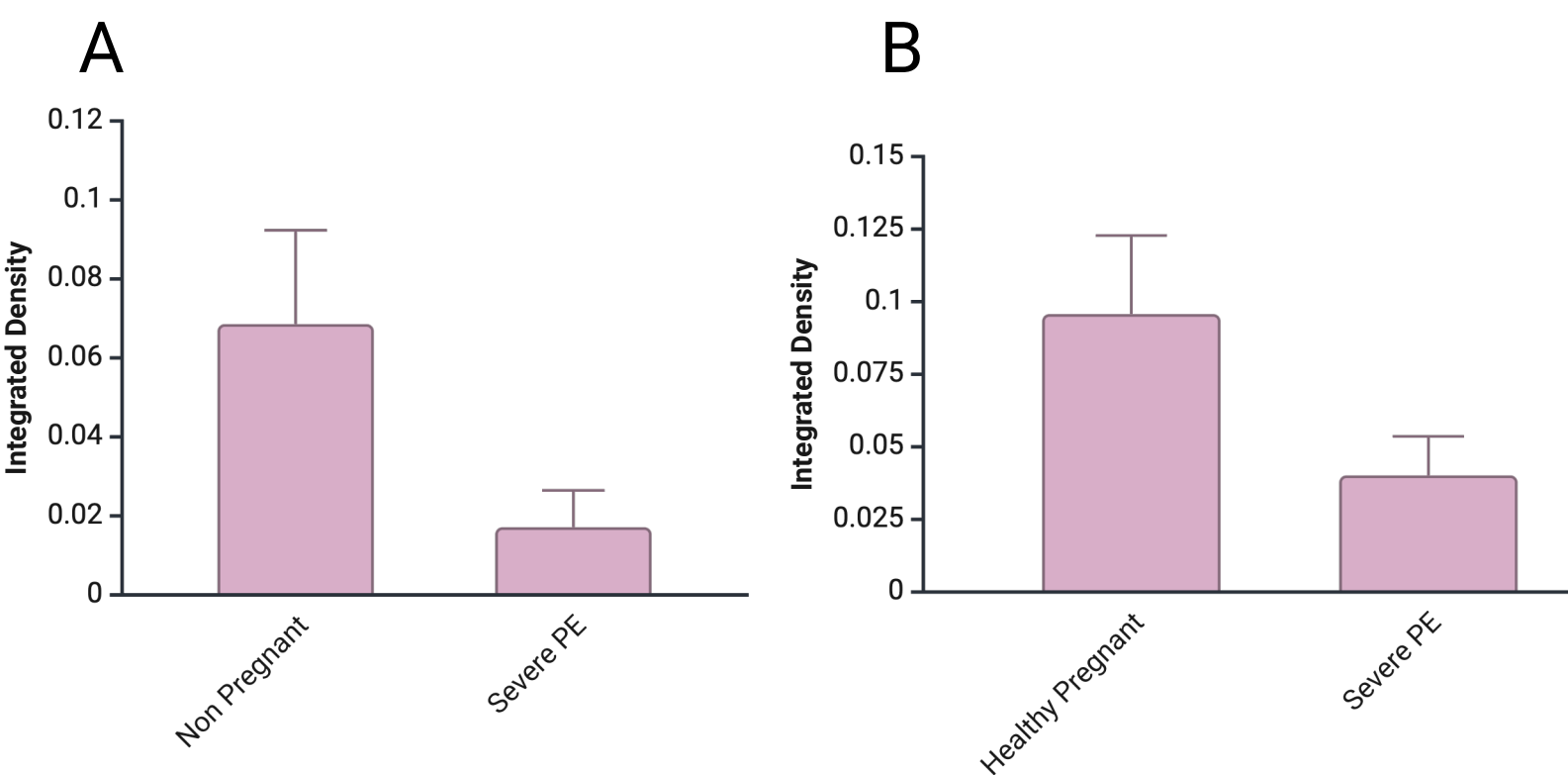- The membranes were scanned using florescent imaging and **quantified** in ImageJ.

Figure #2: Quantified dot blot data measuring the integrated density of dots. Bars show standard error of the mean. A) Uromodulin, comparing healthy non-pregnant and severe PE. B) Perlecan, comparing healthy pregnant and severe PE.
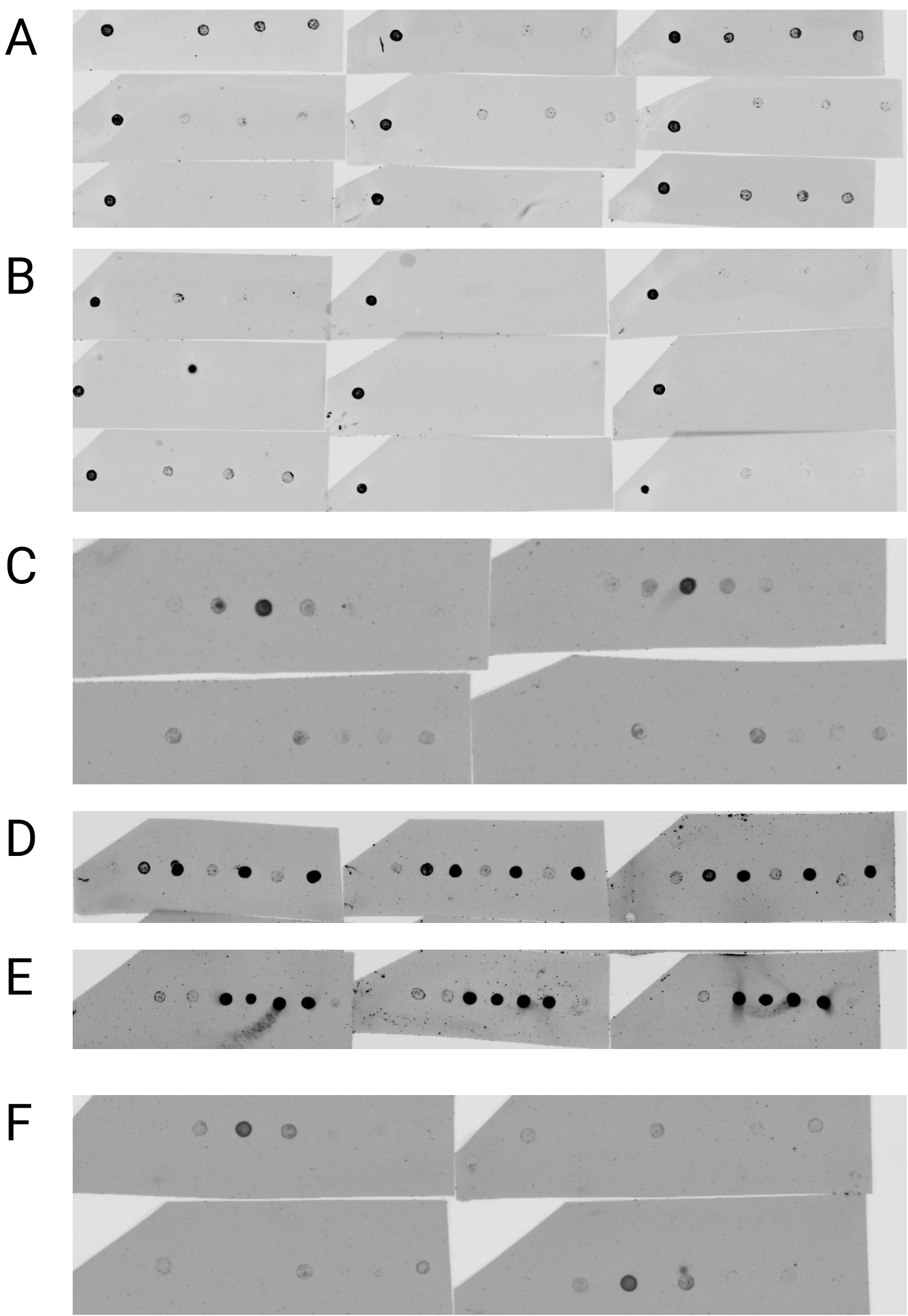

Figure #3: Dot blot scans measuring uromodulin and perlecan across cohorts. A) Uromodulin+healthy non-pregnant. B) Uromodulin+severe preeclampsia. C) Perlecan+healthy non-pregnant. D) Perlecan + healthy pregnant. E) Perlecan+high risk pregnant. F) Perlecan+severe preeclampsia.

PULL-DOWN MASS SPECTROMETRY

Pull-down assay is used to confirm the physical interaction between proteins. We used pull-down assay to extract proteins that have interactions with uromodulin and analyzed them with **mass spectrometry** to determine the identities.
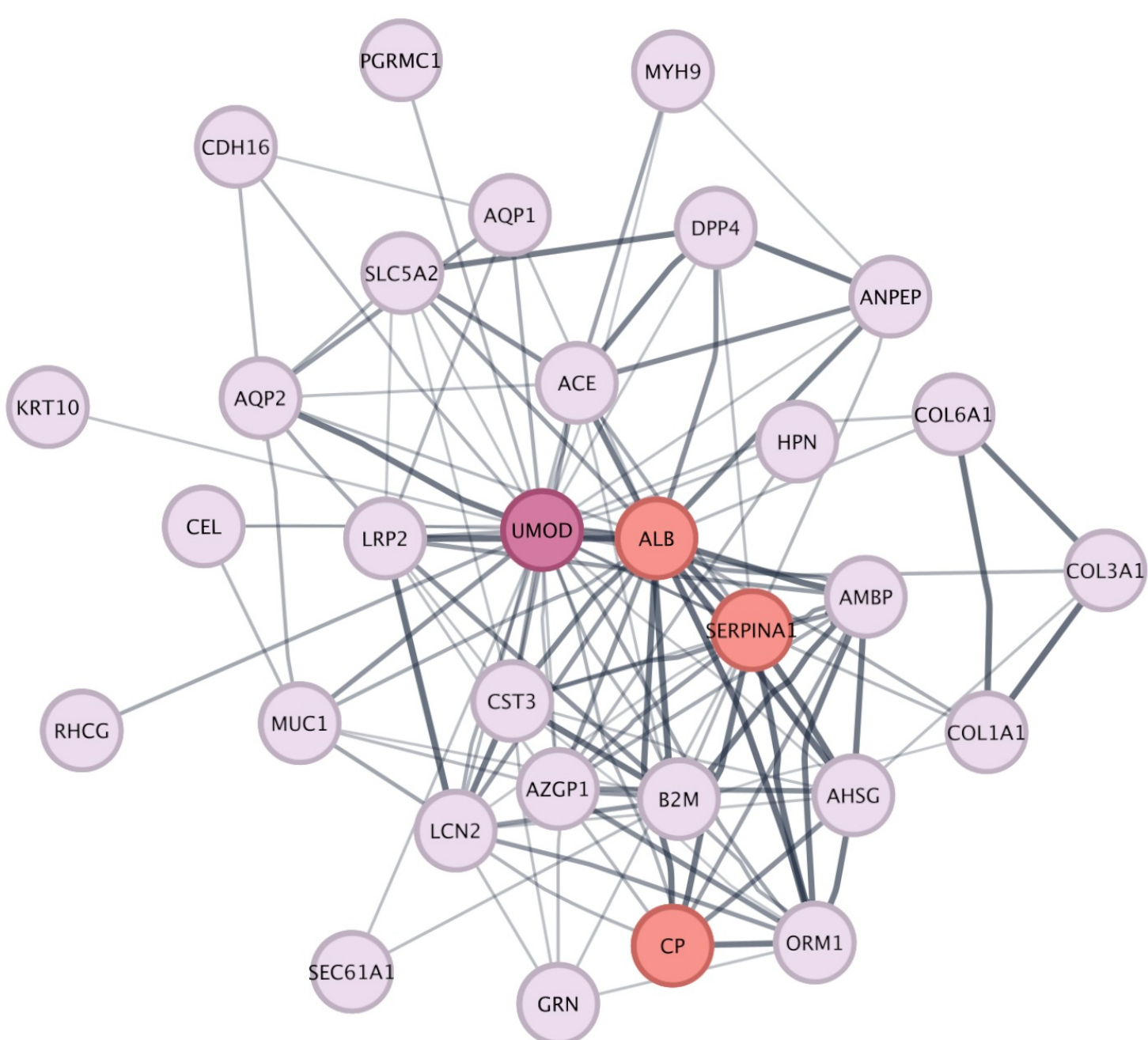

Figure #4: STRING network depicting proteins known to colocalize with uromodulin

Proteins	Fold Enrichment	
SERPINA 1	29-fold	Amyloid fibril candidate
Isoform SERPINA 1	89-fold	Amyloid fibril candidate
Ceruloplasmin	88-fold	Amyloid fibril candidate
Transthyretin	16-fold	Amyloid fibril candidate
Serum Albumin	8-fold	Amyloid fibril candidate
Perlecan	16-fold	Co-localizes with amylid beta
PTGDS	510-fold	Major apoptotic factor in Alzheimer's disease plasma

Figure #5: Chart listing proteins that appeared significantly higher on the preeclamptic uromodulin mass-spec pull down compared to healthy pregnant pulldown.

CONCLUSIONS

- Uromodulin levels are not shown to be significantly greater in preeclamptic urine.
- Perlecan levels are not shown to be significantly greater in preeclamptic urine.
  - Preliminary evidence suggests perlecan levels are lower in the preeclamptic urine.
- Pull-down mass spectrometry reveals that some proteins implicated in neurodegenerative diseases increase in the urine of women with preeclampsia.
  - Among those proteins, SERPINA1, Albumin, and Ceruloplasmin are predicted to have associations with uromodulin.
  - Transthyretin, perlecan, and PTGDS are not currently predicted to have associations with uromodulin.
- We suggest that these interactions are caused by amyloid fibrils being trapped in uromodulin's net-like structure.
  - This indicates a relationship between these proteins' roles in preeclampsia and neurodegenerative disease and is a promising avenue for further work.

REFERENCES

1. Buhimschi, Irina A et al. "Protein misfolding, congophilia, oligomerization, and defective amyloid processing in preeclampsia." *Science translational medicine* vol. 6,245 (2014): 245ra92. doi:10.1126/scitranslmed.3008808
2. Carty, David M et al. "Urinary proteomics for prediction of preeclampsia." *Hypertension (Dallas, Tex. : 1979)* vol. 57,3 (2011): 561-9. doi:10.1161/HYPERTENSIONAHA.110.164285
3. Khan, Bisma et al. "Preeclampsia Incidence and Its Maternal and Neonatal Outcomes With Associated Risk Factors." *Cureus* vol. 14,11 e31143. 6 Nov. 2022, doi:10.7759/cureus.31143
4. Lavorgna, Tessa R et al. "Perlecan: a review of its role in neurologic and musculoskeletal disease." *Frontiers in physiology* vol. 14 1189731. 30 May. 2023, doi:10.3389/fphys.2023.1189731
5. Tong S, Hastie R. Preeclampsia and the Risk of Young-Onset Dementia. *JAMA Netw Open.* 2024;7(5):e2412780. doi:10.1001/jamanetworkopen.2024.12780
6. Weiss, Gregor L et al. "Architecture and function of human uromodulin filaments in urinary tract infections." *Science (New York, N.Y.)* vol. 369,6506 (2020): 1005-1010. doi:10.1126/science.aaz9866

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