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Background

White matter hyperintensities (WMH) and cerebral microbleeds (CMB) are common cerebrovascular lesions in Alzheimer's disease (AD) and contribute to cognitive decline. However, the molecular processes underlying these vascular injuries and their relationship to AD pathology remain poorly characterized, limiting understanding of mixed vascular-AD pathology.

We aim to investigate CSF proteomic signatures of WMH and CMB, and their dependence on amyloid, tau and cognitive status.

The flowchart illustrates the study design and participant classification. It is divided into three main sections: 1. COHORT, 2. DATA ACQUISITION, and 3. PARTICIPANT CLASSIFICATION.

1. COHORT

- EMIF-AD MBD** (represented by an icon of three people)
- N = 217**
- Mean age 67.0 ± 8.1**
- 50.7% females**
- 49.3% males**

2. DATA ACQUISITION

- Brain MRI** (represented by an icon of a brain scan)
- NP tests** (represented by an icon of a clipboard and a person's head)
- CSF** (represented by an icon of a brain with a keyhole)
- Immunoassays** (CSF Aβ42, CSF p-tau)
- TMT spectrometry** (CSF proteomics)

3. PARTICIPANT CLASSIFICATION

- WMH** (White Matter Hyperintensities)
 - Abnormal if Fazekas ≥ 2*
 - n WMH- = 155 (71.4%)
 - n WMH+ = 44 (20.3%)
 - 18 missing WMH (8.3%)
- CMB** (Cerebral Microbleeds)
 - Abnormal if ≥ 1 microbleeds*
 - n CMB- = 131 (60.4%)
 - n CMB+ = 40 (18.4%)
 - 46 missing CMB (21.2%)
- CSF Aβ42 (A)**
 - Local GMM cut-offs*
 - n A- = 92 (44.4%)
 - n A+ = 125 (57.6%)
- CSF p-tau (T)**
 - Local cut-offs*
 - n T- = 116 (53.5%)
 - n T+ = 101 (46.5%)
- Cognitive status**
 - Based on NP tests*
 - n Normal = 85 (39.2%)
 - n MCI = 132 (60.8%)

CSF proteomics
*Linear regression
adjusted for age and sex*

ONLY FOR WMH & CMB:

- Comparison of proteomic signatures
- Functional enrichment using Gene Ontology

FOR ALL:

Multi-group intersection to identify the dependence of the proteomic signatures of WMH and CMB on amyloid, tau and cognitive status.

Comparison
WMH+ vs WMH-
CMB+ vs CMB-
A+ vs A-
T+ vs T-
MCI vs Normal

CSF proteomic signatures of WMH and CMB, and their overlap:
WMH: 123 dysregulated proteins enriched mainly for extracellular matrix remodeling, angiogenesis, and complement activation (Figure 1A).
CMB: 35 dysregulated proteins enriched for oxidative stress and hematopoiesis (downregulated), and ECM remodeling (Figure 1B).
Overlap: WMH- and CMB-associated proteins were largely distinct (Figure 1C).

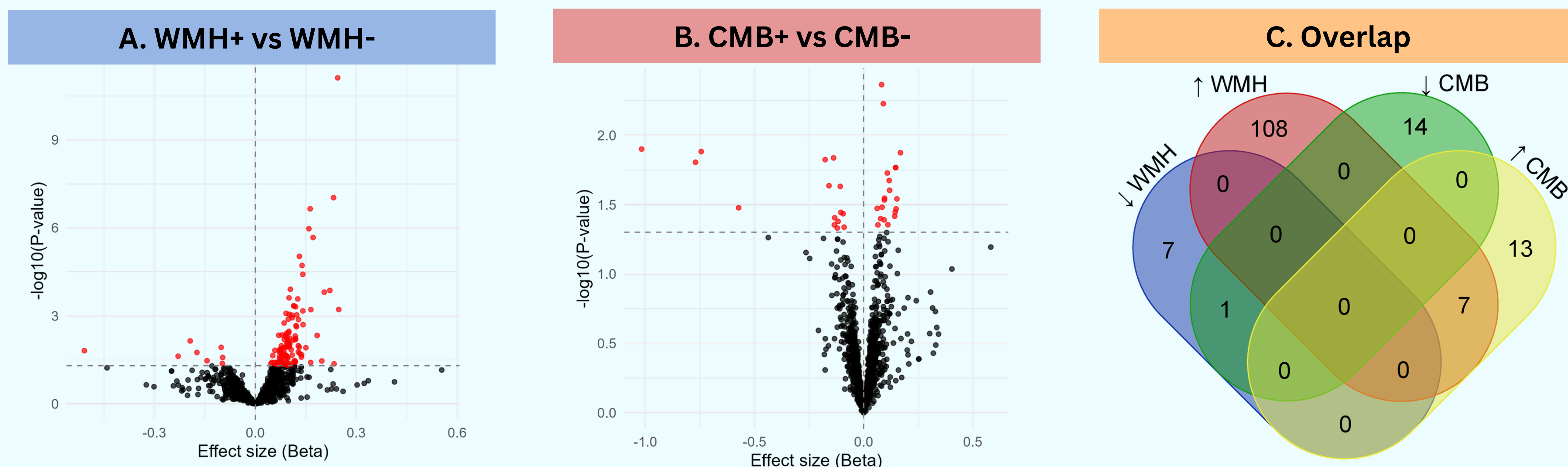


Figure 1. CSF proteomic signatures associated with white matter hyperintensities (WMH) and cerebral microbleeds (CMB). (A) Volcano plot showing the proteomic profile of WMH+ versus WMH- with age and gender adjustment. (B) Volcano plot showing the proteomic profile of CMB+ versus CMB- with age and gender adjustment. (C) Venn diagram showing the number of proteins unique to WMH, unique to CMB, and shared between WMH and CMB.

WMH: Associations largely independent of amyloid and cognitive status, but partly dependent of tau (Figure 2A)
CMB: Associations largely independent of amyloid and cognitive status, but partly dependent of tau (Figure 2B)

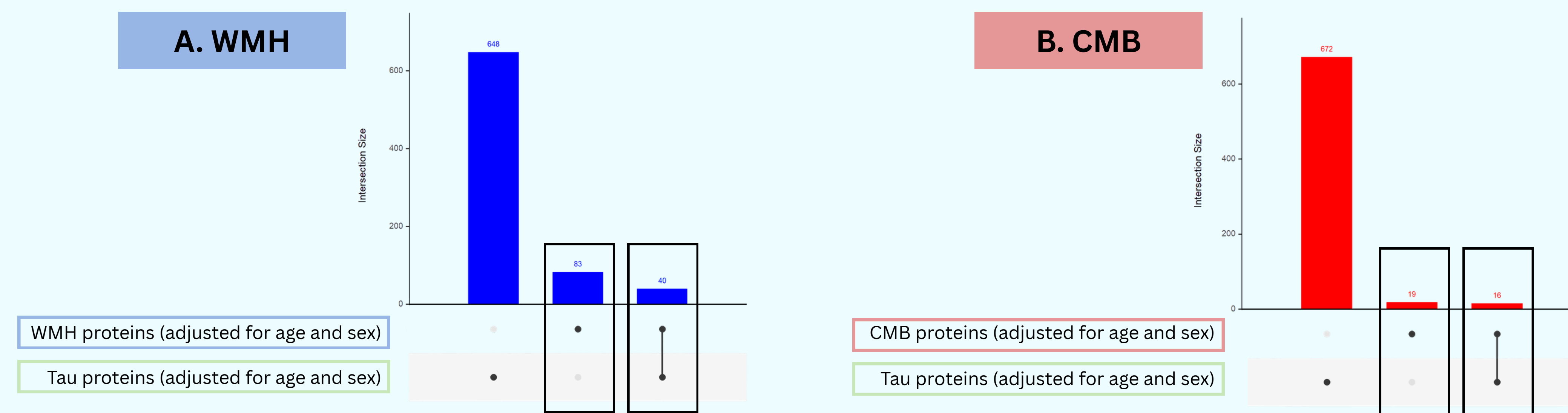


Figure 2. Overlap of WMH- and CMB-associated proteins with tau-related CSF proteomic changes. (A) Upset plot showing the number of WMH-associated proteins that overlap with tau-related proteins and those unique to WMH. (B) Upset plot showing the number of CMB-associated proteins overlapping with tau-related proteins and those unique to CMB.

WMH and CMB showed distinct CSF proteomic signatures largely independent of amyloid and cognitive status, with a subset overlapping tau-related changes, suggesting tau pathology may be partially linked to vascular injury. Considering WMH and CMB in AD trials may improve stratification and outcome interpretation by identifying participants with coexisting vascular and amyloid–tau pathology.

