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HUMAN NEUROPATHOLOGY

Stroke-Induced Gut Dysbiosis Increases Alzheimer's Disease Risk: Insights from Immunohistochemistry and Single-Cell Spatial Transcriptomics

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Abstract

Background: Stroke increases the risk of Alzheimer's disease (AD), but underlying mechanisms remain unclear. This study investigates whether gut dysbiosis (imbalance in gut microbes) from acute ischemic stroke worsens AD pathology. Using fecal microbiota transplantation (FMT) from stroke patients into 3xTg-AD mice, we examine its impact on neuroinflammation and AD markers (Total Tau, GFAP, and IBA1). By integrating immunohistochemistry (IHC) and single-cell spatial transcriptomics, we assess dysbiosis-driven changes in neuroinflammation, AD pathology, cell typing, and targeted gene expression in the brain to elucidate the gut-brain axis and explore therapeutic strategies.

Method: Stool samples from stroke patients ($n = 8$) and age-matched healthy controls ($n = 8$, aged 55-80 years) were used for FMT in three-month-old 3xTg-AD mice. Mice were randomized into naïve control (male: $n = 5$, female: $n = 8$), Healthy-FMT (male: $n = 12$, female: $n = 15$), and Stroke-FMT (male: $n = 14$, female: $n = 17$) groups. FMT followed a one-week antibiotic treatment. IHC assessed Total Tau (hippocampus and cortex), GFAP, and IBA1 (hippocampus) and CosMx Spatial Molecular Imaging (SMI) analyzed cell type-specific changes and targeted gene expression in the whole brain of mice.

Result: IHC analysis revealed a significant increase in neuroinflammation and AD pathology in Stroke-FMT mice, with Total Tau levels significantly elevated in the hippocampus and cortex ($p < 0.001$) (Figure 1a, c), and more pronounced increase in males ($p < 0.01$) (Figure 1b, d), also shown the representative IHC images for all groups (Figure 1e–g). GFAP expression in astrocytes (Figure 2a, b) and IBA1 expression in microglia (Figure 2c, d) were significantly higher in Stroke-FMT than Healthy-FMT ($p < 0.05$) in hippocampus, indicating glial activation and neuroinflammation. CosMx-

SMI cell typing showed increased astrocyte and microglia density in Stroke-FMT, consistent with gut dysbiosis-induced neuroinflammation compared to Healthy-FMT in UMAP clustering (Figure 3a, c). Stroke-FMT mice exhibited dysregulated expressions of ApoE, GFAP, APP, PSEN1, BIN1, and SORL1 across astrocytes, microglia, CA3, and DG, suggesting intensified neuroinflammation and synaptic impairment compared to Healthy-FMT, potentially exacerbating AD pathology (Figure 3b, d).

Conclusion: Stroke donor FMT-induced gut dysbiosis exacerbates AD pathology, emphasizing the critical role of the gut-brain axis in linking stroke and AD. Targeting gut dysbiosis may offer a novel therapeutic strategy for stroke-related AD progression.

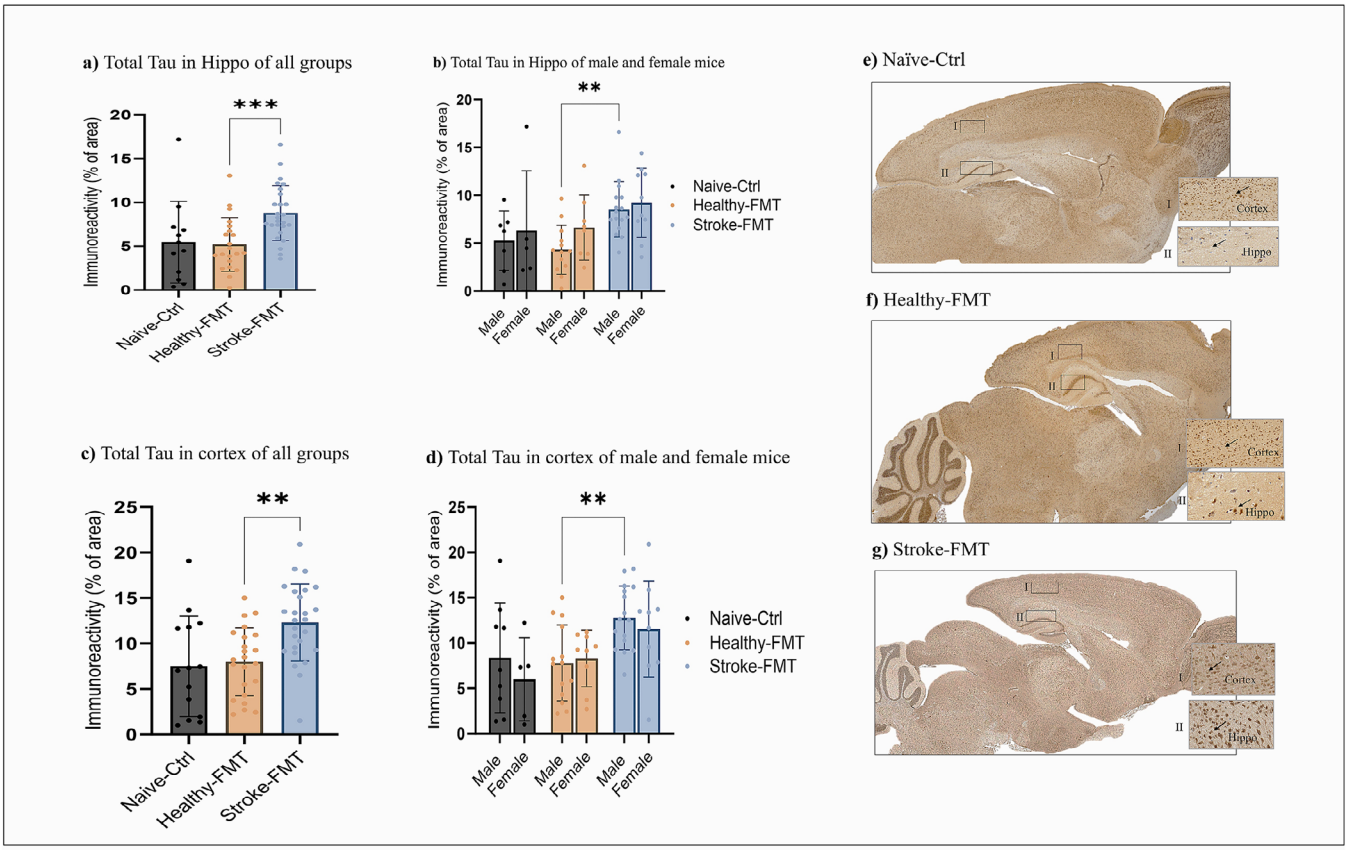

Fig. 1: IHC analysis of neurofibrillary tangles in the Hippo and Cortex in 3xTg-AD mouse brains; a) Immunoreactivity of antibodies against Total Tau in Hippo of naïve, Healthy-FMT, and Stroke-FMT mice. b) IHC of Total Tau in Hippo from male and female mice from all groups. c) IHC of Total Tau in CC in Naïve ctrl, Healthy-FMT, and Stroke-FMT groups. d) IHC of Total Tau in CC from male and female mice with all groups. e-g) Representative sagittal brain section (100 um) for Naïve-Ctrl, Healthy-FMT, and Stroke-FMT (I- Cerebral Cortex (CC) and II- Hippocampus (Hippo)). *p < 0.05; **p < 0.01; *** p < 0.001.

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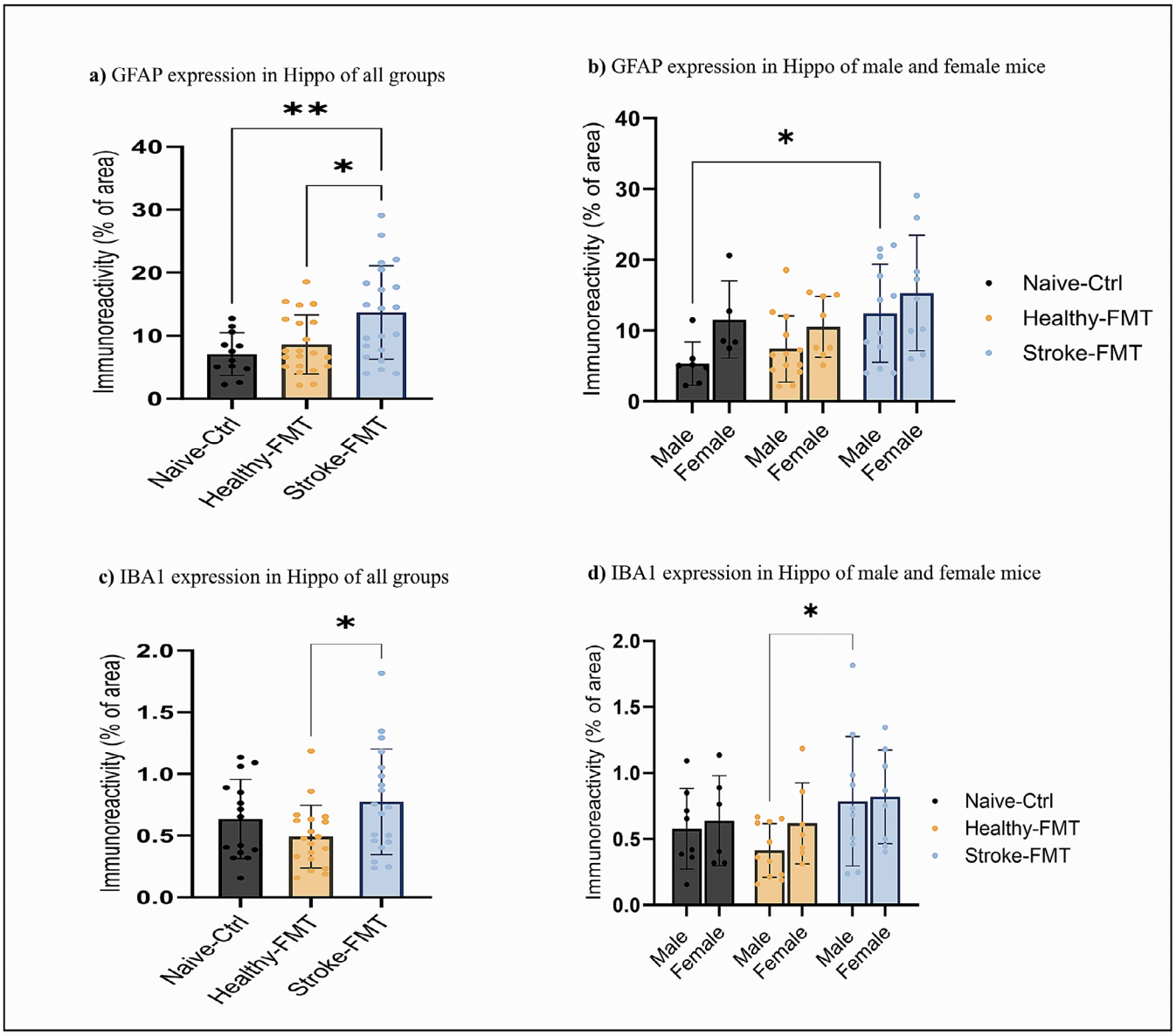

Fig. 2: IHC analysis of Astrocytes biomarker GFAP and Microglial biomarker IBA1 in 3xTg-AD mice Hippocampus. a) Immunoreactivity (% of area) of antibodies against GFAP for astrocytes in Hippo of Naïve-Ctrl, Healthy-FMT, and Stroke-FMT mice. b) IHC of GFAP in Hippo from male and female mice with all groups. c)IHC analysis of Microglial biomarker IBA1 in Hippo of Naïve-Ctrl, Healthy-FMT, and Stroke-FMT mice. d) IHC of IBA1 in Hippo in male and female mice from all groups. *$p < 0.05$, **$p < 0.01$.

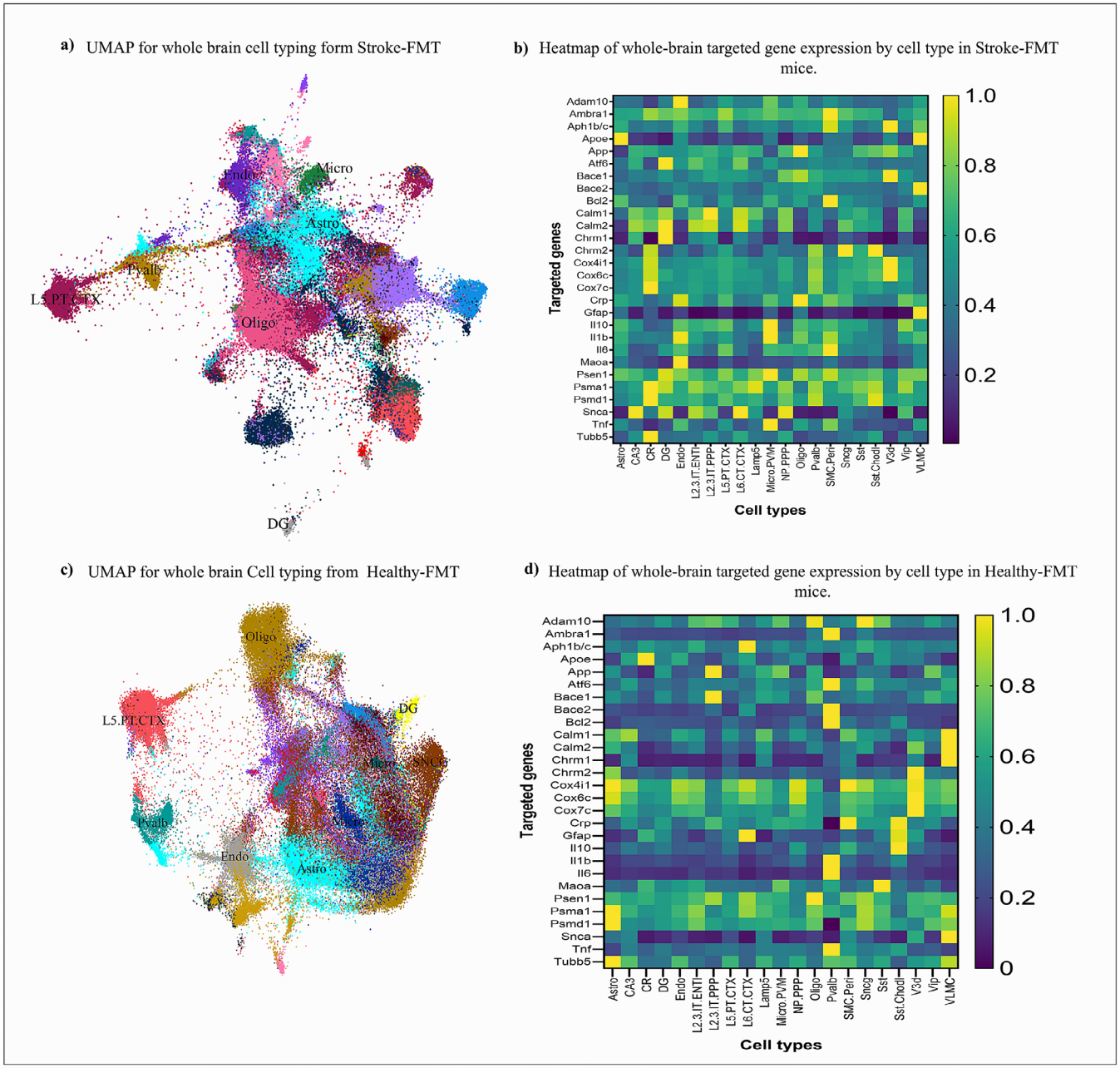

Fig. 3: CosMx SMI technology for cell typing and targeted gene expression; a) UMAP for supervised In Situ cell typing in Stroke-FMT mice brain; b) Heatmap showing expression of the targeted genes with respect to cell typing in Stroke-FMT mice brain; c) UMAP for supervised In Situ cell typing in Healthy-FMT mice brain; d) Heatmap showing expression of targeted genes with respect to cell typing in Healthy-FMT mice brain.