Transcriptomic Profiles of AKAP12 Deficiency in Mouse Corpus Callosum

Bioinformatics and Biology Insights
Volume 18: 1–5

DOI: 10.1177/11779322241276936

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ABSTRACT: A-kinase anchor protein 12 (AKAP12), a scaffold protein, has been implicated in the central nervous system, including blood-brain barrier (BBB) function. Although its expression level in the corpus callosum is higher than in other brain regions, such as the cerebral cortex, the role of AKAP12 in the corpus callosum remains unclear. In this study, we investigate the impact of AKAP12 deficiency by transcriptome analysis using RNA-sequencing (RNA-seq) on the corpus callosum of AKAP12 knockout (KO) mice. We observed minimal changes, with only 13 genes showing differential expression, including *Akap12* itself. Notably, *Klf2* and *Sgk1*, genes potentially involved in BBB function, were downregulated in AKAP12 KO mice and expressed in vascular cells similar to *Akap12*. These changes in gene expression may affect important biological pathways that may be associated with neurological disorders. Our findings provide an additional data set for future research on the role of AKAP12 in the central nervous system.

KEYWORDS: AKAP12, knockout mice, corpus callosum, RNA-seq

RECEIVED: March 28, 2024. **ACCEPTED:** August 6, 2024.

TYPE: Short Communication

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a fellowship from the Uehara Memorial Foundation (to T.H.), funding from the National Institutes of Health (NIH) (to K.A.), and Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS) KAKEN (to H.T.).

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Introduction

A-kinase anchor protein 12 (AKAP12) is a scaffold protein that interacts with several key signaling molecules, including protein kinase C, protein kinase A, protein phosphatase 2B, calmodulin, cyclins, F-actin, lipids, and Src, and is involved in various intracellular and extracellular processes.[1] The AKAP12 plays a crucial role in the central nervous system,[2] widely expressed in the gray and white matter of the brain and spinal cord,[2-4] predominantly in pericytes and endothelial cells but also astrocytes, oligodendrocytes, and neurons.[2,5-7] In addition, AKAP12 has been reported to be involved in angiogenesis,[8,9] and AKAP12 is essential for the formation of the blood-brain barrier (BBB).[10] We have found that AKAP12 expression increases in endothelial cells after ischemic stress, and its deficiency leads to increased endothelial permeability through the disruption of tight junction proteins.[6] In addition, AKAP12 in pericytes regulates the secretion of several trophic factors to support oligodendrocyte differentiation in adult white matter, and its deficiency results in cognitive impairments.[5] As AKAP12 is more abundant in the corpus callosum than in the cortex and its expression gradually decreases with age,[7] AKAP12 may be involved in neurological diseases, such as stroke and vascular dementia. However, the effects of AKAP12 deficiency in the corpus callosum remain largely unknown. Therefore, in this study, we investigated how AKAP12 deficiency affects the transcriptome of the mouse corpus callosum using middle-aged wild-type (WT) and AKAP12 knockout (KO) mice, considering that aging is a major risk factor for neurological diseases.

Methods

Animals, Tissue Sampling, and RNA Extraction

All experimental procedures followed the National Institutes of Health (NIH) guidelines and were approved by the Massachusetts General Hospital Institutional Animal Care

and Use Committee. The AKAP12 KO mouse line (C57BL/6J background)[11] was provided from the Gelman Lab at Roswell Park Comprehensive Cancer Center and was maintained/expanded in the animal facility at Massachusetts General Hospital. All mice were housed in a specific pathogen-free with a 12-hour light/dark cycle and had unrestricted access to food and water. For genotyping, we used the forward primer 5'-CGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCG-3' and the reverse primer 5'-CTCAGCCTTTGCCAGAATAGGCACTGCCCC-3' to detect the KO allele and used the forward primer 5'-CGCTGTACTACTAAGGAGAGTGTTACGC-3' and the reverse primer 5'-CCTC-CTGGGTCTCAGCCAGTTTCTCAGGGG-3' to detect the WT allele. Only male mice were used in this study to avoid potential confounding effects of hormonal changes during the estrous cycle in females, especially given our limited sample size. Sample sizes were determined based on our previous experience and experimental feasibility.

After euthanasia, 8-month to 10-month-old male AKAP12 KO mice (n = 4) and WT mice (n = 3) were perfused with pre-chilled phosphate-buffered saline (PBS) (RNase-free). Collecting of the corpus callosum from the brains was as previously described.[12] All sample collection was conducted 3 to 5 hours after the onset of the light cycle. For RNA isolation, we used QIAzol (QIAGEN, Venlo, The Netherlands, #79306) according to the manufacturer's instructions.

RNA-sequencing and Bioinformatics Analysis

The library using the RNA samples was prepared using an rRNA depletion technique, and RNA-sequencing (RNA-seq) was performed on Illumina HiSeq4000 (paired-end; 2 × 150 bp), carried out by Genewiz, Inc (South Plainfield, New Jersey). The FASTQ files were mapped with STAR (version: 2.7.10a; mm10) and quantified using RSEM (version: 1.3.3). R (version 4.3.1) with packages such as DESeq2 (version 1.42.0) was used to detect differential expression genes (DEGs), |log-2fold change (FC)| > 0.58, adjusted *P*-value (padj) < .05, mean base >10, using normalized counts, and transcripts per kilobase million (TPM) values for plotting.

Results and Discussion

Corpus callosum samples from AKAP12 KO mice and WT mice were collected as previously described,[12] and RNA-seq

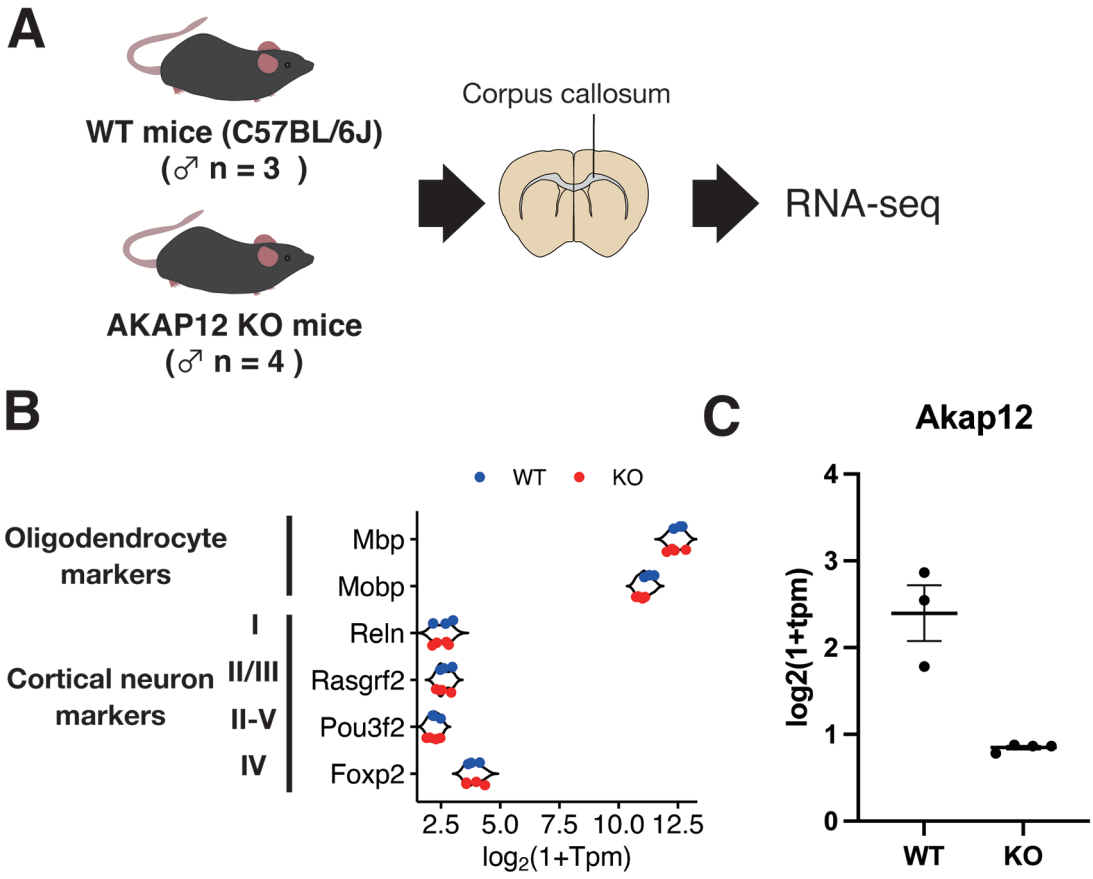

Figure 1. Sample collection of the corpus callosum from AKAP12 KO mice: (A) Schematic representation illustrating the collection of the corpus callosum in wild-type (WT) and AKAP12 KO mice. The isolated RNA was processed for RNA-seq. (B) Violin plots representing the expression of oligodendrocyte markers (*Mbp* and *Mobp*) and cortical neuron markers (*Reln* for layer I, *Rasgrf2* for layer II/III, *Pou3f2* for layer II-V, and *Foxp2* for layer IV). (C) Bar plot showing the *Akap12* gene expression in WT and AKAP12 KO mice. Error bars indicate SEM.

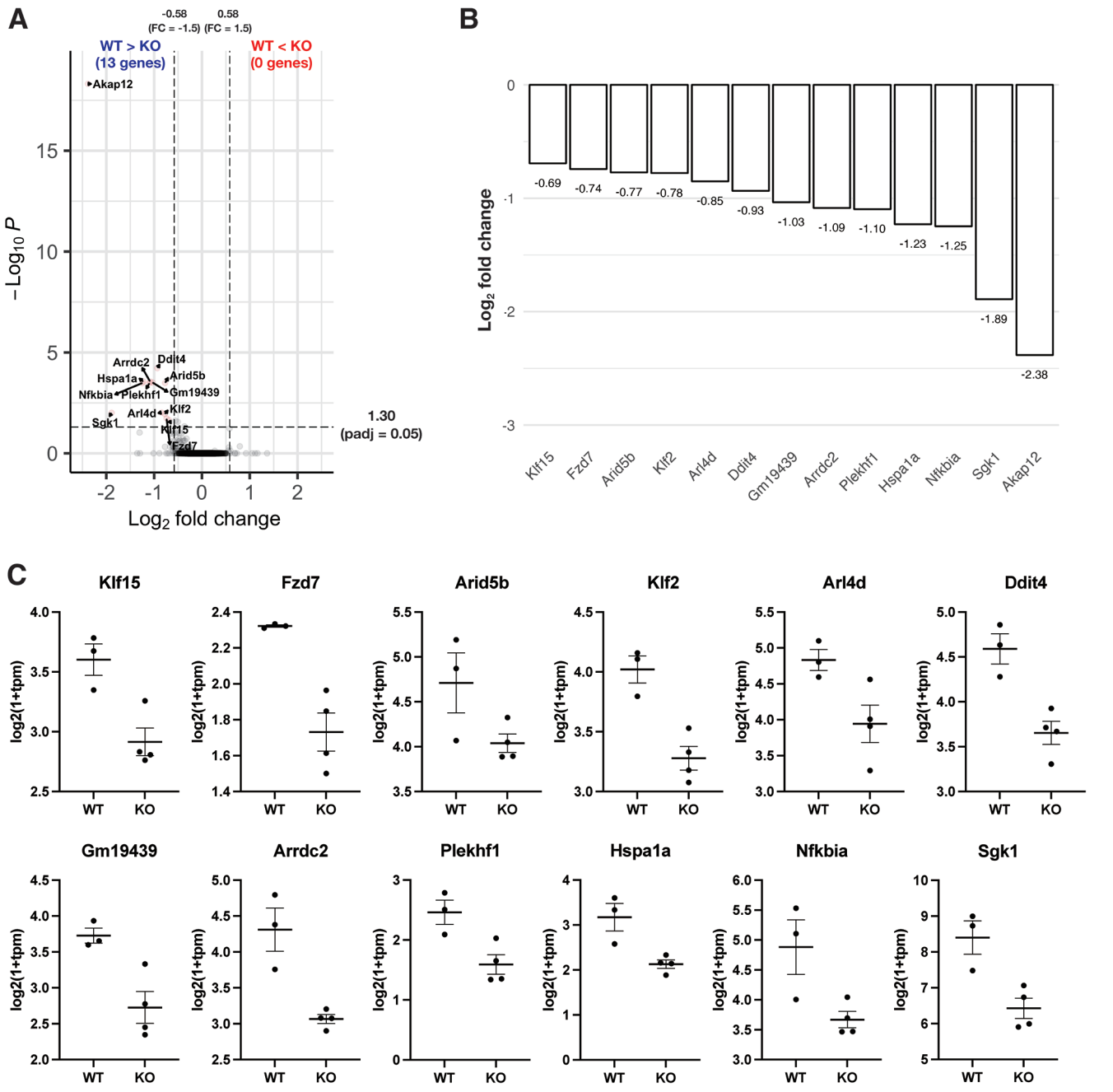

Figure 2. Transcriptome profiling of the corpus callosum in AKAP12 KO mice: (A) Volcano plot illustrating DEGs (a total of 13 genes) between WT and AKAP12 KO mice, |log2fold change (FC)| > 0.58, adjusted *P*-value (padj) < .05, mean base >10. (B) Bar plot showing fold changes of the DEGs in AKAP12 KO mice compared with WT mice. (C) Bar plots (with dot plots) comparing gene expression between WT and AKAP12 KO mice (For *Akap12*, see Figure 1C). Error bars indicate SEM.

was performed (Figure 1A). We checked for oligodendrocyte markers (Figure 1B), and higher expression of oligodendrocyte markers indicated corpus callosum purity. We also confirmed an 82.3% decrease in *Akap12* expression in AKAP12 KO mice (Figure 1C).

Next, we examined the effects of AKAP12 KO on gene expression in the corpus callosum by detecting DEGs. The effect of AKAP deficiency was minimal, and a total of 13 genes were identified as DEGs (Figure 2A to C). These DEGs, ie, 13 downregulated genes compared with WT, were *Akap12* (log2FC = −2.38, padj = 4.8425E-19), *Sgk1* (log2FC = −1.89, padj = 9.8321E-03), *Nfkbia* (log2FC = −1.25, padj = 3.2707E-04), *Hspa1a* (log2FC = −1.23, padj = 2.7872E-04), *Plekhf1* (log2FC = −1.10, padj = 3.2636E-04), *Arrdc2* (log2FC = −1.09, padj = 2.7872E-04), *Gm19439* (log2FC = −1.03, padj = 3.0945E-04), *Ddit4* (log2FC = −0.93, padj = 5.9829E-05), *Arl4d* (log2FC = −0.85, padj = 9.5153E-03), *Klf2* (log2FC = −0.78, padj = 1.1247E-02), *Arid5b* (log2FC = −0.77, padj = 3.2636E-04), *Fzd7* (log2FC = −0.74, padj = 1.8457E-02), and *Klf15* (log2FC = −0.69, padj = 2.2603E-02) (Figure 2B and C). The

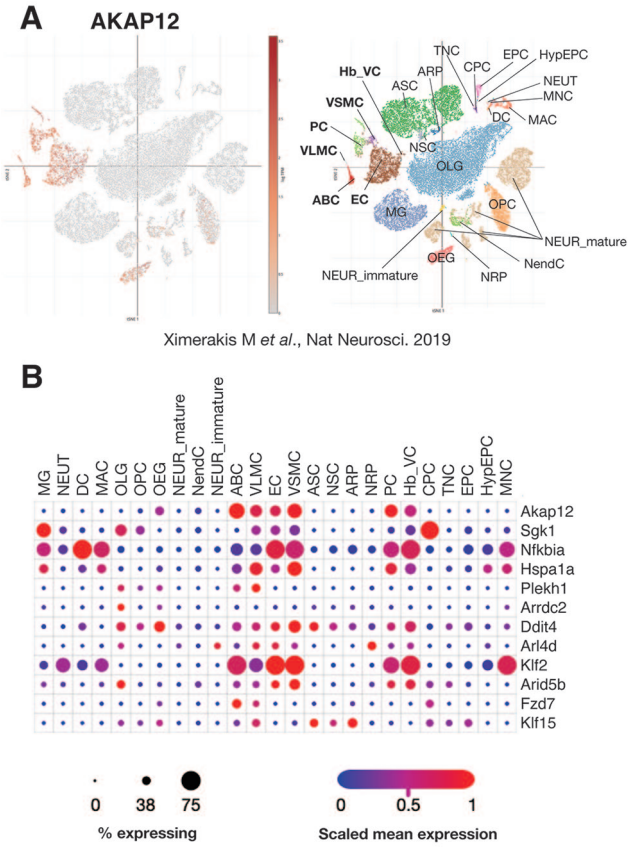

Figure 3. Cell-type distribution of *Akap12* and DEGs in AKAP12 KO mice: (A) Classification of *Akap12* using public single-cell RNA-seq data.[13] (B) Classification of DEGs (*Gm19439* was not registered in this database) using public single-cell RNA-seq data[13] (for detailed explanations of cell type abbreviations used in this figure, please refer to Ximerakis et al.[13]).

limited number of DEGs may be explained by the expression pattern of AKAP12 in the corpus callosum; it is expressed in only a small subset of cells (eg, vascular cells, some oligodendrocyte lineage cells), whereas most cells in this region (oligodendrocytes) express very little AKAP12. This cellular composition, combined with our bulk RNA-seq approach, likely diluted cell-specific changes. Therefore, future studies using single-cell analysis of the corpus callosum may reveal more pronounced transcriptional changes and provide additional insight into cell type-specific effects of AKAP12 deficiency. In addition, the small number of DEGs may indicate the importance of further proteomics and post-translational modification studies for in-depth insights. Furthermore, as AKAP12 expression gradually decreases with age,[7] the use of relatively older mice in this study may have influenced our findings of the limited number of DEGs. Therefore, future studies using younger mice may help to fully understand the impact of AKAP12 deficiency and determine whether AKAP12 deficiency affects the transcriptome profile more strongly in younger brains. Notably, all identified DEGs were downregulated in AKAP12 KO mice. This trend could be attributed to the role of AKAP12 as a signaling scaffold protein, possibly acting as an upstream regulator of these genes. However, it should also be noted that AKAP12 has been suggested to suppress hypoxia-inducible factor 1-alpha (HIF-1α) transactivation and vascular endothelial growth factor (VEGF) expression.[2] Therefore, under hypoxic conditions, AKAP12 deficiency may lead to upregulation of HIF-1/VEGF-related genes, highlighting the need for future comparative studies using disease-specific mouse models, such as ischemic stroke.

We further examined the identified DEGs in more detail using the public single-cell analysis data.[13] *Akap12* is predominantly expressed in vascular cells such as endothelial cells, vascular smooth muscle cells (VSMCs), and pericytes (Figure 3A), suggesting that its deficiency could cause changes in these cells. Indeed, when we examined the cell-specific expression of the identified DEGs in the public single-cell analysis data, genes such as *Sgk1*, *Nfkbia*, *Hspa1a*, *Arl4d*, *Ddit4*, and *Klf2*, were found to be highly expressed in these vascular cells (Figure 3B). Krüppel-like factor 2 (KLF2), a transcription factor, plays an important role in endothelial cells.[14] The KLF2 regulates the tightness of the BBB by upregulating tight junction proteins and has been reported to be a neuroprotective factor in the ischemic stroke model.[15] In addition, serum/glucocorticoid-regulated kinase 1 (SGK1) contributes to the mechanism of angiogenesis in endothelial cells[16] and provides neuroprotection in the stroke model.[17] These reports may implicate these genes in the increased endothelial permeability observed in AKAP12 deficiency.[6] Notably, SGK1 is also expressed in oligodendrocyte lineage cells (Figure 3B) and has been reported to increase in the corpus callosum under acute stress.[18] Considering the cell-cell trophic coupling in the oligovascular niche,[19] the reduction of SGK1 by AKAP12 deficiency may affect the interactions between OPCs and vascular cells and their respective roles. Indeed, *Arrdc2*, a gene predominantly expressed in oligodendrocytes (Figure 3B), was also downregulated in AKAP12 KO mice (Figure 2A to C). This underscores the need to further investigate how AKAP12 deficiency affects vascular and oligodendrocyte function in the cerebral white matter.

In summary, this study provides an additional resource to study the function of AKAP12 in the corpus callosum. Although the transcriptome changes in the corpus callosum are limited in AKAP12 KO mice, our findings are somewhat consistent with previous reports showing white matter dysfunction in AKAP12 KO mice.[5] As AKAP12 expression would increase after pathological conditions, such as stroke,[6] future studies are warranted to investigate the roles and mechanisms of AKAP12 in neurological disorders.

Acknowledgements

The authors thank Dr. Eng H. Lo for many helpful discussions.

Author Contributions

TH, HT, and KA conceptualized and designed the study the study methodology. HT, HI, and GH provided essential resources for the research. SK, NF, JHP, HS, AS, K-WK, IHG, and JL provided critical reading and scientific discussions. TH and HN performed the bioinformatics analysis. TH contributed to the visualization, and prepared and assembled the figures. TH and KA wrote the manuscript. KA was involved in funding acquisition. All authors reviewed and approved the final version of this manuscript.

Ethics Approval

All procedures were approved by the Institutional Animal Care and Use Committee at MGH, under the NIH guidelines.

Data Availability

The RNA-seq data (FASTQ) have been deposited in the public repository under BioProject accession PRJNA1093100.

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