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Targeted Downregulation of *kdm4a* Ameliorates Tau-engendered Defects in *Drosophila melanogaster*

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Disclosure
The authors have no potential conflicts of interest to disclose.

ABSTRACT

Background: Tauopathies, a class of neurodegenerative diseases that includes Alzheimer's disease (AD), are characterized by the deposition of neurofibrillary tangles composed of hyperphosphorylated tau protein in the human brain. As abnormal alterations in histone acetylation and methylation show a cause and effect relationship with AD, we investigated the role of several Jumonji domain-containing histone demethylase (*JHDM*) genes, which have yet to be studied in AD pathology.
Methods: To examine alterations of several *JHDM* genes in AD pathology, we performed bioinformatics analyses of *JHDM* gene expression profiles in brain tissue samples from deceased AD patients. Furthermore, to investigate the possible relationship between alterations in *JHDM* gene expression profiles and AD pathology in vivo, we examined whether tissue-specific downregulation of *JHDM Drosophila* homologs (*kdm*) can affect tau^{R406W}-induced neurotoxicity using transgenic flies containing the UAS-Gal4 binary system.
Results: The expression levels of *JHDM1A*, *JHDM2A/2B*, and *JHDM3A/3B* were significantly higher in postmortem brain tissue from patients with AD than from non-demented controls, whereas *JHDM1B* mRNA levels were downregulated in the brains of patients with AD. Using transgenic flies, we revealed that knockdown of *kdm2* (homolog to human *JHDM1*), *kdm3* (homolog to human *JHDM2*), *kdm4a* (homolog to human *JHDM3A*), or *kdm4b* (homolog to human *JHDM3B*) genes in the eye ameliorated the tau^{R406W}-engendered defects, resulting in less severe phenotypes. However, *kdm4a* knockdown in the central nervous system uniquely ameliorated tau^{R406W}-induced locomotion defects by restoring heterochromatin.
Conclusion: Our results suggest that downregulation of *kdm4a* expression may be a potential therapeutic target in AD.

Keywords: *Drosophila melanogaster*; Tauopathy; Alzheimer's Disease; Heterochromatin; *JHDM*; *kdm*

INTRODUCTION

Tauopathies, a class of neurodegenerative diseases that includes Alzheimer's disease (AD), are characterized by the deposition of neurofibrillary tangles composed of hyperphosphorylated tau protein in the human brain.[1] However, the underlying mechanism of tauopathy remains unclear, and deciphering the process is crucial for developing effective

Author Contributions

Conceptualization: Chun YS, Park SY. Data curation: Park SY, Seo J. Formal analysis: Seo J. Funding acquisition: Chun YS, Park SY. Investigation: Chun YS. Methodology: Park SY, Seo J. Supervision: Chun YS. Writing - original draft: Park SY. Writing - review & editing: Chun YS.

biomarkers and identifying potential therapeutic targets of AD. Abnormal alterations of histone acetylation and methylation have been documented in patients with AD and in a transgenic animal model of AD.[2] In early development, several epigenetic alterations may contribute to AD, whereas certain epigenetic shifts may occur downstream of AD pathology.[2] In particular, histone acetylation is indicative of late onset cognitive loss, and has been used to develop biomarkers of AD.[3]

Tau phosphorylation is an important pathogenic event in AD. Targeted expression of human tau in the central nervous system (CNS) of *Drosophila* recapitulates several characteristics in human AD, such as neuronal loss in the form of prominent vacuoles, compromised life span, and locomotor and cognitive impairments.[4] Therefore, to provide a useful AD model, transgenic *Drosophila* were generated using an FTDP-17 mutant form of human tau (tau^{R406W}) that was subcloned downstream of the yeast upstream activator sequence (UAS),[5] tissue-specific expression of the transgene was achieved by regulating the availability of Gal4 transcription factor with different Gal4 drivers.[6]

Analyses of tissues from AD patients and a transgenic mouse model of AD consistently show decreased histone acetylation.[7] Accordingly, histone deacetylase (HDAC) inhibitors have been proposed as potential procognitive agents for the treatment of AD.[8-11] Indeed, Tip60 histone acetyltransferase (HAT) in human tau overexpressing *Drosophila* has been proposed to play a neuroprotective role in impaired cognition during early development of AD.[12-14] Reciprocally, *Drosophila* genetic screens have shown that HDAC6 null mutation and pharmacological inhibition can rescue tau-induced microtubule defects in muscles and neurons.[15] Furthermore, histone methylation has a marked influence on synaptic plasticity and cognition.[8,10,16]

Recently, Mastroeni and colleagues reported increased histone H3K4 trimethylation in the cytoplasm and significant colocalization with hyperphosphorylated tau tangles, but decreased in the nuclei of AD brains compared to non-demented controls.[17] Moreover, the total levels of histone H3K9 dimethylation and HP1α were decreased and heterochromatin relaxed, resulting in aberrant neuronal gene expression in *Drosophila*, mouse, and human tauopathies.[5] However, no studies have investigated the involvement of histone demethylases in tauopathies.

In this study, bioinformatics analyses revealed significantly higher expression of several Jumonji domain-containing histone demethylase (*JHDM*) genes[18-21] in postmortem brain tissue from patients with AD than from non-demented controls. In addition, our results suggest that the downregulation of *kdm4a* (*JHDM3A*) expression may be a potential therapeutic target in AD, providing new insight into the role of histone demethylases in tauopathies.

METHODS

Bioinformatics analyses

Expression levels of histone demethylases in postmortem brain tissue from patients with AD were evaluated using the publically available Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo, GSE33000) (**Table 1**). The dataset included patients with AD (n = 310) and non-demented controls (n = 157). *JHDM1A* (10025907153_at probe), *JHDM1B* (10025912776_at probe), *JHDM2A* (10023810558_at probe), *JHDM2B* (10025911102_at probe), *JHDM3A*

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Table 1. GSE33000 subject information

Classification	Control subjects	AD subjects
Tissue source	Prefrontal cortex	Prefrontal cortex
No.	157	310
Age, yr	63.0 ± 10.2	80.0 ± 9.8
Sex, male/female	123/34	135/175

AD = Alzheimer's disease.

(10025904057_at probe) and *JHDM3B* (10025924555_at probe) mRNA expression levels were compared between groups using unpaired, two-sided Student's *t*-tests.

Drosophila strains and genetics

Fruit flies were maintained on standard media, and genetic crosses were performed at 25°C unless specified otherwise. All genetic crosses were performed at least in triplicate, and percentages represent the means of the replicates. The following *Drosophila* strains were used: UAS-*kdm2* RNAi (P{KK101783}VIE-260B) (Vienna *Drosophila* RNAi Center [VDRC], Vienna, Austria), UAS-*kdm3* RNAi (y[1] v[1]; P{y[+t7.7] v[+t1.8] =TRiP.HMJ22328}attP40) (Bloomington *Drosophila* Stock Center, Indiana University, Bloomington, IN, USA), UAS-*kdm4a* RNAi lines (w^{1118}; P{GD9133}v32652 hereafter referred to as UAS-*kdm4a* RNAi and w^{1118}; P{GD9133}v32650/CyO hereafter referred to as UAS-*kdm4a* RNAi[2]) (VDRC), UAS-*kdm4b* RNAi (P{KK102089}VIE-260B) (VDRC), *elav*-Gal4 lines (P{w[+mW.hs]=GawB}elav[C155] and P{w[+mC]=GAL4-elav.L}2/CyO, #B8765) (Bloomington *Drosophila* Stock Center), UAS-*kdm4a*-HA_1FLAG_2 (w; P{w[+mC]=[UAS-*kdm4a*-HA_1FLAG_2]} hereafter referred to as UAS-*kdm4a*$^+$) (Jerry L. Workman, Stowers Institute for Medical Research, Kansas, MO, USA),[22] BL2 chromatin reporter (y^1·w*/Dp(3;Y)BL2, P{HS-lacZ.scs}65E) (Bloomington *Drosophila* Stock Center), UAS-tau^{R406W} (Mel B. Feany, Harvard Medical School, Boston, MA, USA),[5] *GMR*-Gal4 (Deborah A. Hursh, CBER/FDA, Silver Springs, MD, USA).[23] The wild-type strain was *w1118* (Seungbok Lee, Seoul National University, Seoul, Korea).[24]

Locomotor behavioral assay

Flies were collected in vials on the day of eclosion and then transferred without anesthesia to fresh vials every other day. The locomotor assay was performed in the afternoon on day 10. Ten flies were placed in each plastic vial marked with 15 cm height and gently tapped to the bottom. The percentage of flies that climbed to the height of 15 cm in 10 seconds at each consecutive 5-day interval was calculated for each genotype unless specified otherwise. The assay was performed for 20 days after eclosion.

Histochemical detection

For histochemical detection of β-galactosidase activity, larvae were heat-shocked for 45 minutes at 37°C, followed by recovery for 1 hour at room temperature. Larval brain lobes were dissected in phosphate-buffered saline (PBS), fixed in 3.7% formaldehyde in PBS for 5 minutes, washed in PBS, transferred to X-gal solution,[25] and incubated overnight at 37°C. Larval brain lobes were mounted in 80% glycerol and examined using an Olympus DP71 microscope.

Statistical analyses

All data were analyzed using Microsoft Excel 2007 and expressed as the means and standard deviations. Continuous variables were analyzed using Student's *t*-tests when data were normally distributed. All statistical tests were two-sided. *P* values < 0.05 were considered to indicate statistical significance.

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RESULTS

Increased expression of several *JHDM* genes in postmortem brain tissue from patients with AD

To investigate the potential relationship between alterations in the expression of *JHDM* genes and AD pathology in vivo, we used bioinformatics analyses to examine differences in the expression levels of *JHDM1A/1B*, *JHDM2A/2B*, and *JHDM3A/3B*, which have *Drosophila* homologs (**Tables 1** and **2**). The expression levels of *JHDM1A*, *JHDM2A/2B*, and *JHDM3A/3B* were significantly higher in postmortem brain tissue from AD patients than from non-demented controls (average $\log_2$ value, 0.0221 ± 0.00337 vs. −0.0333 ± 0.00451; 0.0064 ± 0.00516 vs. −0.0794 ± 0.00543; 0.00837 ± 0.00276 vs. −0.0179 ± 0.00342; 0.0471 ± 0.00251 vs. 0.0256 ± 0.00326; 0.1403 ± 0.0155 vs. −0.0220 ± 0.0171, respectively; $P < 0.001$), whereas the *JHDM1B* mRNA levels were significantly downregulated in brain tissues from AD patients (average $\log_2$ value, −0.1300 ± 0.00603 vs. −0.0161 ± 0.00826; $P < 0.001$) (**Fig. 1**).

Table 2. List of genes for the study

Fly symbol	Fly gene ID	Human symbol	Human gene ID	DIOPT[a] score	Weighted score	Rank
kdm2	cg11033	JHDM1A/KDM2A	22992	14 of 15	13.85	High
		JHDM1B/KDM2B	84678	11 of 15	10.97	Moderate
kdm3	cg8165	JHDM2A/KDM3A	55818	9 of 15	8.75	Moderate
		JHDM2B/KDM3B	51780	10 of 15	9.76	High
kdm4a	cg15835	JHDM3A/KDM4A	9682	10 of 15	9.74	High
		JHDM3B/KDM4B	23030	10 of 15	9.79	High
kdm4b	cg33182	JHDM3A/KDM4A	9682	10 of 15	9.74	High
		JHDM3B/KDM4B	23030	9 of 15	8.79	Moderate

[a]Drosophila RNAi Screening Center Integrative Orthologue Prediction Tool (ver 7.1).

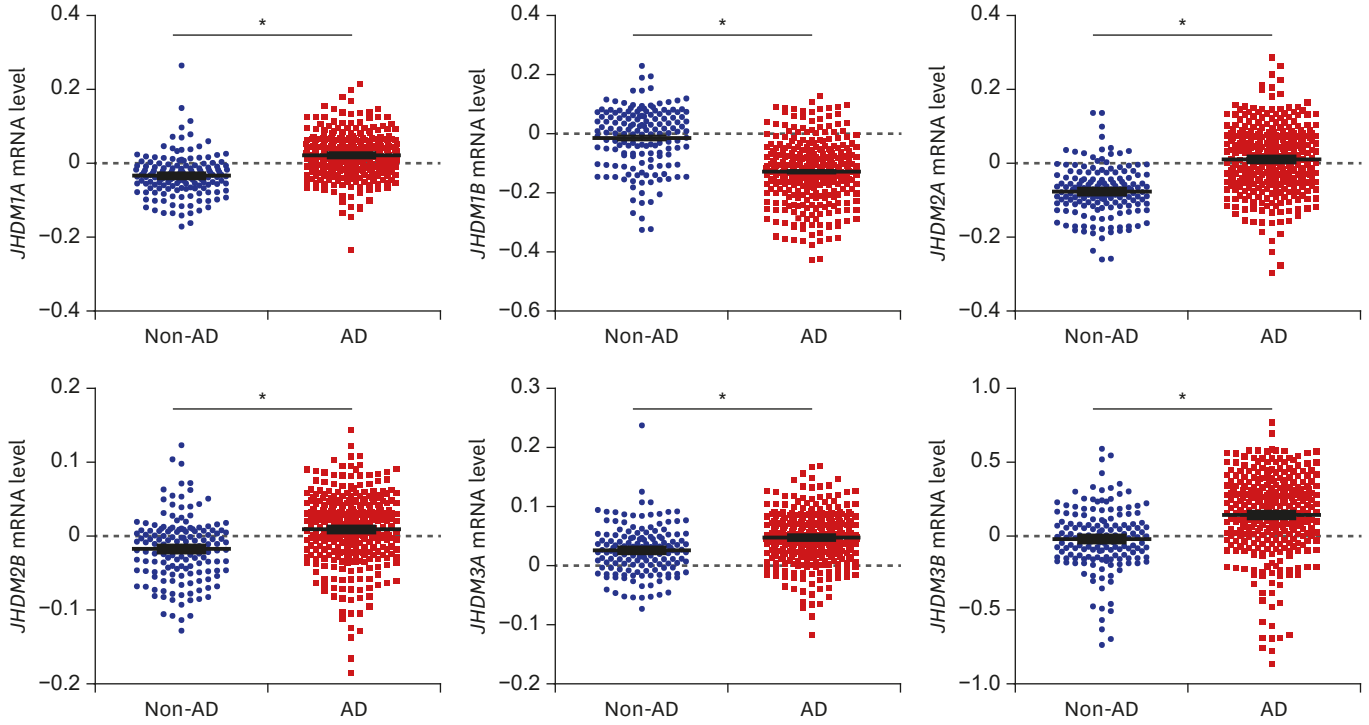

Fig. 1. Increased expression of *JHDM* genes in postmortem brain tissue from patients with AD. Scatter diagrams representing mRNA expression levels of *JHDM* genes in postmortem brain tissue from patients with AD. Dot plots showing *JHDM1A/1B*, *JHDM2A/2B*, and *JHDM3A/3B* mRNA levels in non-demented healthy controls and AD patients.
JHDM = Jumonji domain-containing histone demethylase, AD = Alzheimer's disease.
*$P < 0.001$ between the indicated groups.

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Knockdown of *JHDM* genes ameliorates the morphological deficit induced by overexpressing tau^{R406W} in *Drosophila* eyes

To examine the effect of *JHDM* gene knockdown in an AD background, tau^{R406W}-expressing flies were crossed with UAS-RNAi strains expressing RNAi specific for each of the *JHDM* fly homologs (*kdm2, JHDM1*; *kdm3*, *JHDM2*; *kdm4a*, *JHDM3A*; and *kdm4b*, *JHDM3B*) (**Table 2**) in *Drosophila* eye using the *GMR*-Gal4 driver. Neurodegeneration can be easily monitored when tau^{R406W} is overexpressed in the fly eyes, resulting in eye morphological defects such as reduced size, loss of bristles, disordered ommatidia and roughened eye surfaces.[26,27] The observed eye defects can be categorized into four classes according to their severity: class 1, normal; class 2, roughness in the eyes with size reduction and some ommatidial disruption; class 3, collapsed eye tissue with size reduction; and class 4, black spots of apoptotic tissue in the collapsed eye (**Fig. 2A**). Approximately 74% of flies overexpressing tau^{R406W} have class 3 + 4 eyes. In contrast, knockdown of *kdm2*, *kdm3*, *kdm4a* or *kdm4b* genes in tau^{R406W}-overexpressing flies ameliorated the tau^{R406W}-engendered eye defects, resulting in only 8%, 37%, 40%, or 21% class 3 + 4 eyes, respectively (**Fig. 2B**). No eye defects were detected in

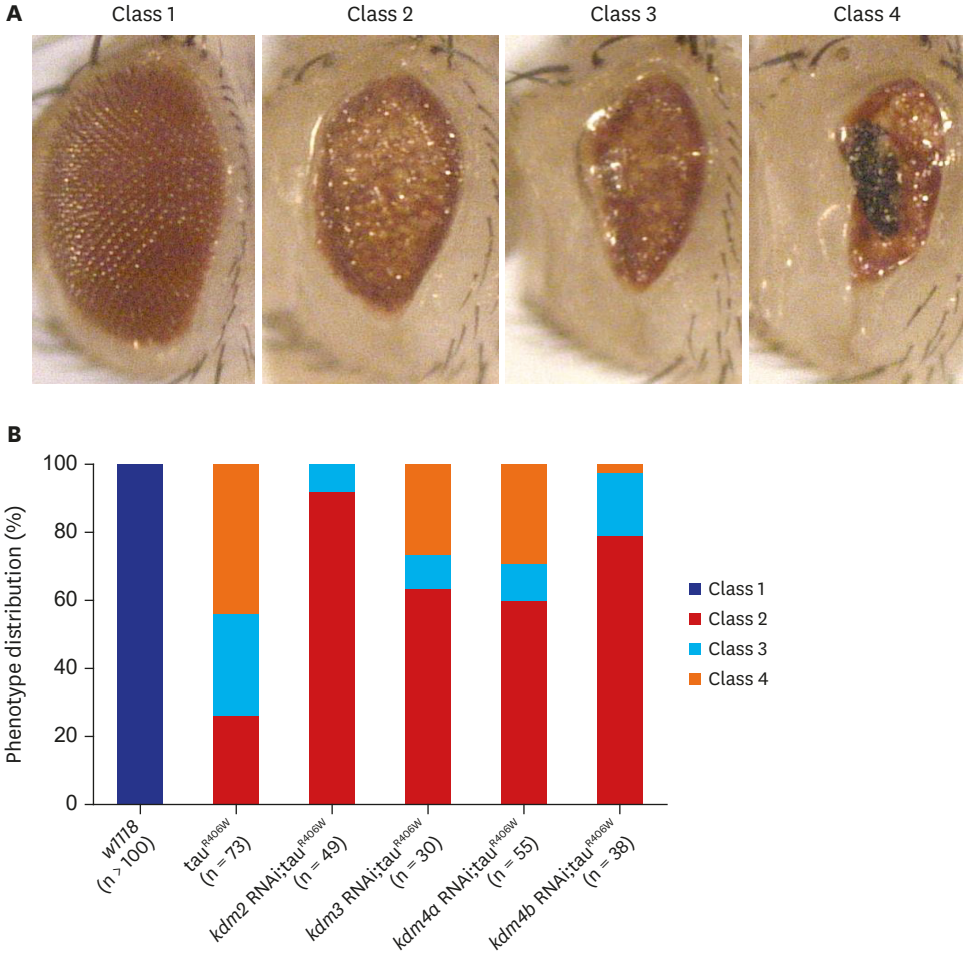

Fig. 2. Knockdown of any of the *JHDM* genes ameliorates the morphological defects induced by overexpressing tau^{R406W} in *Drosophila* eyes. (**A**) Bright field images of 10-day-old adult *Drosophila* eyes of control (*w1118*) and tau^{R406W} transgenic flies heterozygous for knockdown of any of the *kdm* genes using the *GMR*-Gal4. Images were taken using an Olympus SZ61 stereo zoom binocular microscope equipped with an eXcope XCAM1080 digital camera. (**B**) *kdm* knockdown flies that overexpressed tau^{R406W} showed a shift toward less severity in the distribution of phenotype categories in a population of flies (n = 30–73).
JHDM = Jumonji domain-containing histone demethylase.

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flies overexpressing each of the UAS-RNAi strains of *kdm* alone (data not shown). Therefore, knockdown of the *kdm* genes ameliorated the tau^{R406W}-induced eye phenotype, resulting in less severe phenotypes.

Targeted downregulation of *kdm4a* ameliorates tau^{R406W}-induced locomotion defect

To evaluate the neuronal effect of *kdm* gene knockdown in flies overexpressing tau^{R406W} in their CNS via pan-neuronal *elav*-Gal4, we performed the climbing assay, a behavioral readout used for monitoring neurotoxic deficits in *Drosophila*.[28-32] Whereas *w1118* control flies retained 90% of their climbing ability on day 10, only 45%–50% of tau^{R406W}-expressing flies climbed up. Inconsistent with the eye analysis results, knockdown of either *kdm2* or *kdm3* in flies overexpressing tau^{R406W} had no significant effect on the climbing ability of flies overexpressing tau^{R406W} alone (**Fig. 3A and B**). Interestingly, flies that overexpressed *kdm2* RNAi alone exhibited a locomotion defect, showing 60% climbing ability compared to 90% climbing ability in control flies. This finding indicates that *kdm2* itself may play a role in neuronal function. However, knockdown of *kdm4a* in tau^{R406W}-overexpressing flies caused a mild but significant amelioration of the locomotion defect, with flies showing 80% climbing ability compared to the 50%–60% climbing ability of flies overexpressing tau^{R406W} alone (**Fig. 3C**). Importantly, overexpression of *kdm4a* RNAi alone had no significant effect on locomotion, indicating that the neuroprotective effect may be specific to the context of tauopathy. In contrast, knockdown of *kdm4b* in flies that overexpressed tau^{R406W} exacerbated the locomotion defect, and they showed 30% climbing ability compared to 50% in flies overexpressing tau^{R406W} alone. Moreover, flies expressing *kdm4b* RNAi alone exhibited the locomotion defect, showing 60% climbing ability compared to 90% in control flies, indicating that *kdm4b* itself may be involved in neuronal function (**Fig. 3D**). Therefore, tau^{R406W} overexpression may aggravate the neuronal defect in *kdm4b*-deficient flies during development.

To ascertain the neuronal protective effect of *kdm4a* knockdown in tau^{R406W}-overexpressing flies, we performed the climbing assay with different *kdm4a* RNAi strain or wild-type *kdm4a* transgenic flies crossed to tau^{R406W}-overexpressing flies. Crossing the *kdm4a* RNAi[2] line with flies overexpressing tau^{R406W} in their CNS ameliorated the locomotion defect, as they showed 70% climbing ability compared to 50% in flies that overexpressed tau^{R406W} alone (**Fig. 3E**), whereas exogenously overexpressing wild-type *kdm4a* in tau^{R406W}-overexpressing flies exacerbated the locomotion defect, as they showed 10% climbing ability compared to 40% in flies overexpressing tau^{R406W} alone (**Fig. 3F**). Accordingly, in the eye, *kdm4a* knockdown with the RNAi[2] strain in tau^{R406W}-overexpressing flies resulted in 58.5% class 3 + 4 eyes compared to 87% class 3 + 4 eyes in flies overexpressing tau^{R406W} alone, whereas overexpressing *kdm4a* in tau^{R406W}-overexpressing flies showed no significant difference in eye morphology compared to those overexpressing tau^{R406W} alone (**Fig. 3G**). Taken together, *kdm4a* knockdown ameliorated tau^{R406W}-engendered deficits in both neurons and eyes.

Targeted downregulation of *kdm4a* reduces heterochromatic loss induced by tau^{R406W} overexpression

Given that tau promotes neuronal death through heterochromatin loss, global chromatin relaxation, and aberrant transcriptional activation in *Drosophila*, mouse, and human tauopathies,[5] we investigated whether knockdown of *kdm* genes alters heterochromatin loss in flies overexpressing tau^{R406W} in their CNS via pan-neuronal *elav*-Gal4. We measured reporter expression in larval brain lobes using a transgenic fly strain (BL2 reporter) that has a *LacZ* reporter gene embedded in and silenced by heterochromatin.[5,33] After 45 min heat

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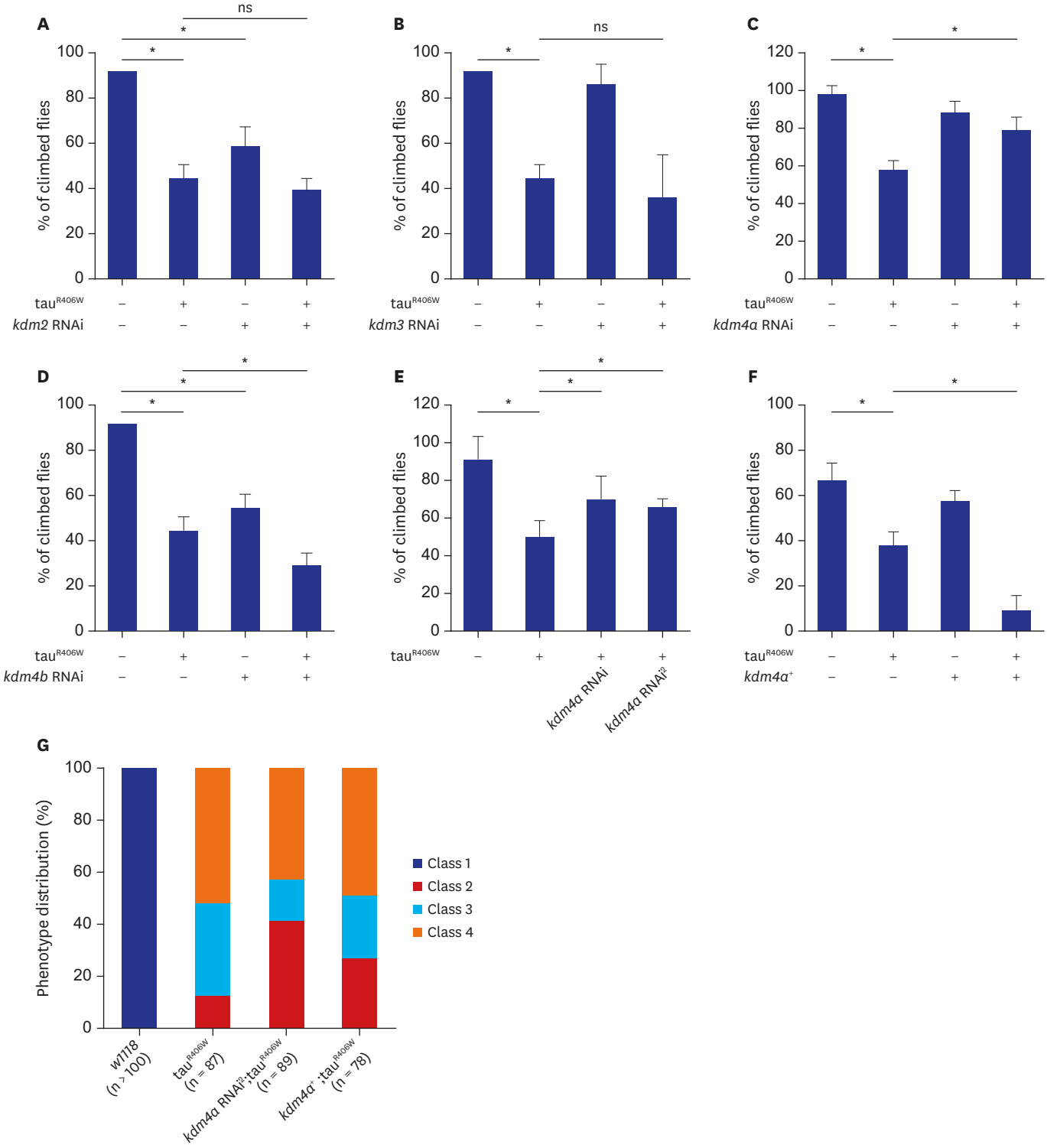

Fig. 3. Targeted downregulation of *kdm4a* ameliorates tau^{R406W}-induced locomotion defect. (**A–E**) Locomotor activity in control and tau^{R406W} transgenic flies heterozygous for knockdown of any of the *kdm* genes using pan-neuronal *elav*-Gal4 (#8765) was measured on day 10 post-eclosion (10 flies and 10 repeats per group, respectively; 15 cm/10 sec). (**F**) Locomotor activity in control and tau^{R406W} transgenic flies heterozygous for overexpression of wild-type *kdm4a* transgene using pan-neuronal *elav*-Gal4 (#c155) was measured on day 7 post-eclosion (10 flies and 10 repeats per group, respectively; 8 cm/20 sec). (**G**) A shift in the distribution of eye phenotype categories in populations of flies with *kdm*4a knockdown or overexpression in tau^{R406W}-overexpressing flies using *GMR*-Gal4 (n = 78–89).
$^{*}P < 0.05$ and n.s. denotes $P > 0.05$ between the indicated groups.

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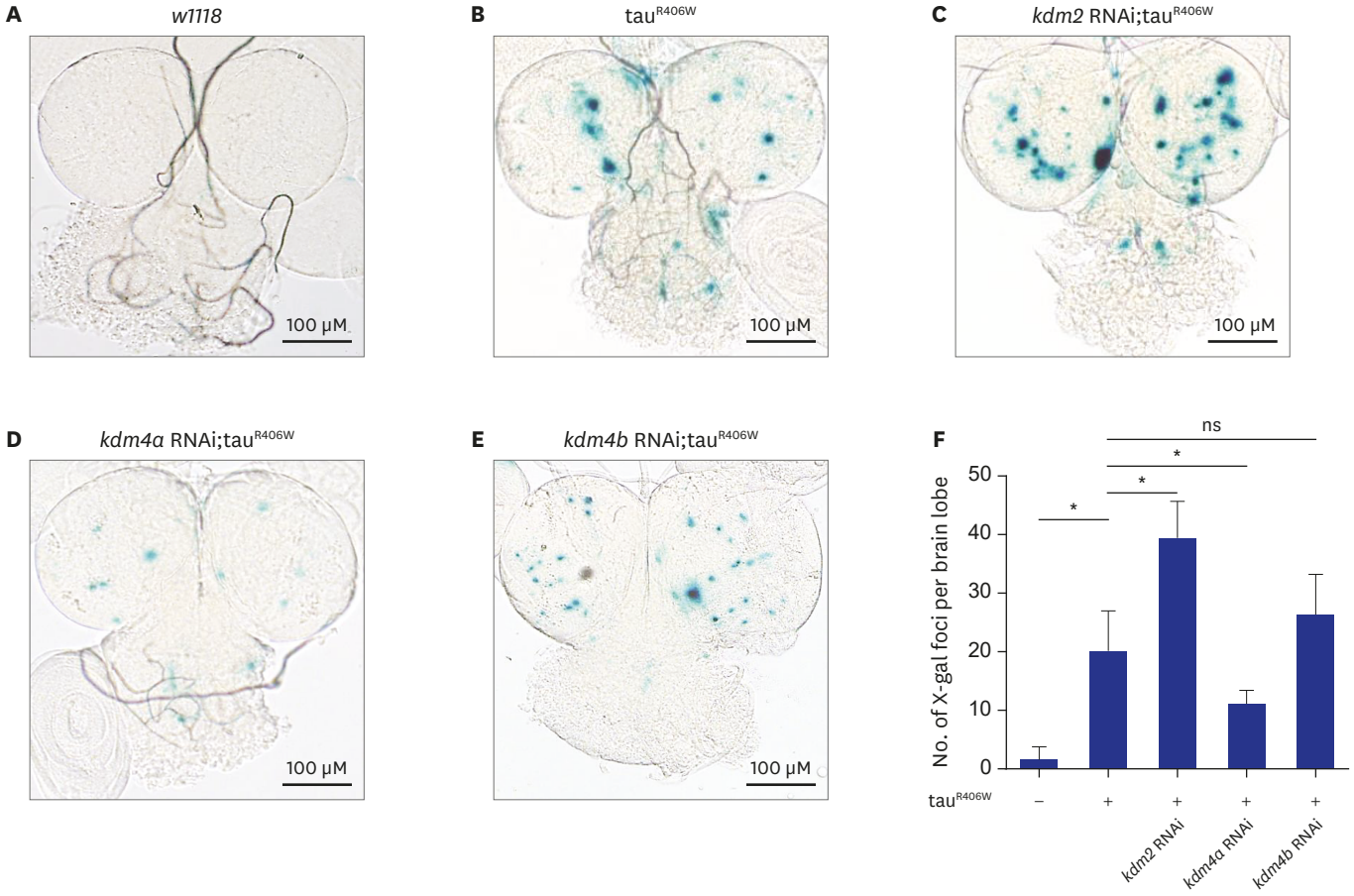

Fig. 4. Targeted downregulation of *kdm4a* reduces heterochromatin loss induced by tau^{R406W}overexpression. (**A–E**) The *lacZ* expression (BL2 reporter) embedded in and silenced by heterochromatin displayed in larval brain lobes of each of the indicated genotypes driven by pan-neuronal *elav*-Gal4 (#8765) under heat shock pulse (45 min at 37°C), using X-gal staining. (**F**) Quantification of BL2 chromatin reporter expression of indicated genotypes (n = 5–10).
*P < 0.05 and n.s. denotes P > 0.05 between the indicated groups. Scale bar = 100 μM.

shock pulse at 37°C, this *LacZ* reporter was silenced in controls (**Fig. 4A**), but abnormally expressed in tau^{R406W}-overexpressing fly brain due to heterochromatin loosening (**Fig. 4B**). *kdm2* knockdown enhanced the expression of the heterochromatin-embedded reporter compared to overexpressed tau^{R406W} alone (**Fig. 4C and F**). Expectedly, RNAi-mediated reduction of *kdm4*a decreased the number and intensity of the expression foci of the heterochromatin-embedded reporter in tau^{R406W}-overexpressing flies (**Fig. 4D and F**), whereas *kdm4b* knockdown had no significant alteration on the expression of the heterochromatin-embedded reporter in flies overexpressing tau^{R406W} alone (**Fig. 4E**). Therefore, neuronal-specific downregulation of *kdm4*a may suppress tau^{R406W}-engendered locomotion impairment by restoring heterochromatin.

DISCUSSION

We used bioinformatics analyses to detect alterations in *JHDM* gene expression in AD pathology. The expression levels of *JHDM1A*, *JHDM2A/2B*, and *JHDM3A/3B* were significantly higher in postmortem brain tissue from AD patients than from non-demented controls. In *Drosophila*, CNS-specific knockdown of *kdm4a* (*JHDM3A*) ameliorated tau^{R406W}-induced locomotion defects, in agreement with eye analysis results.

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Recently, Frost et al.[5] reported that oxidative stress and subsequent DNA damage can substantially alter chromatin structure, inducing heterochromatin loss and aberrant transcriptional activity, causing neurodegeneration in tauopathies. The authors reported that tau^{R406W}-overexpressing adult fly brain had decreased total levels of H3K9 dimethylation and heterochromatin protein 1α (HP1α), which are normally enriched in heterochromatin. In addition, loss of function mutations in HP1α or *Su(var)3-9*, which encodes a histone methyltransferase responsible for H3K9 dimethylation, exacerbated tau^{R406W}-engendered locomotion deficits. Given that KDM4A also directly interacts with HP1α and is essential for heterochromatin organization and function via both enzymatic and structural mechanisms in *Drosophila*,[22,34,35] it is plausible that KDM4A may be associated with heterochromatin alteration in tauopathies. Intriguingly, *kdm4a* knockdown with either of two RNAi strains ameliorated tau^{R406W}-induced deficits in the eye and in neurons. Conversely, wild-type *kdm4a* overexpression in flies overexpressing tau^{R406W} in the CNS exacerbated tau^{R406W}-induced locomotion defects. Importantly, overexpression of *kdm4a* RNAi alone had no significant effect on locomotion in flies, indicating that the neuroprotective effect was specific for the AD pathological context. Consistent with these results, Lorbeck et al.[36] reported that flies containing the P-element suppressor mutant of *kdm4a* (*kdm4a*$^{P\text{-}supp}$) showed no significant loss in climbing ability. According to the BL2 transgenic reporter assay, RNAi-mediated reduction of *kdm4a* in the CNS reduced heterochromatin relaxation, thus ameliorating the tau^{R406W}-engendered locomotion defect. Further studies are needed to elucidate the underlying mechanism of KDM4A involvement in the heterochromatic organization and transcriptional regulation of tauopathies.

kdm4b knockdown in the CNS exacerbated the tau^{R406W}-induced locomotion defect. Interestingly, flies that overexpressed *kdm4b* RNAi alone exhibited the locomotion defect, showing 60% climbing ability compared to 90% in control flies. This finding indicates that KDM4B may play a role in neuronal function in *Drosophila*. Consistent with this result, neuronal-specific *kdm4b*-deficient mice exhibit hyperactive behavior, sustained hyperactivity in a novel environment, deficits in working memory, and spontaneous epileptic-like seizures, indicating impaired neurodevelopment.[37] This finding suggests that KDM4B plays a crucial role in the formation of functional neuronal networks. Therefore, neuronal defects in *kdm4b*-deficient flies during development may exacerbate tau^{R406W}-engendered locomotion impairment. However, the underlying mechanism does not appear to involve altered heterochromatin structure, as *kdm4b* knockdown had no significant effect on heterochromatin relaxation induced by tau^{R406W} overexpression.

Inconsistent with the eye analysis results, knockdown of either *kdm2* or *kdm3* in tau^{R406W}-overexpressing flies had no significant effect on climbing ability in flies that overexpressed tau^{R406W} alone. However *kdm2* knockdown tended to slightly exacerbate the tau^{R406W}-induced locomotion defect, particularly in young flies (2–3 days after eclosion), but not significantly in older flies (data not shown). Furthermore, flies expressing *kdm2* RNAi alone exhibited the locomotion defect, showing 60% climbing ability compared to 90% in control flies, indicating that KDM2 may play a role in neuronal function. In agreement with this result, *kdm2* null mutant adult flies displayed defects in circadian locomotor behavior.[38] Thus, a neuronal defect in *kdm2*-deficient flies during development may exacerbate the tau^{R406W}-engendered locomotion impairment in young flies. Furthermore, *kdm2* knockdown in the larval brain lobe can enhance the number and intensity of expression foci of the heterochromatin-embedded reporter in tau^{R406W}-overexpressing flies. These results indicate that neuronal-specific downregulation of *kdm2* may exacerbate tau^{R406W}-induced locomotion

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impairment by enhancing heterochromatin loss during early development; however, the underlying mechanism remains to be elucidated.

Using bioinformatics analyses, we revealed significantly increased expression of *JHDM1A*, *JHDM2A/2B*, and *JHDM3A/3B* in postmortem brain tissue from patients with AD compared to non-demented controls, whereas *JHDM1B* mRNA levels were downregulated in the brains of AD patients. Using tau^{R406W}-induced transgenic flies as an AD model, we showed that *kdm4a* knockdown in the CNS ameliorated tau^{R406W}-induced locomotion defects by restoring heterochromatin. These results suggest that the downregulation of *kdm4a* expression could be a potential therapeutic target in AD.

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