Gene-expression control in early and late-onset dementia

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Jiayin Zhou
from Liaoning, China

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Thesis Committee:

Prof. Dr. André Fischer, Systems Medicine and Epigenetics in Neurodegenerative Diseases, German Center for Neurodegenerative Diseases, Göttingen

Prof. Dr. Bernd Wollnik, Institute of Human Genetics, University Medical Center, Göttingen

Prof. Dr. Alexander Flügel, Institute for Neuroimmunology and Multiple Sclerosis Research, University Medical Center Göttingen

Members of the Examination Board:

Prof. Dr. Ralf Heinrich, Department of Cellular Neurobiology, Georg-August-University Göttingen, Göttingen

Prof. Dr. Tiago Outeiro, Experimental Neurodegeneration Department, University Medical Center, Göttingen

Prof. Dr Oliver Wirths, Department of Psychiatry, Division of Molecular Psychiatry University Medical Center Göttingen

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To my parents.

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Introduction

Dementia

Dementias are a group of acquired brain diseases that share deficits in memory, communication and language, focus and attention, reasoning and judgment, and visual perception[1]. Alzheimer's disease (AD) has been the most common form of dementia, accounting for approximately 60–80% of cases[2]. Neuropathologically, it is characterized by the buildup of parenchymal plaques composed predominantly of amyloid peptide (A) protein and intraneuronal tangles of the hyperphosphorylated microtubule-associated protein tau[3,4]. Additionally, vascular cognitive impairment is common, accounting for at least 10% of dementia cases and frequently co-occurring with AD. It is a neurological manifestation of cardiovascular disease that occurs as a result of limited blood flow to the brain[5]. Although vascular cognitive impairment is not a significant cause of dementia, it affects several million individuals globally[6]. Dementia with Lewy bodies is characterized by aggregates of Alpha-synuclein (SNCA) protein throughout the brain, accounts for between one in thirteen and one in twenty-five cases of dementia, and can share motor symptoms and neuropathology with Parkinson's disease, particularly in the latter stages of both conditions[7]. Frontotemporal dementia (FTD) is a clinically defined disease that is characterized by the coexistence of numerous separate pathological entities generally referred to as frontotemporal lobar degeneration[8]. There has been some overlap between frontotemporal lobar degeneration and amyotrophic lateral sclerosis, both of which can exhibit TDP43 accumulation and associated pathology on autopsy[9]. Both are uncommon in comparison to AD. Frontotemporal lobar degeneration has an incidence of 15-22 per 100,000 while amyotrophic lateral sclerosis has a prevalence of roughly four per 100,000[10]. These dementias are frequently not detected in so-called community-based autopsy samples of elderly adults (as opposed to cohorts from dementia clinics). By contrast, histologically defined diseases such as hippocampal sclerosis and primary age-related tauopathy affect approximately 15% of any large elderly population and may have a significant impact[11]. However, the precise relationship, if any, between

these late-life alterations in neuroanatomy and serious loss of cognitive function is unknown. For instance, basic age-related tauopathy is rarely associated with significant cognitive deterioration. Thus, while it is classified as a pathology, it may not be a kind of dementia in and of itself. Apart from their clinical characteristics, dementias exhibit a range of onset ages. Frontotemporal lobar degeneration often manifests itself between the ages of 50 and 70 years, although vascular cognitive impairment and dementia are more prevalent in the elderly (age 80 years)[12,13]. AD is unique in that its incidence grows with age, but then plateaus or even declines in those aged 85 and older[14]. Overall, AD and FTD are the two main causes of dementia.

Dementia-related genes

Numerous attempts have been made to unravel the genetic causes of dementia, with varying degrees of success. Pedigree studies identified autosomal dominant forms of AD, FTD, and amyotrophic lateral sclerosis, which were later linked to specific genes (e.g., presenilin and APP in AD C9orf72 in amyotrophic lateral sclerosis and FTD). However, autosomal dominance accounts for a modest number of dementia cases (about 5%)[15]. The most frequently observed relationship between a gene and dementia is that of the APOE4 allele and AD. Although this link is robust, it accounts for no more than 20%-25% of all dementia cases (about 30%-35% of AD cases)[16]. Several studies attempted to determine the genetic and non-genetic risk factors for AD. This massive effort brought together multiple sources of tissue and data collecting, resulting in an immense resource pool for gene association research. AD has been linked to SORL118 and NOS3 variations[17]. Regrettably, none of the genetic correlations revealed had a larger effect on risk than previously known associations, and none approached the level of influence associated with the APOE genotype. There is no comparable effort for other types of dementia, however, genome-wide meta-analyses have revealed several connections between specific types of dementia and gene variations[18–20]. There are numerous potential genes for neurological dementias. APOE4 and variants in the microtubule-associated protein tau (MAPT) gene have also been connected with

vascular cognitive impairment[21,22]. The genetics of Lewy body dementia appear to be somewhat more distinct from those of AD than those of vascular cognitive impairment. There have been associations with variations in the SNCA27 and SNCB28 genes, as well as the leucine-rich repeat kinase 2 (LRRK; gene name PARK8) gene[23]. Even the most prevalent genetic variant associated with AD-APOE4-may be lacking in some cultures. Additionally, even if they share the APOE4 allele, monozygotic twins can be discordant for AD. Many studies demonstrated that environmental factors have a significant impact on the progression of AD[24,25]. These studies stressed the fact that AD is the product of intricate interactions between genes and the environment. Nonetheless, gene association studies are valuable for identifying potentially significant functional pathways that may be perturbed by the environment and thus produce pathogenetic results that are sufficiently similar to those of a genetic disorder (e.g., sporadic *vs* autosomal dominant AD), such that insights gained from studying the latter may help inform effective treatment or prevention of the former. Thus, the question is what physical process leads to dementia disorders as a result of gene-environment interactions.

The accumulating evidence indicates epigenetic mechanisms[26,27]. Epigenetics refers to modifications to the protein components of chromatin (histones) and to the DNA strand's sidechains that do not alter the underlying DNA base sequence. It also comprises non-coding RNA-mediated regulation of genes. In a clinical setting, long-term alterations in the amounts of specific proteins and peptides, such as SNCA in Lewy body dementia, and MAPT in AD, frontotemporal lobar degeneration, and other dementias require an explanation in terms of expression. The effects of epigenetic modifications on gene expression have been well established over a long period of time. In dementia cognition is impaired and memory is affected[28–30].

Memory and aging

The structure and cognitive capacities of the brain are not static but are subject to

ongoing dynamic change throughout the lifespan. Aging is thus a process that is tightly related to physiological deterioration and is a normal and complicated natural biological process that includes cytogenetic, lifestyle, and environmental variables[31]. The process of aging is reflected at the beginning of deterioration in the organism's function to maintain homeostatic balance in the body, and the decline in physiological and biochemical capacity increases the individual's susceptibility to age-related diseases that affect many systems, including the nervous system. As a result, aging is seen as a key risk factor for the development of neurodegenerative disorders, with elderly persons exhibiting a decline in memory and cognitive performance as they age[32]. It is worth mentioning that, the development of memory loss is subtle, thus age-related memory loss has also been described as 'normal aging' in contemporary aging society[33]. Multiple evidence shows in both humans and rats, normal aging is accompanied by molecular alterations in brain function[34,35]. However, not every elderly person with memory loss leads to dementia. Although the mechanisms are different, the pathological features are similar. Thus, it's difficult to distinguish the exact link between cognitive dysfunction and healthy aging memory decline.

The majority of existing research evidence comes from both cross-sectional and longitudinal approaches to studying the interaction between aging and memory. Reveals there is no obvious neuron loss during the normal aging process, but the number of neurons, dendritic length, and dendritic spine density decrease with age[36,37]. Evidence from post-mortem studies supports a positive correlation between the number of hippocampal cells lost and age in the brains of healthy aging[38]. Interestingly, early AD also targets hippocampal damage at the beginning, reflecting that age-related hippocampal-dependent memory is highly linked to memory loss in both AD and healthy older adults[39].

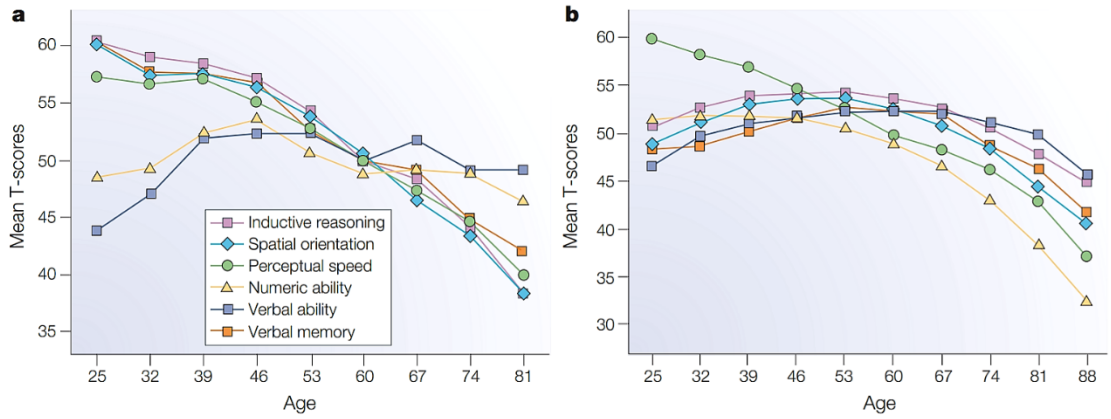


Fig.1 Age-related cognitive changes. (a) Cross-sectional data represent the declines are visible in all domains except verbal and numeric ability. **(b)** Longitudinal data from the same study show a significant decline in all domains after the age of 55. Figure adapted from Hedden et al[40].

There are a lot of unique hallmarks of aging in the brain at the cellular level[41]. Over the last decades, numerous studies have indicated that the progression of neurodegeneration is highly linked to the molecular changes in the following mechanisms (1) Mitochondrial dysfunction; (2) Aberrant neural network activity; (3) Dysfunctional oxygen metabolism; (4) Intracellular oxidatively damaged; (5) Inflammation; (6) Repair of damaged DNA; (7) Neuronal Ca2+ signal dysregulation and so on[42]. Aging has an impact on the brain's ability to scavenge free radicals, and the constant production of reactive oxygen species (ROS) reduces antioxidant defense function. In contrast, ROS in neurons is mostly produced by mitochondrial respiratory activities. Oxidative stress and mitochondrial dysfunction are therefore closely related[43]. Mitochondria are present in the dendrites and axons of neurons and play a crucial role in cellular energy consumption and Ca2+ homeostasis regulation[44]. Furthermore, mitochondrial membrane permeability transition pores are involved in programmed cell death, which is commonly observed in a range of neurodegenerative diseases[45]. There is evidence that both altered mitochondrial morphology and functional deficits in the brain of animals are age-related, for example, enlargement or fragmentation of mitochondria can significantly shorten lifespan[46]. Studies have also shown that hippocampal neurons in aged mice have increased susceptibility to Ca2+, that aging leads to abnormally high intracytoplasmic Ca2+ levels, and that restoring neuronal

Ca2+ homeostasis significantly improves cognitive deficits in aged mice[47]. Simultaneously, disturbances in the neural network cause an imbalance in excitability and an inflammatory response in the elderly brain, with aberrant activation of microglia and the generation of significant amounts of pro-inflammatory cytokines, which cause oxidative damage to neurons and the neuroinflammatory process is aggravated by astrocyte damage[48]. As described above, these hallmarks and molecular changes accelerate the aging process and raise the chance of cognitive impairment.

Learning and Memory

Learning is a process of conscious or unconscious acquisition of new understandings, behaviors, and skills. Human learning begins at birth and continues until death, as a result of the constant interaction between humans and the environment. Learning can be translated into memory, so the underlying mechanisms of learning and memory are a hot topic of research in many fields, including neuroscience and psychology. Memory is an important function of the brain. It represents the accumulation of a person's impressions of past activities, feelings, and experiences. It is the neurological system's ability to store prior events. It is the process through which the human brain identifies, memorizes, maintains, and reproduces what has happened. It also serves as the foundation for higher organisms to engage in complex mental functions like thinking and imagination[49]. The basic process of memory development, according to the current understanding of memory formation mechanisms, is made up of three links: recognition, retention, and recall[50]. The process also changes dynamically over time. Memory is the basic function of people's learning and work. It influences human ideas and serves as a bridge between people and society. Our life will be worthless if we lose our memories.

Hippocampus in learning and memory

Studies in primates and rodents have verified that the hippocampus is a very important brain area for acquiring and consolidating memory, and it is the central area of learning and memory[51]. The hippocampus is located beneath the temporal lobe and the cerebral

cortex and is mainly composed of the dentate gyrus (DG) and the cornus ammonis (CA), which the latter can be further subdivided into three regions CA1-CA3. Together these structures make the anatomical shape of the hippocampus similarly to the letter "C"[52]. The mature hippocampal signal is a one-way output, which means that the signal provided by the DG travels via multiple CA regions to reach various brain locations before being received by the entorhinal cortex[53]. In addition, different areas of the hippocampus also have different functions. The dorsal area dominates spatial learning and memory, and the ventral area connects to the amygdala, which is related to fear and stress[54]. Moreover, the hippocampus is closely associated with AD as it is one of the first areas of the brain to suffer cognitive damage and is primarily characterized by a progressive loss of hippocampus-dependent memory capacity. This is not only reflected in normal recognition memory and spatial memory deficits, but also interferes with the formation of new memories. It is well documented that the hippocampus plays an irreplaceable and important role in learning memory[55,56].

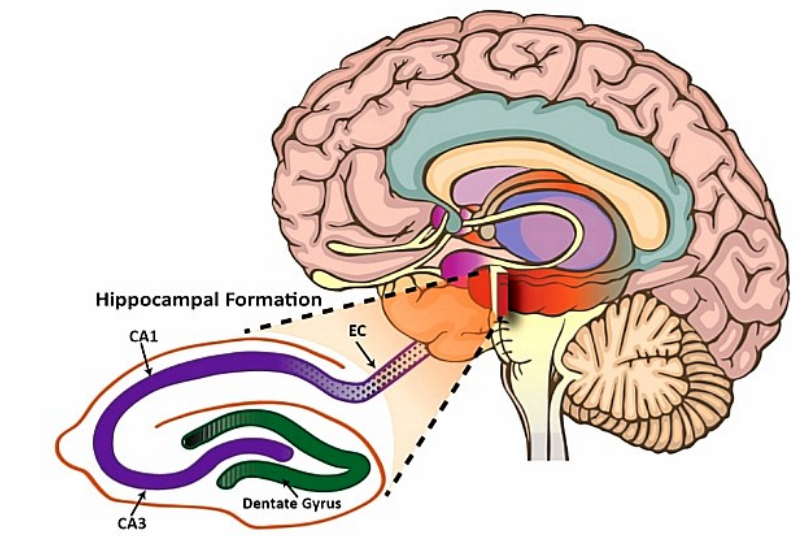


Fig.2 Hippocampal structure. Figure adapted from Craig. M.V et al[57].

Molecular mechanisms of learning and memory

Neurophysiologically, different forms of memory are related to the functions of different brain regions, synaptic plasticity, and neuronal networks. Therefore, it is reasonable to assume that memory storage involves multiple cellular mechanisms[58]. It

is widely acknowledged that short-term memory (STM) is primarily governed by temporal alters within synapses, but long-term memory (LTM) is influenced by changes in gene expression, which result in long-term cellular changes[59,60]. STM has a limited capacity, demanding the use of cellular processes that may be engaged instantly upon request. In the STM process, the molecular alterations that occur in memory formation neurons are due to the existing proteins being covalently modified by different kinases. Relatively, LTM requires more extensive modifications starting with protein synthesis[61]. Studies found if neurons were treated with a moderate, low-frequency electric pulse, their responses remained steady without any changes in synaptic transmission strength, while tonic stimulation causes neuronal hyperexcitation increasing the postsynaptic potential (EPSP) which will remain elevated over time. This response is termed long-term potentiation (LTP) and is recognized as the main mechanism in the memory process at present[62]. It requires the involvement of two major ionotropic glutamate receptors, N-methyl-D-aspartate receptors (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA). The activation of NMDA leads to calcium influx in hippocampus and while calcium influx occurs can stimulate the long-term plasticity of synapses and trigger lasting changes in neuronal structure. In turn, this process will further initiate an enzymatic cascade, which is ultimately responsible for synaptic transmission through the enhancement of AMPAR and the increase of transmitters[63–65]. In addition, once the concentration of calcium influx exceeds a certain threshold, it will activate CaM-dependent adenylate cyclase to produce cAMP and activate the transcription factor cAMP-responsive element-binding protein (CREB) through the PKA/MAPK pathway[66,67]. CREB can maintain LTP over time and consolidate long-term memory formation. Phosphorylated CREB activates the transcription of plasticity-related genes by binding to the response element cAMP, triggering the generation of new synapses[68,69].

Chromatin plasticity

In molecular cell biology, histone protein determines the structure of chromatin in the

nucleus of eukaryotic cells. The epigenetic process is centered on histone modifications in addition to the two major processes of DNA methylation and non-coding RNA[70]. DNA with a length of 147 base pairs is wrapped in a histone octamer, which constitutes the nucleosome, the fundamental unit of chromatin. And each octamer is composed of two sets of four histone dimer pairings, two H2A/H2B and two H3/H4. This relationship is due to the fact that positively charged histones can bind negatively charged DNA[71].

Histones are susceptible to post-translational modifications such as methylation, acetylation, ubiquitylation, phosphorylation, etc based on different amino acid residues in the N-terminal tail. In turn, these modifications can weaken the positive charge carried by histones and thereby weaken the attraction between DNA[72]. This kind of interaction between the histone-DNA can occur on the promoter. As a result, the active chromatin transitions from a relaxed to a compact heterochromatin state, making it easier for transcription factors to reach the DNA for activation or repression. One classic example is acetylation, which often occurs on histone lysine residues and acts as an activator of genes during transcription. The key to maintaining acetylation homeostasis on chromatin is through cooperation between the catalytic function of histone acetyltransferase (HAT) and the inhibition of transcriptional activity by histone deacetylase (HDAC). Both HAT and HDAC are highly specialized and diversified enzyme families and their acetylation or deacetylation is targeted at the tagging or removal of specific amino acid residues from the histone tails[73,74].

Histone modifications have an impact on gene splicing. The histone reader is able to recognize existing histone modification tags, while the writer can mark the tag, the eraser can in turn remove it to create a different pattern of expression[75]. For example, the methyltransferase KMT2A adds methylation to histone 3 lysine 4 residues to activate the gene, in turn, HDAC can inactivate gene expression by erasing the acetyl group[76]. Different stimuli lead to histone modifications that are variable and essential

for memory formation. In fact, many studies to date have demonstrated that histone modifications in the brain particularly in the hippocampus area have crucial effects on learning and memory. Pavlov's fear conditioning experiments provide strong evidence of increased levels of histone methylation and acetylation in the hippocampus of mice following stimulation. Moreover, the reversal of fear memory also depended on the regulation of histone modifications[77]. Another supporting study showed that interfering with chromatin modifications affects the formation of LTP[78,79]. Upregulation of histone acetylation levels with HDAC inhibitors enhances LTP for consolidated memory formation[80]. In addition to acetylation, histone methylation is also inextricably linked to memory and plays an important role, which will be described in detail below.

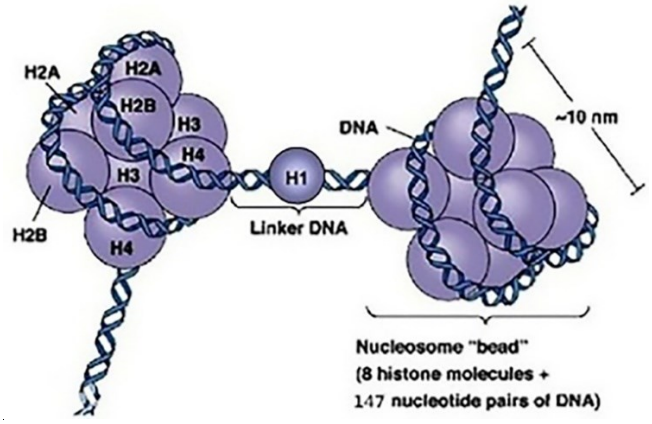


Fig.3 Nucleosome structure. Figure adapted from Meng. Y.L et al[81].

Histone Methylation

Histone methylation is the process of transferring methyl to histone amino acids that form nucleosomes, which can regulate gene expression and allow various genes to be expressed by different cells. This process can both enhance and reduce the transcription of genes, depending on the location of the methylated amino acids in the histone and the number of attached methyl groups[82]. Similar to acetylation, methylation also happens at lysine residues in the histone tails and is regulated by histone methyltransferase (HMT) and histone demethylase (HMD) to enhance or counteract activity. A feature that distinguishes it from acetylation, however, is that histone

acetylation tends to add only one residue, whereas histone methylation can be monomethylated, dimethylated and trimethylated, produced by specialized enzymes[83]. It is worth noting that regardless of the status of histone lysine methylation, it can be applied in either a non-progressive or progressive manner. Thus, methylation is a highly complicated process that is linked to both active euchromatin and the formation of heterochromatin, which is linked to gene silence[84].

Furthermore, histone methylation is involved in transcription. For example, trimethylation of histone H3 at lysine 4 (H3K4me3) is for labeling active transcripts. However, dimethylation of histone H3 at lysine 9 (H3K9me2) serves to repress transcription[85]. Activation of further inhibition of transcription depends on the addition of methyl groups to histones by histone methyltransferases. Animal models have demonstrated that histone methylation is closely linked to aging and neurodegenerative diseases[86]. This epigenetic mechanism modifies the structure and properties of nucleosomes, influencing their interactions with proteins and, in particular, the transcription process for genes.

H3K4 Methylation in learning and memory

As previously stated, histone methylation allows for changes in chromatin structure without affecting the underlying genetic sequence, whereas lysine methylation in the histone tail is an essential part of the field of neuroepigenetics. Rather than changing histone charge characteristics, the methylation process mostly changes hydrophobicity. Specifically, H3K4 methylation is frequently active at transcriptional initiation sites of transcribed genes to facilitate transcriptional regulation and is therefore commonly identified as a hallmark of transcriptional activity[87]. As for the mechanism by which H3K4 methylation might work, there are two main speculations. One is that the process of H3K4 methylation will gradually change the structure of higher chromatin, and the other is that methylation will interfere with the binding of effector proteins and thus has an impact on the process that mediates downstream signals[88,89].

In addition, H3K4 methylation was graded to produce mono-, di- or trimethylated H3K4 (H3K4me1, H3K4me2 and H3K4me3, respectively) as well as unmethylated H3K4 to form distinct histone configurations[90]. In view of the diversity of methylation, the function of H3K4 methylation is also different. H3K4me1 has been widely used in many studies to identify or predict enhancers of cell-type-specific gene expression based on their binding chromatin label and protein loci. Comparatively, H3K4me2 is uniformly distributed in the coding area and is primarily responsible for the expression of the active genes, as well as playing an important role in transcriptional memory. This is mostly due to the fact that H3K4me2 remains in the gene following fast transcription. H3K4me3 is abundant near the transcription start site, promoting gene expression, and is tightly connected to active promoters and nuclear processes such as pre-mRNA splicing after receiving the stimulus[91–93].

Evidence revealed mutations in multiple genes producing H3K4 methylation modifiers have been linked to learning, memory and cognitive impairment in many animal models. Meanwhile, it's implicated in the regulation of neural memory formation. For example, when animals that had received foot shocks were given the relevant signals again, H3K4me3 levels in critical output sub-regions of the hippocampus CA1 region were considerably higher than in control rats. Interestingly, knockdown of a specific methyltransferase decreased the level of H3K4me3 and inhibited the recovery of this memory, suggesting a causal relationship between H3K4me3 and memory[94]. Clinical findings showed that H3K4 methylation patterns had changed in ASD post-mortem brain tissue and other human brain ChIP-seq data also indicated H3K4me3 marks are found in approximately 30,000 peaks across the genome[95]. Although its function in the neurological system is very weakly known, at least the behavioral and cognitive deficits caused by H3K4 methylation are related to its associated enzymes in certain, which is an important direction for future research.

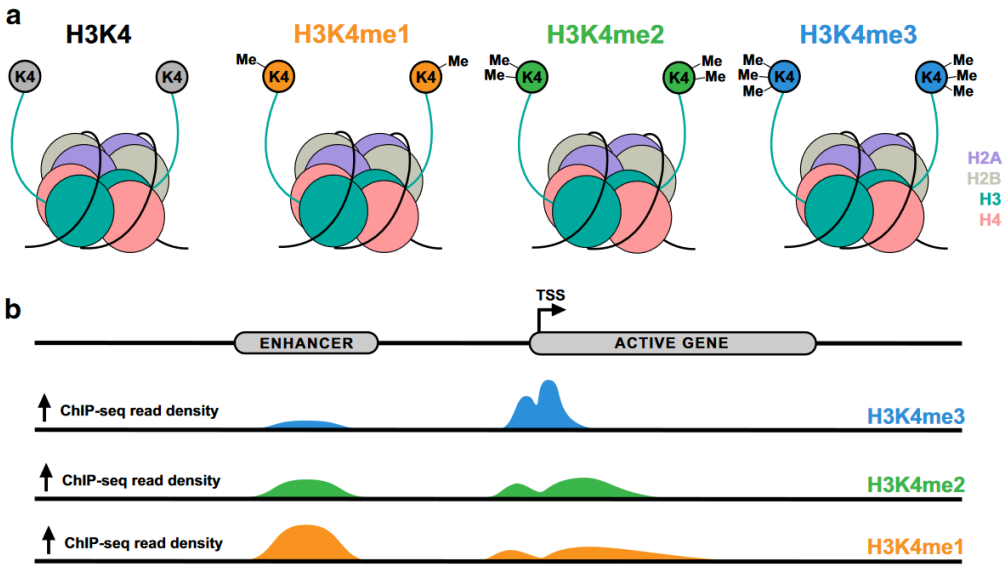


Fig.4 H3K4 methylation. (a) Histone octamers can be mono-, di-, or tri-methylated. **(b)** H3K4me1, H3K4me2, and H3K4me3 are in different regions of the genome. Figure adapted from Bridget E. Collins et al[90].

Histone dynamic controllers: methyltransferases and demethylases

H3K4 methylation is not a static process and its epigenetic control is balanced by the reverse regulation of a series of enzymatic members, the lysine methyltransferase (KMT) 'writers' and the lysine demethylase (KDM) 'erasers'. Loss of either underlying enzyme activity will result in altered levels of H3K4 methylation throughout the genome[96]. However, the continuity of the dynamic processes and the specific mechanisms involved in methylation by individual enzymes are still unclear.

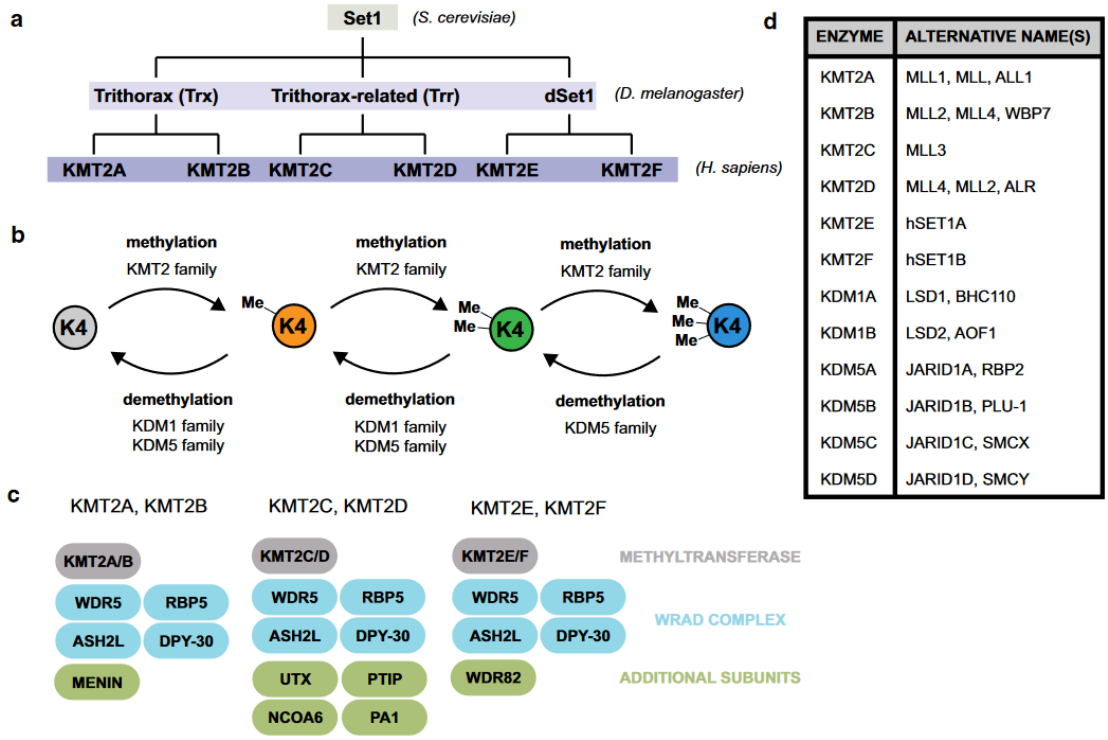
Initially, the first H3K4 methyltransferase which only encodes Set1 was identified in a COMPASS (Complex of Proteins Associated with Set1) complex in yeast. Set1 is an enzymatically active component of the COMPASS complex and is the only methylase that can regulate H3K4 mono-, di-, and trimethylation[97]. Three Set1 homologs can be presented in Drosophila, termed Trithorax (Trx), Trithoraxrelated (Trr), and dSet1 proteins. Higher mammals such as humans can express six Set1 homologs that can methylate H3K4. In line with it, KMT2A and KMT2B are a group of Drosophila Trx homologs, KMT2C and KMT2D are a pair of Drosophila Trr homologs, whereas KMTSet2E and KMT2F in mammals are homologs to dSet1 of Drosophila. Notably,

the SET1 structural domain is typically conserved in both yeast and humans[98,99].

Structurally, all H3K4 methylases are composed of a C-terminal catalytic SET domain for histone methylation, a PHD domain that participates in the interaction between proteins, and an HMG region used for DNA binding. Moreover, there is a 'WRAD complex' made up of four shared components (WDR5, DPY-30, ASH2L, and RBP5 respectively) similar to the COMPASS complex that may contribute to directing the enzyme's affinity towards certain genomic regions[100]. For example, MENIN itself as a tumor suppressor protein also is a part of the KMT2A and KMT2B complexes. Similarly, WDR82 is a key component of the other complexes[90]. These methyltransferases in humans have been demonstrated to have distinct targets in line with these diverse assemblies, implying that structural differences lead to corresponding functional differences. Furthermore, any complex component deletion causes a large drop in H3K4 methylation.

Many people believe that histone methylation is an irreversible 'permanent' epigenetic modification that can only be reversed by exchanging histones. This inherent impression lasted until 2004 when the first Lysine (K)-specific demethylase 1A (KDM1A) appeared[101]. Following this fundamental finding, people realized that unrestricted H3K4 methylation, like over-restricted methylation, can generate a lot of nuclear resentment. There are mainly two families of H3K4 demethylases in humans, KDM1 and KDM5, with six members namely KDM1A, KDM1B, KDM5A-KDM5D. Interestingly, because of substrate variations and differential catalytic mechanisms, the KDM1 family is only able to delete methyl groups from H3K4me1 and H3K4me2, unlike the KDM5 enzymes, which also remove methyl from H3K4me3[90]. The KDM1 family of enzymes was subsequently characterized as being dependent on flavin adenine dinucleotide (FAD) and amine oxidase domain interactions for demethylation. This dependence requires a pair of electrons on the nitrogen of the lysine side-chain to oxidize the single bond of the methyl group. Therefore, trimethylated lysine cannot be

removed because KDM1 family members lack a lone electron pair in side-chain nitrogen. In contrast, KDM5 family enzymes rely on iron and the N-terminal catalysis of α-ketoglutarate. The JmjC structural domain does not need a nitrogen-carbon double bond and it thus can remove trimethylating lysine[100,102].



ENZYME	ALTERNATIVE NAME(S)
KMT2A	MLL1, MLL, ALL1
KMT2B	MLL2, MLL4, WBP7
KMT2C	MLL3
KMT2D	MLL4, MLL2, ALR
KMT2E	hSET1A
KMT2F	hSET1B
KDM1A	LSD1, BHC110
KDM1B	LSD2, AOF1
KDM5A	JARID1A, RBP2
KDM5B	JARID1B, PLU-1
KDM5C	JARID1C, SMCX
KDM5D	JARID1D, SMCY

Fig.5 Enzymology of H3K4 methylation and demethylation. (a) H3K4 methyltransferase evolutionary tree diagram. **(b)** H3K4 methylation and demethylation reaction pathways. **(c)** Each KMT2 family member forms a bubble diagram of COMPASS-like complexes. **(d)** Alternative names of H3K4 enzymes. Figure adapted from Bridget E. Collins et al[90].

H3K4 demethylases preferentially operate on specific genomes to regulate their activity, although the basis for this preference is unknown. For example, the KDM1 family is essential in the process of embryogenesis and cell proliferation. The lack of any KDM1 enzyme can result in the death of embryonic mice. Another example is that neurological research has discovered that KDM5C functions primarily in mature neurons and regulates their basic activity by changing the methylation levels of their enhancers. Meanwhile, the absence of KDM5C function is connected to the occurrence and development of X chromosome-linked mental retardation[103,104]. KDM5B works

similarly to other demethylases in the KDM5 family, both of which are involved in developmental processes. According to research, KDM5B interacts with PcG protein, which is involved in transcriptional inhibition. Further, injection of KDM5B into reconstructed embryos significantly decrease the level of H3K4me3 and rescued the expression of several developmentally relevant genes. According to the latest research, KDM5B enhances immune evasion by enlisting the help of SETDB1 to silence retroelements[105,106].

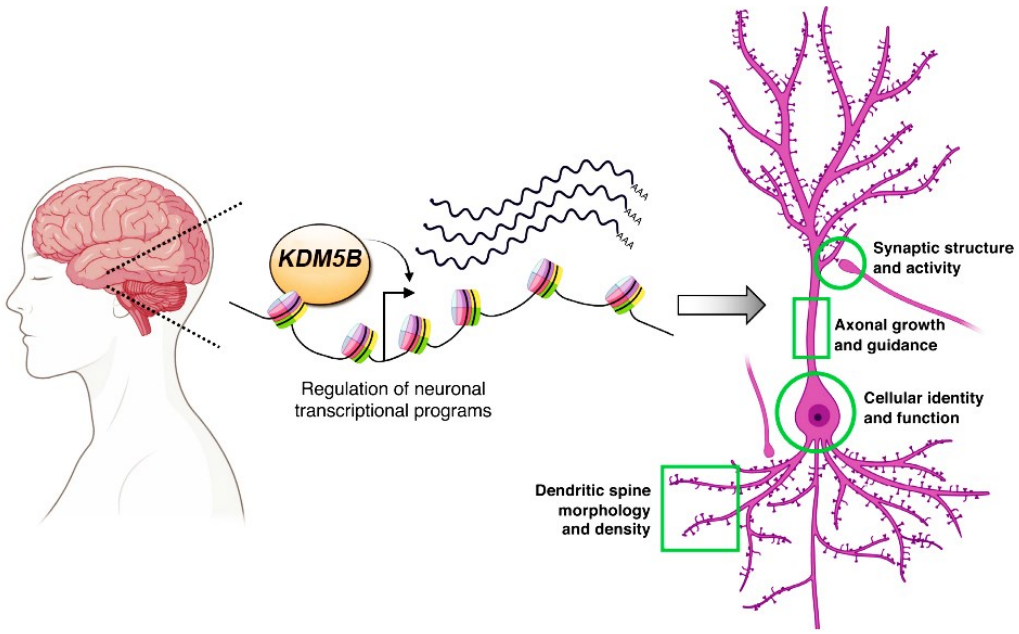


Fig.6 Neuronal functions of KDM5B. Figure adapted from Hayden A. M. H et al[107].

In this thesis, I will be focusing on two distinct types of dementia, namely AD and FTD. I will detail the genetics and molecular pathogenesis of AD in the first half of the introductory section, and then discuss FTD and how the small non-coding RNAome plays a role in the pathogenesis of FTD disease in the second section.

Alzheimer's Disease

Dementia is not a unique illness in the scientific sense, but rather a collection of brain disorders symptoms that occur as a result of the disease. It will have a severe and negative impact on a person's memory, logical thinking, personality, and social

behavior, interfering with their daily life and putting an unbearable financial strain on the family[1]. According to ADI (an International Alzheimer's Association) statistics by 2021, more than 55 million people globally have dementia at present, with the number growing every day reach to 78 million by 2030. Among all different types of dementia, AD, as an irreversible and highly age-related neurodegenerative disease, will gradually destroy brain function with the continuous increase of age, currently becoming the most common type of dementia, accounting for the total incidence of 60-80%[108]. It is also the third leading cause of death in the elderly after heart disease and cancer.

In terms of disease progression, AD is a slowly progressive neurodegenerative disease that can be broadly divided into several main stages[109]. The first stage is from healthy aging to the pre-dementia stage, which is commonly described as a very mild cognitive impairment that begins within the first five years of a clinical diagnosis of dementia. During this period, there is no noticeable worsening in the patient's activities of daily living. The second stage is the transition from pre-dementia to mild cognitive impairment (MCI), which is characterized by a lower quality of life, a tendency to depression, some spatial disorientation as well as avoidance of social interaction. But the majority of the time, patients can live on their own. So, in this stage, if the development of MCI can be delayed in time, the prevalence of dementia and the cost of treatment will be considerably reduced. However, because the symptoms of MCI are very similar to age-related memory disorder, thus it is hard to diagnose and easily misses the ideal opportunity for treatment. The third stage is the Moderate dementia stage, including indifferent expression, and severe memory loss from recent and unpredictable behavior[110]. As many as 20% of patients experience hallucinations, which may be closely related to Cholinergic defects and at the same time, the patient showed a certain aggressiveness. Eventually, in the later stages of the disease, the patient suffers significant impairment of almost all cognitive processes. They will lose the ability to care for themselves completely[111]. It is therefore an extremely serious progressive neurodegenerative disease that occurs in the elderly.

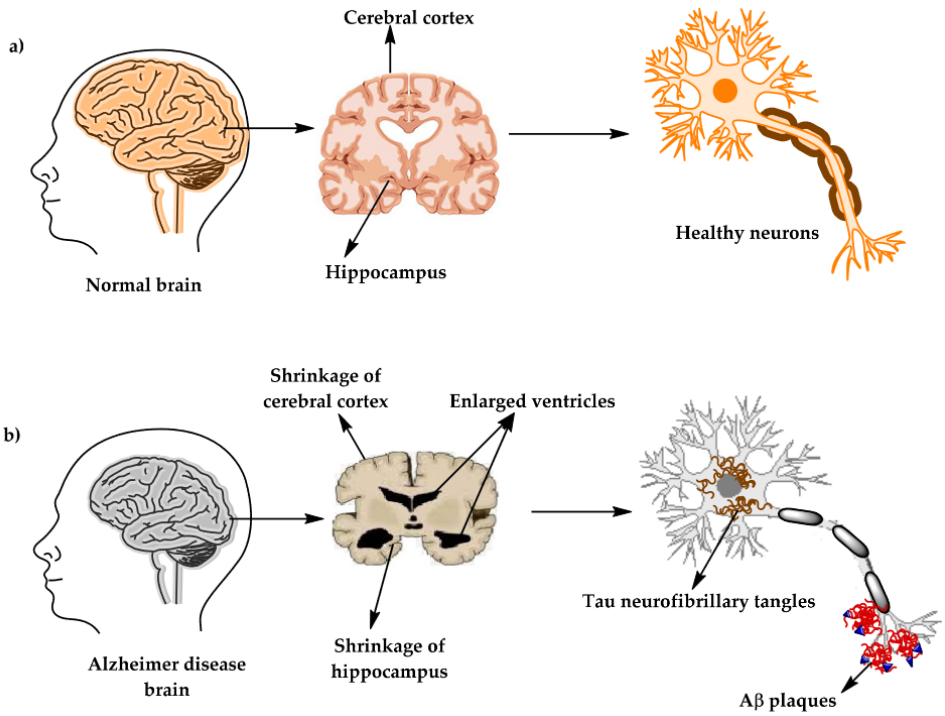


Fig.7 (a) healthy brain and (b) Alzheimer's disease brain. Figure adapted from Zeinab. B et al[110].

Genetics of AD

AD is a multifactorial disease, with genetic risk accounting for 70-80% of the incidence and external environmental factors accounting for only about 20%[24]. Genetic factors include genes associated with familial AD and sporadic AD. Broadly speaking, the genes closely associated with early-onset familial AD are amyloid precursor protein (APP), and presenilin (PSEN), while the gene that is closely involved in common late-onset sporadic AD is APOE (See table below)[112]. The widely known APOE ε4 allele describes a significant portion of the heritability of AD (but not entirely), and a genome-wide study showed that the APOE ε4 allele increased the risk of AD by 3-4 times[18]. Furthermore, there are approximately 40 other genetic variants that also contribute to the increased risk of AD[113]. Interestingly, the function of these risk loci reveals that in addition to the influence of β-amyloid metabolism, a number of immune cause-related lipid metabolic functions, endocytosis, and vascular factors (e.g. TREM2, ABCA1, ABCA7, and SORL1) also play an important regulatory role in the development of AD. Studies have found that AD patients express protein damage

variants of these genes, implying that the undamaged protein products of these genes are equally crucial for maintaining brain health[114–117].

Gene	Protein	Chromosome	Mutations	Molecular phenotype
APP	Amyloid β (A4) protein precursor	21q21	24 (duplication)	Increased $A\beta_{42}/A\beta_{40}$ ratio Increased Aβ production Increased Aβ aggregation
PSEN1	Presenilin 1	14q24	185	Increased $A\beta_{42}/A\beta_{40}$ ratio
PSEN2	Presenilin 2	1q31	14	Increased $A\beta_{42}/A\beta_{40}$ ratio

Table.1 Familial Alzheimer's disease genes and pathogenic effects. Table adapted from Rudolph E. Tanzi[112].

Pathophysiology of AD

Scientists specify that at the level of molecular markers and pathology, AD is associated with abnormal protein aggregation. Extracellular Amyloid-ß plaque (Aß) deposits, as well as misfolded intracellular neurogenic fibers (or tau tangles) caused by neuroinflammation, aging, vascular disease, etc., are common in AD patients' brains[118]. Furthermore, the brain's nerve cells (neurons) and synapses connections are lost. These brain traits are regarded to be some of the most prominent characteristics of AD. Meanwhile, changes in microglia and astrocytes can also lead to the insidious progression of AD. Microscopic pathological changes can gradually induce the macroscopic atrophy of the hippocampus, amygdala, and neocortex[119,120]. According to the progress of its pathological development and the severity of symptoms, AD can be described and divided into different grades by the Braak stage. Evidence showed slight cortical and hippocampal lesions will appear in the early stages, the abnormal deposition of tangles usually starts in the internal olfactory area (stage I-II) then spreads to cerebral cortex areas (stage III-IV), and ultimately reaches the neocortex (stage V-VI)[121].

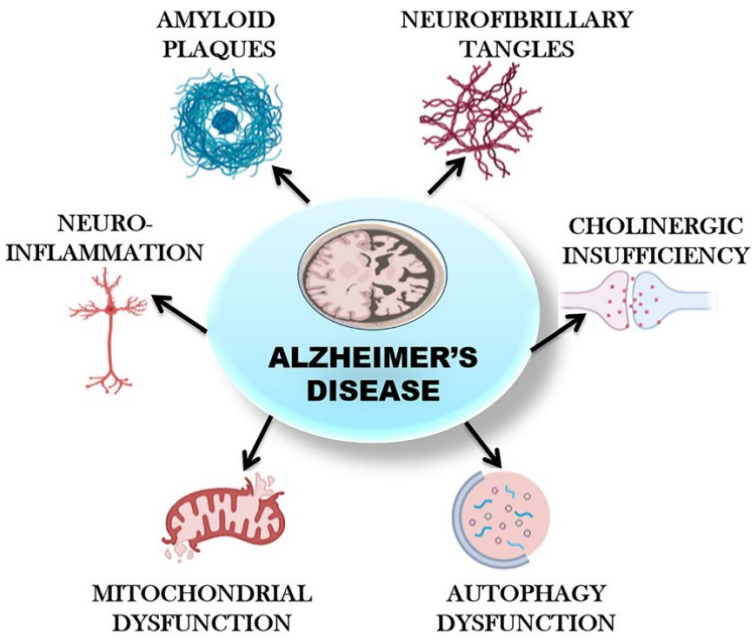


Fig.8 Pathophysiology of Alzheimer's disease. Figure adapted from Rishika. et al[122].

Epigenetics factors in AD

For the past few years, epigenetics has become a research hotspot in central nervous system diseases and cancer. Moreover, the functions of epigenetics have been confirmed in numerous fields of biology, revealing the interaction between genes and the expression of genotypes. DNA methylation, histone changes, chromatin remodeling, and non-coding RNA regulation are all epigenetic factors. Nowadays, many researchers are examining the possible significance of epigenetics in the pathophysiology of AD[123].

DNA methylation: DNA methylation alters cytosine residues by adding methyl groups to areas rich in cytosine/guanine, such as CpG islands. DNA methyltransferases (DNMT), including DNMT1, DNMT2, DNMT3a, and DNMT3b, initiate the process. This section explores the notion that AD is caused by aberrant DNA methylation in a few specific genes and the possible significance of methylation as a biomarker for AD. For example, it was discovered that some cytosines in the promoter region of the APP gene, notably those between 207 and approximately 182, are mostly methylated and that their demethylation with age may result in A deposition in the aging brain. Methylation of the microtubule-related protein tau (MAPT) gene may also inhibit

MAPT expression, hence lowering tau protein levels[124]. Mano et al. discovered that BRCA1 expression was considerably elevated in postmortem brain tissues from AD patients, owing to its hypomethylation[125]. A recent study discovered that DNA methylation (CpG5) of the BDNF gene promoter and a tag SNP (rs6265) have a substantial role in the etiology and progression of amnestic mild cognitive impairment (aMCI)[126]. Additionally, a five-year longitudinal study utilizing multivariate Cox regression analysis found that increased methylation of the CpG5 promoter region of BDNF was a significant independent predictor of AD conversion. This shows that increasing levels of BDNF promoter methylation in the peripheral blood may serve as an epigenetic biomarker for the progression of aMCI to AD[127]. Kobayashi et al. analyzed the COASY and SPINT gene promoter areas for DNA methylation and discovered that DNA methylation in both regions was considerably higher in AD and aMCI compared to controls[128]. Di Francesco et al. discovered that global DNA methylation levels were significantly higher in the peripheral blood mononuclear cells of LOAD patients than in healthy controls and that these higher DNA methylation levels were associated with the presence of the APOE 4 allele ($p=0.0043$) and APOE 3 carriers ($p=0.05$) in the global population. This study demonstrated that global DNA methylation in peripheral blood samples is a helpful diagnostic for identifying persons at risk of acquiring AD[129].

Histone modification and chromatin remodeling: Chromatin is a complex structure composed of genomic DNA, histone proteins, and other factors. Chromatin can be dynamically altered through various histone alterations. Additionally, nucleosome relocation, chromatin remodeling, and nuclear compartmentalization are also conceivable changes. Histones are fundamental proteins that serve as the nucleosome's building blocks. Their tails are amenable to a variety of changes. Together, these modifications form the extremely complicated histone code[130]. Among the alterations, acetylation is without a doubt the most well-characterized post-translational modification of core histones. It is well established that acetylation of histones promotes

gene transcription, whereas deacetylation suppresses gene expression by compressing the chromatin[131]. Details for the histone acetyltransferases and deacetylases have been described in the previous section.

Non-coding RNA: ncRNA is not derived from protein-coding genes but is present in the cell. Less than 2% of the mammalian genome is known to code for proteins. Most are spliced or reduced into smaller products in some way[132]. Indeed, most human genome programming relies on facilitating different programs by regulating the structure, splicing, and stability of RNA. Based on the length of the molecule, ncRNA can be divided into small size (< 400 nucleotides) and long size (> 400 nucleotides). They each perform a different function such as the ribosomal RNAs and transfer RNAs are responsible for translation, the small nuclear RNAs for splicing, and the small nucleolar RNAs for modification. It is well established that RNA processing is involved in many diseases and that ncRNAs are involved in epigenetic regulation associated with aging[133].

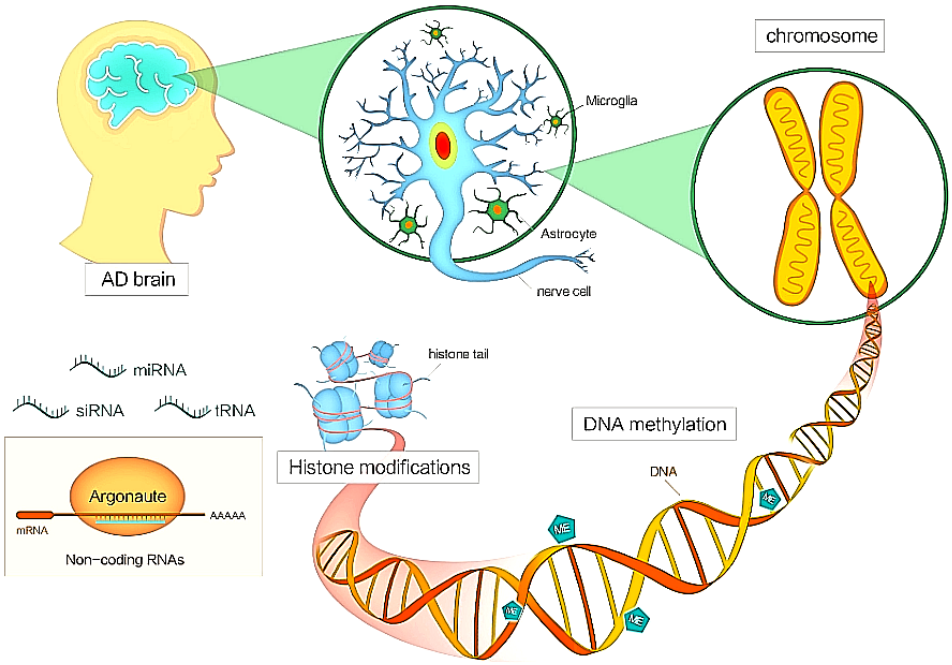


Fig.9 Epigenetics factors in AD. Figure adapted from Xiao. L.L et al[123].

AD affect memory

Researchers have identified common areas of cognitive impairment for people with AD including memory, language, visuospatial, attention, and emotion. Of these problems, memory impairment is the key problem[134]. Thus, understanding how people with AD experience memory deficits as the disease progresses is essential for assessing the severity of cognitive impairment and developing novel medications. Neuroscientists have described six main types of everyday human memory, including working memory, procedural memory, simple classical conditioning, semantic memory, and episodic memory[108]. In AD patients, some of these memories are severely impaired, while others are relatively preserved. A large body of data suggests that the function of semantic memory is the most severely damaged part in AD, with patients exhibiting a variety of specific deficits in naming. This is usually attributed to lesions in the temporal and frontal lobes of the brain resulting in a loss of neuronal dendritic function in cortical areas[135]. In addition, due to lesions in the hippocampus, conditioned reflexes such as the amygdala-dependent fear and the blink conditioning are impaired in AD patients. Of the memory systems mentioned above, episodic memory impairment has the highest clinical relevance for people with AD and is one of the earliest symptoms of AD. This usually manifests itself as a blurred memory of recent events compared to previous events[136]. Neuroimaging revealed that episodic memory is mainly supported by the hippocampus. Significant differences in hippocampal surface structure and volume in AD patients compared to normal subjects[137]. As a result, the hippocampus has been considered one of the most critical sites of injury in AD.

Current status of AD treatment

Currently, AD has been considered a complex illness with a variety of risk factors, including aging, gender, genetic factors, cardiovascular disease, inflammation, lifestyle, and environment. Apart from that, there are also many different pathogenic mechanisms involved in AD. Due to the interference of these multiple factors, the development of effective medicines for the treatment of AD has been extremely challenging. So far, only two traditional medicines, cholinesterase inhibitors, and N-methyl d-aspartate

(NMDA) antagonists are licensed for clinical use in the treatment of AD[138]. But unfortunately, these two types of drugs only have the effect of delaying the progression of the disease, but not curing, reversing, or preventing it. In the past ten years, only a few clinical studies for AD have been undertaken, but the outcomes have been disastrous. Growing molecular pathological evidence suggests that histopathological changes in the brain of patients with AD begin to occur years before the clinical syndrome appears[139]. These molecular changes rely on epigenetic mechanisms and, therefore, it is very important to accurately detect AD based on molecular biological markers in the early stage and use drugs to intervene in time.

Frontotemporal Dementia

Frontotemporal dementia (FTD), a group of neurodegenerative diseases caused by hereditary or primary (unexplained) disorders, was first described by a Czech scientist named Arnold Pick in 1892 who found a patient was losing his ability to communicate due to localized degeneration of the frontal and temporal lobes[140]. Later in 1911 that another scientist, Alois Alzheimer, carried out a histopathological analysis of the clinical case provided by Pick, which revealed silver-loving cytoplasmic inclusions within the swollen neurons. Subsequently, Pick's students Onari and Hugo had assigned the label 'Pick's disease' to frontotemporal lobar atrophy and it was not until 1994 that a group of researchers from Sweden and the UK coined the term 'frontotemporal dementia' to replace 'Pick's disease'[8,141].

In recent years, with the deepening of research, scientists have found that FTD is a highly heritable disease associated with a combination of genetic and environmental factors. But on the other hand, more than half of persons with FTD, do not have a history of dementia in their family. However, compared to AD, which mainly occurs in the aging stage of humans, FTD belongs to early/young-onset dementia. The age of onset is mainly concentrated in the age of 45-65, and the impact on men and women is roughly equal[142]. The International Classification of Diseases certifies that the disease

causes psychological and behavioral disorders in humans and it turns to be the second most common cause of dementia after AD, which is characterized by frequent mood changes, screaming, uncontrollable behavior, social impairment, language dysfunction, and other typical patterns of social expression[143].

Neuropathology of FTD

From a macroscopic perspective, the principal symptom of FTD is a functional impairment marked by atrophy of the frontal and temporal lobes of the brain and these macroscopic changes usually appear in stage 1. It then progresses to the basal nucleus and posterior temporal lobes and is referred to as stage 2. Subsequently, atrophy of the frontotemporal lobe leads to white matter degeneration (stage 3). Over time, eventually, areas of the frontotemporal lobe, hippocampus, and basal ganglia become severely atrophied (stage 4)[144]. In terms of micro-changes, FTD is accompanied by changes such as astrocytic gliosis and synaptic loss, followed by massive neuronal death due to excessive deposition of particular molecular proteins in neurons. These specific aggregates have a variety of morphologies and preferential targets, including cortical layers, neurons, and glial cells[145]. In addition, most of them are directly correlated to the different pathological subtypes of frontotemporal lobar degeneration, such as Tau (τ), TAR DNA-binding protein 43 (TDP-43), and fusion sarcoma (FUS)[146].

FTD-Tau: Tau protein is a microtubule protein that plays an essential role in the stability of cell structure and cellular transport, as well as some signaling pathways. Over 50 mutations were found to be associated with FTD-tau pathology. One of the primary pathological causes of neurodegenerative diseases is hyperphosphorylation of Tau proteins. Neuronal fiber tangles in AD are mostly made up of 3R and 4R tau inclusions, whereas FTD is mostly made up of 3R tau aggregates and is associated with pathogenic mutations in MAPT[147].

TDP-43: A heterogeneous nuclear ribonucleoprotein that plays a critical role in the

processing, splicing, translation, and stabilization of thousands of micro-RNA. When TDP-43 accumulates excessively in the cytoplasm, it induces phosphorylation and ubiquitination, leading to FTD. In 2011, TDP-43 was divided into four different subtypes (TDP43 A-D) depending on how common they are in clinical. Notably, the most representative TDP-43 A phenotype, whose inclusion bodies are usually present in the cortical layer, is highly associated with pathogenic mutations in GRN. Meanwhile, TDP-43 B type is closely related to pathogenic mutations in C9orf72[144].

FUS: like TDP-43, is also a nuclear binding protein that accumulates in the cytoplasm in FTD. However, most of the FUS is sporadic and not related to mutations. Generally accompanied by a younger (20-40 years old) trend[148].

Immunohistochemistry revealed that almost all FTD cases were positive in the major proteome for Tau (45%), TDP-43 (45%), or FUS (5%), only with very few cases presenting with other pathological subtypes or unknown[149].

Clinical features of FTD

Based on clinical symptoms, the core of FTD spectrum impairment is classified into three different subtypes. Behavioral variant frontotemporal dementia (bvFTD), which manifested as executive dysfunction. Nonfluent aphasia or semantic dementia (nfPPA), presents language dysfunction. And semantic variant PPA (svPPA), which is a gradual identification and semantic knowledge disorder. Among them, bvFTD is the most common type of FTD, which is closely related to Pick body[8]. Pick bodies are spherical structures that are unique to the cytoplasm of damaged cells and consist of a variety of protein components such as tau protofibrils and other associated microtubule proteins. This type of patients has relatively complete memory preservation, but their personality is inconsistent with daily routines and changes significantly. They are usually emotionally impulsive or become reticent. Meanwhile, a small proportion of patients will gradually develop motor neuron disease as the disease progresses. Progressive

nonfluent aphasia is characterized by a decline in hearing and speech, whereas semantic dementia is described by relatively unaffected speech but varying degrees of comprehension deficits[150].

The features of the three clinical types of FTD might converge as the disease advances, as a localized degeneration becomes more widespread and affects broad areas of the frontal and temporal lobes. Thus, usually in the later stages of FTD, various clinical phenotypes will appear at the same time.

Genetics of FTD

There are many reasons leading to FTD but the specific mechanism is still unclear. It has been reported that up to 40% of people with FTD have a family history of the condition. Of these, 60% of familial heritability cases had mutations in microtubule associated protein Tau (MAPT), Progranulin (GRN), and chromosome 9 open reading frame 72 (C9orf72)[151].

MAPT: The gene MAPT was the first to be confirmed linked to FTD[152]. As previously stated, pick bodies are made up of multiple microtubule proteins and tau, implying that there is a direct neuropathological link between various types of tau mutations and gene mutations. Through the association of FTD with the tau locus region on the chromosome, it was found that mutations in the MAPT gene encoding the tau protein on chromosome 17 can form neuronal tangles, destroy brain cells, thereby causing frontotemporal dementia and Parkinson's disease[153]. Furthermore, alterations in the tau exon 10 splice site result in aberrant tau deposition within neurons[154]. Tau deposition inside glial cells is similarly variable in other mutation families. Patients with MAPT genetic mutations have different clinical presentations depending on whether they have bvFTD, nfPPA, et. This means the same mutation may lead to different phenotypes. This also confirms that FTD can be linked to tau polymorphism, and Tau dysfunction alone is enough to induce neurodegeneration in the absence of amyloid pathology.

GRN: which was found in neurons in 2006 and was also located on chromosome 17. It encodes transcription factors that are secreted and plays a role in inflammation[155]. The distance between the GRN gene and the tau gene is only two centimorgans. Due to its close proximity, cloning posed a significant challenge to existing linkage studies. Another further study has found that haploinsufficiency causes neurodegeneration. Patients with GRN genetic mutations have a delayed presence compared to MAPT due to the incomplete penetrance, even at the age of 70, one-tenth of carriers are asymptomatic. GRN-FTD is typically inherited as an autosomal dominant trait. According to data, 95% of patients with GRN mutations have family genetics and a history of FTD. GRN mutations reduce the production of granule protein precursor proteins and induce TDP-43 errors in brain cells, which may contribute to the emergence of frontotemporal lobe disorders[156–158].

C9orf72: One of the most prevalent genetic causes of FTD is mutations in the C9orf72 gene, which account for around a quarter of all family cases[159]. C9orf72 is a set of hexanucleotide GGGGCC repeats found in the non-coding region of chromosome 9. It is harmful when more than 30 repeats are present, most patients exhibit thousands of repeat lengths. According to research, the repeats' length in the brains of FTD patients is negatively correlated with their survival rate, which means that the longer the repeats, the shorter the survival[160]. Furthermore, another study that looked at C9ORF72 amplification in clinical FTD patients discovered that all C9-positive patients had bvFTD and motor neuron disease syndromes. Patients with C9+bvFTD, on the other hand, have more frequent delusions of grandeur, as well as greater memory deficits. Although C9-positive individuals are uncommon in dementias such as FTD or AD, it is certain that repeat amplification of the C9ORF72 hexanucleotide has the potential to develop FTD and therefore this could also contribute to the diagnostic treatment of FTD in the clinic[161,162].

Rare genetics: During the last few years, more and more genes have been found to be

closely linked to autosomal dominant FTD, including CHMP2B (2005), FUS (2009), TBK1 (2015), TIA1 (2017), *etc.* However, they cumulatively account for less than 5% of FTD overall, with TBK1 being identified as the fourth most common genetic mutation at 2%[151,163].

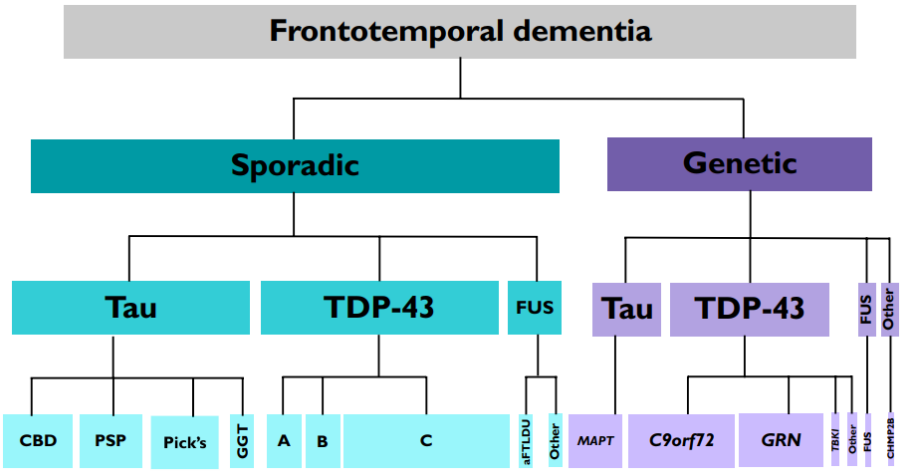


Fig.10 Genetics of FTD. Figure adapted from Caroline V. G.et al[151].

Epigenetics of FTD

With the discovery of the structure of DNA in the mid-twentieth century, the concept of DNA as a carrier of genetic information became generally recognized. Genetic sequencing enables the detection of hereditary disorders as well as previously unknown genetic traits. Thus, controlling gene expression programs is critical for cellular function. Once the gene is abnormally expressed it can lead to numerous pathophysiologically related diseases and the most directly relevant processes that impact gene expression are epigenetic mechanisms[164,165]. The term epigenetics was first coined by biologist Conrad Waddington in 1957. It is used to describe the causal relationship between an individual's genotype and its phenotype[166]. For example, initially, each cell shares a single genome, and when particular genes are activated or repressed they further differentiate into a specific cell type, generating an entirely new gene expression profile that is maintained throughout the cell division process[167]. These mechanisms have since been identified as heritable changes that do not rely on DNA

sequence alterations, including DNA methylation, Histone-modification and Non-coding RNA (ncRNA)[168].

Small RNAs (sRNAs) and long noncoding RNAs (lncRNAs) are examples of ncRNAs. And sRNAs are further divided into short interfering (si) RNAs and micro (mi) RNAs. Most ncRNAs are known to be functional, and their signaling and editing have been shown to play important roles in nuclear and chromatin structure. In particular, ncRNAs function through repression or control to carry out epigenetic regulation[169]. It is now widely accepted that alterations in RNA processing may involve a number of aging-related disorders, including AD, PD, and FTD[170]. Interestingly, miRNAs were found to be expressed at different levels in different stages of pathology in brain tissue and blood samples from deceased FTD patients[171].

Biomarkers: microRNAs

MicroRNA (miRNA) is a short single-stranded non-coding RNA molecule found in living organisms that regulates gene expression post-transcriptionally under a variety of situations. It can destabilize mRNA by basic matching with complementary mRNA sequences to cleave or shorten poly(A) tails, thereby reducing the translation efficiency of proteins, inducing neurological signaling, and promoting different pathogenic mechanisms[172]. Interestingly, the targeting between miRNAs and mRNAs is not singularly ordered, but instead interacts with multiple targets to form a complex network of regulatory genes[173]. According to research, hundreds of proteins can be inhibited by a single miRNA. Although miRNAs can target nearly 60% of mammalian genes, their evolution has been highly conserved. Nearly a hundred miRNA families have been preserved in ancient mammalian ancestors, and these conserved miRNAs have crucial biological activities in numerous mammalian cell types[174].

The human genome currently encodes more than 2000 miRNAs. However, miRNAs are expressed differently in different tissues and cells, and abnormal miRNA expression

can cause a variety of diseases. Therefore, targeting and regulating specific miRNAs is a hot topic in medical treatment[175]. Chronic lymphocytic leukemia was the first disease that has been recognized by modern medicine to be associated with miRNA dysregulation and miRNAs serve as both proto-oncogenes and tumor suppressors in this disease[176]. In addition, as the field of research expands, the regulatory role of miRNAs in genetic diseases, cancer, heart disease, and neurological disorders is becoming more and more prominent[177].

In the brain, a specific miR-124 has been shown to be involved in repressing CREB-1 transcriptional function, thereby affecting mammalian synaptic plasticity and reducing its memory levels[178]. Another study on memory in the Drosophila brain similarly confirmed that miRNAs mediate neural development and memory formation[179]. Subsequently, the research reported conditional knockdown of specific miRNAs improves learning memory, enhances synaptic plasticity, and regulates dendritic spine formation in behavioral tests in mice[180,181]. Another study revealed that when miR-34c levels are elevated in the hippocampus of aged mice, dysregulation of this miRNA directly contributes to the development of age-dependent cognitive dysfunction[182]. Many miRNAs have been recognized as being involved in the progress of neurological diseases in recent years. Hence, all the above evidence suggests that the expression and regulation of miRNA are essential for maintaining a stable gene network and a healthy organism.

microRNAs in FTD

Increasing evidence suggests that miR-134, miR-137, and miR-9, as well as a range of other miRNAs, play a key role in neurodevelopment, synaptic plasticity, and dendritic spine formation[183–185]. Meanwhile, miRNAs were also found to be dysregulated in AD, Parkinson's disease, Huntington's disease, and FTD[186]. For instance, the aforementioned miR-124 has been shown to be involved in memory levels in mammals, miR-124 was also found actively involved in regulating social behavior in FTD model

mice in another study[187]. Additionally, miR-132 and miR-212 mimics were delivered into HEK cells via lipid nanoparticles transfection, and both miRNAs decreased TMEM106B mRNA levels[188]. Furthermore, miR-107 significantly decreased the GRN expression in HeLa cells[189]. A recent review provides a very comprehensive summary of FTD-related miRNA biomarkers published in the last decade. All data were obtained from the plasma, blood serum, and cerebrospinal fluid of subjects in FTD tissue. The table below details the significant regulatory effects and potential options for miRNA biomarkers in FTD patients (See alterations of miRNAs expression in table 2: FTD blood plasma and serum; table 3: in FTD CSF and brain tissue; Candidates list for miRNA biomarkers in FTD as shown in table 4)[171]. Intuitively, it strongly demonstrates that miRNAs are intimately involved in the development of FTD.

Author	Method of miRNA analysis	Comparison	miRNA analysis
Blood plasma			
Kmetsch et al., 2020	miRNA sequencing	Symptomatic mutation carriers[1] *vs.* HC	Upregulated: miR-34a-5p,-345-5p Downregulated: miR-200c-3p,-10a-3p
Kmetsch et al., 2020	miRNA sequencing	Presymptomatic mutation carriers[2] *vs.* HC	Upregulated: miR-34a-5p
Kmetsch et al., 2020	miRNA sequencing	Symptomatic mutation carriers *vs.* presymptomatic mutation carriers	Upregulated: miR-345-5p Downregulated: miR-200c-3p,-10a-3p
Siedlecki-Wullich et al., 2019	RT-PCR	AD *vs.* HC	Upregulated: miR-92a-3p,-181c-5p,-210-3p
Siedlecki-Wullich et al., 2019	RT-PCR	MCI *vs.* HC	Upregulated: miR-181c-5p,-210-3p
Siedlecki-Wullich et al., 2019	RT-PCR	FTD *vs.* HC	No significant differences in miR-92a-3p,-181c-5p,-210-3p
Grasso et al., 2019	RT-PCR	FTD *vs.* HC	Downregulated: miR-663a,-502-3p,-206
Grasso et al., 2019	RT-PCR	bvFTD *vs.* PPA	No significant differences in miR-663a,-502-3p,-206
Grasso et al., 2019	RT-PCR	Male FTD *vs.* HC	Downregulated: miR-663a,-502-3p,-206
Grasso et al., 2019	RT-PCR	Female FTD *vs.* HC	Downregulated: miR-663a,-502-3p, let-7e-5p
Piscopo et al., 2018	RT-PCR	FTD *vs.* HC	Downregulated: miR-127-3p
Piscopo et al., 2018	RT-PCR	FTD *vs.* AD	Downregulated: miR-127-3p
Piscopo et al., 2018	RT-PCR	Male FTD *vs.* HC	Downregulated: miR-127-3p
Piscopo et al., 2018	RT-PCR	Female FTD *vs.* HC	Downregulated: miR-127-3p
Piscopo et al., 2018	RT-PCR	Male FTD *vs.* AD	Downregulated: miR-127-3p
Piscopo et al., 2018	RT-PCR	Female FTD *vs.* AD	Downregulated: miR-127-3p
Sheinerman et al., 2017	RT-PCR	FTD *vs.* HC	The ratios miR-9-3p/let-7e, miR-7/miR-451, miR-335-5p/let-7e distinguished FTD from HC
Sheinerman et al., 2017	RT-PCR	FTD *vs.* AD	The ratios miR-125b/miR-29a, miR-125b/miR-874, miR-107/miR-335-5p distinguished FTD from AD
Sheinerman et al., 2017	RT-PCR	FTD *vs.* ALS	The ratios miR-129-3p/miR-206 and miR-338-3p/let-7e distinguished FTD from ALS
Sørensen et al., 2016	RT-PCR	AD *vs.* other dementia types (vascular, FTD, DLB)	Upregulated: miR-590-5p,-142-5p but not significant by Benjamini-Hochberg Downregulated: miR-194-5p but not significant by Benjamini-Hochberg
Sheinerman et al., 2012	RT-PCR	MCI *vs.* HC	The ratios miR-128/miR-491-5p, miR-132/miR-491-5p, miR-874/miR-491-5p, miR-134/miR-370, miR-323-3p/miR-370, miR-382/miR-370 distinguished MCI from HC
Blood serum			
Denk et al., 2018	RT-PCR	bvFTD *vs.* HC	Upregulated: miR-143-3p,-197-3p,-27a-3p,-338-3p,-491-5p,-7b-5p,-7g-5p,-106a-5p,-106b-5p,-18b-5p,-223-3p,-26a-5p,-26b-5p,-301a-3p,-30b-5p Downregulated: miR-100-5p,-335-5p,-99a-5p,-146a-5p,-15a-5p,-22-3p,-320a,-320b,-92a-3p,-1246
Galimberti et al., 2014	RT-PCR	AD *vs.* NINDC	Downregulated: miR-125b,-23a,-26b-5p
Galimberti et al., 2014	RT-PCR	AD *vs.* FTD and INDC	No significant differences in miR-125b,-23a,-26b-5p

AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; bvFTD:behavioral variant FTD; DLB: dementia with Lewy bodies; FTD: frontotemporal dementia; HC: non-demented healthy controls; INDC: inflammatory neurologic disease controls; MCI: mild cognitive impairment; NINDC: non-inflammatory neurologic disease controls; PPA: primary progressive aphasia; RT-PCR: real time polymerase chain reaction. [1]Symptomatic mutation carriers consisted of 15 FTD, 4 FTD/ALS, 3 ALS patients carrying a *C9orf72* expansion; [2]Presymptomatic mutation carriers were 46 asymptomatic first-degree relatives of *C9orf72* patients in which a pathogenic expansion was found.

Table.2 Alterations of miRNA expression in FTD blood plasma and serum. Table adapted from Bridget Ma. et al[171].

Author	Method of miRNA analysis	Comparison	miRNA analysis
CSF			
Schneider et al., 2018	RT-PCR	Symptomatic mutation carriers[1] *vs.* presymptomatic mutation carriers[2]	Downregulated: miR-204-5p,-632 in exosomes
Schneider et al., 2018	RT-PCR	Symptomatic mutation carriers with either *GRN* or *C9orf72* mutations *vs.* presymptomatic mutation carriers	Downegulated: miR-204-5p in exosomes
Schneider et al., 2018	RT-PCR	Symptomatic mutation carriers with GRN but not with *C9orf72* mutations *vs.* presymptomatic mutation carriers	Downregulated: miR-632 in exosomes
Schneider et al., 2018	RT-PCR	bvFTD *vs.* presymptomatic mutation carriers	Downregulated: miR-204-5p,-632 in exosomes
Schneider et al., 2018	RT-PCR	FTD *vs.* HC	Downregulated: miR-632 in exosomes
Schneider et al., 2018	RT-PCR	FTD *vs.* AD	Downregulated: miR-632 in exosomes
Derkow et al., 2018	RT-PCR	AD *vs.* HC	Upregulated: let-7e
Derkow et al., 2018	RT-PCR	MDE *vs.* HC	Upregulated: let-7e
Derkow et al., 2018	RT-PCR	FTLD *vs.* HC	Upregulated: miR-124
Derkow et al., 2018	RT-PCR	FTLD *vs.* AD	Upregulated: miR-124
Derkow et al., 2018	RT-PCR	FTLD *vs.* MDE	Upregulated: miR-124
Denk et al., 2018	RT-PCR	bvFTD *vs.* HC	Upregulated: miR-124-3p,-125a-5p,-223-3p Downregulated: miR-15a-5p
Denk et al., 2018	RT-PCR	bvFTD *vs.* AD	Downregulated: miR-140-3p,-30a-5p,-30e-5p,-22-3p
Sørensen et al., 2016	RT-PCR	AD *vs.* other types of dementia	The ratio miR-29c-3p/miR-15a-5p distinguished AD from other types of dementia
Galimberti et al., 2014	RT-PCR	AD *vs.* NINDC	Downregulated: miR-125b,-26b
Galimberti et al., 2014	RT-PCR	AD *vs.* NINDC	No difference in miR-23a
Galimberti et al., 2014	RT-PCR	AD *vs.* FTD and INDC	No differences in miR-125b,-26b
Brain tissue			
Jawaid et al., 2019	RT-PCR	ALS *vs.* HC	Downregulated: miR-183/96/182 in frontal cortex
Jawaid et al., 2019	RT-PCR	FTLD *vs.* HC	Downregulated: miR-183/96/182 in frontal cortex
Hébert et al., 2013	RT-PCR	AD *vs.* HC	Downregulated: miR-132-3p,-100 in temporal cortex
Hébert et al., 2013	RT-PCR	FTD *vs.* HC	Upregulated: possibly miR-100 in temporal cortex Downregulated: miR-132-3p in temporal cortex
Hébert et al., 2013	RT-PCR	PSP *vs.* HC	Downregulated: miR-132-3p in temporal cortex
Hébert et al., 2013	RT-PCR	FTD *vs.* AD	Upregulated: possibly miR-100 in temporal cortex
Chen-Plotkin et al., 2012	RT-PCR	GRN(-)FTLD-TDP *vs.* HC	Downregulated: miR-132-5p,-132-3p,-212 in frontal cortex
Chen-Plotkin et al., 2012	RT-PCR	GRN(+)FTLD-TDP *vs.* HC	Downregulated: miR-132-5p,-132-3p,-212 in frontal cortex

AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; bvFTD: behavioral variant FTD; CSF: cerebrospinal fluid; FTD: frontotemporal dementia; FTLD: frontal temporal lobar degeneration; INDC, inflammatory neurologic disease control; MDE: major depressive episode; NINDC: non-inflammatory neurologic disease control; PPA: primary progressive aphasia; PSP: progressive supranuclear palsy; RT-PCR: real time polymerase chain reaction. [1]Symptomatic mutation carriers consisted of *GRN*, *C9orf72*, *MAPT* mutation carriers and consisted of 12 bvFTD, 1 nfvPPA, 1 svPPA, 1 dementia not otherwise specified; [2]Presymptomatic mutation carriers consisted of 23 patients.

Table.3 Alterations of miRNA expression in FTD CSF and brain tissue. Table adapted from Bridget Ma. et al[171].

Author	Number of FTD patients, gender, ages	Subjects for comparison, number, gender, ages	Sample assayed	Altered miRNA expression	Related pathway to FTD
bvFTD vs. HC					
Denk et al., 2018	48 bvFTD[1], 30M/18F, 65±9.2 yr (48 serum)	44 HC, 20M/24F, 64±11.3 yr (38 serum)	Serum	miR-223-3p upregulated	Possible protection of surviving neurons by miR-223-3p. MiR-223-3p targets *G6PT* gene
				miR-15a-5p downregulated	A positive linkage of miR-15a with amantadine, which is associated with the treatment of behavioral disturbances
					MiR-15a-5p targets *BDNF* gene
				miR-22-3p downregulated	MiR-22-3p targets *BDNF*, *PTEN*, and *SIRT1* genes
Denk et al., 2018	48 bvFTD[1], 30M/18F, 65±9.2 yr (48 CSF)	44 HC, 20M/24F, 64±11.3 yr (44 CSF)	CSF	miR-223-3p upregulated	Possible protection of surviving neurons by miR-223-3p. MiR-223-3p targets *G6PT* gene
				miR-124-3p upregulated	The target gene of miR-124 is AMPAR. AMPA receptors are associated with the regulation of social behavior
				miR-15a-5p downregulated	A positive linkage of miR-15a with amantadine, which is associated with the treatment of behavioral disturbances
					MiR-15a-5p targets *BDNF* gene
Denk et al., 2018	48 bvFTD[1], 30M/18F, 65±9.2 yr (48 CSF)	48 AD[1], 22M/26F, 65±9.3 yr (48 CSF)	CSF	miR-22-3p downregulated	MiR-22-3p targets *BDNF*, *PTEN*, and *SIRT1* genes
FTLD vs. HC					
Derkow et al., 2018	8 FTD, 3M/5F, 64±11.5 yr	10 HC, 7M/3F, 58.3±11 yr	CSF	miR-124 upregulated	The target gene of miR-124 is *AMPAR*. AMPA receptors are associated with the regulation of social behavior
FTLD vs. AD					
Derkow et al., 2018	8 FTD, 3M/5F, 64±11.5 yr	12 AD, 2M/10F, 71.5±8.5 yr	CSF	miR-124 upregulated	The target gene of miR-124 is *AMPAR*. AMPA receptors are associated with the regulation of social behavior

AD: Alzheimer's disease; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CSF: cerebrospinal fluid; F: female; FTD: frontotemporal dementia; bvFTD: behavioral variant FTD; FTLD: frontotemporal lobar degeneration; HC: healthy controls; M: male; yr: years. [1]A total of 41 of the 48 bvFTD and 20 of the 48 AD cases were tested negative for the most prominent gene *C9orf72*, and no mutations in the genes *MAPT* and *GRN* were identified in the tested AD (n=11) and bvFTD (n = 11) cases.

Table.4 Possible miRNA biomarker candidates in FTD. Table adapted from Bridget Ma. et al[171].

Treatment and future directions

Although FTD and Amyotrophic lateral sclerosis (ALS) are two different progressive diseases, over the last few decades there has been increasing evidence that most patients diagnosed with ALS often also present with signs of FTD. Through further genetic screening and pathological analysis, scholars have found that the similar set of features exhibited by FTD and ALS is due to the overlap in the underlying mechanisms and genetic characteristics between the two diseases. This is the reason why up to 50% of patients develop the disease in combination[190]. Therefore, the development of new therapeutic strategies through effective biomedical technologies is of great importance for both diseases. However, at present, there is no FDA-approved specific drug for the treatment of FTD currently. The medications that have been marketed for AD have not had a similar significant positive effect on FTD. For instance, patients with FTD can tolerate Memantine (a classical drug for the treatment of moderate to severe AD) and their cognitive and behavioral deficits are not effectively improved[191]. There is also evidence have shown that acetylcholinesterase inhibitors such as Donepezil (treatment of mild to moderate AD) can exacerbate FTD-type behavioral abnormalities[192]. Moreover, low doses of atypical antipsychotics are widely believed that can help to control emotion and reduce aggression. In reality, it should be used with caution in elderly patients, where its extrapyramidal side effects can increase the risk of heart disease[193].

Although there are no certified therapeutic drugs, the development of effective potential therapeutic targets is under active experimentation based on the FTD mechanisms. For example, there is clinical research that focuses on tau pathology, with therapies aimed at avoiding tau accumulation, maintaining tau microtubules, and deleting tau via tau-targeted antibodies[194]. There are also studies underway that are attempting to address haploinsufficiency in GRN genomic by employing various techniques to boost progranulin levels[195]. The most interesting is the fact that molecular-based antisense oligonucleotide (ASO) therapies are closer to success than ever before[196,197]. Nowadays,

miRNA in cerebrospinal fluid or tumor marker testing is widely used in clinical practice[198]. Of note, many biotech companies around the world are based on the development of miRNA therapy, and in 2018 the FDA approved the first clinical trial study of miRNA[199]. Although little is known about biomarkers for the diagnosis of early dementia, it is also proven that targeting microRNAs to intervene in FTD is the most potential therapeutic approach, which is of great clinical significance and provides us the future directions.

Scope of this thesis

Part 1: Study on histone lysine-specific demethylase KDM5B in *vitro* and *vivo*.

In the first chapter of my Ph.D., I have described the effects of knocking down KDM5B expression on both molecular and behavioral levels, thereby providing potential targets for intervention in the most common neurodegenerative diseases, headed by Alzheimer's disease. KDM5B is a histone demethylase that is closely involved in H3K4 methylation. According to unpublished data, KDM5B levels were observed to be much higher in the brains of Alzheimer's decedents than in the non-demented population. In addition to this, animal experiments have shown that KDM5B levels are significantly reduced in the hippocampus of mice with high cognitive abilities. Thus, KDM5B is strongly linked to neurodevelopmental diseases and intellectual disorders. However, the specific functional analysis of its involvement in the nervous system is poorly explored. Therefore, my major goals were to (1) establish an in *vitro* knockdown model of KDM5B and testing of the associated changes in its transcriptome, (2) assess the effects of KDM5B inhibition on neuronal and synaptic function (3) evaluate behavior changes in the animal model after enzyme loss.

Part 2: Investigate the role of miR-129-5p in Frontotemporal dementia.

In the second part, I investigated the underlying mechanism of miR-129-5p in Frontotemporal dementia (FTD), which is one of the most common neurodegenerative diseases that can typically strike the younger age group. Numerous reports have revealed altered microRNA signatures were identified in FTD patients' blood, plasma, and cerebrospinal fluid. Although in molecular, microRNA is well known for its unique capacity to regulate multiple genes in the same pathway and play an epigenetic role, the specific target to regulate FTD is still unclear. Thus, our aims for this part were to (1) Find specific target genes in FTD to differentiate from AD, (2) analyze the transcriptome involved in the target gene, (3) combine in *vitro* and in *vivo* studies to validate the regulatory role and biological functions of the target gene.

Chapter 1-Targeting the histone demethylase KDM5B as a treatment for cognitive and synaptic dysfunctions in mouse models of Alzheimer's disease

Detailed Author contribution of Jiayin Zhou

Experimental work

- Performing primary neuronal culture and siRNA transfection
- Performing Dil dye labelling and Imaging
- Performing RNA isolation and Protein isolation
- Performing q-PCR and Western blotting
- Performing Genotyping
- Performing Stereotactic surgery on mice
- Performing Behavioral experiments on mice

Data presentation

- Manuscript editing
- Preparing figures

Data analysis

- Assisting basic data analysis

Targeting the histone demethylase KDM5B as a treatment for cognitive and synaptic dysfunctions in mouse models of Alzheimer's disease

Jiayin Zhou[1,$], Lalit Kaurani[1,*,$], Susanne Burkhardt[1], Ranjit Pradhan[1], Aditi Methi[1], Marina Saad[1], Dennis M. Krüger[1], Andre Fischer[1,2,3**]

[1]Department for Systems Medicine and Epigenetics, German Center for Neurodegenerative Diseases (DZNE), Von Siebold Str. 3a, 37075, Göttingen, Germany

[2]Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, Von Siebold Str 5, 37075 Göttingen, Germany

[3]Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Göttingen, Germany

*To whom correspondence should be addressed: lalit.kaurani@dzne.de

**To whom correspondence should be addressed: andre.fischer@dzne.de

$ These authors contributed equally to this work

Abstract

Mutations in genes that control epigenetic gene expression, especially the machinery that controls Histone 3 lysine 4 (H3K4me) methylation, are over-represented in intellectual disability disorders. It is mediated by both lysine methyltransferases and demethylases. Dysregulation of these enzymes is closely associated with cognitive dysfunction in humans. Moreover, there is evidence that H3K4me3 levels decrease in neurodegenerative diseases such as Alzheimer's disease and previous studies demonstrated H3K4me demethylases (KDMs) are the important targets for cognitive functions. In this study, we have specifically tested the therapeutic potential of H3K4me demethylases (KDMs) in *vitro* and in *vivo.* Our data suggest that decreasing the levels of KMD5B can improve neuronal synapse plasticity and reduce inflammatory responses. When we downregulated the KDM5B in aged mice, it helps to rescue their learning and memory abilities. Furthermore, inhibition of KDM5B in mouse models for age-associated memory decline or amyloid deposition also ameliorated memory impairment. Our data strongly suggest that H3K4me demethylases, represented by KDM5B, have great potential to become therapeutic targets for the treatment of cognitive disorders.

Keywords: H3K4me demethylases, KDM5B, learning & memory, RNA-seq, cognitive disease.

Introduction

Cognitive impairment is a main pathological feature of neurodegenerative diseases, and the degree of cognitive impairment correlates positively with the passage of time[1–4]. Typically, the early clinical symptoms of patients with a true age-associated cognitive dysfunction are nearly identical to the benign memory decline caused by natural, normal aging, making it difficult to distinguish between dementia and non-dementia in the early stage[5–7]. By the time the disorder can be diagnosed using brain imaging techniques, it has usually progressed to an advanced pathological stage and the best time to intervene has been missed, which is considered the most important reason for the unsatisfactory treatment of neurodegenerative disorders so far[8–10]. Although there are numerous mechanisms and causes that induce neurodegenerative diseases, it is widely acknowledged that epigenetic changes are a major cause of many human diseases, and epigenetic alterations have become a hot topic of research in both the central nervous system and cancer areas in recent years[11–18]. Additionally, epigenetic functions have been established in a variety of disciplines of biology, indicating the relationship between genes and genotype expression[19,20]. As a result, targeting the epigenetic regulation of specific molecular markers can meet the urgent need to intervene at an early stage of the disease to reduce the risk and achieve the efficacy of delaying or even reversing cognitive impairment[21–24].

DNA methylation, histone modification, and non-coding RNA regulation are all epigenetic factors. And among them, histone modification is a classic epigenetic mechanism that has been implicated in learning and memory in numerous studies, and its dysregulation is closely related to cognitive dysfunction[25–29]. Histone methylation is a dynamic process that depends on the regulation of lysine methyltransferases (KMTs) and lysine demethylases (KDMs)[30,31]. Loss of any one enzyme activity will alter methylation levels across the genome, and such mutations are strongly associated with cognitive phenotypes[32]. Basically, they are all linked to Alzheimer's disease accompanied by reduced neuronal H3K4me3[33,34].

Our group already provides strong evidence suggesting that deletion of KMTs in mice can lead to severe memory impairment[35,36]. We are interested in whether the activity of KDMs, a regulatory enzyme with an opposite activity to KMTs, is also closely involved in and regulates cognitive performance and whether it can play a role in alleviating cognitive dysfunction. For this, we conducted a preliminary analysis and some unpublished data from the lab indicate the level of KDM5B is up-regulated in postmortem brain tissues of AD patients. In addition, another animal project revealed mice that performed better in the behavioral tests had lower levels of KDM5B in the hippocampus. According to these, we assumed that KDM5B has negatively related to memory. And the low expression of KDM5B might be helpful in the memory process. Therefore, in this study, we conducted a series of in *vitro* and in *vivo* experiments to specifically revealed the regulation mechanism of learning and memory by KDM5B, suggesting that have the significant potential to become a therapeutic target for the treatment of cognitive impairment such as AD.

Results

Reduced level of Kdm5b modulates the expression of plasticity-related genes and fine-tunes the cell cycle regulators during neuronal maturation.

Since the development of dementia is closely linked to the molecular level, molecular detail alterations can be used to identify early dementia[37,38]. Thus, the first aim of our study is to identify the effects of Kdm5b on neural function at the molecular level. For this reason, we probed the epigenetic impact on gene regulation in primary neurons **(Fig.1A)**. After transfection, we performed qPCR probe test to examine the Kdm5b expression level and observed that neurons transfected with the Kdm5b antisense oligo decreased 50% of Kdm5b expression compared with the control group **(Fig.1B)**. Furthermore, we also found the inhibitor group showed a substantial decrease of Kdm5b in protein level **(Fig.1C)**. Subsequently, RNA sequencing data revealed that 488 genes were upregulated and 259 genes were downregulated in the Kdm5b antisense oligo siRNA treatment group **(Fig.1D)**. The mapped volcano plots are more visually

showing these differentially expressed genes when compared with the control data. For this, we selected genes with logFC±0.5, adjusted P-value<0.05, and basement>=50 as the candidates, and the colorful genes in figures indicate the significant deregulation of transcripts after analysis **(Fig.1E)**. We thus restricted the further analysis to differential genes that met the criteria, for the correlated biological pathway analysis using ClueGO (a Cytoscape plugin). The functional analysis confirmed that downregulated genes were enriched for inflammation, whereas upregulated genes showed enrichment for cell cycle, synaptic plasticity, and brain development related pathways **(Fig.1F)**.

In conclusion, on this basis, we found that knockdown Kdm5b contributes to synaptic function, which is known to be critical for the formation of high cognitive function and memory, as well as closely related to the severity of cognitive impairment.

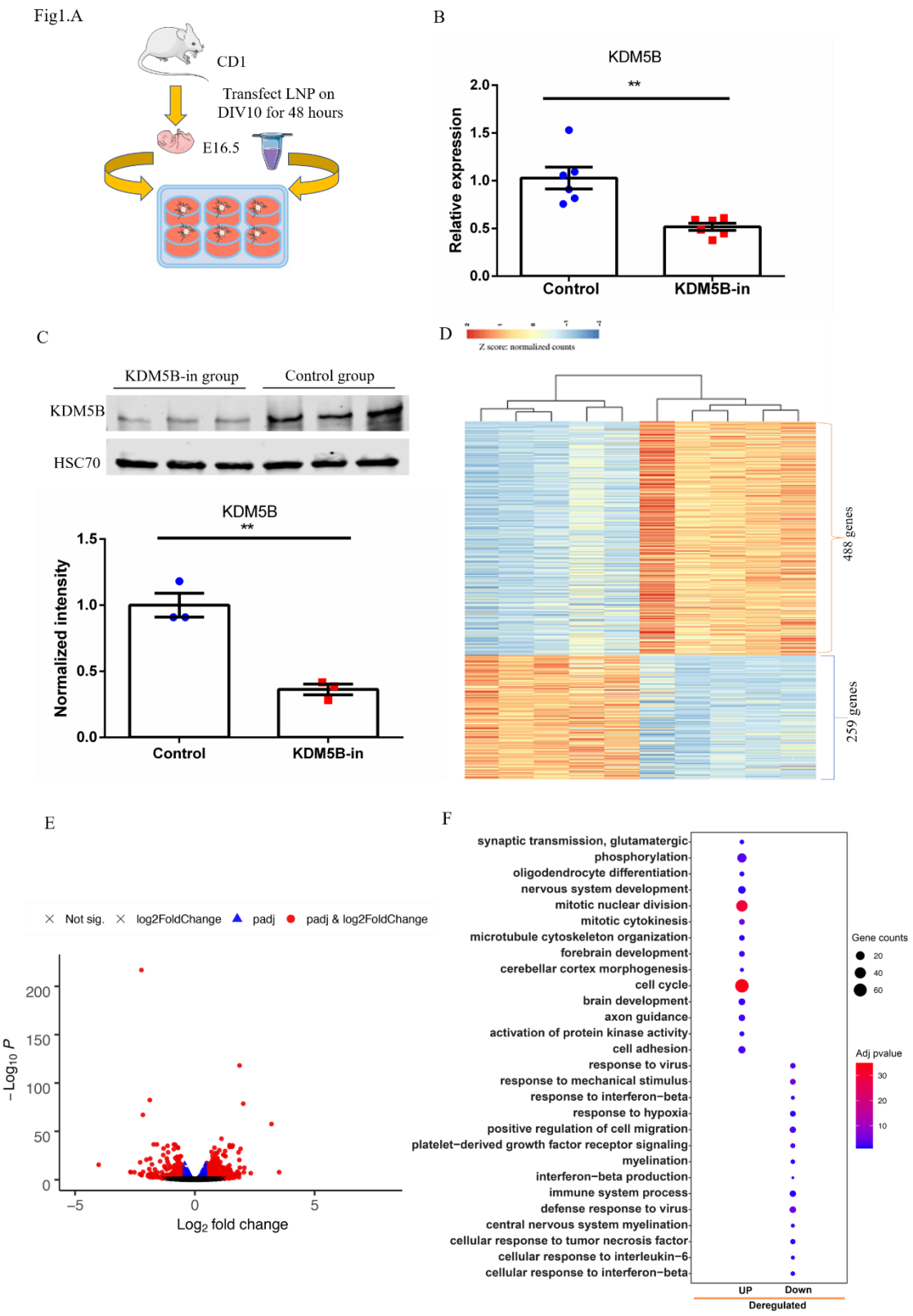


Fig. 1 Analysis of the effects of Kdm5b downregulation on primary neurons and related pathways in vitro. (A) Schematic diagram of the in vitro neuronal culture and lipid nanoparticle (LNP) transfection protocol. **(B)** mRNA levels of Kdm5b after the inhibitor treatment in primary neurons were expressed lower than the negative control (n=6, unpaired t-test: **p<0.01). **(C)** Western Blot analysis of Kdm5b protein levels in

primary neurons. Kdm5b protein was significantly decreased in inhibitor group (n=3, unpaired t-test: **p<0.01). **(D)** Heatmap describes the number of deregulated genes in Kdm5b inhibitor group compared with controls (n=5). **(E)** Volcano plot description differentially expressed genes (FDR<0.05) compared with control. The colorful dots indicate the significant deregulation of transcripts (p-value<0.05). And the red genes represent more upregulated or downregulated than the blue ones. **(F)** Dot plot depicting the functional pathways influenced by deregulated genes in the Kdm5b knockdown group. Error bar means Mean±SEM.

Decreased level of Kdm5b contributes to increased dendritic spine density and enhances synaptic plasticity.

Our sequencing data indicated that inhibition of Kdm5b upregulated the synaptic transmission pathway. In order to verify this result, we performed dendritic spine analysis and first treated primary neurons with a nano lipid plasmid-packed Kdm5b antagonist as previously described, then stained them with a special crystal "DiI" to achieve visualization and quantification of dendritic spines[39] **(Fig.2A)**. As a result, we observed that the number of dendritic spines in neuronal cells treated with Kdm5b inhibitor was significantly increased than the non-treatment group **(Fig.2B)**, which might be associated with enhanced synaptic plasticity. We thus performed the qPCR to test the expression level of some specific synaptic related markers. As shown in **Fig.2C-D**, BDNF, as strong evidence of neuronal plasticity tag, was obviously increased, while NLGN1, which plays a role in neuronal remodeling and synaptic plasticity, was significantly decreased in the Kdm5b knockdown group than in the control. Since bursting was shown to play an essential role in synaptic plasticity, on the other hand, we further examined the role of Kdm5b inhibitors in synapses plasticity and organization in primary neurons by means of a Multielectrode array (MEA) to verity the qPCR results[40]. And we observed that low levels of Kdm5b contributed to stimulating bursts to occur, increasing the number of spikes during bursts to achieve enhancing synaptic plasticity **(Fig.2E-H)**.

Dendritic spines are major structures in the brain connections that are involved in learning and memory processes and are closely related to synaptic plasticity.

Neurodegenerative disorders can be caused by both dendritic spine and synaptic plasticity damage. According to the findings of this subsection, low levels of Kdm5b contribute to an increase in dendritic spine density while enhancing synaptic plasticity. This has notable implications for improving the development of cognitive dysfunction and the enhancement of neural networks.

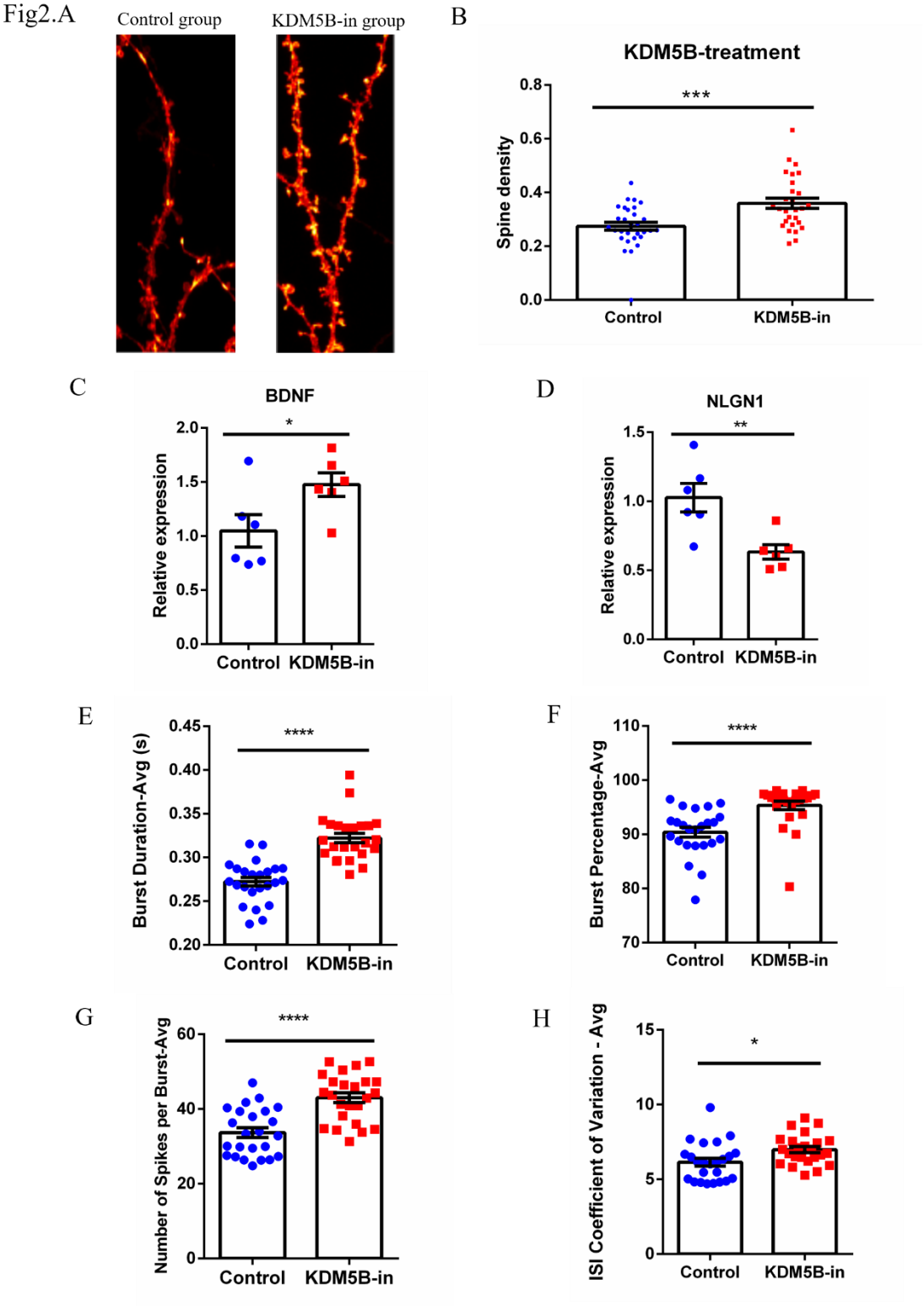


Fig. 2 Reduced level of Kdm5b promotes dendritic spine density and synapse number in primary neurons. (A) Confocal images of WT primary neurons and neurons with Kdm5b KD treated with lipophilic dye 'DIL' at DIV 12 (scale bar: 5 μm). **(B)** The number of spines per 1 μm dendrite length is shown in panel B. Spine density is substantially increased in the Kdm5b-inhibitor treated neurons compared with the control group (n=28/30 images, ***p<0.0001). **(C)** The relative expression of synaptic

related marker BDNF in the Kdm5b knockdown group was increased by qPCR confirmation (n=6, unpaired t-test: *p<0.05). **(D)** qPCR shows the significant differences in synaptic related marker expression in the Kdm5b inhibitor group compared to the control group (n=6, unpaired t-test: **p<0.01). **(E-H)** The electrophysiological activity in primary neurons was recorded by the MEA system after transfection, the Kdm5b knockdown group showed an increased number of bursts compare to the control (n=24, unpaired t-test: ****p<0.0001, *p<0.05). Error bar means Mean±SEM.

Inhibiting Kdm5b improves anxiety-like behavior in aged mice.

In order to perform a series of behavioral tests to assess the potential effects of Kdm5b inhibition on cognitive deficits in aging wildtype mice at 17 months of age, we first injected Kdm5b inhibitor or the scramble LNP (as negative control) into the CA1 region of the dorsal hippocampus in aged mice **(Fig.3A)**. Then we tested whether there was a difference in anxiety-like behavior and general locomotor between the inhibitor group and the control group by conducting an open field experiment[41] (OF) **(Fig.3B)**. Our result found that the Kdm5b inhibitor group showed significantly different levels of comparison with the control group in exploratory behavior. Aged mice spent more percentage of time exploring the middle area of the OF after knocking down Kdm5b levels **(Fig.3C)**. However, the total distance traveled in OF and the average speed of movement in this test was similar for both groups of subjects, indicating that neither group of animals had severe motor impairment **(Fig.3D-E)**. Combined with the statistical chart of residence time in the central area, it is obvious that the anxiety-like performance of the control mice group is more pronounced than the inhibitor group. To further confirm whether low levels of Kdm5b can improve anxiety-like behavior in aged mice, we subsequently performed an elevated plus maze test[42] (EPM) **(Fig.3F)**. Similarly, the traveled path and average speed of the mice in all groups did not differ **(Fig.3H-I)**. But we observed that mice injected with Kdm5b inhibitor spent significantly more time in the open arms which might be an indication of reduced anxiety compared with the control mice **(Fig.3G)**.

Overall, the results from EPM were highly consistent with the OF results. These

findings are sufficient to suggest that inhibition of Kdm5b can effectively improve anxiety-like behaviors in aged mice (normal aging).

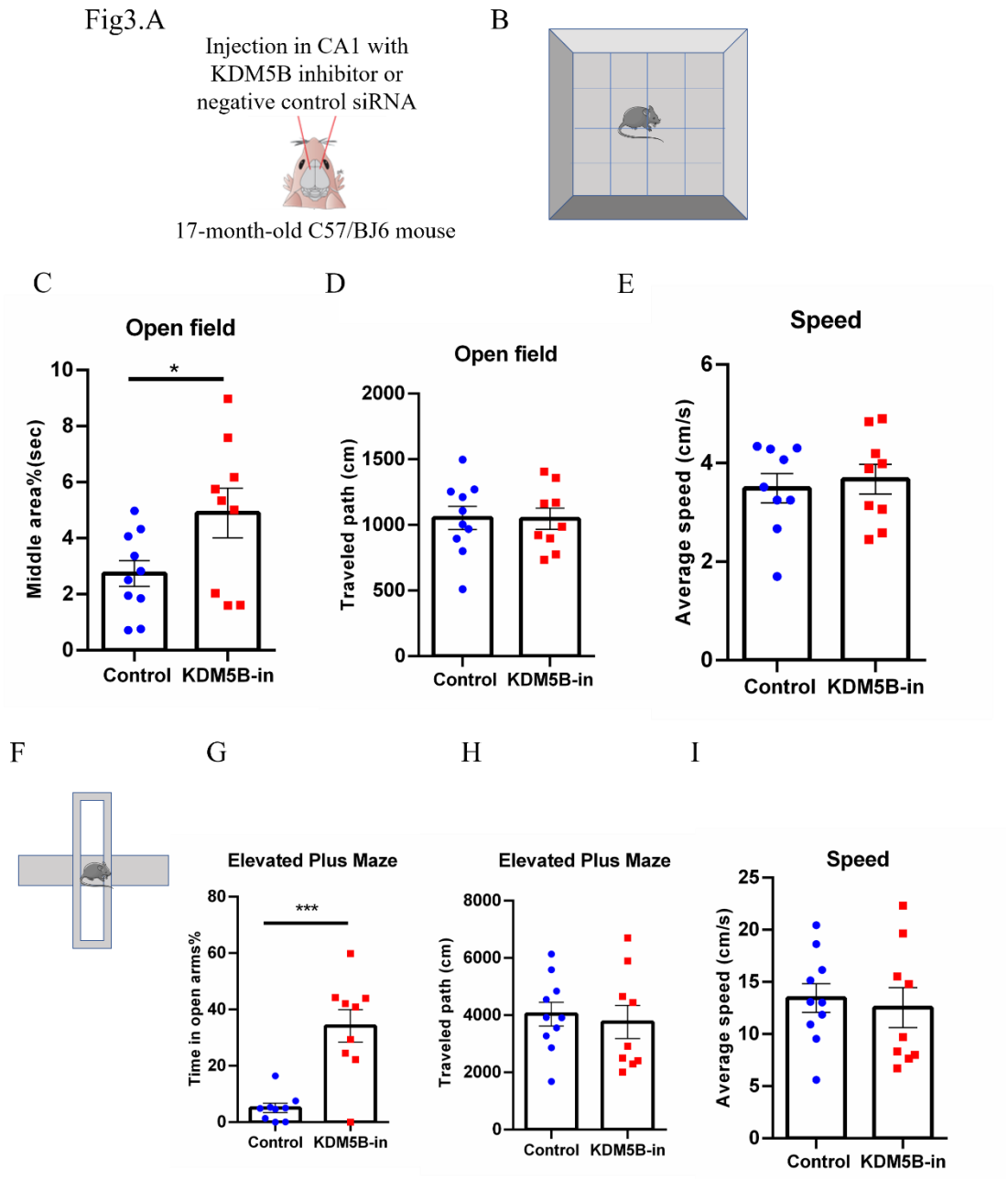


Fig. 3 Behavior data in Kdm5b inhibitor/Vehicle treated wildtype mice (male) at 17 months of age. (A) Scheme of the experimental design. Kdm5b vehicle or inhibitor oligonucleotides were injected into the CA1 region of the dorsal hippocampus via the pre-pulled glass micropipettes prior to the behavior experiments. **(B)** Scheme of the open field setup. **(C)** Percentage of time spent in the middle area of the open field (n=9/10, unpaired t-test: *p<0.05). **(D)** The total traveled path in open field was similar among two groups (n=9/10, unpaired t-test: p-value=0.9616). **(E)** Speed in the open field didn't differ in both groups (n=9, unpaired t-test: p-value=0.6679). **(F)** Scheme of

the Elevated plus maze setup. **(G)** Mice injected with Kdm5b inhibitor spent more time than control group in open arms in elevated plus maze (n=9; unpaired t-test: ***p<0.0001). **(H)** The traveled path was similar between two groups in elevated plus maze (n=9/10, unpaired t-test: p-value=0.6986). **(I)** The speed of two groups in the elevated plus maze was similar (n=9/10, unpaired t-test: p-value=0.6982). Error bar means Mean±SEM.

Inhibiting Kdm5b reduces cognitive impairment in aged mice.

To further establish the effect of regulating Kdm5b on learning and memory in normal aged mice, we conducted an additional study to see if it also could help repair cognitive deficits. Thus, we applied the most widely used Morris water maze[43] (MWM) test to examine the aged mice's hippocampus-dependent spatial learning memory ability. During the training phase, animals were taught to locate on a hidden platform in the water maze using special visual cues of various colors and shapes **(Fig.4A)**. Although our data showed there was no strict statistical difference between the two groups' comparison, we found that the spatial learning memory ability of all mice improved significantly over time as indicated by the escape latency curves **(Fig.4B)**. And it was extremely clear that the inhibitor group's performance was better than the control group from the first day to the end of the training day due to they spent less time searching the target region. So far, to investigate this finding in further detail, we used the more sensitive MUST-C algorithm[44] to analyze the water maze tracking data. The strategy plot showed the overall trend of swimming strategies used by the two groups of mice over the training phase (day 1-7). We observed that the Kdm5b inhibitor group increasingly preferred cognitively challenging as described "direct", "corrected", "short-chaining" and "focused" strategies, while the control mice exhibited more of "long-chaining", "focused false", "circling", "random", "thigmotaxis", and even "passivity" strategies which was never selected by inhibitor group. Comparatively, mice in the control group performed poor cognitive challenging strategies **(Fig.4C)**. In addition, the cognitive scores clearly showed the corresponding cognitive scores for each group of animals **(Fig.4D, Supplement 5-9)**. The average cognitive score for the Kdm5b inhibitor group was higher than the control group, indicating they were smarter

and in general closer to the target region during the swimming test. Even though after the hidden platform was removed, the mice in the control group spent slightly more time in the target quadrant in probe test, there was no statistical difference in target quadrant exploration between the two groups **(Fig.4E)**.

In summary, these findings are sufficient to suggest that inhibition of Kdm5b can effectively improve spatial memory and learning abilities in aged mice, and have a positive effect on cognitive function.

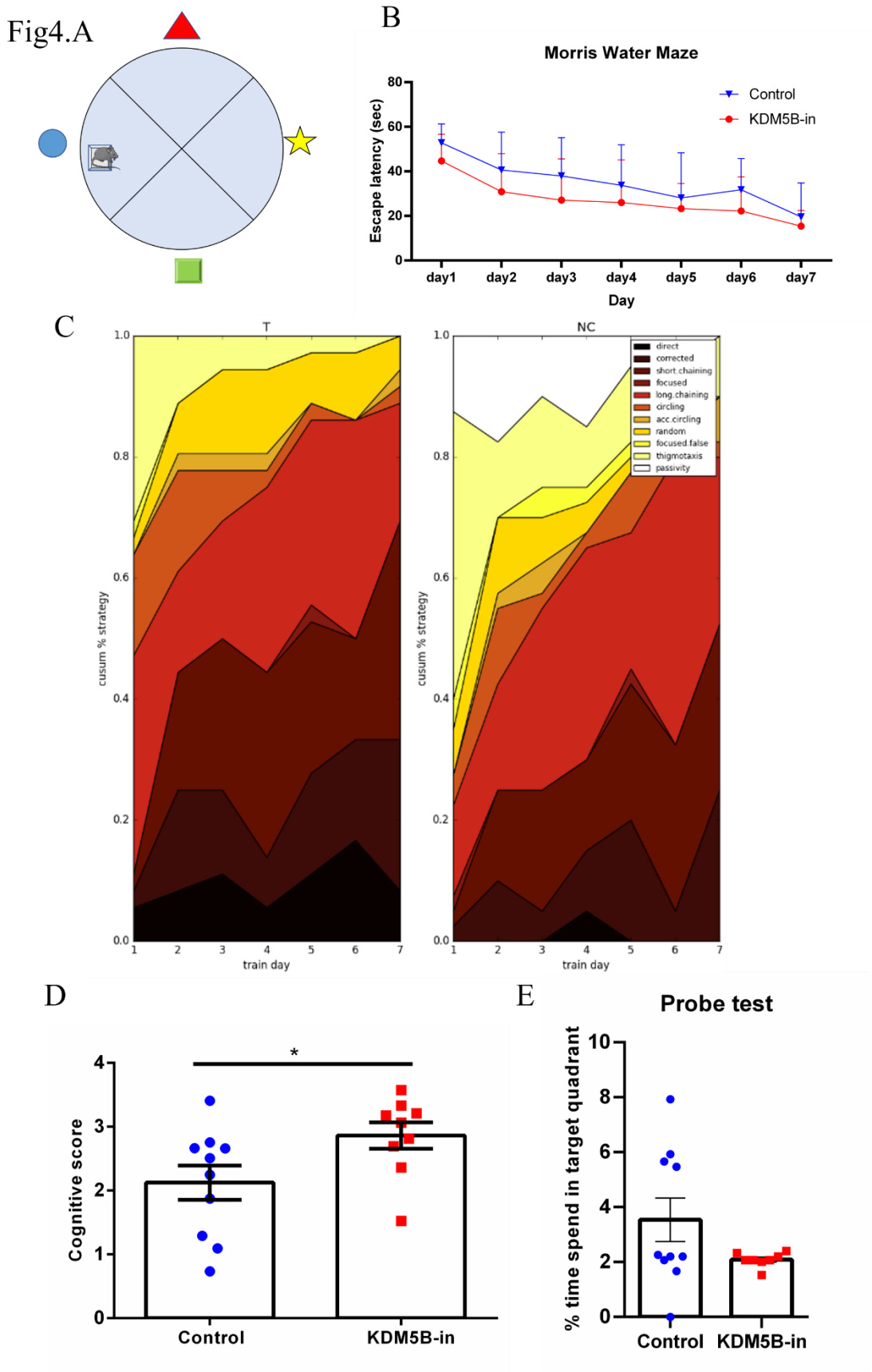


Fig. 4 Water maze data from wildtype aged mice treated with Kdm5b inhibitor/Vehicle. (A) Scheme of the Morris water maze setup. **(B)** Escape latency in

control and inhibitor treatment mice (Control group: n=10, Kdm5b inhibitor group: n=9). **(C)** Strategy analysis during the Morris water maze training sessions. (Control group: n=10, Kdm5b inhibitor group: n=9). **(D)** Higher cognitive scores in the Kdm5b inhibitor group (n=9/10, unpaired t-test: *p<0.05). **(E)** Percentage of time spent in the target quadrant during the probe test (n=8/10, unpaired t-test: p-value=0.1242). The error bar represents the Mean±SEM.

Inhibition of the H3K4 demethylase Kdm5b enzyme in 9-month-old 5xFAD model mice does not alter anxiety-like behavior.

According to our previous behavioral analysis of wildtype aged mice, we further validated the possibility of Kdm5b-mediated cognitive rescue with 5xFAD transgenic mice. For this purpose, three experimental groups were set up: wildtype (no treatment during the surgery), transgenic vehicle (inject scramble LNP), and transgenic Kdm5b inhibitor groups (inject Kdm5b inhibitor LNP) **(Fig.5A)**. The same behavioral tests were subsequently performed on each subject in turn. In the OF test **(Fig.5B)**, all groups spent essentially the same amount of time in the center **(Fig.5C)**. However, as the results shown in the EPM test **(Fig.5D)**, compared to the negative control group, the inhibitor group mice performed similarly in both distance traveled and speed of movement. But when compared with the wildtype group, all transgenic mice showed different degrees of mobility impairment, and were relatively slower after being treated with the inhibitor **(Fig.5F-G)**. This also revealed the reason why the inhibitor group spent the longest time exploring the side of the open arm, which could not be ruled out due to the slow movement of the 5xFAD phenotype **(Fig.5E)**.

Thus, overall, there was no significant positive effect of Kdm5b inhibition treatment on anxiety-like behavior in 5xFAD transgenic mice in both OF and EPM tests.

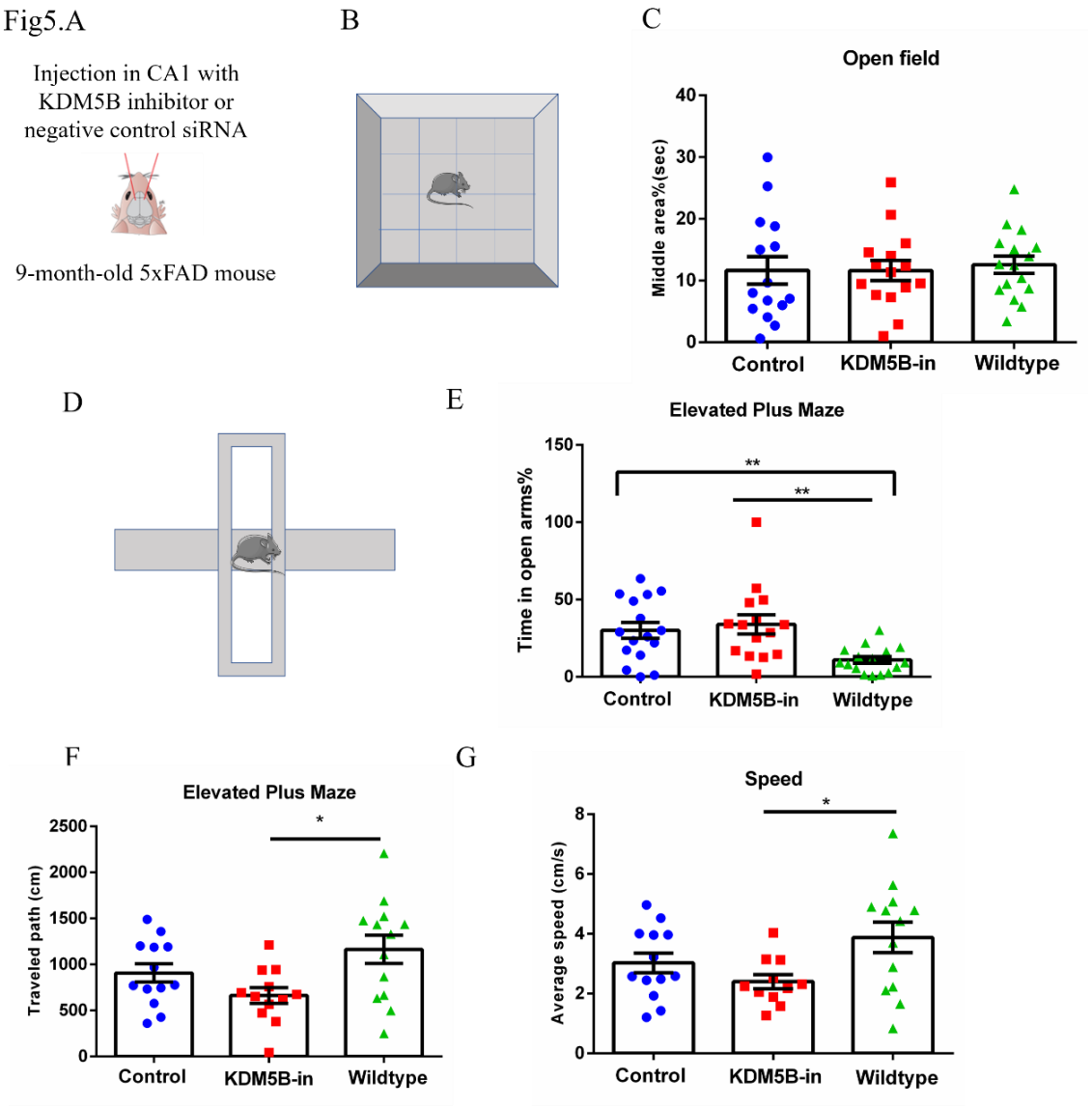


Fig. 5 Inhibit the Kdm5b enzyme does not rescue anxiety-like behavior in 9-month-old 5xFAD mice. (A) Scheme of the behavioral test design. Kdm5b vehicle or inhibitor oligonucleotides were injected into 5xFAD mice CA1 region of the dorsal hippocampus via the glass micropipettes prior to the behavior tests. **(B)** Scheme of the open field setup. **(C)** Percentage of time spent in the center in open field (n=15/16). **(D)** Scheme of the Elevated plus maze setup. **(E)** Mice injected with Kdm5b vehicle or inhibitor spent more time in open arms than wildtype mice in the elevated plus maze (n=15/16, unpaired t-test: **p<0.01). **(F)** The traveled path was similar between control and wildtype groups, and the Kdm5b inhibitor group showed less traveled distance than the wildtype mice in elevated plus maze (n=12/13; unpaired t-test: Control *vs* Wildtype, p-value=0.1728; *p<0.05). **(G)** The speed of three groups in the elevated plus maze. (n=11/13, unpaired t-test: Control *vs* Wildtype, p-value=0.1740; *p<0.05). This part was performed with gender mix mice and found no gender effect in any of the mouse behavior battery of tests. The error bar represents the Mean±SEM.

Inhibit H3K4 demethylases Kdm5b enzyme ameliorates cognitive deficits in 5xFAD model mice.

The further analysis interestingly revealed the significant group differences started to reflect in the following test of hippocampal-dependent spatial learning and memory. Combined with the above data analysis, although the transgenic mice were slower than the wildtype group mice in terms of movement, the learning ability and spatial memory of the inhibitor group in the water maze training phase tended to be consistent with the wildtype group **(Fig.6B)**. Since the LNP effect stays up to 12-14 days after injection, surprisingly, in the first two days of training, mice treated with inhibitors showed the shortest escape latency. Despite the fact wildtype mice were able to locate the hidden platform faster starting on the third day, the difference between the inhibitor group and the wildtype group was shortened day by day, demonstrating that the spatial memory ability of inhibitor group mice was gradually improved. If the inhibitor group and the transgenic control group are analyzed in comparison, it is obvious that the cognitive deficits in the Kdm5b inhibitor group were rescued back to normal levels on day 6. This finding was also intuitively verified in the probe test. After the hidden platform was removed, the inhibitor-treated group spent much more time investigating the target quadrant and had a significantly larger number of target region explorations than the transgenic control group, and performed in line with the wildtype group **(Fig.6C-D).**

In conclusion, while inhibiting the H3K4 demethylases Kdm5b enzyme had no significant positive effect on anxiety-like behavior due to the 5xFAD model mice phenotype, it was effective in improving spatial learning ability and alleviating cognitive deficits in 5xFAD mice. It has even been shown to salvage cognitive deficits to a near-normal level.

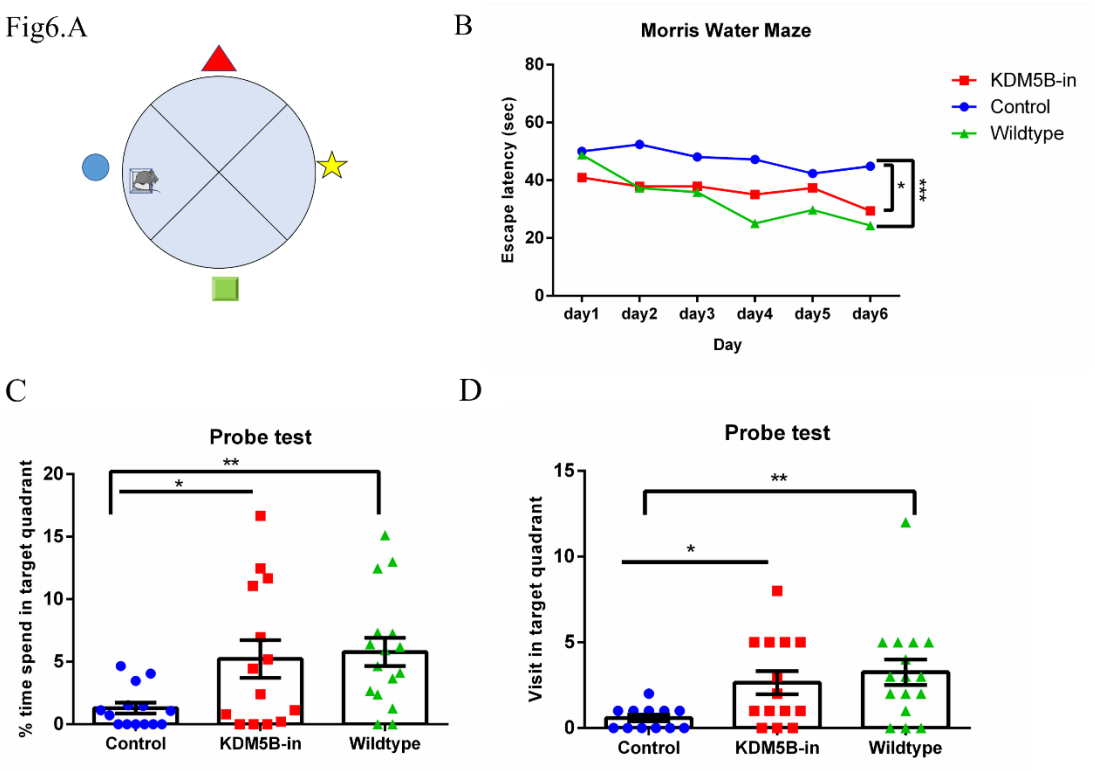


Fig. 6 Inhibit Kdm5b enzyme ameliorate cognitive deficits in 9-month-old 5xFAD mice. (A) Scheme of the Morris water maze setup. **(B)** Escape latency in the treated mice and the wildtype mice (n=15/16, 2way ANOVA, *p<0.05, ***p<0.0001). **(C)** Percentage of time spent in the target quadrant in the probe test (n=14/16, unpaired t-test: *p<0.05, **p<0.01). **(D)** The number of visit times in the target quadrant (Control group: n=12, Kdm5b inhibitor group: n=14, Wildtype group: n=16; unpaired t-test: *p<0.05, **p<0.01). This part was performed with gender mix mice and found no gender effect in any of the mouse behavior battery of tests. The error bar represents the Mean±SEM.

Discussion

Epigenetic modifications are widely known to permit variations in chromatin structure without affecting the fundamental genome sequence, thereby inducing numerous disease manifestations such as neurodegenerative diseases and cancer, etc [13,18,19,45]. Increasing evidence from animal research strongly shows that dysregulation of histone modification pathways directly affects behavior cognition and has been closely involved in neurological disorders including AD, indicating that appropriate epigenetic control is required for the processes underlying learning and memory[46,47]. H3K4 methylation, an important component of epigenetics control, accumulates in the hippocampus as memories are formed[25]. It has been confirmed by human clinical data to be involved in neurodevelopmental disease or neurodegenerative disease and to play an important role in the cognition process[48–50]. Indeed, H3K4 methylation is balanced by the dynamic regulation of methyltransferases and demethylases[51,52]. Although the particular mechanisms involved in methylation by individual enzymes and the continuity of the dynamic processes are still unknown. It is certain that the loss of anyone potential enzyme activity will eventually impact H3K4 methylation levels throughout the genome. Therefore, we are interested in deciphering the specific mechanisms behind the regulation of histone methylation by a range of enzymes and the potential role on cognitive function, particularly in learning and memory.

Interestingly, previously published research from our laboratory observed that the deletion of two members of the methyltransferase Set1 family, Kmt2a and Kmt2b, affects H3K4 methylation levels in different genomes through multiple molecular pathways, further affecting neuronal plasticity and leading to hippocampus-dependent memory impairment[36]. Additionally, we have recently shown postnatal Setd1b deletion has been linked to severe learning impairment, indicating that SETD1B-mediated control of H3K4me levels is crucial for cognitive function[53]. After identifying the potential mechanisms involved in the epigenetic regulation of histone methylation by methyltransferases, we developed the alternative hypothesis that enzymes with opposite

functions to methyltransferase would also play a regulatory role in cognitive processes. Among the demethylases, H3K4 methylation is carried out by two distinct enzyme families. The KDM1 family can only remove methyl from H3K4me1 and H3K4me2, due to substrate differences and different catalytic processes, whereas members of the KDM5 family can also remove the methyl group from H3K4me3. Evidence shows the KDM1 family is essential for cell proliferation and development in the embryo[54]. Loss of KDM5C function induces X-chromosome-linked mental retardation[55]. In the meantime, KDM5A has been found as an autism spectrum disorder[56]. So far, there is a relative lack of knowledge about KDM5B. For this reason, we pre-analyzed AD patient data and revealed KDM5B level in the AD brain is overexpressed and significantly correlates with cognition. In addition to this, animal data suggest that low Kdm5b levels are accompanied by higher cognitive scores. To this end, this study aims to further elucidate the potential mechanisms of histone demethylase Kdm5b involvement in cognitive function and its impact on behavioral performance in mice.

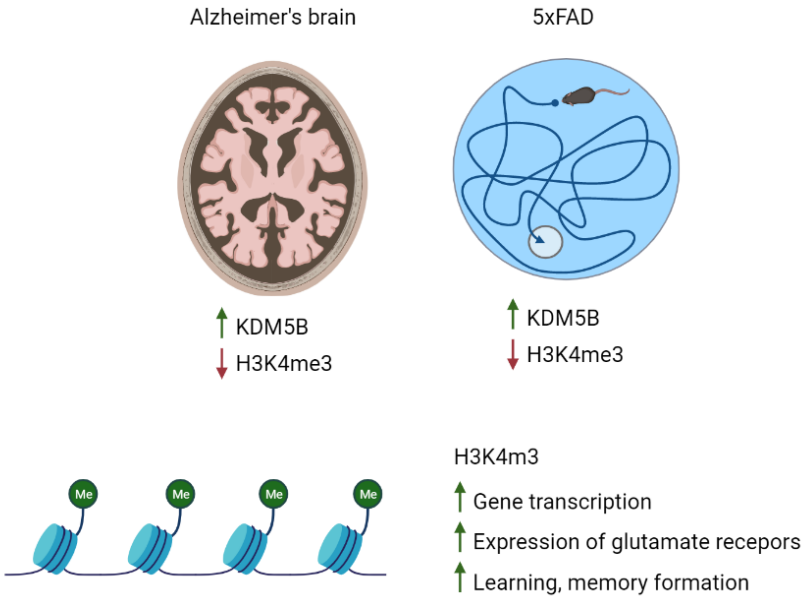


Fig.7 In AD patients' post-mortem brains, H3K4me3 decreased, while the KDM5B enzyme increased. The 5xFAD mouse model corresponds to the results found in humans. 5xFAD animals also exhibit impaired spatial memory and learning, shown by an increased time to locate the target quadrant in the Morris Water Maze test. Created with BioRender.com.

AD is the most common form of dementia and is usually characterized macroscopically by shrinkage of brain volume. Microscopically, magnetic resonance imaging reveals pathology characterized by Aβ deposition and neurogenic fiber tangles, accompanied by neuronal loss and synaptic deficits[57]. To identify this, we established in *vitro* model construction combined with high-throughput sequencing data and observed that reduced Kdm5b gene level correlated with plasticity-related gene expression, including enhanced synaptic plasticity, promoting nervous system development, and upregulating cell cycle pathways. On the other hand, accompanied by downregulation of inflammation-enriched pathways (Supplement 2-4). In line with previous studies, these processes changes are similar to or overlap with the mechanisms involved in regulating and mitigating the neurodegenerative diseases represented by Alzheimer's[58,59].

Of note here, in the nervous system, neurons form neural circuits through synaptic interconnections, and dendritic spines, the main structure of brain connections, are directly related to synaptic plasticity[60–62]. Neuroscience has demonstrated that neurons in the brain with neurodegenerative diseases have significantly reduced numbers of dendritic spines and are morphologically distorted or atrophied. Concurrently, the majority of the mutated genes encode proteins in signaling pathways that regulate synaptic plasticity, implying that dendritic spines and synaptic failure are the direct cause of the cognitive decline and memory impairment in AD[63]. The results of our dendritic spine visualization analysis are highly consistent with the above statement that knockdown of Kdm5b contributes to an increase in dendritic spine density, which has significant implications for the development of reinforcing neural networks. Correspondingly, we also observed a significant increase in the classical marker BDNF associated with synapses. Since synaptic plasticity and dendritic spine density depend on relative differences between the NLGN1 genes rather than an absolute increase or decrease in its expression, this also accounts for the lower levels of NLGN1 seen in this study allowing for scenarios of increased neurons plasticity and dendritic spines[64]. Since it was discovered that bursting is required for synaptic plasticity[65]. We provided

evidence from the multi-electrode array and suggests that low levels of Kdm5b contribute to stimulating the bursting of neuronal action potentials and this increase in burst frequency and the number of spikes during the burst is beneficial in increasing neuronal excitability, thus laying the foundation for synaptic plasticity (Fig.2, Supplement 1).

The early clinical signs of AD are indistinguishable from age-related normal aging. Both present with varying degrees of cognitive impairment and diminished learning and memory capacity[66–68]. Although our in *vitro* experiments have provided insights into the mechanism of Kdm5b. However, validation from in *vivo* part is lacking. To this question, we bred 5xFAD transgenic mice as an aggressive amyloid model, overexpressing human APP/PS1, with five familial AD (FAD) mutations to imitate AD-like cognitive deficits. Also set up aged wildtype mice to imitate a normal aging model, and perform stereotaxic brain surgery in both. Our data revealed that inhibiting Kdm5b not only significantly improved anxiety-like behavior and rescued cognitive dysfunction in wildtype aged mice, but also partially improved their learning and memory. On account of 5xFAD mice begin to show visible Aβ deposition at 2 months of age and develop hippocampal-dependent memory loss at around 5 months of age. The appearance of these behavioral phenotypes is consistent with the synaptic dysfunction in hippocampal. At 9 months of age, 5xFAD mice exhibit severe memory deficits due to Aβ pathology, accompanied by marked synaptic degeneration. In line with our study, our transgenic mice similarly developed significant motor impairment at 9 months of age. Although this kind of behavioral phenotype restricts their mobility, the learning and memory abilities of the inhibitor group of mice were still shockingly rescued and even restored to normal levels under this circumstance. Our in *vivo* data strongly suggest that Kdm5b is negatively associated with memory in the hippocampus, and the low expression of Kdm5b may contribute to memory processes and rescue cognition in both normal aging and AD-like phenotype (Fig.3-6).

In conclusion, we provide evidence that H3K4me demethylases, represented by KDM5B, have substantial regulatory roles in cognition specifically in learning and memory in mice. Meanwhile, downregulation of Kdm5b suppresses inflammatory responses and activates plasticity-related pathways to enhance nervous system development. Inflammation and synapses, as one of the important mechanisms of pathogenesis of AD and dynamic unit structures, play a non-negligible and irreplaceable role in disease progression at the molecular level. Although more clinical studies are needed in the future to confirm the efficacy of KDM5B epigenetic regulatory mechanisms in humans. We propose some initial ideas on how it could be used as a target. This is essential in identifying how it regulates pathogenic mechanisms in specific. Secondly, Kdm5b is not the only H3K4 demethylase, and more research is needed to see if other individuals share similar mechanisms or what specific roles each enzyme plays in epigenetic regulation. This section of the analysis will be performed at a later stage. This part of the study aims to explore the great potential of Kdm5b as an RNA therapeutic target for the treatment of cognitive disorders such as AD.

Materials and Methods

Animals

Male C57BL/6J mice were aged 16-17 months and mixed-gender 5xFAD mice aged 9 months were used in this study for behavioral testing. All animals were housed in an indoor laboratory on a 12-hour light/dark cycle. Food and water were given ad libitum. To adapt to the environment and avoid aggressive behavior in the same cage during the behavioral test, all mice were individually housed in standard cages two weeks before the behavioral test. Each behavioral test was performed in the same order as the open field test, elevated plus maze, and Morris water maze to ensure that the experimental circumstances were consistent. After the test, mice were sacrificed by cervical dislocation according to an approved protocol, and the CA1 region was stripped in Dulbecco's Phosphate Buffered Saline (DPBS, PAN-biotech GmbH) at 4°C without EDTA protease inhibitor cocktail (Roche) and used liquid nitrogen to transfer to -80°C for long-term storage for a series of follow-up experiments. In addition, pregnant mice used for cell culture were purchased from Janvier (CD1-SWISS, E15). All animal experiments involved in this research were carried out in strict accordance with the German Federal Act on the Protection of Animals and were approved by the competent authorities of Lower Saxony.

Bilateral ventricle injection

Mice were anesthetized with 10 μL of xylazine/ketamine mixture per 1 g body weight, 50 μL of the analgesic carprofen was pre-injected before surgery, and the eyes were protected with bepanthen cream. The scalp was incised and the CA1 region was located using a digital stereotaxic instrument (Leica Microsystems) based on the coordinates 1.75 mm posterior bregma, ±1 mm lateral, and 1.5 mm dorsoventral to drill the hole. Anti-Kdm5b siRNA or scramble was injected with the speed of 0.35 μL/min via the glass micropipette (1.5 μL/side). After surgery, the wounds are closed with medical sutures and the experimental animals are placed on a thermostatic heat pad until they wake up and fed a special soft food and soft bedding.

Behavioral tests

Open Field Test: It is widely used to assess motor function and anxiety behavior in subjects. Mice were placed in an arena (length 1 m; width 1 m; height 20 cm) to explore freely for 5 minutes and all the process was recorded and analyzed by VideoMot2 (TSE Systems). The arena was divided equally into 16 quadrants and the time spent exploring the central 4 quadrants reflects the anxiety level of the mice. Furthermore, the overall distance that mice traveled in this open field reflects their motor function. Before placing each mouse, the test area should be cleaned with 70% ethanol to remove the traces of odor.

Elevated plus maze: A special plastic device for testing anxiety-like performance consisting of two open and two closed arms. The animals were placed in the central area of the device facing the direction of the closed arms and allowed to explore freely for 5 minutes. The test was recorded by the VideoMot2 software and the elevated plus maze was cleaned with 70% ethanol before each experimental animal entered. The relative time that the animal stayed on the side of the open arms was counted as the standard for judging the degree of anxiety.

Morris water maze: Lab animals are forced to swim and explore the hidden platform in the water in this classic experiment. It is commonly used to examine the abilities of animals to learn and retain spatial position and orientation in investigations of learning and memory, Alzheimer's disease, the hippocampus, cognition, and aging. The system consists of a pool with opaque water (20°C-22°C, 1.2 m diameter), a camera, and a behavior trajectory analysis system. The pool is separated into four quadrants, each with a different color and shape of visual signal (triangle, circle, square and star) and place a fixed hidden plastic platform (11 x 11 cm) in the target quadrant. During the training day, mice were randomly placed in the pool from different quadrants and went swimming for 1 minute to find the platform, once they found it and stayed for 2 seconds, they were regarded to have accomplished the assignment. If mice are unable to identify

the platform within 1 minute they need to be guided and stayed for 15 seconds on the platform. During the training period, the position of the platform visual cues should not be changed. On the last day of the probe test, the hidden platform of the target quadrant was removed, mice were placed in the pool from the opposite side away from the target quadrant and allowed to swim for 1 minute. The spatial memory and learning abilities of mice were analyzed according to the percentage of time they explored the target quadrant and the number of times they crossed target platforms. To accurately analyze the behavioral data of the mice, a modified version of the MUST-C algorithm was employed to score the water maze data for unbiased cognitive traits. Higher levels of cognitive strategies will result in higher scores. The specific scoring rules are as follows: passivity=1, randomly=2, chaining=3, focused=4, corrective=5, and direct=6. Performance as direct, corrective, focused, and short-chain strategies were associated with higher cognitive, whereas recurrent, long-chain were seen as poor hippocampal spatial memory performance.

Lipid nanoparticle packaging of siRNA

The Neuro9™ siRNA Spark™ Kit (2 nmol, NWS0001) was purchased from Precision NanoSystems. For examining gene knockdown in neuronal cultures, the siRNA can be packed into nanoparticles by the kit. Prepare a final concentration of 130-160 μg/mL by mixing the siRNA containing the reconstituted nucleic acid with Buffer 1. Then mixed it with Buffer 2 and placed all the stuff in the spark chamber to generate siRNA-containing lipid nanoparticles by NanoAssemblr Spark equipment. The whole process must be carried out under sterile conditions and the packaged siRNA lipid nanoparticles were kept at 4°C.

5xFAD Genotyping

Isolate DNA from ear: A small piece of mouse ear tissue was stored in a 1.5 mL tube (Eppendorf, Germany) and 197 μL of DirectPCR® Lysis Reagent (ear) (Viagen, USA) was added together with 3 μL of Proteinase K (0.3 mg/ml. Roth, Germany). Biopsies

were lysed in a Thermomixer Comfort (Eppendorf, Germany) at 55°C shaking for 4 hours at 650 rpm then increased to 85°C and incubated for a further 45 minutes. The extracted DNA can be stored at room temperature or at 4°C for a long time.

Polymerase Chain Reaction: The PCR mix consists of a 25 µL system, the cycling conditions and the primer details are shown in the table. All primers were purchased from Sigma (Germany) and diluted to a final concentration of 10 µM. Samples were run for 50 min at 130V in 1xTAE buffer on a 1.5% agarose gel (Roth, Germany) together with 0.06 µL/mL Midori Green dye (Takara CloneTech, Japan). Thermo Scientific GeneRuler 1kd ladder was utilized as a molecular weight standard. The FAS V Gel documentation System (Nippon genetics, Japan) was used to visualize the fragments.

Table 1: Reaction mixes for genotyping PCRs.

Component	Amount per reaction (µL)
10x Dream Taq Green buffer (Thermo Scientific, USA)	2.5
dNTP Mix 10 mM (Bioline, Lot. DM 1-414211)	2
App mix (Sigma, Germany)	0.5
PS1 Mix (Sigma, Germany)	0.5
Dream Taq DNA Polymerase (Thermo Scientific, USA)	0.13
Water (Sigma, Germany)	18.87
DNA	0.5

Table 2: Primer sequences.

Primer	Sequence
APP_F	5'-AGGACTGACCACTCGACCAG
APP_R	5'-CGGGGGTCTAGTTCTGCAT
PS1_F	5'-AATAGAGAACGGCAGGAGCA
PS1_R	5'-GCCATGAGGGCACTAATCAT

Table 3: Protocol for genotyping.

Step	Temperature	Duration	Cycles
Denaturation	95°C	3 min	1
Amplification	95°C	30 sec	x 35
	58°C	1 min	
	72°C	1 min	
Elongation	72°C	5 min	1
Cooling	8°C	forever	1

Primary neuronal culture

Prepare Processing media (PM) and Maintenance media (MM). The components of PM are DMEM, 10% FBS, 1% Penicillin/streptomycin and 1% Glutamax. The stuffs that make up MM are Neuroabasal media, 1% penicillin/streptomycin, 2% B27 and 1% Glutamax. All the above reagents were from Gibco Company. Pre-warm PM, MM, 2.5% Trypsin (Gibco, USA), DNase and PBS at 37°C before culture the neurons. The entire hippocampal cortex of E16.5 CD1 mouse embryos was microscopically dissected and minced into 4.5 mL of pre-warmed PBS, added with 500 µL of 2.5%-Trypsin and digested at 37°C for 13 minute. The reaction was then stopped by adding 5 mL PM and 10 µL DNase and the cells were resuspended in PM and centrifuged at 300 rcf for 5 minutes to obtain the cell pellet after three washing. The pellet was resuspended in MM and filtered through a 70 µM cell strainer (Falcon, USA), the number of cells was counted and plated in a PDL pre-coated plate, incubated at 37°C with a 30% change of MM every 3 days, and the transfection was started on day 10 for 48 hours.

Dendritic spine labeling

Dendritic spines were identified using fluorescent Dil marker (Invitrogen, USA). Briefly, the transfected neurons were fixed with fresh 4% paraformaldehyde (Sigma, Germany) for 15 min**.** Then gently washed once with ice PBS and added approximately 2-3 Dil crystals to each well with forceps. A minimal amount of PBS was administered

at the border of the wells to avoid dehydration of the cells. To reduce dye clumping, extra attention was taken in delivering the tiniest crystals. The neurons with crystals were set on the shaker for 10 min at 120 rpm for complete staining. The plate was then washed again with ice PBS until no residual crystals were visible in the wells. The neurons should be incubated in PBS overnight at room temperature in the dark for dye diffusion. The next day, the plate was washed 3 times with pure water for 5 min each. All images should be taken within a week after staining to prevent fading. A confocal STEDYCON microscope was used to visualize the dendritic spines (Abberior Instruments GmbH, Germany). A 63x/1.4 oil immersion lens was used to capture all the images. Serie stacks could be collected from the bottom to the top, with an electrooptic slice thickness of 0.25 μM. The final image was reconstructed to discover the underlying protrusions using intensity Z-stack projections. FJ Image was used to measure spine density and total spine length. Spine density was obtained by comparing the overall number of spines per dendritic segment length and then dividing by the average number of spines per μM.

MEA assay

Cultured primary neurons in 6 well plate as described. Meanwhile, dropped 5 μL PDL solution to each well in MEA plate (Axion Biosystems, USA) and incubated the plate at 37°C for 1 hour. After the pre-treatment, washed the plate 4 times with 300 μL sterile water and allowed it dry overnight. Then seeded 5 μL neuron suspension onto the MEA electrode recording zone and incubated 1 hour in the incubator. Gently added 1000 μL maintenance media into the plate and exchanged 40% of the meida every 3 days. Neural spikes were detected after 21 days by MAESTRO PRO.

RNA Isolation

RNA was extracted from neuronal cells and brain tissue using TRIZOL kits purchased from Invitrogen for qPCR and next generation sequencing. In brief, cells were washed and lysed with an equal volume of TRI reagent (100 μL per 24 wells, 200 μL per

12 wells and 400 µL per 6 wells). Brain tissue needs to be homogenised in TRI. Incubate at room temperature for 15 min and then add 0.2xV chloroform and centrifuge at 12000 rcf for 15 min at 4ºC. Collect the aqueous phase and add 0.5xV Isopropanol and kept at 4 ºC for 30 min centrifuged at 12000 rcf. The RNA pallet was resuspended in 30 µL PCR grade water after washed twice with 75% EtOH. Afterwards, the final concentration of RNA was determined in NanoDrop 2000 (Thermo Scientific, USA).

cDNA preparation

1 µg of total RNA reverse transcription was performed with Transcriptor First Strand cDNA Synthesis Roche Kit (Roche, Germany). Prepare the reaction mixture according to the manufacturer's instructions. The mixture should be incubated for 10 min at 25ºC and then for 30 min at 55ºC. The reverse transcriptase was then inactivated by a final 5 mins incubation at 85ºC in the Mastercycler. The prepared cDNA was kept in a 96 well plates at -20°C.

Quantitative real-time PCR

Primer's design was carried out using the online tool provided by Sigma and the quantitative real-time PCR was performed with SYBR Green PCR Master Mix (Roche, Germany). Before the qPCR assay, the cDNA-containing mixes should be added to a 96-well plate in the dark and short spun down for 20 seconds, then placed the plate in a standard Light Cycler program to start the reaction. All primer's sequence, mixes system and the qPCR protocol were shown in table 4-6. Internal control was provided by the housekeeping gene Hprt1.

Table 4: Primer sequences.

Primer	Sequence
Kdm5b_F_mm	5'-GACTGGGTTCAGGATGAGGA
Kdm5b_R_mm	5'-TGTCTCTAACACTGGCACACG
HPRT1_F_mm	5'-CCTCCTCAGACCGCTTTTT
HPRT1_R_mm	5'-AACCTGGTTCATCATCGCTAA

Primer	Sequence
BDNF_F_mm	5'-TGCAGGGGCATAGACAAAAGG
BDNF_R_mm	5'-CTTATGAATCGCCAGCCAATTCTC
NLGN1_F_mm	5'-CCATCCACGGACATCACTCT
NLGN1_R_mm	5'-GAGAAGGGGCTTGGTTGTTG

Table 5: Reaction mixes for qPCR.

Component	Amount per reaction (μL)
LC480 SYBR Green buffer	7.5
Primer_F_mm (Sigma, Germany)	0.5
Primer_R_mm (Sigma, Germany)	0.5
Water (Sigma, Germany)	5.5
cDNA	1

Table 6: Protocol for qRT-PCR.

Step	Temperature	Duration	Cycles
Denaturation	95°C	5 min	1
Amplification	95°C	10 sec	x 45
	60°C	30 sec	
	72°C	10 sec	
Cooling	40°C	30 sec	1

Protein extraction

Protein extraction with TX buffer together with a protease inhibitor is from Thermo. Add 80 μL per 6 well dishes and incubate on ice for 15 min, followed by scraping the cells. For tissues, they were homogenized in 500 μL isolation buffer and sonicated in a Bioruptor (Diagenode, Belgium) (25 min, HIGH, 30 sec on/off). Afterward, centrifuged the samples at 4ºC for 15 minutes at 13000 rpm and collected supernatant for the subsequent research. Pierce™ BCA Protein Assay Kit (Thermo Scientific, USA) was

used to determine the protein concentration. Bovine serum albumin was diluted to different concentrations according to the instructions to produce a standard curve representing protein concentration versus optical density. The samples were mixed with two working solutions from the kit and incubated at 37ºC for 30 min before being tested for concentration using the Omega MARS software.

Western Blotting

loading 30 µg of protein on 4-15% mini-PROTEAN® SDS-acrylamide gels (Bio-Rad, USA) and running 4 hours at 120V. The transfer was carried out with the TransBlotâ Turboä Transfer system and the Midi-nitrocellulose transfer packs (Bio-Rad). Following that, the membrane was blocked with 5% BSA for at least 16 hours of incubation with target antibodies at 4°C, see Table 7. On the second day, membrane should be washed 3 times (5 min/time) and incubated with respective corresponding secondary antibodies (IRDye 800, 1:5000). The immunoblots were then visualized and quantified using Odyssey Imager.

Table 7: Antibodies in western blot.

Target protein	Dilution	Company	Product number
anti-Kdm5b	1:500	Bethyl	A-301-813A
anti-HSC70	1:1000	Abcam	19136
IRDye 800CW	1:5000	Licor	926-32230

RNA sequencing

Based on the manufacturer´s protocol (Illumina, USA) performed the Next-generation sequencing of the total RNA from collected cells and tissues with TruSeq RNA Library Preparation Kit. To identify a differential analysis, RUVSeq was used to detect and reduce unwanted variation (RUVs). DESeq2 was then used for analyzing differential gene expression. Adjusted (Benjamini-Hochberg) p-values <0.05 were considered to be significantly deregulated.

Statistics

GraphPad Prism was used for statistical analysis of behavioral and molecular studies. FIJI image was used for dendritic spines analysis. The statistical tests used were one-way, two-way, and t-tests, which were all depending on the data structure.

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Author contribution

JZ initiated the project as part of her Ph.D thesis, conducted genotyping, behavior, qPCR, western blot and cell culture experiments, analyzed behavior data, and drafted the paper. LK performed behavior experiment, analyzed and interpreted RNA-seq data, designed all experiments, supervised JZ, and drafted the paper. SB provided technical support. RP performed MEA assay. AM provided bioinformatics support. MS performed imaging. DMK provided bioinformatics support. AF arranged funding, designed and supervised the study, drafted and revised the final version of the manuscript. All authors read and approved the final version of the paper.

Competing Interests

The authors state that there is no conflict of interest.

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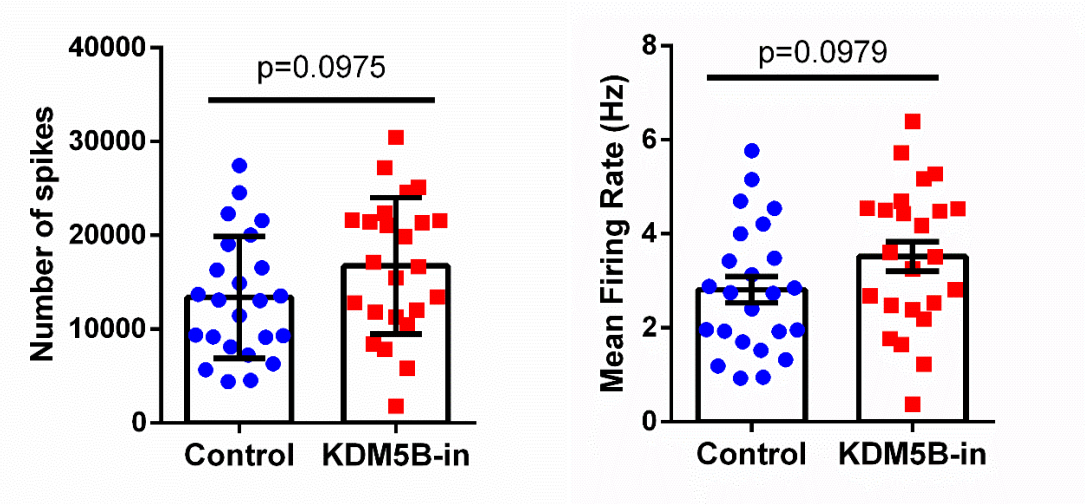
Supplementary

Supplement 1: The number of spikes and mean firing rate (Hz) in primary neurons after knockdown of Kdm5b.

A. B.



Supplementary Figure 1. (A-B) The electrophysiological activity in primary neurons was recorded by the MEA system after transfection, the Kdm5b knockdown group showed an increased number of spikes and firing rate than the control group (n=24, unpaired t-test). Error bar means Mean±SEM.

Supplement 2: List of top 30 differentially up-regulated genes after knockdown of Kdm5b in primary neurons of wildtype mice.

Gene	log2FoldChange	Adjusted p-value
Igfbp2	3.198233433	2.64E-58
Afap1l2	2.067128728	3.68E-13
Pcdh9	2.019773636	2.27E-79
Rfx4	1.907562741	1.05E-32
Spata6	1.877299161	3.78E-13
A2m	1.865287868	7.66E-119
Col16a1	1.855132938	3.70E-21
Eda	1.825223982	4.95E-29
Fmo1	1.77684019	5.82E-09
Igf1r	1.581285627	3.76E-36
Cd109	1.553479835	4.52E-06
Irak1	1.552655842	9.31E-35
Col4a5	1.479003137	9.39E-11
Col11a2	1.471416731	6.06E-10
Aldh1l1	1.459908405	2.29E-11
Acss2	1.449225746	1.67E-36
Cyld	1.446687691	1.24E-34
Col11a1	1.446411402	3.41E-05
Pdlim5	1.442595783	1.09E-15
Bub1b	1.423840128	4.88E-10
Il17rd	1.366498015	4.49E-14
Apln	1.359830673	5.44E-12
Dock1	1.345779541	4.36E-06
Cdc20	1.345699598	6.26E-10
Focad	1.281391315	5.73E-25
Ggt7	1.263576112	2.14E-30
Anxa11	1.253590112	3.80E-11
Hjurp	1.221423219	5.93E-26
Pbk	1.203368393	2.30E-07

Supplement 3: List of top 30 differentially down-regulated genes after knockdown Kdm5b in primary neurons of wildtype mice.

Gene	log2FoldChange	Adjusted p-value
Adgrg6	-2.283187573	1.06E-18
Trank1	-2.231836004	1.43E-217
Plp1	-2.170022034	8.16E-68
Ptgds	-2.021730967	1.79E-21
Serpina3h	-1.90250839	9.34E-12
Arhgef12	-1.880145217	3.95E-83
Xist	-1.863795178	1.34E-22
Fbln2	-1.791121618	1.14E-16
Oasl2	-1.772284517	0.004359971
Slc17a5	-1.723022769	2.96E-37
Trim56	-1.666710203	2.19E-11
S1pr3	-1.586039636	2.96E-37
Fam199x	-1.451411334	8.40E-20
Ifitm3	-1.390890567	0.018523618
Parp9	-1.371701477	1.72E-08
Pid1	-1.321522237	2.88E-26
Vcam1	-1.306650257	4.37E-33
Hspb8	-1.269775764	8.90E-15
Hsd17b11	-1.242125845	4.17E-13
Apobec1	-1.194541471	0.00012854
Parp3	-1.183039936	7.19E-06
Plin3	-1.171389465	2.10E-11
Slc25a18	-1.169817486	8.99E-23
Cxcr4	-1.141008349	1.58E-19
Kdm5b	-1.138737541	9.28E-36
Zkscan2	-1.116006089	4.14E-29
Pdpn	-1.097325423	1.43E-16
Ddx58	-1.086929697	8.66E-06
Parp12	-1.072737576	1.30E-05

Supplement 4: List of deregulated pathways after knockdown of Kdm5b in primary neurons of wildtype mice.

Up-regulated	Down-regulated
Cell cycle	TNF signaling pathway
ECM-receptor interaction	Biosynthesis of unsaturated fatty acids
Protein digestion and absorption	Pathways in cancer
Focal adhesion	RIG-I-like receptor signaling pathway
Oocyte meiosis	Neurotrophin signaling pathway
PI3K-Akt signaling pathway	Influenza A
MAPK signaling pathway	Fatty acid metabolism
Progesterone-mediated oocyte maturation	Fatty acid elongation
p53 signaling pathway	Focal adhesion
Fanconi anemia pathway	Herpes simplex infection
DNA replication	Cytosolic DNA-sensing pathway
One carbon pool by folate	B cell receptor signaling pathway
Hippo signaling pathway	Viral carcinogenesis
Arrhythmogenic right ventricular cardiomyopathy	Axon guidance
Amoebiasis	Small cell lung cancer
Platelet activation	Epstein-Barr virus infection
Hypertrophic cardiomyopathy (HCM)	Hepatitis C
Dilated cardiomyopathy	Bladder cancer
Viral carcinogenesis	ErbB signaling pathway
Hepatitis B	Chemokine signaling pathway
Nitrogen metabolism	
HTLV-I infection	
Pathways in cancer	
FoxO signaling pathway	

Supplement 5: Behavior data for aged wildtype mice (outliers were removed).

5-1: Time spent in the middle area (seconds)

Control	Kdm5b-in	Table Analyzed	Open Field
1.85	2.04	Column B	Kdm5b-in
3.37	5.01	vs.	vs.
0.72	1.61	Column A	Control
0.76	6.18	Unpaired t test	
1.95	7.59	P value	0.0392
2.51	1.6	P value summary	*
4.07	5.75	Significantly different? (P < 0.05)	Yes
2.83	5.35	One- or two-tailed P value?	Two-tailed
4.33	8.98	t, df	t=2.234 df=17
4.98			

5-2: Traveled Path (cm)

Control	Kdm5b-in	Table Analyzed	Open Field
1497.16	896.99	Column B	Kdm5b-in
1270.97	733.8	vs.	vs.
968.76	922.37	Column A	Control
800.47	1406.03	Unpaired t test	
1253.51	775.6	P value	0.9616
896.29	1164.81	P value summary	ns
1106.96	987.52	Significantly different? (P < 0.05)	No
1213.2	1170.57	One- or two-tailed P value?	Two-tailed
1004.36	1359.51	t, df	t=0.04881 df=17
510.82			

5-3: Average speed (cm/s)

Control	Kdm5b-in	Table Analyzed	Open Field
4.31	3.14	Column B	Kdm5b-in
3.25	2.45	vs.	vs.
2.67	3.07	Column A	Control
4.29	4.84	Unpaired t test	
3.25	2.59	P value	0.6679
4.34	3.89	P value summary	ns
4.07	4.2	Significantly different? (P < 0.05)	No
3.52	3.99	One- or two-tailed P value?	Two-tailed
1.7	4.9	t, df	t=0.4371 df=16

5-4: Time spent in open arm (percentage)

Control	Kdm5b-in	Table Analyzed	EPM
7.57	43.97	Column B	Kdm5b-in
0	42.09	vs.	vs.
5.01	44.24	Column A	Control
4.89	22.2	Unpaired t test	
1.34	59.82	P value	0.0002
5.27	40.92	P value summary	***
4.64	0.04	Significantly different? (P < 0.05)	Yes
16.42	24.52	One- or two-tailed P value?	Two-tailed
0.08	29.38	t, df	t=4.855 df=16

5-5: Traveled Path (cm)

Control	Kdm5b-in	Table Analyzed	EPM
3277.62	2913.67	Column B	Kdm5b-in
1682.24	4657.91	vs.	vs.
4548.71	5893.78	Column A	Control
3926.01	6700.4	Unpaired t test	
4844.18	2295.68	P value	0.6986
3556.95	2504.76	P value summary	ns
2864.57	2012.87	Significantly different? (P < 0.05)	No
6133.75	2407.1	One- or two-tailed P value?	Two-tailed
5593.16	4443.02	t, df	t=0.3939 df=17
3911.42			

5-6: Average speed (cm/s)

Control	Kdm5b-in	Table Analyzed	EPM
10.92	9.71	Column B	Kdm5b-in
5.61	15.53	vs.	vs.
15.16	19.65	Column A	Control
13.09	22.33	Unpaired t test	
16.15	7.65	P value	0.6982
11.86	8.35	P value summary	ns
9.55	6.71	Significantly different? (P < 0.05)	No
20.45	8.02	One- or two-tailed P value?	Two-tailed
18.64	14.81	t, df	t=0.3944 df=17
13.04			

5-7: Morris water maze escape latency (seconds)

Control group (n=10)

No.	1	2	3	4	5
day1	36.48	46.885	53.385	41.7775	60
day2	25.76	40.065	21.91	47.71	60
day3	17.305	28.865	33.4075	36.5975	60
day4	10.3875	18.98	14.885	24.925	60
day5	17.4225	11.1175	6.1725	16.1375	60
day6	12.9075	20.185	34.925	14.435	60
day7	13.2675	24.0825	20.455	9.9125	60
No.	6	7	8	9	10
day1	60	60	52.6	60	57.305
day2	60	46.0725	12.8825	60	32.2425
day3	52.8825	51.955	15.1025	60	23.2475
day4	23.155	54.82	37.735	55.665	37.1225
day5	18.7825	60	13.2025	46.0025	32.025
day6	39.52	39.2875	38.1575	28.1375	30.07
day7	11.07	17.98	7.57	19.865	11.1875

Kdm5b-in group (n=9)

No.	1	2	3	4	5
day1	50.66	24.7725	60	29.1275	51.3225
day2	11.7025	44.64	49.0225	10.6375	48.485
day3	5.2025	17.28	31.63	45.815	60
day4	10.865	7.9	27.755	47.3475	60
day5	14.0825	22.13	17.62	41.035	40.57
day6	9.45	15.2	18.775	21.185	60
day7	11.7225	22.2275	25.06	14.8975	24.4825
No.	6	7	8	9	
day1	54.5675	51.0525	37.9425	43.105	
day2	28.47	45.5025	31.4375	8.3875	
day3	7.515	24.91	12.9375	38.4375	
day4	9.77	13.6375	41.49	15.7925	
day5	19.905	20.3725	26.42	7.315	
day6	15.315	28.7875	20.965	10.7075	
day7	4.7575	10.42	14.3275	10.785	

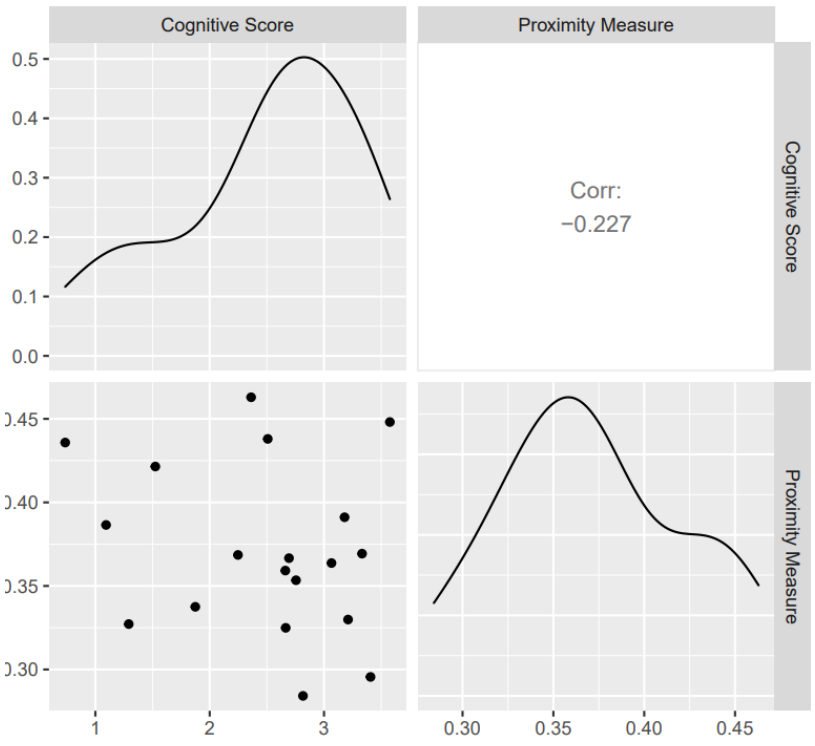
No significant

5-8: Probe test (Percentage of time spent in the target quadrant)

Control	Kdm5b-in	Table Analyzed	probe test_time%
5.93	2.01	Column B	Kdm5b-in
1.67	2.4	vs.	vs.
5.66	2.33	Column A	Control
2.2	2.2	Unpaired t test	
0	1.53	P value	0.1242
2.26	2.07	P value summary	ns
7.93	2.07	Significantly different? (P < 0.05)	No
2.2	2.07	One- or two-tailed P value?	Two-tailed
2.07		t, df	t=1.622 df=16
5.47			

5-9: Cognitive Scores

Sample	Cognitive_Score_weighted	Sample	Cognitive_Score_weighted
Control1	3.406830711	Kdm5b-in1	3.576065832
Control2	2.507837853	Kdm5b-in2	3.332633009
Control3	2.664863935	Kdm5b-in3	2.362391683
Control4	2.755227289	Kdm5b-in4	2.693739264
Control5	0.735948436	Kdm5b-in5	1.524494065
Control6	1.874158985	Kdm5b-in6	3.179951038
Control7	1.291875684	Kdm5b-in7	2.815951794
Control8	2.66157826	Kdm5b-in8	3.065509841
Control9	1.093486454	Kdm5b-in9	3.210237292
Control10	2.247218575		



Supplement 6: Behavior data for 9-month-old 5xFAD mice (outliers were removed).

6-1: Time spent in the middle area (seconds)

Control	Kdm5b-in	Wildtype	Table Analyzed	Open field
6.78	20.67	24.81	Column A	Control
15.01	7.31	13.96	Column B	Kdm5b-in
5.45	12.64	10.41	Column C	Wildtype
7.08	14.57	3.4	Unpaired t test	
8.03	12.39	8.72	A *vs* B (P value)	0.9966
19.47	1	9.47	One- or two-tailed P value?	Two-tailed
6.02	9.54	8.53	t, df	t=0.004330 df=28
25.29	2.9	12.53	A *vs* C (P value)	0.7245
2.71	9.44	5.77	One- or two-tailed P value?	Two-tailed
9.67	16.06	15.07	t, df	t=0.3559 df=29
18.82	7.68	19.12	B *vs* C (P value)	0.6642
15.55	8.93	18.24	One- or two-tailed P value?	Two-tailed
29.99	14	12.64	t, df	t=0.4385 df=29
4.1	25.91	16.08	P value summary	ns
0.62	11.37	6.89	Significantly different? (P < 0.05)	No
		15.37		

6-2: Time spent in open arm (percentage)

Control	Kdm5b-in	Wildtype	Table Analyzed	EPM
17.29	33.66	16.45	Column A	Control
37.9	33.85	5.52	Column B	Kdm5b-in
63.62	48.01	19.15	Column C	Wildtype
53.29	25.26	11.93	Unpaired t test	
22.07	16.99	1.4	A *vs* B (P value)	0.6337
55.52	49.78	1.36	One- or two-tailed P value?	Two-tailed
0.07	12.67	30.2	t, df	t=0.4817 df=29
30.11	14.59	2.83	A *vs* C (P value)	0.0016
4.48	100	13.14	P value summary	**
1.2	57.37	8.03	Significantly different? (P < 0.05)	Yes
29.08	28.53	0.72	One- or two-tailed P value?	Two-tailed
23.47	38.12	17.35	t, df	t=3.474 df=30
14.1	1.86	9.32	B *vs* C (P value)	0.0011
49.04	34.34	21.93	P value summary	**
53.63	13.49	6.36	Significantly different? (P < 0.05)	Yes
26.12		9.32	One- or two-tailed P value?	Two-tailed
			t, df	t=3.607 df=29

6-3: Traveled Path (cm)

Control	Kdm5b-in	Wildtype	Table Analyzed	EPM
577.72	940.51	634.35	Column A	Control
970.11	694.72	251.42	Column B	Kdm5b-in
778.42	616.75	667.85	Column C	Wildtype
733.74	568.21	499.47	Unpaired t test	
362.4	45.69	1524.39	A *vs* B (P value)	0.0773
772.54	380.38	867.14	One- or two-tailed P value?	Two-tailed
427.72	474.63	2207.75	t, df	t=1.849 df=23
1359.34	945.82	1691.58	A *vs* C (P value)	0.1728
747.41	759.26	1478.14	One- or two-tailed P value?	Two-tailed
1203.91	675.93	1109.38	t, df	t=1.405 df=24
1188.03	1212.69	1434.73	B *vs* C (P value)	0.0106
1489.9	652.77	1329.4	P value summary	*
1191.87		1437.45	Significantly different? (P < 0.05)	Yes
			One- or two-tailed P value?	Two-tailed
			t, df	t=2.781 df=23

6-4: Average speed (cm/s)

Control	Kdm5b-in	Wildtype	Table Analyzed	EPM
1.93	3.13	2.11	Column A	Control
3.23	2.32	0.84	Column B	Kdm5b-in
2.59	2.06	2.23	Column C	Wildtype
2.45	1.89	1.66	Unpaired t test	
1.21	1.27	5.08	A *vs* B (P value)	0.1493
2.58	1.58	2.89	One- or two-tailed P value?	Two-tailed
1.43	3.15	7.36	t, df	t=1.494 df=22
4.53	2.53	5.64	A *vs* C (P value)	0.174
2.49	2.25	4.9	One- or two-tailed P value?	Two-tailed
4.01	4.04	3.7	t, df	t=1.401 df=24
3.96	2.18	4.78	B *vs* C (P value)	0.0217
4.97		4.43	P value summary	*
3.97		4.79	Significantly different? (P < 0.05)	Yes
			One- or two-tailed P value?	Two-tailed
			t, df	t=2.471 df=22

6-5: Morris water maze escape latency (seconds)

Wildtype group (n=16)

NO.	1	2	3	4	5	6	7	8
day1	57.005	54.4025	52.8725	60	60	48.915	55.0125	41.6775
day2	60	38.765	30.44	40.845	60	47.495	33.8375	46.685
day3	60	60	11.9025	39.025	60	35.525	16.45	55.2625
day4	20.44	60	6.9825	11.26	60	46.0025	21.9825	60
day5	18.02	45.6525	10.24	52.2025	60	60	12.16	46.9125
day6	11.48	39.735	6.62	44.21	60	29.3275	8.48	60
NO.	9	10	11	12	13	14	15	16
day1	56.0025	60	37.71	28.54	46.46	47.05	29.43	46.48
day2	34.5075	60	11.21	19.15	26.41	37.93	13.48	37.58
day3	36.1175	47.0325	20.8	27.67	29.57	27.36	18.38	29.83
day4	15.6525	8.65	20.83	12.5	22.73	14.82	7.31	11.97
day5	19.965	34.3975	23.46	13.06	38.55	22.31	7.75	12.05
day6	13.15	18.59	31.1	8.88	10.35	13.73	8.08	26.52

Control group (n=16)

NO.	1	2	3	4	5	6	7	8
day1	49.835	60	60	39.895	47.8525	46.0325	37.3375	57.1125
day2	35.6125	60	53.005	46.655	60	48.6525	60	59.4925
day3	17.69	24.6775	60	48.0025	60	46.4725	60	32.46
day4	52.3025	45.8025	50.4025	54.3225	60	28.2275	60	28.32
day5	26.54	39.7975	29.5775	60	60	58.9725	31.29	33.97
day6	39.87667	57.2125	51.8325	60	60	27.28	60	18.21
NO.	9	10	11	12	13	14	15	16
day1	48.8325	60	17.21	57.15	45.44	60	54.12	60
day2	49.9425	60	51.23	60	55.47	59.56	32.29	46.55
day3	44.0575	60	42.84	58.02	60	60	35.22	60
day4	42.815	60	60	43.09	60	60	26.18	24.24
day5	28.1325	60	54.3	33.68	60	60	16.06	26.06
day6	26.47	60	53.01	36.57	60	60	24.08	24.18

Kdm5b-in group (n=15)

No.	1	2	3	4	5	6	7	8
day1	35.2875	45.5125	59.0525	45.5125	32.8975	37.8975	23.66	30.7075
day2	25.7075	15.49	60	38.9875	37.6275	45.6525	45.9225	44.005
day3	36.405	54.7825	48.5225	25.8275	31.16	53.6075	43.445	43.605
day4	17	45.5125	28.0075	33.575	31.725	14.775	60	45.6025
day5	14.38	60	54.2225	16.2	27.9175	43.575	60	60
day6	17.55	32.175	23.7675	19.3325	60	18.4225	36.505	21.1575

No.	9	10	11	12	13	14	15
day1	45.5425	47.73	38.71	50.61	26.01	49.89	45.74
day2	60	42.3	27.45	28.51	28.12	47.63	20.82
day3	45.8375	14.17	18.08	34.49	39.62	60	20.34
day4	59.1925	19.18	36.65	35.65	27.07	51.1	21.48
day5	50.3425	12.06	18.53	10.35	55.03	60	17.93
day6	59.7325	9	12.48	25.39	38.96	59.86	7.66

Table Analyzed:Morris Water Maze; Two-way RM ANOVA; Matching: Stacked; Alpha 0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	3.599	0.0235	*	Yes
Time	7.338	< 0.0001	****	Yes
Column Factor	12.77	0.002	**	Yes
Subjects (matching)	38.98	< 0.0001	****	Yes

6-6: Probe test (Percentage of time spent in the target quadrant)

Control	Kdm5b-in	Wildtype	Table Analyzed	Probe test
4.66	11.67	3.67	Column A	Control
1.47	0	4.66	Column B	Kdm5b-in
0	2.4	13	Column C	Wildtype
0	6.97	6.4	Unpaired t test	
0	0.8	0	A *vs.* B (P value)	0.0191
1.07	1.13	0	P value summary	*
1.13	4.46	6.2	Significantly different? (P < 0.05)	Yes
4.06	0	2.39	One- or two-tailed P value?	Two-tailed
1.53	11.07	2.67	t, df	t=2.499 df=26
0	0	7.26	A *vs.* C (P value)	0.0015
0	5.2	5.93	P value summary	**
0	12.47	4.13	Significantly different? (P < 0.05)	Yes
3.47	0.2	7.33	One- or two-tailed P value?	Two-tailed
0.73	16.67	12.47	t, df	t=3.517 df=28
		15.13	B *vs.* C (P value)	0.7632
		1.27	P value summary	ns
			Significantly different? (P < 0.05)	No
			One- or two-tailed P value?	Two-tailed
			t, df	t=0.3042 df=28

6-7: Probe test (Visit # of target quadrant)

Control	Kdm5b-in	Wildtype	Table Analyzed	Probe test
2	3	3	Column A	Control
1	0	2	Column B	Kdm5b-in
0	1	0	Column C	Wildtype
0	5	3	Unpaired t test	
0	1	0	A *vs.* B (P value)	0.0116
1	1	0	P value summary	*
1	5	5	Significantly different? (P < 0.05)	Yes
1	0	2	One- or two-tailed P value?	Two-tailed
0	8	2	t, df	t=2.732 df=24
0	0	3	A *vs.* C (P value)	0.0052
0	2	5	P value summary	**
1	5	4	Significantly different? (P < 0.05)	Yes
	1	5	One- or two-tailed P value?	Two-tailed
	5	5	t, df	t=3.054 df=26
		12	B *vs.* C (P value)	0.5535
		1	P value summary	ns
			Significantly different? (P < 0.05)	No
			One- or two-tailed P value?	Two-tailed
			t, df	t=0.5998 df=28

Chapter 2-Deregulated miR-129-5p in Frontotemporal Dementia Patients leads to tripartite synapse loss and cognitive impairment

Detailed Author contribution of Jiayin Zhou

Experimental work

- Performing primary neuronal culture
- Performing transfection to knockdown target gene expression
- Performing RNA isolation and q-PCR
- Performing Dil dye labelling and Imaging
- Performing Stereotactic surgery on mice
- Performing Behavioral experiments on mice

Data presentation

- Partial editing of the manuscript

Data analysis

- Assisting basic data analysis

Deregulated miR-129-5p in Frontotemporal Dementia Patients leads to tripartite synapse loss and cognitive impairment

Lalit Kaurani[1*†], Jiayin Zhou[1†], Ranjit Pradhan[1], Aditi Methi[1], Susanne Burkhardt[1], Raquel Pinto[2], MD Rezaul Islam[1], Sophie Schröder[1], Peter Heutink[3], Farahnaz Sananbenesi[1**], Andre Fischer[1,4,5***]

[1]Department for Systems Medicine and Epigenetics, German Center for Neurodegenerative Diseases (DZNE), Göttingen, Germany

[2]GGNB Neurosciences-International Max Planck Research School

[3]German Center for Neurodegenerative Diseases, Tübingen, Germany

[4]Cluster of Excellence"Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells"(MBExC), University of Göttingen, Germany

[5]Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, Göttingen, Germany

*Corresponding author; E-mail: lalit.kaurani@dzne.de

**Corresponding author; E-mail: fsananb@gwdg.de

***Corresponding author; E-mail: andre.fischer@dzne.de

[†]These authors contributed equally to this work

Abstract

Cognitive dysfunction is a key pathological marker for a variety of psychiatric and neurodegenerative disorders. As a result, there is an urgent need for biomarkers that can predict the risk of cognitive deficits in the future. miRNAs have been identified as being involved in the aging process and as potential biomarkers affecting the progression of neurodegenerative diseases. Frontotemporal degeneration as the second most common type after Alzheimer's disease, sharing pathophysiological mechanisms and genetic origins with some dementia-specific disorders. Mining FTD-associated microRNAs can be used to distinguish FTD from other dementia-specific disorders. In this study, we established an in-depth smallRNAome sequencing analysis of frontal and temporal cortex tissue to identify specific microRNAs that showed dysregulation in a group of FTD patients. Further analysis was performed by manipulating one of these signatures, miR-129-5p, to reflect the molecular changes that occur during brain pathology in *vitro*. The impact of inhibition of miR-129-5p markers on cognitive impairment was also revealed in animals, suggesting its use as a powerful pathogenetic indicator of FTD-related disorders.

Keywords: FTD, miR-129-5p, biomarker, small RNA-seq, cognitive disease.

Introduction

Frontotemporal lobar degeneration (FTLD) is a set of degenerative brain disorders characterized by prefrontal and anterior temporal lobe atrophy. Clinical manifestations include behavioral and personality changes, linguistic difficulties, and, in certain cases, motor neuron disease or parkinsonism. This category of disorders accounts for 5-15% of all dementia cases, second only to Alzheimer's disease (AD), which accounts for 50%-70% of all dementia cases and is now the leading cause of early-onset dementia among adults under the age of 60[1,2]. FTLD is regarded to be synonymous with frontotemporal dementia (FTD), which is classified into two major clinical subtypes: behavioral variation (bvFTD) and primary progressive aphasia (PPA). The first subtype is defined by behavioral symptoms, whereas the second subtype is divided into a semantic variant PPA (svPPA) and a nonfluent variant PPA (nfvPPA), both of which are defined by progressive language and speech impairment[1]. The bvFTD accounts for more than 50% of cases[3]. The onset of the first symptom might occur as early as 30 years of age or as late as 60 years or more of age (UCSF Weill Institute for Neurosciences). Currently, there is no cure for FTD.

Genetic, epigenetic, and environmental variables are all thought to play a role in the development of FTD disease[4]. Around 40% of patients with FTD have a positive family history of dementia, and roughly 25% of people with FTD have an identifiable hereditary variant of the disease[1,5]. Numerous genes, including those encoding the microtubule-associated protein tau (MAPT), progranulin (GRN), chromosome 9 open reading frame 72 (C9orf72), TAR DNA-binding protein 43 (TDP-43), fused in sarcoma-binding protein (FUS), valosin-containing protein (VCP), and charged multivesicular body protein 2B (CHMP2B), were found to be mutated[6,7]. Screening for mutations in these genes enables the study of the disease at its prodromal phase. However, individual research groups have been able to analyze just a small number of hereditary FTD cases. The vast majority of cases of sporadic FTD are unknown in their cause, however, genetic abnormalities may be expected[8]. Some FTD symptoms are

detectable in approximately 50% of individuals with amyotrophic lateral sclerosis (ALS) (FTD-ALS), and these two diseases share pathophysiological mechanisms and genetic origins[9,10].

Currently, FTD is diagnosed mostly by medical history, neuropsychological testing to better assess an individual's pattern of cognitive decline and to rule out other neurodegenerative conditions, as well as neuroimaging to determine the location and amount of atrophied brain regions. FTD manifests mostly as personality and behavioral disturbances or increasing aphasia that may be mistaken as a psychiatric disease[3]. Furthermore, clinical diagnosis of FTD is complicated by significant overlap of clinical symptoms and neuropsychological profiles between and across subtypes, as well as with AD and other neurodegenerative diseases (NDs), which frequently results in misdiagnoses[11,12]. At postmortem examination, 10-30% of patients presenting with an FTD clinical presentation were confirmed to have AD[13]. There is no single diagnostic test that can be used to confirm or exclude a diagnosis of FTD. We urgently require cost-effective and specific biofluid biomarkers that can aid in the early detection of FTD and can corroborate MRI neuroimaging findings[14]. Notably, circulating miRNAs have been identified as prospective biomarkers for neurodegenerative diseases and processes affecting the central nervous system, particularly during aging.

MicroRNAs (miRNAs) are single-stranded non-coding RNA molecules that recognize sequences in the 3'-untranslated regions of target messenger RNAs and either promote their degradation or impede their translation[15–17]. MiRNAs have been shown to be dysregulated in a range of neurodegenerative diseases (NDs), including AD, ALS, Parkinson's disease, Huntington's disease, age-related macular degeneration, and multiple sclerosis, and to play a critical role in FTD. miR-29b, miR-107, and miR-659 have been shown to regulate the progranulin gene post-transcriptionally. A recent study found that TMEM106B, a risk gene for Frontotemporal Dementia, is regulated by the miR-132/212 cluster.

While these investigations identified a number of miRNAs related to FTD, they do not give mechanistic evidence that these FTD-associated microRNAs were useful for distinguishing between FTD patients and those with other dementia-specific disorders such as AD. We conducted an in-depth smallRNAome sequencing analysis on frontal and temporal cortex tissue from a well-characterized cohort in this study. MiRNAs were shown to be dysregulated in FTD patients when compared to a cohort of non-demented controls and AD patients. The purpose of this study was to profile miRNA in peripheral, non-invasive biomarkers for FTD and to provide mechanistic functioning in major CNS cell types that reflect molecular changes occurring during pathological processes in the brain and serve as a robust indicator of FTD-related disease pathogenesis.

Results

smallRNAome data for frontotemporal dementia (FTD).

We analyzed data from frontal and temporal brain tissue obtained from diseased patients carrying mutations in the MAPT (n=12), GRN (n=5) or C9orf72 (n=8) genes, as well as from non-demented controls (n=9). The FTD groups had a lower average age than the healthy controls. Except for patients with C9orf72 gene mutations, all other subjects were of similar gender proportion. Patients carrying mutations in the C9orf72 gene had more females than males **(Fig.1A-C).** We generated smallRNAome data and adjusted for batch, age, and gender effects.

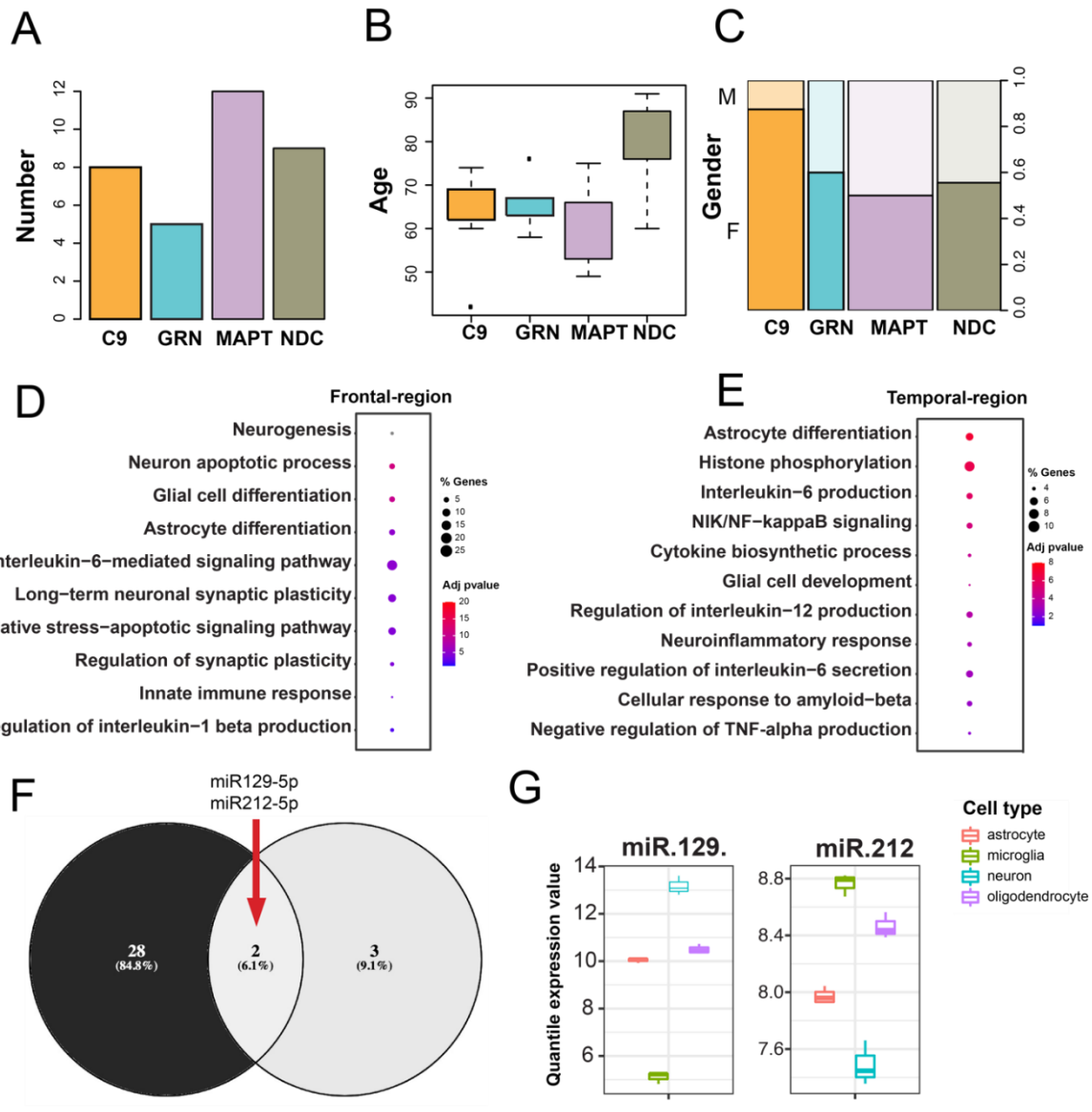


Fig.1 Sample characteristics and enrichment analysis at the cellular and pathway levels. (A) Sample number used in this study. **(B)** Age distribution among FTD cohort. **C)** Gender distribution of the FTD cohort. (**D-E)** A dot plot displays the functional analysis of commonly deregulated miRNA target genes in the frontal region (for each mutation in three main genes commonly associated with FTD: MAPT, GRN, and C9orf72). Significant expression levels are color-coded, whereas the proportion of genes associated with enriched biological processes is size-coded. MicroRNAs with a basemean greater than 50, log2foldchange+-0.5, and an adjusted p-value of 0.05 were evaluated for the analysis. The investigation was restricted to the miRTarBase-verified microRNA target genes (version 8). Based on the analysis performed with the gene ontology tool (http://geneontology.org/), statistically significant GO enrichment terms for these genes were identified (FDR=0.05, Benjamin–Hochberg correction). The significant GO terms related to brain functioning were filtered based on earlier curation of GO annotation pertinent to dementia research. GO semantic similarity was used to integrate comparable GO phrases into clusters, and the parent GO term was highlighted for visualization. Similar to the frontal region, the temporal region's functional analysis of miRNA target genes is represented by a dot plot. Similarly, only microRNAs from the temporal region with basemean>=50, log2foldchange+-0.5, and an adjusted p-value<0.05 were included in the analysis. The analysis was restricted to the miRTarBase-verified microRNA target genes (version 8). Using the gene ontology tool (http://geneontology.org), statistically significant GO enrichment terms were identified for these genes (FDR=0.05, Benjamin-Hochberg correction). (**F)** Venn diagram illustrating miRNAs shared by the frontal and temporal regions of the FTD cohort when patients with each mutant gene were compared to control samples. **(G)** Cell type-specific expression of miR-129-5p and miR-212-5p in rat central nervous system (CNS) brain cells. Under physiological conditions, rat CNS brain cell types express miR-129-5p. miR-129-5p expression is consistently enriched in neuronal cells within nervous system tissues, whereas miR-212-5p expression is consistently enriched in microglia (n=3-4 rat; mean±SEM). Data information: *P<0.05, **P<0.01, ***P<0.001. Bars and error bars indicate mean±SEM. Centre line denotes median, while 75th and 25th percentiles are shown by lines on either side of it in boxplots. The whiskers indicate the minimum and maximum values within the interquartile range.

Commonly deregulated microRNAs in FTD cohort.

We initially examined the deregulated microRNAome in frontal and temporal regions independently for each patient group (i.e., patients bearing mutations in the MAPT, GRN, and C9orf72 genes) *vs* non-demented controls. Using Benjamin-Hochberg-adjusted p-value criteria, the similarity and dissimilarity of differentially expressed microRNAs were assessed, with the directionality of the expression differences also being verified. There were several expression differences in

the miRNAome, notably within temporal regions based on the number of dysregulated miRNAs. Nonetheless, we determined that many of the strongest effects are reproduced in each brain region.

Since all three mutation subtypes in FTD patients result in a similar endophenotype, we sought to investigate the similarity of differentially expressed miRNAs in frontal and temporal brain regions among the three patient groups. In the frontal brain region, we identified 30 miRNAs significantly differentially expressed among all three patient groups (adj.p-value 0.05, basemean>=50, log2FC=-0.5), whereas in the temporal brain region, we identified 5 miRNAs significantly differentially expressed among all three patient groups (adj.p-value 0.05, basemean>=50, log2FC=-0.5). As demonstrated in Table 1 of the Supplement, the directionality of the expression changes was comparable for all commonly dysregulated microRNA in the patient group. Next, we independently performed target mining of deregulated miRNAs in the frontal and temporal brain regions. The miRTarbase database was utilized to identify gene targets. The GTEx portal was used to examine their expression in the brain, and only targets with moderate expression in the brain were chosen **(see methods)**. Target mining revealed 275 brain-enriched targets for the frontal brain miRNAs that are dysregulated **(Supplementary table 1)**. The enrichment analysis of these targets demonstrated that neuroinflammation, neurotransmission, synaptic plasticity (long-term potentiation), and glial cell development (astrocyte differentiation and glia cell differentiation) are tightly interconnected GO biological processes **(Fig.1D)**. In contrast, target mining found 88 brain-enriched miRNA targets in the temporal brain region **(Supplementary table 2)**. The enrichment analysis of these targets indicated comparable pathways associated to the frontal brain area. Neuroinflammation, synaptic plasticity (long-term potentiation), glial cell development (astrocyte differentiation and glia cell differentiation), and histone phosphorylation are tightly interconnected GO biological processes **(Fig.1E).**

Next, we investigated deregulated miRNAs shared by the frontal and temporal areas of the brain. This study identified two miRNAs, miR-129-5p and miR-212-5p **(Fig.1F)**. To investigate the cell type-specific enrichment of these two miRNAs, we performed a thorough investigation of their expression in several brain cell types. We identified that miR-129-5p expression is enriched in neurons, whereas miR-212-5p expression is enriched in microglia **(Fig.1G)**. Due to the fact that the diagnosis of the FTD spectrum of diseases is reliant on clinical symptoms, there is a high rate of misdiagnoses, with a number of patients presenting with an FTD clinical syndrome being discovered to have Alzheimer's disease (AD) upon autopsy. To investigate the role of miRNAs capable of differentiating FTD from AD, we examined whether these two miRNAs are dysregulated in AD using our in-house dataset of post-mortem brain tissues from AD patients and healthy controls. In addition, we conducted a literature search to determine whether or not the deregulation of these miRNAs is associated with AD. Our miRNAome data on the post-mortem brains of AD patients, as well as a literature review, revealed that miR-212-5p is dysfunctional in AD. miR-129-5p was not shown to be dysregulated in AD. Since disruption of miR-212-5p was observed in AD, we focused on the potential role of miR-129-5p in the synaptic disorder FTD.

Knockdown of miR-129-5p elevates neuroinflammation and is associated with microglia mediated astrocyte activation.

To investigate the activity of miR-129-5p in *vitro*, we transfected mouse primary neurons with antisense oligonucleotides encoded by locked nucleic acid (LNA). We evaluated the miR-129-5p levels 48 hours after transfecting neurons with an inhibitor targeting miR-129-5p or a nontargeting scrambled sequence (as negative control). After transfection, knockdown of miR-129-5p was evident **(Fig.2A)**. To investigate further the effect of miR-129-5p KD on the transcriptome, RNA sequencing was performed on cultured neurons from both groups. Analysis of gene expression revealed 333 deregulated genes (adj.p-value<0.05, log2foldchange=+-0.4, basemean>=50), 155 up-regulated and 188 down-regulated **(Fig.2B)**. Neuroimmune response, active type I

interferon (IFN) signature, and astrocyte development and proliferation were overrepresented in the gene ontology (GO) analysis of up-regulated genes. GO analysis of down-regulated genes revealed associations with cell polarity, oligodendrocyte development, and neural precursor cell proliferation **(Fig.2C)**. A previous study demonstrated that silencing miR-129-5p increased the expression of proinflammatory cytokines (ref). We observed that inhibiting miR-129-5p increased neuroinflammation and downregulated cell polarity-related activities. Neuroinflammation plays a role in altering synaptic plasticity, as established by previous scientific investigations. Thus, we decided to investigate the expression of critical synaptic plasticity and inflammatory markers in primary neuronal cultures following miR-129-5p knockdown. qPCR confirmation of synaptic and inflammatory marker expression status in miR-129-5p treated neurons *vs* scrambled sequence treated neurons is depicted in **Fig.2D**. As illustrated in **Fig.2D**, miR-129-5p silencing inhibits the expression of genes involved in synaptic plasticity while increasing the expression of genes involved in inflammation. These results motivated us to investigate synaptic morphology as a result of the downregulation of miR-129-5p. LNPs containing anti-miR-129-5p and a scrambled sequence were administered to primary cortical neurons. After 48 hours of incubation, cells were examined for dendritic spine density and mature synapses. For the measurement of dendritic spines, Dil staining was used **(Fig.2E)**. Quantitative analysis revealed that miR-129-5p inhibitor treated neurons had significantly lower spine density compared to scrambled control neurons.

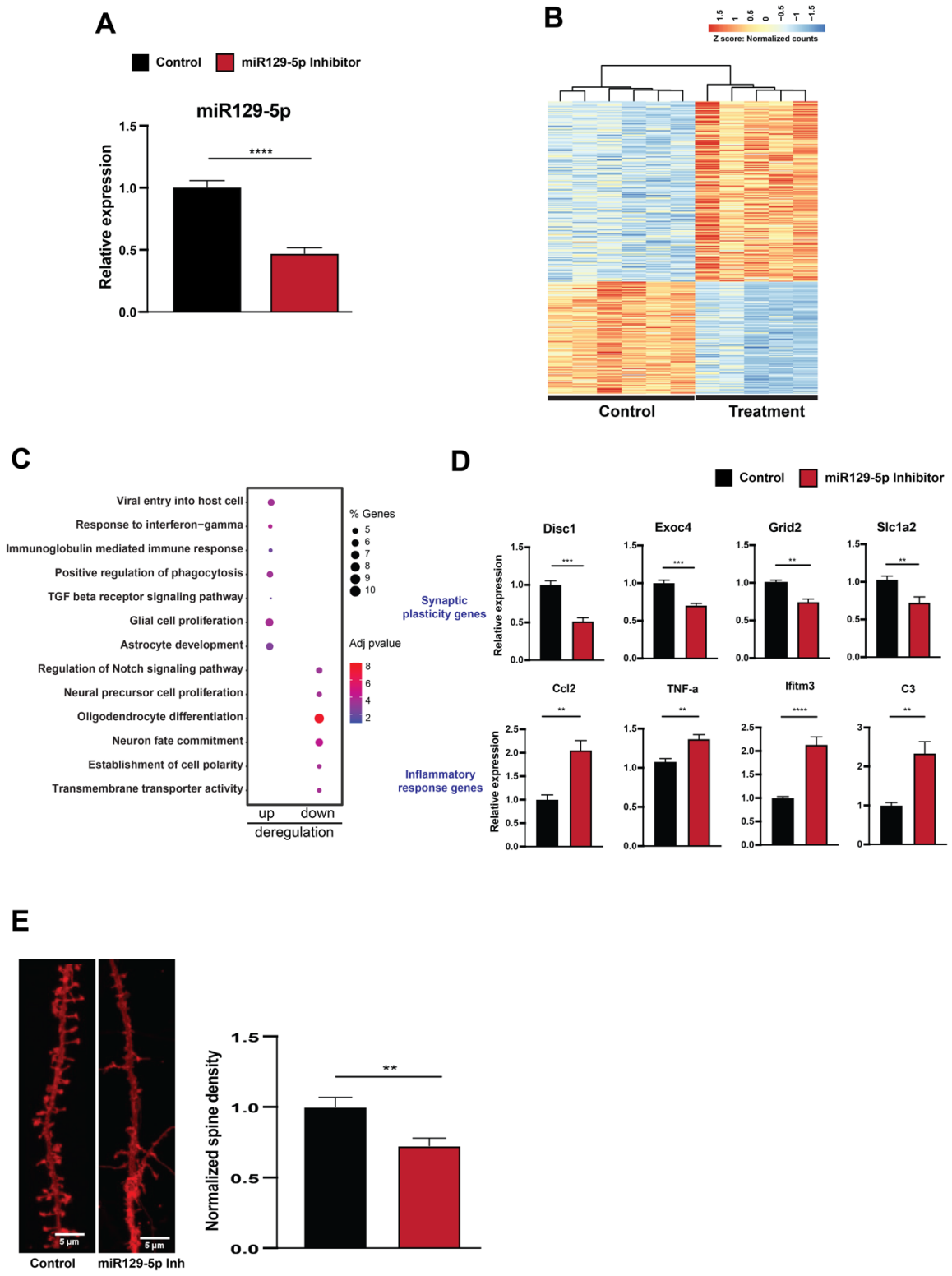


Fig.2 miR-129-5p silencing enhances neuroinflammatory responses. (A) qPCR analysis of mmu-miR-129-5p expression in primary neuronal cultures. Preparation of lipid nanoparticles using a scrambled sequence (as negative control) and a mmu-miR-129-5p inhibitor sequence. At DIV10, primary neurons were cultured and treated with a scrambled sequence and an inhibitor of mmu-miR-129-5p. After 48 hours of transfection, cells were harvested, RNA was extracted, and qPCR was performed. The relative expression of miR-129-5p compared to scramble was calculated. The expression of miR-129-5p was normalized to U6 expression. *P<0.05 based on a

one-sample, two-tailed t-test compared to the control conditions. (n=6 in each group) **(B-C)** A heatmap illustrates gene expression analyses in primary neurons with low miR-129-5p levels. The color intensity shows the status of the expression. Increased expression is marked by red color, whereas blue represents decreased expression. A dot plot represents the functional analysis of dysregulated genes in primary neuronal cultures. Significant expression levels are color-coded, whereas the proportion of genes associated with enhanced biological activities is size-coded. The analysis included genes with a basemean>=100, log2foldchange+-0.5, and an adj.p-value 0.05. ClueGO, a plugin for cytoscape, was used for GO enrichment analysis. For these genes, statistically significant GO enrichment terms were identified. The significant GO terms pertaining to brain functioning were filtered based on earlier curation of GO annotation pertinent to dementia research. GO semantic similarity was used to integrate comparable GO phrases into clusters, and the parent GO term was highlighted for visualization. **(D)** A qPCR experiment for multiple synaptic genes (Disc1, Exoc4, Grid2, and Slc1a2) and inflammatory response genes (Ccl2, TNF, Ifitm3, and C3) reveals that silencing miR-129-5p decreases synaptic plasticity (n=6, one-sample, two-tailed t-test). Informational data: *P<0.05, **P<0.01, ***P<0.001 The bars and error bars reflect the mean and standard error of the mean. **(E)** Representative confocal pictures of dendrites from neurons treated with miR-129-5p inhibitor or scramble control. Spine density of neurons treated with an inhibitor of miR-129-5p compared to control neurons. Random dendritic segments were used for images. The y-axis represents the total number of spines per unit length of a selected dendritic segment. Number of examined dendritic segments: control siRNA=31, miR-129-5p inhibitor=32. Unpaired two-tailed t test. The error bars represent the mean±SEM.

Inhibition of miR-129-5p in the mouse hippocampus induces cognitive impairment.

To test further detail in memory based on the role of miR-129-5p on neuronal function, we injected miR-129-5p inhibitor into the hippocampus of 3-month-old male mice. A series of behavioural tests were used to analyze the changes in learning memory and cognitive abilities of the mice after the surgery. Through the OF, mice with lower levels of mmu-miR129-5p exhibited no differences in locomotion as compared to control mice **(Fig.3A)**. However, the inhibitor group spent more time in the middle area and open arm during the OF and EPM tests, indicating decreased level of mmu-miR129-5p didn't influence animal general activity levels but significantly reduced the explorative in mice, a performance that defies nature is reflected anxiety-like behavioural phenotypes **(Fig.3B-C).** To identify the effect of regulating miR-129-5p on cognition, we further applied the MWM test to examine the hippocampus-dependent spatial

learning memory ability of mice. Animals were trained to locate a hidden platform in the water maze using distinct visual signals of varied colors and forms throughout the training phase. Our data clearly showed that the knockdown group spent more time searching the target quadrant than the control group from the first training day to the end **(Fig.3D)**. The Probe test further revealed a significant reduction in the relative time taken by the knockdown group to traverse the target quadrant at the end of training **(Fig.3E)**. Together, our behavioural data provide sufficient evidence that low levels of miR-129-5p lead to hippocampus-dependent learning memory impairment, which manifests as cognitive dysfunction. To this end, we analyzed and evaluated cognitive function in mice using a hippocampus-independent search strategy. The terms "direct," "corrected," and etc, imply a higher cognitive challenge. It is worth noting that mice with low miR-129-5p expression did not tend to make such advanced choices, but rather performed poorer cognitive strategies, such as passivity or randomness, more often **(Fig.3F)**. Therefore, we scored subjects based on their different behaviours and found that mice with low miR-129-5p expression had significantly impaired cognitive function **(Fig.3G-H)**. Overall, the expression level of miR-129-5p was positively correlated with cognitive performance.

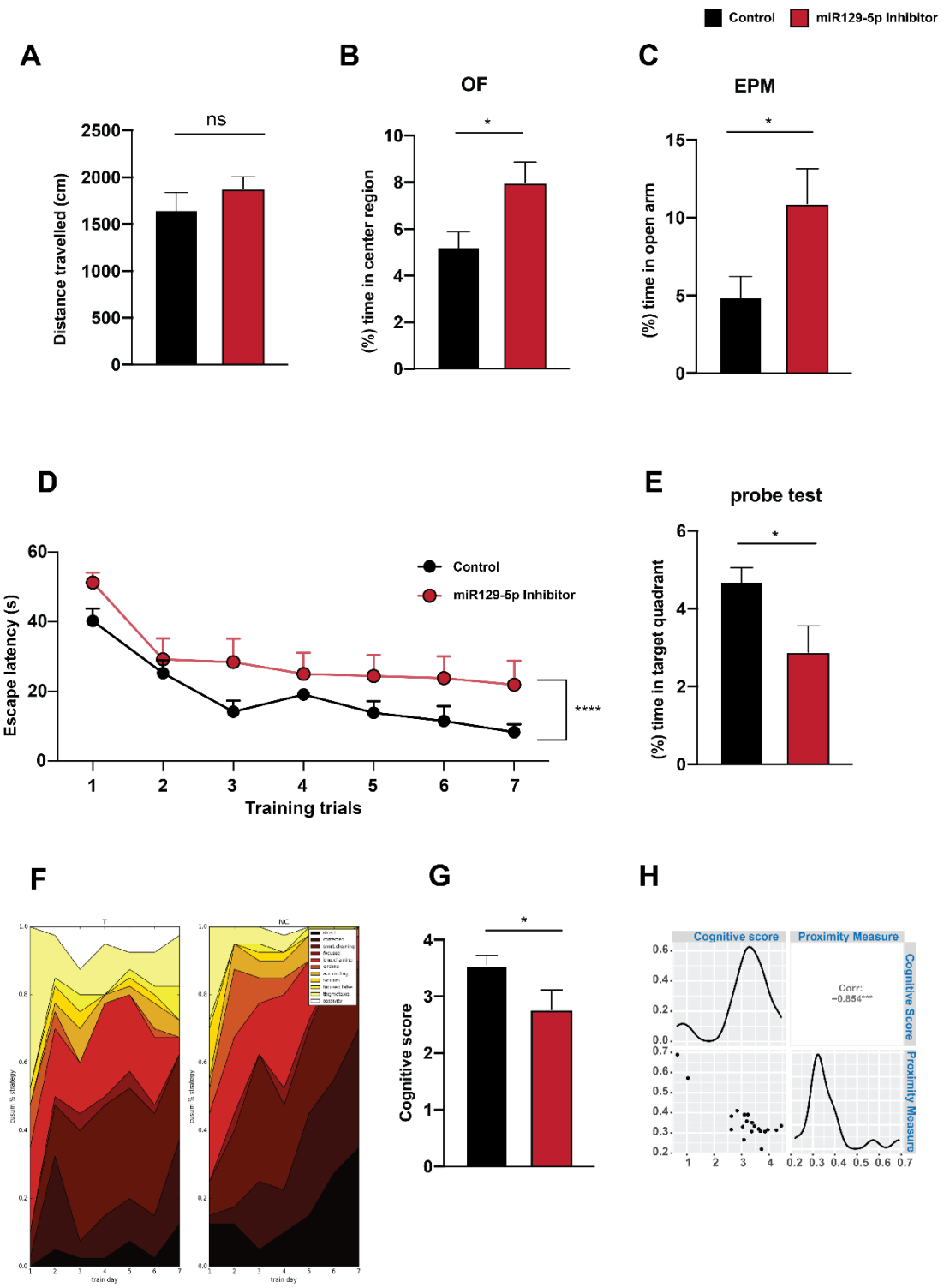


Fig.3 miR-129-5p silencing in mice leads to cognitive impairment.

(A) Mice with decrease level of mmu-miR129-5p do not show difference in locomotion compared with control group (*P<0.05, unpaired Student's t test). **(B)** Mice with decrease level of mmu-miR129-5p show reduced explorative behavior (*P<0.05, unpaired Student's t test). **(C)** Mice with decrease level of mmu-miR129-5p show anxiety like phenotype (*P<0.05, unpaired Student's t test). **(D)** Escape latency during the 7 days of training. Two-way ANOVA revealed a significant difference amongst groups (P=0.0001, F=7.7). **(E)** The time spent in the target quadrant during a probe test

performed after 7 days of training was significantly decreased (19.86±3.14; mean±SEM) in the mice with decrease expression of miR-129-5p (n=10/group, unpaired t test, parametric, two-tailed, *P<0.05). (**F)** Analysis of the different search strategies during the water training sessions. Note that especially the mice with low expression of miR-129-5p adopt hippocampal independent search strategies indicative of impaired cognitive function. (**G-H)** The cognitive score calculated for each day on the basis of hippocampal-dependent strategies. Cognition was significantly impaired when comparing mice with low expression of miR-129-5p to their performance with control group. Data are normalized to control group (n=10/group, unpaired t test, parametric, two-tailed, *P<0.05).

Discussion

Frontotemporal dementia (FTD) is a form of early-onset which has become the second most common cause of dementia after Alzheimer's disease (AD)[18–20]. Researchers have discovered that FTD is a highly heritable disease caused by an interplay between genetic and environmental aspects in recent years[21,22]. However, FTD is difficult to be diagnosed accurately and cured because they have similar genetic origins or overlapping pathological mechanisms to other atopic neurodegenerative diseases (e.g., AD, ALS)[23]. As a result, a single, low-cost diagnostic marker is urgently needed to confirm or rule out FTD and so intervene early in the disease process. Notably, miRNAs have been shown to be dysregulated in a variety of neurodegenerative disorders in latest years, including AD, Parkinson's disease, ALS, and Huntington's disease. Similarly, they also have an essential role in FTD[24–30]. Although these studies have identified many miRNAs associated with FTD, they do not provide potential targets that can be used to differentiate patients with FTD from others with dementia.

To this end, we collected post-mortem brain tissue from FTD patients, AD patients, and non-demented patients at the brain bank. The FTD group had samples carrying three mutations in the MAPT, GRN, and C9orf72 genes. We subsequently performed an in-depth small RNA group sequencing analysis of two different regions including frontal and temporal lobes. Our data showed the average age of FTD onset was lower than other neurodegenerative diseases, revealing and validating its early-onset signature[21,31,32]. Meanwhile, we observed the occurrence of MAPT and GRN mutations was not affected by gender, but the proportion of women with C9orf72 mutations was significantly higher than the men **(Fig.1A-C)**. In addition, we examined the expression of frontal and temporal lobe dysregulated miRNAs in patients with the three mutations to controls to see how similar or different they appeared. We found 30 miRNAs in the frontal region that was significantly expressed in all mutation groups, and 5 in the temporal region. This is highly consistent with the overlapping nature of the phenotypes resulting from the three mutations described above. In parallel, we provided all

commonly dysregulated miRNAs in FTD **(Supplementary Table 1)** and looked for targeted dysregulated microRNAs independently in frontal and temporal brain regions. Through miRTarbase database matching, we revealed over 270 dysregulated miRNA targets in the frontal brain, and enrichment analysis revealed that these targets are strongly associated with neuroinflammation, synaptic plasticity, and glial cell development **(Fig.1D)**. Similarly, **Supplementary table 2** shows that temporal brain regions are enriched for brain miRNA targets and that these targets are enriched in pathways that communicate with the frontal lobes **(Fig.1E)**. Among these targets, we identified miR-129-5p and miR-212-5p shared in the frontal and temporal lobes **(Fig.1F)**. As the brain contains many different cell types, we investigated two cell types that were specifically enriched for miRNAs. Interestingly, our analysis showed miR-129-5p was enriched in neurons, whereas miR-212-5p was more frequently expressed in microglia **(Fig.1G)**. And this result has important implications for the in *vitro* validation of different microRNAs.

This study was designed to explore particular biomarkers of FTD to distinguish AD. Therefore, we examined whether these two miRNAs were dysregulated in AD using our in-house dataset. In combination with the available literature, we found that miR-212-5p was dysfunctional in AD[33,34]. However, miR-129-5p was not. Therefore, miR-129-5p is the best candidate for a potential role in FTD.

According to our analysis, miR-129-5p is enriched in neurons, so we knocked down its expression in neurons in *vitro* in order to investigate the effect of miR-129-5p on the transcriptome, and our results revealed a total of 333 dysregulated genes in the KD group **(Fig.2B)**. Related literature reports that silencing miR-129-5p increases the expression of pro-inflammatory cytokines[35,36]. It is consistent with our results that upregulated genes are mainly involved in neuroimmune responses, while downregulated genes are associated with oligodendrocyte development, among others **(Fig. 2C)**. It is known that neuroinflammation can alter synaptic plasticity[37–39]. And

synaptic plasticity is critical to the connectivity and cognitive ability of neural network function[40–42]. Thus, we examined the expression of synaptic plasticity and inflammatory markers in the miR-129-5p KD group. The results showed that low levels of miR-129-5p increased the expression of inflammatory genes while suppressing synaptic plasticity genes **(Fig.2D).** Additionally, we visualized and quantified dendritic spine morphology and our data showed a significant reduction in spine density in neurons treated with miR-129-5p inhibitors compared to controls **(Fig.2E)**, demonstrating that low levels of miR-129-5p can trigger inflammatory responses and have deleterious effects on neuronal synapses.

Based on the in *vitro* data, we performed the surgery on mice to test the role of miR-129-5p in memory. Interestingly, silencing miR-129-5p not only increased the anxiety phenotype of the subject mice, but also reduced their spatial learning memory capacity, leading to cognitive impairment **(Fig.3)**. Overall, the expression level of miR-129-5p was positively correlated with cognitive performance.

In summary, we screened FTD biomarker miR-129-5p by analysis and provided the mechanistic functions of its major cell types, reflecting the molecular changes that occur during pathology, revealing the association between silent miR-129-5p and cognitive impairment. In the future. miR-129-5p has the potential to become a powerful indicator of FTD pathogenesis.

Materials and Methods

High-throughput sequencing of smallRNAomes

The NEBNext® small RNA library preparation kit was employed to generate smallRNAome libraries from total RNA in accordance with the manufacturer guidelines. All smallRNAome libraries for human subjects were generated using 150 ng of total RNA. In brief, total RNA was employed as the initial material. The first strand of cDNA was synthesized, then amplified using PCR. The libraries were pooled and PAGE was run to determine the optimal size. For library purification and quantification, a 150-bp RNAome band was extracted and used. Two nanomolar libraries were used for sequencing. Sequencing was performed on Illumina HiSeq 2000. A 50-bp single-read setup was utilized for the sequencing. Demultiplexing was performed with Illumina CASAVA 1.8. Adapters for sequencing were removed using cutadapt-1.8.1. FastQC was used to evaluate the quality of sequence data (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The total number of reads, percentage of GC content, N content per base, sequence length distribution, duplication levels, overrepresented sequences, and Kmer content were used to estimate the sequencing quality.

Processing and quality control

The miRdeep2 package was used to align high-quality reads to the Homo sapiens. GRCh38.p10 genome assembly (hg38) (https://www.mdc-berlin.de/content/mirdeep2-documentation). The UCSC Genomic Browser provided access to genome sequences (https://genome.ucsc.edu/). The Bowtie-build tool (version 1.12) was used with the default option to map reads. For mapping and generating raw counts for miRNAs, the MirDeep2 package's Perl-based scripts were employed. The mapper.pl script was utilized to map reads to HG38. We removed reads with fewer than 18 nucleotides. These reads were then employed to quantify known miRNAs. Quantifier.pl from the miRDeep2 package was used to quantify known miRNAs.

Differential expression (DE)

We used raw counts for DE analysis. Before DE analysis, the raw read counts were log2-transformed and normalized for library size. Each sample was assigned a quality z-score, and samples of low quality (Z>2.5) were considered outliers and excluded from further analysis. Read counts of 5 in at least 50% of the studied samples were utilized for further DE analysis. The RUVSeq package6 was used to account for hidden batch effects and reduce unnecessary variation. Age and gender corrections have been made to the data. Using DESeq27, differential expression analysis was performed. MiRNAs with a basemean larger than 100, fold changes greater than or equal to 30 percent, and an adjusted p value of 0.05 was considered differentially expressed.

WGCNA analysis

Weighted gene co-expression network analysis (WGCNA) tool (version 1.61) was used to analyze the miRNAome co-expression module. We first removed age, gender, and other latent variables from the sequencing data using regression analysis. The normalized count values were then log (base 2) transformed. Next, pair-wise bi-weighted midcorrelations between miRNAs were calculated using the modified data. In order to develop a signed miRNA network, a soft threshold power of 8 was selected based on approximate scale-free topology and used to determine pair-wise topological overlap between miRNAs. Using the cutreeDynamic function with the parameters method="hybrid", deepSplit=3, pamRespectsDendro=T, and pamStage=T, modules of co-expressed miRNAs with a minimum module size of 20 were subsequently discovered. With a dissimilarity correlation criterion of 0.15, closely related modules were combined. Different modules were summarized as networks of modular eigengenes (MEs), The module membership (MM) of miRNAs was defined as the correlation between miRNA expression profiles and MEs, and a correlation coefficient threshold of 0.60 was established to identify module-specific miRNAs.

Gene ontology enrichment and pathway analysis

To build the Gene Regulatory network (GRN) for miRNA-target genes, validated miRNA targets were extracted from miRTarBase (v 7.0) (http://mirtarbase.mbc.nctu.edu.tw). The microRNA target genes were further selected on the basis of their brain expression. Cytoscape 3.2.1's ClueGO v2.2.5 plugin was utilized to determine the biological processes and pathways within the miRNA-target genes. ClueGo plugin10 calculated the significance of each term using a two-sided hypergeometric test and Benjamini-Hochberg for P-value adjustment. The pathway study utilized the KEGG (https://www.genome.jp/kegg/) and Reactome (https://reactome.org/) databases. Cytoscape 3.2.1's ClueGO v2.2.5 plugin was utilized to generate GRN for substantially deregulated mRNAs. Those biological processes and pathways having a 0.05-adjusted p value were chosen for further evaluation.

Quantitative PCR

The miScript II RT Kit (Qiagen, Germany) was used to synthesize cDNA according to the manufacturer's instructions. Briefly, for cDNA synthesis, 200 ng of total RNA were employed. The same cDNA was used so that it could be used for quantitative PCR analysis of both mRNA and miRNA (qPCR). For quantification, a miRNA-specific forward primer and a universal reverse primer were used. As a control, the U6 small nuclear RNA gene was used. Gene-specific forward and reverse primers were employed to quantify mRNA. The relative quantities of mRNA were normalized against GAPDH. The $2^{-\Delta\Delta Ct}$ technique was used to calculate the fold change for each miRNA and mRNA. We used Light Cycler® 480 Real-Time PCR System (Roche, Germany) to perform the qPCR.

Preparation of microRNA and mRNA lipid nanoparticles

miR-129-5p inhibitor sequences were used to inhibit miR-129-5p expression. Antisense oligos (ASO), inhibitors, and negative control sequences were purchased from Qiagen. Using a proprietary blend of lipids comprising an ionizable cationic lipid, the Neuro9™ siRNA Spark™ Kit was able to produce a lipid nanoparticle (LNP) formulation for

miRNA inhibitors or ASOs (5 nmol). On the NanoAssemblr^{TM} Spark^{TM} technology, miRNA inhibitors or ASOs were encapsulated utilizing a microfluidic device for controlled mixing conditions (Precision Nanosystems, Canada). The experiment was performed according to the methodology provided by the manufacturer. For instance, 5 nmol of lyophilized miRNA inhibitors or ASOs were reconstituted in formulation buffer 1 (FB1) to provide a final concentration of 2 nmol. This solution was diluted further to a concentration of 930 μg/mL. Using the NanoAssembler Spark system, formulation buffer 2 (FB 2), miRNA inhibitor/ASOs in formulation buffer 1 (FB1), and lipid nanoparticles were added to the cartridge and encapsulated.

Primary neuronal culture

Primary neuronal cultures were prepared from E17 pregnant mouse of CD1 background (Janvier Labs, France). Mice were sacrificed by cervical dislocation, and the brains, meninges, and cortex of embryos were removed and dissected. The cortex is rinsed with PBS (Pan Biotech, Germany). After incubation with trypsin and DNase and subsequent disintegration, single-cell suspensions were produced. On poly-D-lysine-coated 24-well plates containing Neurobasal media (Thermo Fisher Scientific, Germany) supplemented with B-27, 130000 cells were plated per well (Thermo Fisher Scientific, Germany). At DIV10-12, primary cortical neurons were employed in experiments.

Dendritic spine labelling

Primary cortical neurons and primary neurons with astrocyte co-culture were prepared and fixed with 2% PFA. Dendritic spines were labeled as described. Briefly, cells were aspirated and 2-3 crystals of Dil dye (Life Technologies-Molecular Probes) were added to each culture well, followed by a 10-minute shaker incubation at room temperature. The cells were rinsed with PBS until no visible crystals remained, then incubated at room temperature overnight. The cells were cleaned and mounted with mowiol the following day. In order to get images with a high level of magnification, a multicolor confocal STED microscope equipped with a 60-oil objective was used. ImageJ was

utilized to measure spine density and total spine length.

Animal treatments and sample collection

All mice had a genetic background of C57BL/6J. Janvier Labs was the supplier of mice. Mice were housed in the animal facility under standardized conditions, with one mouse per cage, a 12-hour light-dark cycle, a constant temperature (23°C), and free access to food and water. Experiments on mice were conducted in accordance with applicable ethical rules and with the approval of the local ethics committee. All experiments were conducted using male mice aged 3 months. For RNA-seq experiments, the CA1 area was dissected five days after stereotaxic surgery.

Stereotactic surgical procedure

As previously stated, intracerebral stereotaxic injections of the brain were administered. For stereotaxic injections of LNPs into the CA1 region, 3-month-old mice were sedated with ketamine and xylazine prior to the drilling of two tiny holes in the skull. These coordinates were utilized to create holes: 1.75 mm posterior bregma, ±1 mm lateral, and 1.5 mm dorsoventral. LNPs of microRNA inhibitor/negative control were injected bilaterally into mice (0.1 μg/mL for microRNA inhibitor/negative control). Per side, LNPs were injected at a rate of 0.3 μL /min. Only 1.5 μL of LNPs were administered through injection. After surgery, all mice were monitored until complete anesthetic recovery and maintained in uniform settings until full recovery. On day five, behavior experiments were undertaken.

Behavioral phenotyping

All the behavior experiments were done as described before. The locomotory and exploratory functions were assessed by an open-field test. Individual mice were placed in the center of an open arena (length of 1 m, width of 1 m, and side walls were 20 cm high). Five minutes of locomotive movement were captured using the VideoMot2 tracking system (TSE Systems). The elevated plus maze was used to assess baseline

anxiety. Individual mice were placed in the center of a plastic box with two open and two closed arms (10x40 cm each; the walls were 40 cm high). The VideoMot2 equipment was used to record the behavior of mice for 5 minutes. To assess the anxiety phenotype, the time spent in open versus closed arms was assessed.

RNA sequencing

Total RNA was utilized to prepare the library using the TrueSeq RNA library prep kit v2 (Illumina, USA) per the manufacturer guidelines. As a starting material, 500 ng of RNA was used. The Bioanalyzer was utilized to evaluate the library's quality (Agilent Technologies). The QubitTM dsDNA HS Assay Kit was used to determine the library concentration (Thermo Fisher Scientific, USA). Multiplexed libraries were loaded directly onto a Hiseq2000 (Ilumina) using a 50 bp single read setup. Illumina CASAVA 1.8 was used to do demultiplexing. Adapters for sequencing were removed using cutadapt-1.8.1.

Publicly available datasets

This study uses various publically available datasets to investigate the cell type-specific expression of differentially expressed genes. Gene expression specific to neurons, astrocytes, and microglia were investigated using published single-cell data.

Brain smallRNAome data from Alzheimer's patients

The Harvard brain repository provided snap-frozen, age- and gender-matched post-mortem human brain tissue from 24 AD patients and 24 old controls. These were obtained from the Brodmann region BA9. The Harvard University Ethics Committees approved the use of human tissues in our research. As shown above, RNA isolation and smallRNA sequencing were done.

Reproducibility and statistics

The statistical analysis was performed using GraphPad Prism version 8.0. The statistical

measurement is presented as a mean standard error of the mean. Each n denotes a biological sample. The data were analyzed using either a two-tailed unpaired t-test or a two-way ANOVA with Tukey's post hoc test. Analyses of enriched gene ontology and pathways were performed using Fisher's exact test and Benjamini-Hochberg correction.

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Author contribution

JZ performed behavior and cell culture experiments, analyzed behavior data, and drafted the paper. LK performed behavior experiments, analyzed and interpreted RNA-seq data, designed all experiments, supervised JZ, and drafted the paper. SB provided technical support. AM provided bioinformatics support. RP, MRI, SS provided technical support. PH provided samples. FS designed and supervised the study, drafted, and revised the final version of the manuscript. AF arranged funding, designed, and supervised the study, drafted, and revised the final version of the manuscript.

Competing Interests

The authors state that there is no conflict of interest.

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Supplementary

Supplement table 1: Target genes for the frontal brain microRNAs that are dysregulated.

Target Gene	brain_expression	Target Gene	brain_expression
PSAP	269.25	MCL1	25.75
HSP90AA1	248.5	FGFR3	25.75
ATP5B	186.375	SRSF11	25.25
UBC	175.375	PAK1	25.25
APP	145.875	SKI	25.125
LDHB	141.625	SLC2A1	24.875
ATP1B1	139.625	RAB14	24.375
BASP1	101.25	FSCN1	24.25
RHOB	94.875	KHSRP	24
RAC1	83.375	MXI1	24
PKM	80.625	PRDX3	23.375
RHOA	79.75	MT1M	23.375
DPYSL2	75.5	STX16	23.25
PGRMC1	66.125	TNK2	21.5
STMN1	63.875	WNK1	21.375
HNRNPDL	56.75	EWSR1	20.5
ST13	48	LRRC8A	20.25
RNASE1	46.375	PIN1	20.25
NEFM	45.75	CDC34	19.875
PRDX6	44.875	SGSM3	19.875
ABCA2	43.125	FOS	19.75
H3F3A	42.625	MAPK1	19.75
NDFIP1	41.25	SMARCB1	19.625
VIM	37	HMGA1	19
TF	36.25	CD47	18.75
DUSP1	35.25	BCL6	18.625
ARHGDIA	35	EIF2S3	18.375
CTNNB1	34.75	AZIN1	18.25
KAT2A	33.625	TUSC2	17.875
HMGB1	33	RAB5A	17.25
LDHA	32.5	ZFP36L1	17.125
CDKN1B	32.375	MSMO1	16.875
MAP4K4	29.5	PRPS1	16.5
CTDSP2	27.875	ARRB2	16.375
GRB2	26.875	RAP2A	16.125
ZBTB4	26.625	KLF13	15.75
HRAS	26	PARP1	15.625

Target Gene	brain_expression	Target Gene	brain_expression
CDK9	25.875	STAT5B	15.375
MEF2D	15.25	FOPNL	10
ADIPOR2	15	TJP1	10
SERP1	14.875	SRF	10
NCAN	14.875	ARIH2	9.875
ITGB1	14.5	FBXW7	9.875
TOP1	14.5	PPP1R10	9.5
INSIG1	14.375	SNX19	9.375
KAT2B	14.25	ERCC3	9.375
PCNA	14.25	ACADM	9.375
ABCC5	14.125	PTPRF	9.375
APC	14.125	STAT1	9.375
H2AFX	13.875	ZEB2	9.25
HIF1A	13.875	CBX5	9.125
SOX9	13.75	STAT3	9.125
SRCIN1	13.625	NF2	8.875
AMFR	12.875	DEDD	8.875
RAB40C	12.25	IMPDH1	8.875
MAPK4	12.25	PTEN	8.625
BACE1	12.125	SLC20A1	8.625
MLEC	12.125	TFCP2	8.625
SNHG1	11.875	PIK3R3	8.625
MCRS1	11.625	EGR1	8.5
MAP3K5	11.5	KCNH2	8.5
SH3PXD2A	11.375	ACVR1B	8.375
SNX6	11.375	EP300	8.375
MTUS1	11.375	CORO1A	8.375
FURIN	11.25	MECP2	8.25
PRKCA	11.25	PRPF38A	8.25
NCSTN	11.125	CCND2	8.25
BMI1	11.125	RAB13	8.25
GRSF1	11.125	ZNF513	8.125
ALCAM	10.875	SMARCA5	8.125
NDST1	10.75	ZEB1	7.875
NLK	10.625	VPS4B	7.875
HIPK1	10.5	BCAR1	7.875
CTNND1	10.5	PHLPP1	7.75
CAMTA1	10.375	MEN1	7.75
CNDP2	10.125	BAG5	7.75
TMED7	10.125	ARID1A	7.625
ITGAV	10	XBP1	7.625
FNDC3A	7.625	KMT5A	6.25

Target Gene	brain_expression	Target Gene	brain_expression
SLC16A1	7.5	MTOR	6.25
PRMT5	7.5	SSSCA1	6.25
ZMPSTE24	7.5	UBE2F	6.125
FMR1	7.5	PIK3CB	6.125
GADD45A	7.5	KLF6	6
MARK2	7.5	WWP1	6
PDIA6	7.5	NRAS	5.875
PMEPA1	7.5	FEN1	5.75
ATG4D	7.25	LMNB2	5.75
CNST	7.25	MAP3K9	5.75
MLH1	7.25	SFRP1	5.75
PDPK1	7.25	SP1	5.75
BMPR2	7.125	TRIM11	5.75
HIPK3	7.125	KRAS	5.625
MBD6	7.125	LMNB2	5.75
CDKN1A	7.125	MAP3K9	5.75
PDGFRB	7.125	SFRP1	5.75
TOM1	7	SP1	5.75
COPS5	7	TRIM11	5.75
VHL	7	KRAS	5.625
ABCG2	6.875	RAVER2	5.25
CASP3	6.75	AGO4	5.25
NOB1	6.75	DICER1	5.25
HDAC1	6.75	SATB1	5.25
AKT1	6.75	CD34	5.25
P2RX7	6.625	TTC9C	5.25
FAF1	6.625	CCND1	5
MAPK14	6.375	TGFBR2	5
CEBPB	6.375	PRKAA1	5
DHFR	6.25		

Supplement table 2: Target mining found 88 brain-enriched microRNA targets in the temporal brain region.

Target Gene	brain_expression	Target Gene	brain_expression
MALAT1	68.5	FGF2	4.375
VIM	37	MAF	4.375
HMGB1	33	PDCD2	4.125
SLC2A1	24.875	ZNRF3	4
MAPK1	19.75	PDGFRA	3.9875
HNRNPD	16.75	NOTCH1	3.5
ABCC5	14.125	ERBB4	3.25
APC	14.125	FOXO1	3.125
CRK	13.875	ABCG1	3
RAF1	13	TLR4	2.875
YY1AP1	12.25	NFKB1	2.75
MCRS1	11.625	CCNB1	2.375
IRAK1	10.75	SOX4	2.25
CAMTA1	10.375	TRAF6	2.25
TJP1	10	EGFR	2.2375
NOVA1	9.875	ZNF117	2.125
MYO6	9.625	SPRY1	2
CCDC6	8.875	SMAD2	2
PIK3R3	8.625	RARB	1.9375
GSK3B	8.5	AGO3	1.875
RASA1	8.125	MMP16	1.6125
FNDC3A	7.625	CCNA2	1.6125
FMR1	7.5	IRAK4	1.5
PDPK1	7.25	ETV6	1.4875
CDKN1A	7.125	CDK6	1.225
BMPR2	7.125	S100A12	1.175
SPRED1	7.125	RET	1.1375
TJAP1	6.75	SOX5	1.1
UBE2F	6.125	BDNF	0.92857143
ARHGAP32	6	AGO2	0.875
TLN2	5.75	KLHL11	0.825
SP1	5.75	IL1RAP	0.775
GALNT1	5.5	SOX6	0.65
KIT	5.375	COL1A1	0.575
BECN1	4.875	AURKA	0.525
SIRT1	4.75	SLC5A5	0.4875
RB1	4.75	UHRF1	0.4625
ABCB1	4.625	CDH1	0.425
HBEGF	4.5	WNT4	0.4125

Target Gene	brain_expression	Target Gene	brain_expression
NR4A2	4.375	IL1RL2	0.4
IGF1	0.3875	PAX8	0.31666667
BDKRB2	0.35	MMP9	0.2125
IL6	0.325	GDF5	0.125
TWIST1	0.325	IGF2BP3	0.1

Discussion

Dementia is a group of progressive neurodegenerative brain disorders with varying degrees of deficits in memory, language, attention, and communication. Although macroscopically dementia patients are usually characterized by reduced brain volume, molecular and metabolic changes can be traced back several years before the onset of the disease. The markers and cognitive tests that have been identified are only applicable to the diagnosis of advanced and severe memory loss. Meanwhile, the drugs that have been used in clinical trials to improve cognition in dementia patients in recent years have been unsuccessful. Thus, early prevention, timely diagnosis, accurate and effective treatment of dementia remain urgent scientific challenges that require multidisciplinary collaboration and synergy. The study of biomarkers has provided unique advantages in different areas of human disease (e.g., diagnosis of cancer), but the development of cognitively relevant markers to predict core clinical features has been much neglected in comparison. It is worth noting that cognitive markers serve as the foundation for disease diagnosis and therapy. As a result, screening for cognitive markers is an important component of the global strategy for dementia prevention development.

Therefore, in this thesis, I detailed two different types of dementia, late-onset AD, and early-onset FTD in separate sections, and explored the potential role of the histone demethylase KDM5B in cognitive impairment based on the genetics of AD and its molecular pathogenesis. Also, a specific miRNA biomarker in FTD disease was screened to clearly distinguish FTD from AD and to reveal its corresponding molecular mechanisms, thus reaching biological targets that provide potential therapeutic strategies for AD and FTD.

Firstly, AD is the most common type of dementia, accounting for more than two-thirds of the incidence of dementia. In the early stages of AD, the clinical symptoms overlap

with age-related normal aging. Both have varying degrees of cognitive decline as well as decreased learning and memory abilities. Our laboratory studies have shown that the histone modification H3K4me3 is required for memory maintenance in AD, and therefore, low levels of H3K4me3 may underlie cognitive deficits. Since H3K4me3 is regulated by demethylase, so we are interested in whether cognitive impairment can be reversed or improved by regulating the expression level of demethylase KDM5B. It is well known that neurons form neural circuits through connections between synapses and therefore dendritic spines are the main structures of brain connections. Patients with neurodegenerative diseases usually have a large loss of neuronal dendritic spines or severe morphological distortions in the brain. This means that dendritic spine and synaptic failure is the direct cause of the cognitive decline in AD. Interestingly, in the first study, we discovered that low levels of KDM5B altered the cell cycle and increased the dendritic spine density also improved synaptic plasticity. This implies that low levels of KDM5B can promote neurodevelopment and brain development. On the other hand, sequencing data showed an immediate downregulation of inflammatory response after knockdown of KDM5B, as one of the important pathological mechanisms that induce AD, the level of inflammation directly affects cognitive performance. These results were fully validated in our mouse models. In our aged wildtype mice (a model of normal aging), low levels of KDM5B not only significantly improved the mice's anxiety-like behaviors, but also improved their learning and memory abilities. Similarly, our 5xFAD transgenic mice that mimic AD-like cognitive deficits exhibited higher levels of cognition after the knockdown of KDM5B. This implies that H3K4me demethylases, represented by KDM5B, are negatively correlated with memory in the hippocampus and have a significant modulatory effect on both normal aging-induced cognitive decline and AD-like cognitive impairment, especially in learning and memory.

Apart from AD, FTD has become the second most common form of dementia. It is a highly genetically and environmentally susceptible disease with an early-onset, and it shares physiopathological mechanisms with AD, which leads to clinical misdiagnosis.

Therefore, screening for biomarkers that are sensitive and expressed only in FTD but do not significantly act on AD is important for the specific diagnosis of FTD. We compared FTD and AD with non-demented postmortem brain tissue by small RNA sequencing analysis. Three major mutations in genes responsible for the development of FTD were identified. MAPT and GRN mutations were gender-independent, in contrast, the C9orf72 mutation occurred significantly more frequently in females than in males. We also investigated the expression of dysregulated miRNAs in frontal and temporal lobes in subjects with the three mutations to see if there were any similarities or differences. We selected miR-129-5p, which is found in both the frontal and temporal lobes and is highly enriched in neurons, using publicly available smallRNAome database. Importantly, no study has yet proven a convincing association between miR-129-5p dysregulation and AD. This indicates it has the potential to be the best candidate for detecting FTD. We inhibited miR-129-5p expression to better understood the molecular alterations that occur during pathology and discovered that the upregulated genes were mostly inflammatory factors, whereas the developing capacity of glial cells was dramatically reduced. Our qPCR data verified this observation by validating classic inflammation and synaptic markers. Concurrently, we discovered that inhibiting miR-129-5p expression resulted in a considerable reduction in neuronal spine density by visualizing and quantifying dendritic spines, demonstrating the negative consequences on neuronal synapses after provoking an inflammatory response. More intuitive evidence comes from in *vivo* behavioral experiments. Silencing miR-129-5p not only triggered the anxiety phenotype of the tested mice, but also significantly reduced their learning and memory ability and induced the cognitive impairment phenotype, which further revealed the expression of miR-129-5p is positively related to cognitive ability.

In summary, this thesis contains two chapters that examine late-onset (Alzheimer's disease) and early-onset (Frontotemporal dementia) dementia based on epigenetics modifications, respectively. We revealed that silencing the H3K4 demethylase KDM5B

has significant effects in enhancing neural and brain development, reducing inflammation levels, and improving cognitive impairment, suggesting its potential as an RNA target for AD therapy. Meanwhile, we identified miR-129-5p as a specific gene to distinguish FTD from other neurodegenerative diseases (AD) through analytical screening. We also demonstrated that low levels of miR-129-5p can induce cognitive impairment by triggering inflammatory responses. All findings have implications for the screening, diagnosing, and treatment of different types of dementia to provide us the future directions.

List of Abbreviations

AD	Alzheimer's disease
Aß	Amyloid-ß plaque
ALS	Amyotrophic lateral sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APP	Amyloid precursor protei
bvFTD	Behavioral variant frontotemporal dementia
CA	Cornu Ammonis
CHMP2B	charged multivesicular body protein 2B
CREB	cAMP-responsive element-binding protein
C9orf72	Chromosome 9 open reading frame 72
COMPASS	Complex of Proteins Associated with Set1
DG	Dentate gyrus
DNMT	DNA methyltransferases
EPM	Elevated plus maze
EPSP	Postsynaptic potential
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
FUS	Fusion sarcoma
GRN	Progranulin
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HMD	Histone demethylase
HMT	Histone methyltransferase
H3K4me3	Histone H3 at lysine 4
H3K9me2	Histone H3 at lysine 9
KDM	Lysine demethylase
KMT	Lysine methyltransferase

lncRNAs	Long-noncoding RNAs
LTM	Long-term memory
LTP	Long-term potentiation
MAPT	Microtubule associated protein Tau
MCI	Mild cognitive impairment
miRNA	microRNAs
MWM	Morris water maze
ncRNA	Non-coding RNA
NDs	Neurodegenerative diseases
nfPPA	Nonfluent aphasia or semantic dementia
NMDA	N-methyl-D-aspartate receptors
OF	Open field
PSEN	Presenilin
ROS	Reactive oxygen species
SNCA	Alpha-synuclein
sRNAs	Small RNAs
siRNAs	Short interfering
STM	Short-term memory
svPPA	Semantic variant PPA
TDP-43	TAR DNA-binding protein 43

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Declaration

I herewith declare that I have written the dissertation
"Gene-expression control in early and late-onset dementia"
independently with no other aids or sources than quoted.

Jiayin Zhou; 30.05.2022.