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Neurodevelopmental disorders with epigenetic dysregulation and *Drosophila*

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TABLE OF CONTENTS

1. ABSTRACT

Recently, the mechanisms underlying epigenetic dysregulation associated with neurodevelopmental disorders have attracted increasing attention. Although most neurodevelopmental disorders in humans are multifaceted and encompass a wide range of symptoms, a small number of cases linked to specific single gene disruptions have been identified. The *Drosophila* genetic system provides excellent models for such diseases. This review will discuss recent advances in the study of human neurodevelopmental disorders associated with epigenetic dysregulation, particularly monogenic disorders established in relevant *Drosophila* models. Due to the vast range of genes affecting epigenetic dysregulation, we aim to provide a selective review of the disorders caused by aberrant histone modifications, with particular emphasis on enzymes regulating histone acetylation and methylation, in order to give the essential understanding of the nature of the neurodevelopmental disorders for rational therapeutic treatments.

2. INTRODUCTION

Epigenetic regulation can modulate biological functions with no alteration to the DNA sequence itself (1). Various types of mechanisms underlying epigenetic processes have been extensively studied

in recent decades, which are known to be distinct yet highly interrelated in function: DNA methylation, histone modification, noncoding RNA mechanisms, and chromatin (2, 3). It is well-accepted that these epigenetic alterations with consequent disruptions of normal signaling pathways might be important markers of status or progression in various human diseases (4). Such epigenetic dysregulation that triggers abnormal signaling cascades has been reported in the human brain as causing neurological problems (5).

Neurological disorders are characterized by cell loss accompanied by a reduction in cell numbers and consequent aberrant brain function, and have been dichotomized as neurodevelopmental and neurodegenerative disorders. Neurodevelopmental disorders are caused by abnormal brain development or damage at an early age, whereas neurodegenerative diseases arise due to the progressive loss of specific neuronal populations and involve age-related dysfunctions of neuronal maintenance over a lifetime (6). Neurodegenerative diseases are associated with the formation of cellular aggregates of toxic proteins, representative examples of which include Parkinson's disease and Alzheimer's disease (7). On the other hand, neurodevelopmental disorders include attention-deficit hyperactivity disorder, autism, learning disabilities, intellectual disability (ID; also known as mental retardation), conduct disorders, cerebral palsy, impairments in vision and hearing, and other developmental delays (8). Based on a survey conducted in 2006–2008, approximately 15% of children in the United States, aged 3 to 17 years, were affected by neurodevelopmental disorders (9). Most neurodevelopmental disorders are multifactorial and likely result from a combination of genetic and environmental risk factors, as is widely accepted for autism spectrum disorders (ASDs). However, some cases of neurodevelopmental disorders are associated with specific single gene disruptions, and such monogenic disorders are readily modeled for investigations of disease etiology leading to the logical development of therapeutic intervention strategies.

Drosophila is a model organism widely used as a tool for understanding many fundamental biological processes common to higher eukaryotes. Owing to complete sequencing and subsequent annotation of the *Drosophila* genome, the high degree of conservation in developmental processes between *Drosophila* and humans has been revealed (10). Moreover, about 77% of human disease-associated sequences in OMIM have been reported to show strong matches to sequences in the *Drosophila* sequence database (11).

In this review, we discuss monogenic disorders causing neurodevelopmental abnormalities via epigenetic dysregulation, particularly in histone acetylation and methylation, that are conserved between humans and *Drosophila* as shown in Figure 1. In the following sections, we briefly outline general epigenetic regulation mechanisms and describe *Drosophila* models for human neurodevelopmental disorders caused by two major histone modifications, acetylation and methylation, with demonstrative examples of recent discoveries in the fly, emphasizing how *Drosophila* have expanded our knowledge concerning human neurodevelopmental disorders.

3. GENERAL BASIS OF HISTONE ACETYLATION AND HISTONE METHYLATION

The alterations of histones constitute key epigenetic mechanisms in which epigenetic markers are deposited or reversibly removed by specific enzymes (known as "writers" and "erasers," respectively), and subsequently recognized by effector proteins ("readers"). The direct chemical modifications of histones by the three modifiers take place at the posttranslational level. The various posttranslational modifications (PTMs) in histone proteins can form a unique histone code, regulating gene activity at the transcriptional level by modifying DNA-histone interactions, which leads to structural changes and transcriptional activation or silencing (12, 13). Histone PTMs include acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, and citrullination via covalent modifications of specific residues primarily located at histone N-terminal tails (14–16). Among them, histone acetylation and methylation are the two major epigenetic modifications in terms of their roles in deciphering histone codes as well as gene regulation.

3.1. Histone acetylation

Protein acetylation occurs mainly on lysine residues (17). The four core histones, H2A, H2B, H3, and H4, contain numerous lysine residues that are accessible to acetylation. Initially, modification events in the unstructured N-terminal histone tail domains were extensively studied. Thus, histone acetylation was mostly observed in N-terminal tails at histone H2A lysine 5(H2AK5), H2BK12/K15, H3K9/K14/K18/K23/K36, and H4K5/K8/K12/K16, but later acetylation events at H3K56, H4K59, and H4K91 were also identified (18, 19) .

Histone lysine acetylation is regulated by various specific writers and erasers, such as lysine or histone acetyltransferase (HAT/KAT) and deacetylase complexes (HDAC/KDAC), respectively. Most HATs belong to one of four families based on their homologies: the Gcn5-related acetyltransferase (GNAT) family, the p300/CBP family, the SRC/p160 family, and the MYST family (named after its founding members, MOZ, Ybf2/Sas3, Sas2, and Tip60) (20). In mammals, there are

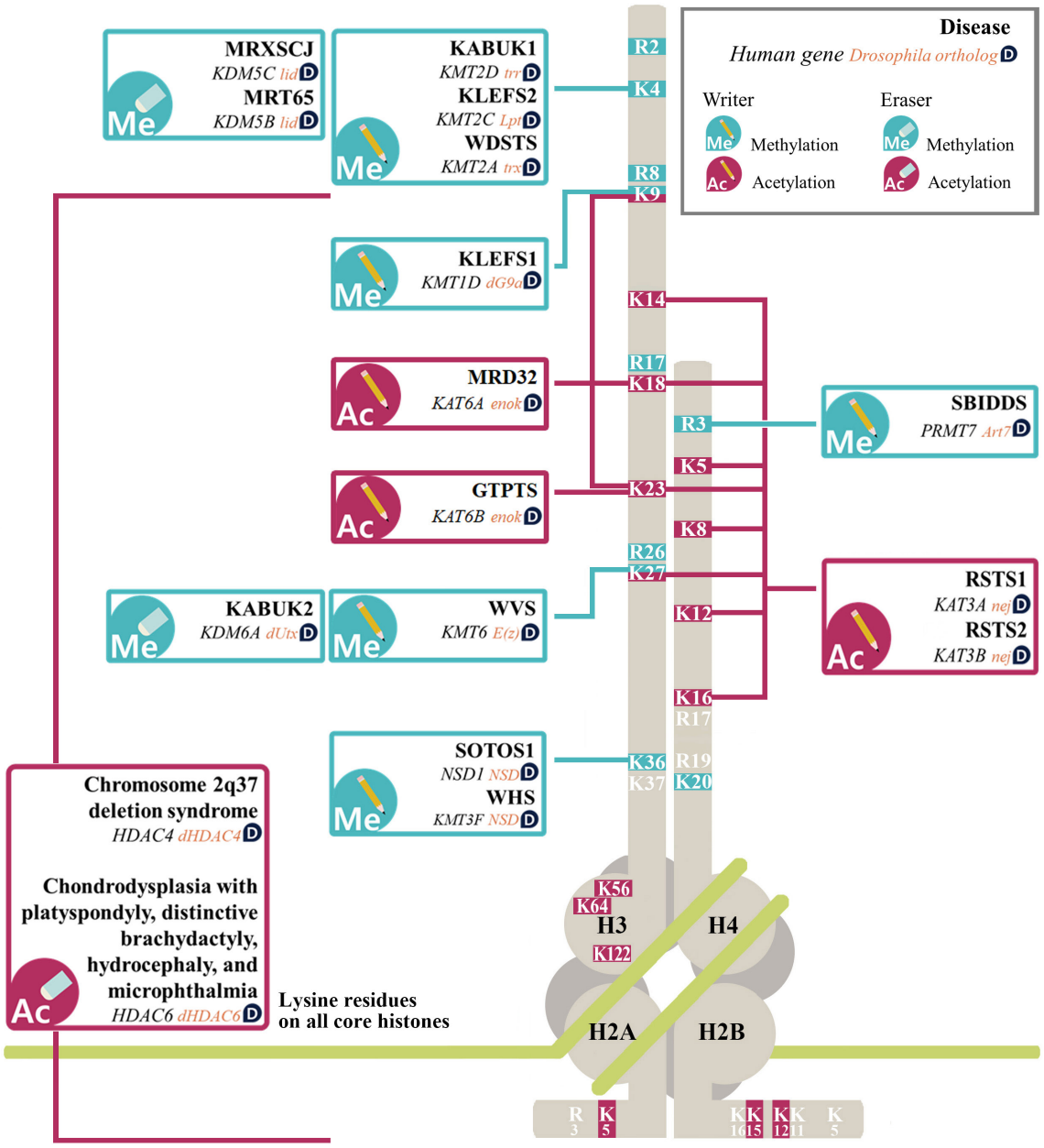

Figure 1. Histone acetylation and methylation residues involved in neurodevelopmental disorders with their relevant models. In this figure, 17 neurodevelopmental disorders associated with aberrant histone acetylation and/or methylation are shown. Arginine and lysine residues at the N-terminal ends of core histone proteins and some disease-associated lysine residues inside are shown. Among them, the residues known to be acetylated or methylated are highlighted in purple or blue squares, respectively. The association of the residues with neurodevelopmental disorders in this review are briefly described in the purple- or blue-lined textboxes depending on the types of histone modifications, acetylation or methylation, inside which the names of disorders, the related human genes and their *Drosophila* orthologs, and modifying activities are indicated. The writers (HAT and KMT) and the erasers (HDAC and KDM) are separately marked with symbols of a pencil and an eraser, respectively. RSTS1, Rubinstein-Taybi syndrome 1; RSTS2, Rubinstein-Taybi syndrome 2; MRD32, Autosomal dominant mental retardation 32; GTPTS, Genitopatellar syndrome; KABUK 1, Kabuki syndrome 1; KABUK 2, Kabuki syndrome 2; KLEFS1, Kleefstra syndrome 1; KLEFS2, Kleefstra syndrome 2; MRXSCJ, Mental retardation, X-linked, syndromic, Claes-Jensen type disorder; MRT65, Autosomal recessive mental retardation 65; SOTOS1, Sotos syndrome 1; WHS, Wolf-Hirschhorn syndrome; WVS, Weaver syndrome; WDSTS, Wiedemann-Steiner syndrome; SBIDDS, Short stature, brachydactyly, intellectual developmental disability, and seizures

18 HDAC enzymes that are divided into four separate categories called classes (Class I, II, III, and IV proteins) based on sequence similarities, all of which use either zinc-dependent (Class I, II, and IV) or NAD^+-dependent (Class III) mechanisms for deacetylation (21, 22). There have been difficulties in deciphering substrate specificity of HDACs due to their functional redundancy, and currently suggested that most HDAC can deacetylate all four core histones due to a lack of except for few cases (21).

So far, histone acetylation is generally known to be associated with transcriptional activation in an untargeted and globalized manner by affecting most nucleosomes, which are repressed by histone deacetylation. It is intriguing that specific acetylatable lysine residues can function as sites which interact with other epigenetic regulatory factors, and that histone deacetylation can be necessary for gene activity (17, 23). A wealth of evidence indicates that HAT and HDAC functions are critical for proper brain development and functionality. Lysine acetylation generally promotes cognitive performance, whereas the opposite process appears to negatively regulate cognition in multiple brain regions; accordingly, inhibition of HDACs has been proposed as a therapeutic approach for various neurological disorders (19, 24).

3.2. Histone methylation

Histone methylation occurs at lysine residues (H3K4/K9/K27/K36/K79, and H4K20) and arginine (histone H3 arginine 2 (H3R2)/R8/R17/R26, and H4R3) (25, 26). Lysine residues on histones are subject to mono-, di-, or trimethylation, whereas arginine residues can be mono- or dimethylated.

Histone methylations of lysine residues are controlled by histone lysine methyltransferases (KMTs) and demethylases (KDMs), aberrant expression of which often plays a significant role in various pathological processes (22). KMTs are classified into two groups, the larger of which is comprised of SET domain-containing KMTs including the SET and MYND domain family and SET domain KMTs. The second group consists of non-SET domain-containing KMTs that are represented solely by KMT4 (Dot1-like KMT) (27). KDMs also have two groups: the lysine-specific demethylase (LSD) and Jumonji C-terminal domain (JmjC) families (22).

In contrast to HDACs that globally regulate gene expression across different cell types, specific modifications by KMTs and KDMs can result in distinct functional outcomes, either transcriptional activation or repression, depending on the site and degree of methylation (28)(29). Key developmental genes carry repressive or activating histone lysine markers, such as trimethylation of H3K27 or H3K4, respectively, conferring a bivalent state to pluripotent embryonic stem cells (30).

Methylation of arginine residues in histones is achieved by the arginine methyltransferase (PRMT) family (31). In mammals, modification by arginine methylation is as common as phosphorylation and ubiquitination (32). Despite the controversy concerning the existence of arginine demethylases (RDMs), it was recently shown that certain histone lysine demethylases (KDMs) also possess arginine demethylation activity *in vitro* (33). The role of arginine methylation in human diseases has been rapidly emerging over the years (34). It has been reported that full deletion of PRMTs is an embryonic lethal mutation, and numerous links between arginine methylation and neurodegenerative diseases have been revealed over the last few years (35).

4. *DROSOPHILA* MODELS FOR HUMAN NEURODEVELOPMENTAL DISORDERS

The functions and the structures of histone-modifying enzymes are well-conserved between *Drosophila* and humans (21, 36). For example, HATs are grouped into 4 main families in humans, members of which have been identified in *Drosophila* (37). Thus, since the roles of histone PTMs are well-known in human neural development (38–40). it is worth utilizing *Drosophila* models to study human neurodevelopmental disorders caused by epigenetic dysregulation. In the following sections, we discuss human neurodevelopmental disorders caused by the aberration of single genes with identified *Drosophila* homologs, which subsequently dysregulate histone PTMs as summarized in Table 1. Among the single gene aberrations, the human disorders associated with dysregulation of two types of histone modifiers is covered; the writers (HATs and KMTs) and the erasers (HDACs and KDMs). Examples of neurodevelopmental disorders caused by dysregulation of non-histone protein modification by histone-modifying enzymes (41) are not included here.

4.1. Drosophila models for histone acetylation dysregulation

4.1.1. Rubinstein-Taybi syndrome 1 and Rubinstein-Taybi syndrome 2

Rubinstein-Taybi syndrome-1 (RSTS1; OMIM 180849) is caused by a heterozygous mutation in the gene encoding for CREB-binding protein (*CREBBP*), also known as *KAT3A* and *CBP*. This disease was named after Rubinstein and Taybi, who first reported a syndrome characterized by ID, broad thumbs and toes, and facial abnormalities (42). KAT3A is a coactivator for the cAMP-responsive transcription factor CREB. Patients with RSTS carry heterozygous point mutations in the *KAT3A* gene, suggesting that the loss of one functional copy of *KAT3A* triggers the developmentally abnormal condition (43). The heterozygous mice that had truncated Crebbp protein (residues 1 to 1084) containing the CREB-binding domain showed clinical features of RSTS (44).

On the other hand, Rubinstein-Taybi syndrome 2 (RSTS2; OMIM 613684) is caused by a heterozygous mutation in the *EP300* gene, also known as *KAT3B*, that encodes the adenovirus E1A-associated cellular

Table 1. List of neurodevelopmental disorders caused by mutations in histone acetylation or methylation-related genes mentioned in this review and the related *Drosophila* genes

Histone Modification	Residue	Disodrder	OMIM	Symptom	Human Gene (synonym)	Function	Target Sites in Histones	Drosophila Ortholog	Similarity *
Acetylation	Lysine	Rubinstein-Taybi syndrome 1	180849	ID, broad thumbs and toes, and facial abnormalities	KAT3A (CREBBP, CBP)	HAT	H3K14/ K18/ K23/ K27, H4K5/K8/ K12/K16	nej	67(a)
		Rubinstein-Taybi syndrome 2	613684	craniofacial abnormalities, postnatal growth deficiency, broad thumbs, broad big toes, ID, and a propensity for the development of malignancies	KAT3B (EP300, p300)	HAT	H3K14/ K18/ K23/ K27, H4K5/K8/ K12/K17		32(a)
		Autosomal dominant mental retardation 32	616268	microcephaly, poor overall growth, and delayed psychomotor development with ID and absent speech	KAT6A (MYST3, MOZ)	HAT	H3K9/K18/ K23	enok	23(a)
		Genitopatellar syndrome	606170	microcephaly, severe psychomotor retardation, ID, genital abnormalities, missing or underdeveloped kneecaps, and other abnormalities	KAT6B (MYST4, MORF)	HAT	H3K23		22(a)
		Chromosome 2q37 deletion syndrome	600430	developmental delay, ID, ASD, brachydactyly mental retardation (BDMR) syndrome, and dysmorphic facial features	HDAC4	HDAC	Lysine residues on all four core histones	dHDAC4	33(a)
		Chondrodysplasia with platyspondyly, distinctive brachydactyly, hydrocephaly, and microphthalmia	300863	intrauterine growth retardation, hydrocephaly, macrocephaly, frontal bossing, and microphthalmia in affected males whereas a milder phenotype involving short stature and sometimes associated with mild ID in affected females	HDAC6	HDAC	Lysine residues on all four core histones	dHDAC6	52.45(n)
		Kabuki syndrome 1	147920	congenital ID syndrome with additional features including postnatal dwarfism, a peculiar facies characterized by long palpebral fissures with eversion of the lateral third of the lower eyelids	KMT2D (MLL2, MLL4)	KMT	H3K4	trr	26(a)
		Kabuki syndrome 2	300867	a variety of phenotypes ranging from typical KABUK to a milder clinical presentation, and more common hypoglycaemia in KABUK 2 than in KABUK 1	KDM6A (UTX)	KDM	H3K27	dUtx	59.12(n)

Methylation	Lysine	Kleefstra syndrome 1	610253	ID without speech development, hypotonia, and characteristic facial features	KMT1D (EHMT1, GLP1)	KMT	H3K9	dG9a	22(a)
		Kleefstra syndrome 2	617768	delayed psychomotor development, variable ID, and mild dysmorphic features	KMT2C (MLL3)	KMT	H3K4	Lpt	32(a)
		Mental retardation, X-linked, syndromic, Claes-Jensen type disorder	300534	severe ID, slowly progressive spastic paraplegia, facial hypotonia, and maxillary hypoplasia	KDM5C (JARID1C, MRXSCJ)	KDM	H3K4	lid	35(a)
		Autosomal recessive mental retardation 65	618109	poor overall growth, neonatal feeding difficulties, dolichocephaly, ID, and moderate learning disabilities	KDM5B (JARID1B, MRT65)	KDM	H3K4		36 (a)
		Sotos syndrome 1	117550	childhood overgrowth, facial dysmorphism, macrocephaly, and non-progressive neurological delay	KMT3B (NSD1, SOTOS1)	KMT	H3K36	NSD	25(a)
		Wolf-hirschhorn syndrome	194190	delayed growth and ID, microcephaly, “Greek helmet” facies, and closure defects	KMT3F, KMT3G (NSD2, WHSC1, MMSET)	KMT	H3K37		23(a)
		Weaver syndrome	277590	considerable phenotypic overlap with SOTOS syndrome	KMT6, KMT6A (EZH2)	KMT	H3K27	E(z)	56.56(n)
		Wiedemann-Steiner syndrome	605130	extremely rare neurodevelopmental condition accompanied by microcephaly, short stature, an autism-like phenotype, and aggression	KMT2A (MLL1, MLL, TRX1)	KMT	H3K4	trx	18(a)
	Arginine	Short stature, brachydactyly, intellectual developmental disability, and seizures	617157	global delayed development, microcephaly, ID, brachydactyly, and short metacarpals	PRMT7	RMT	H4R3	Art7	50.65(n)

p300 transcriptional co-activator protein. RSTS2 is a disorder characterized by craniofacial abnormalities, postnatal growth deficiency, broad thumbs, broad big toes, ID, and a propensity for the development of malignancies. RSTS2 displays a milder phenotype than RSTS1. About 50 to 70% of patients have RSTS1 due to a mutation in the *KAT3A* gene, whereas RSTS2 is much less common; only about 3% of patients have mutations in the *KAT3B* gene. A fraction of the intracellular HDAC1 protein is incorporated in a multiprotein complex containing several components, including KAT3B that can acetylate HDAC1, leading to its inactivation and modulation of transcription (45).

Among the four different HAT families mentioned previously in section 3.1., KAT3B and KAT3A belong to the p300/CBP family, as the names imply. Although sharing 86% amino acid sequence homology (46), the functions of KAT3B and KAT3A are overlapped but distinct; they have significant HAT activity on the same core histone substrates (H3K14/K18/K23/K27 and H4K5/K8/K12/K16) with differences in specificity and selectivity (47, 48). p300/CBP family members are required for proper brain development (49–51).

Drosophila has a single p300/CBP homolog, nejire (nej). It acetylates several nuclear proteins, including histone H3K18, H3K27, and H4K8 (52) (53) (54). It was shown that nej is necessary for the *in vivo* activation of a specific target gene as well as for the global acetylation of H4, suggesting a role in regulating global histone acetylation throughout the developing organism (54). In addition, nej is an intrinsic component

for circadian-controlled transcription and participates in a postsynaptic regulatory system that controls functional synaptic development (55, 56).

4.1.2. Autosomal dominant mental retardation 32 and genitopatellar syndrome

Autosomal dominant mental retardation 32 (MRD32; OMIM 616268) is caused by a heterozygous mutation in the *KAT6A* gene. KAT6A (known as MYST3 and MOZ) is a MYST-family histone acetyltransferase, and reported to acetylate H3K9/K18/K23 residues (57, 58). Common features of MRD32 patients include microcephaly, poor overall growth, and delayed psychomotor development with ID and absent speech. Studies of patient cells showed alterations in the acetylation of H3K9 and H3K18, as well as changes in signaling downstream of p53, suggesting disruption of multiple pathways involved in apoptosis, metabolism, and transcriptional regulation (57).

Genitopatellar syndrome (GTPTS; OMIM 606170) is a rare autosomal dominant disorder caused by a heterozygous mutation in the *KAT6B* gene. Like KAT6A, KAT6B (MYST4 and MORF) is also a MYST-family histone acetyltransferase, and known to acetylate the H3K23 residue (59). Various gene mutations leading to C-terminal truncations in MORF cause the rare genitopatellar syndrome (OMIM 606170), a condition characterized by microcephaly, severe psychomotor retardation, ID, genital abnormalities, missing or underdeveloped kneecaps, and other abnormalities (60, 61). KAT6B is required for RUNX2-dependent transcriptional activation and may be involved in cerebral cortex development.

The two mammalian *KAT6* genes, described above as *KAT6A* and its paralog *KAT6B*, have been identified thus far (62, 63), whereas *Drosophila KAT6*, named Enoki mushroom (*Enok*), has been reported as a critical factor in neuroblast proliferation (64). KAT6 complexes are composed of multisubunits and are highly conserved between flies and mammals, strongly suggesting that KAT6 HATs play crucial and conserved roles in a wide range of species (65). Enok acetylates lysine residues on histones, including H3K23, in order to regulate gene transcription. The disruption of Enok HAT activity in neuroblasts resulted in defective development of the mushroom body of the fly memory center due to an arrest in neuroblast proliferation rather than a failure in either cell fate switching or axon branching (66). Later, it was revealed that the Enok complex promotes G1/S transition by interacting with the proliferating cell nuclear antigen (PCNA) unloader Elg1 complex and inhibiting its PCNA-unloading function.

4.1.3. Chromosome 2q37 deletion syndrome

Chromosome 2q37 deletion syndrome (OMIM 600430) is caused by a contiguous gene deletion of several genes on chromosome 2q37.2, one of which is *HDAC4*. Patients with chromosome 2q37 deletion syndrome show highly variable clinical manifestations likely resulting from deletions of various genes at various sizes. Variable clinical features include developmental delay, ID, ASD, brachydactyly mental retardation syndrome, and dysmorphic facial features.

HDAC4 is highly expressed in the brain (67), and it interacts with multiple transcription factors which are necessary for brain development, including myocyte enhancer factor 2A (MEF2A) (68). In humans, deletion, duplication, and haploinsufficiency of *HDAC4* lead to mental retardation, suggesting that *HDAC4* plays an important role in neurodevelopment which is directly linked to cognitive function (69, 70).

Similar to human *HDAC4*, the repression of *HDAC4* in *Drosophila* results in the impairment of synaptic plasticity as well as learning and memory deficits due to a failure to properly redirect MEF2 (71). In addition, it has been reported that the homeostasis of *HDAC4* is crucial for the maintenance of cognitive function by regulating many other transcription genes involved in synaptic plasticity, neuronal survival, and neurodevelopment (72).

4.1.4. Chondrodysplasia with platyspondyly, distinctive brachydactyly, hydrocephaly, and microphthalmia

Chondrodysplasia with platyspondyly, distinctive brachydactyly, hydrocephaly, and microphthalmia (OMIM 300863) is an X-linked dominant disorder caused by a mutation in the *HDAC6* gene (300272) (73). Affected males show intrauterine growth retardation, hydrocephaly, macrocephaly, frontal bossing, and microphthalmia, whereas affected females have a milder phenotype involving short stature and sometimes associated with mild mental retardation. A study of a family with this disorder demonstrated that it results from mutations in the 3′ untranslated regions of *HDAC6* which suppress miR433-mediated posttranscriptional regulation and cause overexpression of *HDAC6* (74).

Perry *et al.* showed that *Drosophila HDAC6* is expressed in neurons and *dHDAC6* knockdown flies have a learning deficit, suggesting that it plays a role in memory formation, probably by modulating synaptic plasticity through the active-zone scaffold Bruchpilot (75). Using a *Drosophila* Parkinson's disease model constructed by ectopic expression of human alpha-synuclein, it was found that *dHDAC6* plays a critical role in the protection of DA neurons and formation of alpha-synuclein inclusions (76). On the contrary, in a *Drosophila* Alzheimer model with ectopic expression of human tau, a *dHDAC6* null mutation rescued tau-induced microtubule defects in both muscles and

neurons, suggesting that *dHDAC6* may be a unique potential drug target for AD and related tauopathies (77).

4.2. *Drosophila* models for histone methylation dysregulation

4.2.1. Kabuki syndrome 1 and Kabuki syndrome 2

Kabuki syndrome 1 (KABUK 1; OMIM 147920) is a congenital mental retardation syndrome with additional features including postnatal dwarfism, a peculiar facies characterized by long palpebral fissures with eversion of the lateral third of the lower eyelids (78). KABUK 1 is caused by a heterozygous mutation in the *KMT2D* gene (also known as *MLL2* or *MLL4*). Heterozygous autosomal dominant mutations in *KMT2D* were found in more than 50 percent of patients with KABUK1, whereas X-linked mutations in *KDM6A* (also known as *Utx*) were reported to contribute to less than 10 percent of the incidence of this syndrome, known as Kabuki syndrome 2 (KABUK2; OMIM 300867) (79). It was reported that patients with KABUK 2 display a variety of phenotypes, ranging from typical KABUK to a milder clinical presentation. Hypoglycaemia is more common in KABUK 2 than in KABUK 1 (79, 80).

KMT2D methylates H3K4 residues, representing a specific tag for epigenetic transcriptional activation. KMT2D is a prominent mammalian H3K4 mono-methyltransferase that acts on enhancer region (81). KMT2D is widely expressed in adult tissues and is essential for the expression of cell-type-specific genes during neuronal and osteoblast differentiation (82) (83). The trithorax related (*trr*) gene in *Drosophila* is a homolog of human *KMT2D*, but its homology is limited to the C-terminal SET domain of *KMT2D*. Consistent with the role of KMT2D in mammals, trr was shown to regulate H3K4me1 (84) Koemans *et al.* (2017) found that KMT2D binds to the promoters of many genes involved in neuronal processes in the fly brain and that trr-specific knockdown in the mushroom body of the fly brain resulted in impaired short-term memory (85) It is intriguing that H3K4 monomethylation catalyzed by trr is unnecessary for development and viability (86). However, trr mutants displayed subtle developmental phenotypes when subjected to temperature stress, suggesting H3K4me1 may act on cis-regulatory elements in specific settings to fine-tune transcriptional regulation in response to environmental stress.

On the contrary, KDM6A specifically demethylates trimethylated and dimethylated H3K27 residues, but not the monomethylated residues that are a hallmark of silent chromatin (87, 88). It plays a central role in the regulation of posterior development in vertebrates by regulating *HOX* gene expression (87). Since H3K4 methylation is concomitant with H3K27 demethylation (89), the dysregulation of KMT2D and KDM6A may cause two similar syndromes, KABUK1 and 2, respectively. *Drosophila* carries a single KDM6 ortholog (90); thus, the *Drosophila* homolog of KDM6A is dUtx (also known as ubiquitously transcribed tetratricopeptide repeat protein, X chromosome). dUtx is also a JmjC domain-containing protein that specifically demethylates di- and trimethylated H3K27 (90). It has been revealed that dUtx is involved in various biological processes such as autophagic cell death, negative regulation of the Notch signaling pathway, DNA damage response, and wound healing (91–94). *Using Drosophila* mutants expressing an inactive dUtx protein, Copur and Muller (2018) reported that dUtx demethylase activity is essential not only in the earliest embryonic stages, but also to sustain viability in adult flies (95). These *Drosophila* mutants exhibit the same phenotypes shown by animals lacking the Utx protein, such as abnormal regulation of *HOX* gene expression, indicating that dUtx is indeed a functional ortholog of human KDM6A.

4.2.2. Kleefstra syndrome 1

Kleefstra syndrome 1 (KLEFS1; OMIM 610253) is caused by a heterozygous mutation in the *Euchromatin histone methyltransferase 1* gene (*EHMT1*; also known as *KMT1D* or G9a-Like Protein 1 (*GLP1*)). EHMT1 specifically mono- and dimethylates H3K9 in euchromatin, which marks a specific tag for epigenetic transcriptional repression by recruiting HP1 proteins. It also weakly methylates H3K27me. This protein has been known to act in the silencing of MYC- and E2F-responsive genes and therefore could play a role in G0/G1 transition during the cell cycle. Kleefstra *et al.* (2009) reported KLEFS1 patients who have intragenic *EHMT1* mutations with the core phenotype of the deletion syndrome, including mental retardation without speech development, hypotonia, and characteristic facial features (96).

In addition to *EHMT1*, the human genome encodes another *EHMT* gene, *EHMT2* (also known as *KMT1C* or *G9A*), and *dG9a* is a *Drosophila* homolog of both *EHMT* genes. Although *dG9a* is widely expressed in the nervous system, *dG9a*-mutant flies remain viable (97). This gene contributes to multiple processes including dendrite morphogenesis, larval locomotory behavior, and short and long-term memory, and is currently known as a key cognition regulator of an epigenetic program controlling learning and memory genes (98, 99). In *dG9a*-mutant flies, loss of H3K9 dimethylation occurs at 5% of the euchromatic genome and is enriched at the distinct classes of genes that control neuronal and behavioral processes. Like trr, dG9a is also required in the mushroom body for short term memory. Transcriptional profiling of two mutant fly heads, pan-neuronal *trr* knockdown, and *dG9a*-null mutants identified that many misregulated genes are

significantly overlapped, including factors involved in the regulation of synaptic plasticity. It is noteworthy that these findings indicate the molecular convergence between the KMT2 and EHMT protein families, which may contribute to a molecular network involved in both fly and human neurodevelopment (100).

4.2.3. Kleefstra syndrome 2

Kleefstra syndrome 2 (KLEFS2; OMIM 617768) is an autosomal dominant neurodevelopmental disorder characterized by delayed psychomotor development, variable ID, and mild dysmorphic features, and is caused by a heterozygous mutation in the *KMT2C* (also known as *MLL3*) gene. KMT2C exhibits histone methylation activity at H3K4, is involved in transcriptional coactivation, leukemogenesis, and developmental disorders.

The *Drosophila* homolog of *KMT2C*, the Lost plant homeodomains (PHDs) of trr *(Lpt)* gene, encodes a protein highly related to the N-terminus of MLL3/4; this protein is copurified with the trr complex (84). It has been reported that Lpt is required for the activation of targets of the hormone ecdysone and it plays a critical role in development and tissue patterning through regulation of the conserved Decapentaplegic signaling pathway (101). It has been revealed that Lpt is required for proper global trimethylation of H3K4 and that hormone-stimulated transcription requires chromatin binding by Lpt, H3K4 methylation by trr, and H3K27 demethylation by the demethylase Utx (102). These are very interesting results since three epigenetic regulators, Lpt, trr, and Utx, are thought to regulate chromatin structure at transcriptional enhancer regions.

4.2.4. Mental retardation, X-linked, syndromic, Claes-Jensen type disorder and autosomal recessive mental retardation 65

Mammals encode four KDM5 paralogs: KDM5A, KDM5B, KDM5C, and KDM5D. KDM5 family proteins share a similar domain structure that allows them to influence gene expression through several distinct mechanisms. The JmjC domain is the enzymatic core of KDM5 proteins, and the only known role of this domain is to demethylate histone H3 trimethylated at H3K4. In addition to removing H3K4me3, KDM5 proteins have two other domains that recognize the methylation status of H3K4. The C-terminal PHD motif binds to di- and trimethylated H3K4, and the N-terminal PHD recognizes histone H3 that is unmethylated at K4 (103, 104). Mutations in KDM5 family histone demethylases cause ID in humans.

Mental retardation, X-linked, syndromic, Claes-Jensen-type disorders (MRXSCJ; OMIM 300534) are caused by a mutation in the *KDM5C* gene, also known as *JARID1C* or *MRXSCJ*, and phenotypes of such disorders include severe mental retardation, slowly progressive spastic paraplegia, facial hypotonia, and maxillary hypoplasia. KDM5C specifically demethylates trimethylated and dimethylated, but not monomethylated, H3K4. It has been reported that KDM5C participates in transcriptional repression of neuronal genes by recruiting histone deacetylases and RE-1 silencing transcription factor at neuron-restrictive silencer elements.

Autosomal recessive mental retardation 65 (MRT65; OMIM 618109) displays poor overall growth and is caused by a homozygous or compound heterozygous mutation in the *KDM5B* gene that demethylates trimethylated, dimethylated, and monomethylated H3K4. Patients with this disease show neonatal feeding difficulties, dolichocephaly, ID, and moderate learning disabilities. This demethylase acts as a transcriptional corepressor for *FOXG1B* and *PAX9* and positively regulates the proliferation of breast cancer cells by repressing tumor suppressor genes such as *BRCA1* and *HOXA5* (105).

Unlike humans, who have four KDM5 proteins, the *Drosophila* genome encodes a single KDM5 protein known as little imaginal discs (Lid). The Lid protein is a trimethyl H3K4 histone demethylase that regulates transcription through both demethylase-dependent and demethylase-independent mechanisms. So far, dKDM5 has been reported to play a role in regulating various biological processes, such as cell growth, circadian rhythm, stress resistance, hematopoiesis, and fertility. Recently, Zamurrad *et al.* (2018) revealed that the mutant flies with an allele corresponding to human mutant *KDM5C* that causes the MRXSCJ disorder showed impaired learning and memory. This result confirms that KDM5C is a key cause of the cognitive phenotypes associated with this disorder, accordingly exhibiting further use of *Drosophila* as a disease model to better understand human neurodevelopmental disorders caused by epigenetic dysregulation (106).

4.2.5. Sotos syndrome 1 and Wolf-Hirshhorn syndrome

Sotos syndrome 1 (SOTOS1; OMIM 117550) is an autosomal dominant disorder characterized by childhood overgrowth, facial dysmorphism, macrocephaly, and non-progressive neurological delay (107). This disease is caused by heterozygous mutations in *KMT3B*, also known as Nuclear Receptor SET Domain-Containing Protein 1 (*NSD1*) (108). NSD1 methylates H3K36 and is capable of either negatively or positively influencing transcription depending on cellular context. Interestingly, *NSD1* duplication resulted in a phenotypic outcome which

greatly contrasts with that of SOTOS1, exhibiting microcephaly and growth retardation, and indicating the importance of proper *NSD1* expression during brain development (109). On the contrary, deficiency of NSD2 (also known as KMT3F, KMT3G, and MMSET) is associated with Wolf-Hirshhorn syndrome (WHS; OMIM 194190), key features of which include delayed growth and ID, microcephaly, "Greek helmet" facies, and closure defects (110). It is well-established that overexpression of *NSD2* via translocation is involved in the formation of various tumors. It is intriguing that defects in two genes of the same family, *NSD1* and *NSD2*, exhibit oppositional phenotypes, such as microcephaly in WHS and macrocephaly in SOTOS patients.

Mammals encode three NSD paralogs, NSD1, NSD2, and NSD3, while the *Drosophila* genome has a single NSD protein; all of these mono- and dimethylate H3K36 (111). It has been reported that NSD functions in the regulation of several genes, such as opposite modulators in transcript initiation and elongation, a novel insulator-binding protein cofactor, and hHP1a-interacting proteins for heterochromatin enrichment (112, 113). Also, NSD is one of the downstream targets of the DRE/DREF pathway that is associated with various cellular processes in *Drosophila* (114). So far, despite the existence of *Drosophila* NSD as the ortholog of human NSD family members, a *Drosophila* model for the two human diseases described above has yet to be demonstrated. However, ubiquitous overexpression of *NSD* in the fly caused developmental delay and reduced body size at the larval stage, characteristic of *NSD*-overexpressed phenotypes by *NSD1* duplications reported in human disorders with growth retardation (109). The whole-body high expression of *NSD* resulted in pupal lethality due to apoptosis via the activation of Jun-N-terminal kinase, indicating possible molecular mechanisms that may reveal a novel pathway involved in NSD1-related human diseases (115).

4.2.6. Weaver syndrome

Weaver syndrome (WVS; OMIM 277590), which shows considerable phenotypic overlap with SOTOS syndrome, is caused by a mutation in the *KMT6* (also known as the Enhancer of Zeste 2 Polycomb Repressive Complex 2 (PRC2) Subunit (EZH2), or *KMT6A*) gene that mono-, di- and trimethylates H327. PRC2 is one of the two classes of polycomb-group (PcG) proteins and it silences developmental genes to determine specific differentiated cell identities. KMT6 plays a major role in forming trimethylated H3K27, which is required to determine embryonic stem cell identity and achieve proper differentiation. Several studies have shown that *EZH2* deficiencies in animal models induced abnormal neurogenesis during embryonic development as well as adult hippocampal neurogenesis (116, 117). These results suggest that EZH2-induced H3K27 methylation plays an important role in various processes of neurodevelopment, the dysfunction of which might be closely related to ID in patients with WVS. The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two distinct epigenetic repression systems. In addition, it was found that *NSD2* overexpression not only causes a global increase in H3K36 dimethylation but also a reduction in trimethylation on H3K27 across the genome (118), indicating the interplay between NSD2 and EZH2, which may explain why WVS displays similar phenotypes with SOTOS1 despite of the different origins of their affected genes.

The *Drosophila* homolog of KMT6 is called the enhancer of zeste (E(z)) and is capable of methylating H3K27. It was reported that E(z) is the catalytic component of PRC2 in *Drosophila*, indicating evolutionally conserved roles of KMT6 in various functions in terms of fly neurodevelopment. PcG genes are essential for normal neuroblast survival in the postembryonic CNS of *Drosophila*. The absence of E(z) causes various neuronal developmental defects, and the proliferation of postembryonic neuroblast clones is dramatically reduced (119, 120).

4.2.7. Weidemann-Steiner syndrome

Wiedemann-Steiner syndrome (WDSTS; OMIM 605130) is caused by a heterozygous mutation in the *KMT2A* gene (also known as *MLL1*, *MLL*, or *TRX1*) that encodes a catalytic subunit of the MLL1/MLL complex. WDSTS is an extremely rare neurodevelopmental condition accompanied by microcephaly, short stature, an autism-like phenotype, and aggression (121). In the MLL1/MLL complex, KMT2A specifically mediates H3K4 methylation, a specific tag for epigenetic transcriptional activation, and plays an essential role in early development and hematopoiesis (122, 123). Interestingly, the abnormal brain functions of WDSTS patients were recapitulated in KMT2A heterozygous mutant mice, which displayed profound deficits in long-term contextual fear memory (124, 125). However, it has been also reported that the genes affected by decreased H3K4 trimethylation in hippocampal neurons of *Kmt2a*-lacking mice display a significant overlap to the changes observed in the model mouse for Alzheimer's disease (126), suggesting a role of dysfunction of Kmt2a-mediated H3K4 methylation in the pathogenesis of neurodegenerative diseases.

Like KMT2A, the *Drosophila* homolog, trithorax (trx), is a chromatin-modifying enzyme that methylates histone H3K4 (84). This activity promotes further acetylation of a gene, and antagonizes the epigenetic silencing by PRC2 in neuroblast (127). In addition, Trx was shown to contribute to axon growth in

visual system wiring and germ cell migration (128, 129), thus indicating its critical role in fly neurodevelopment.

4.2.8. Short stature, brachydactyly, intellectual developmental disability, and seizures

Short stature, brachydactyly, intellectual developmental disability, and seizures (SBIDDS; OMIM 617157) is characterized by global delayed development, microcephaly, intellectual disabilities, brachydactyly, and short metacarpals (130). SBIDDS is caused by multiple heterozygous mutation in the *PRMT7* gene that encodes an enzyme to mediate the symmetric dimethylation of the arginine residue at H4R3, possibly leading to recruit DNMTs at this site (131, 132). In the cellular differentiation of NT2/D1 stem cells, opposing effects between KMT2D-catalyzed H3K4 methylation and PRMT7-catalyzed H4R3 symmetric dimethylation via trans regulation have been demonstrated on cellular differentiation (82). PRMT7 plays a role in a wide range of biological processes, including neuronal differentiation, small nuclear ribonucleoprotein (snRNP) biogenesis, and regulation of the Wnt signaling pathway via generating methylarginines not only on histone proteins but also other proteins such as snRNPs.

The *Drosophila* Arginine methyltransferase 7 (Art7), is an ortholog of the human PRMT7 (133). The loss of Art7 resulted in pupal lethality, indicating an essential role of the gene for fly development (134). Like PRMT7, Art7 symmetrically dimethylates Sm proteins, a part of snRNPs, in *Drosophila* (134). However, it is not certain yet that Art7 is the functional ortholog of the human PRMT7. Sm protein methylation is a critical requirement for mammalian snRNP biogenesis (135). In contrast, however, *Art7*-depletion in flies displayed reduced Sm protein dimethylation levels similar to the result of the RNAi experiments with S2 cells, but did not affect snRNP assembly (134), indicating that functional differences may exist between *Drosophila* and humans. The histone modification by Art7 as well as its roles on neurodevelopment in *Drosophila* has not been reported, thus further studies on the role of Art7 in brain development using the *Art7*-deleted *Drosophila* model are necessary to identify the epigenetic causes of mental retardation seen in patients with SBIDDS syndrome.

5. PERSPECTIVES

As listed in Table 1, the *Drosophila* genome carries many orthologs that are counterparts of the genes causing neurodevelopmental human disorders. Although it is true that the *Drosophila* model has distinctive advantages as we have described, there are also some limitations to its use in all human disease studies caused by epigenetic mechanisms. For example, *Drosophila* does not produce any of the typical DNA methyltransferases while DNA methylation in Drosophila has been suggested to serve a role in controlling retrotransposon silencing and genome stability due to the obvious DNA methylation activity and the existence of DNA methylation in its genome. Furthermore, the signaling systems and genes involved in *Drosophila* may be completely different from those in humans. Thus, although there are some genes known to promote abnormal neurodevelopment following mutation in *Drosophila*, it would be another matter whether mutations in their pseudogenes lead to neurodevelopmental diseases in humans. In addition, compared with numerous studies on the molecular mechanisms mediated by epigenetically regulated or regulating genes, there are relatively few studies on phenotypes of fly disease models related with these genes. Despite these limitations, however, the *Drosophila* model is very efficient for studying the function of the genes responsible for human diseases, as many of these genes induce similar disorders in *Drosophila*. In the future, more detailed studies on the role of epigenetic regulation in brain development and related phenotypes using *Drosophil*a models will be necessary to identify the causes of mental retardation in patients with various neurodevelopmental disorders.

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