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Original Article

Individuals with reported and novel *KDM5C* variants present with seizures, a feature recapitulated in a *Drosophila* model

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Abstract

Variants that disrupt the function of the chromatin regulator KDM5C cause a rare neurodevelopmental disorder (KDM5C-NDD) characterized by intellectual disability, seizures, and a broad range of systemic features. To better understand this disorder, more detailed and standardized information is required regarding the association between these genetic variants and cognitive and behavioral traits. Utilizing data obtained by the RARE-X KDM5C Data Collection Program, we analyzed survey and genetic data from 31 newly reported individuals. In addition to the expected neurodevelopmental challenges, participants frequently reported growth abnormalities, vision and digestive issues, behavioral concerns, and seizures in nearly half of the cases. Meta-analyses of this data and previously published cases reaffirmed that seizures are a frequent feature in both hemizygous males and heterozygous females with *KDM5C* variants, with over a third of individuals reporting at least one seizure. Based on the prevalence of seizures in the RARE-X and published datasets, we sought to develop robust quantitative assays of KDM5-associated seizure behavior using the model organism *Drosophila*. Reducing the expression of its single *Kdm5* gene in neurons, but not glia, led to spontaneous and stimulus-induced seizures, underscoring a cell-intrinsic requirement for KDM5 in maintaining neuronal stability. Together, these human and fly studies highlight KDM5C as a critical regulator of nervous system function, demonstrating how patient-driven data collection and scalable model systems can be effectively integrated. This work expands our understanding of KDM5C-NDD and lays the groundwork for future therapeutic discoveries.

Keywords: KDM5C; Claes-Jensen syndrome; histone demethylase; epilepsy

Introduction

Neurodevelopmental disorders (NDDs) are a group of heterogeneous conditions that impact the development and function of the nervous system. Worldwide, it is estimated that over 316 million children live with NDDs [1]. Individuals with NDDs present with a spectrum of symptoms, the presence and severity of which vary between individuals with different conditions and even amongst individuals with the same NDD. Genes encoding histone-modifying enzymes are increasingly recognized as a significant genetic cause of NDDs, including the Lysine Demethylase 5 (KDM5) family of histone demethylases [2–4].

In mammals, genetic alterations that impact KDM5A [5, 6], KDM5B [7–11], or KDM5C function [12] have been linked to atypical neurodevelopment, whereas KDM5D, located on the Y chromosome, has not. Of the three *KDM5* genes associated with NDDs, *KDM5C* has the largest number of reported variants, largely due to its X-linked inheritance, although many questions remain regarding how loss of KDM5C alters neuronal function. The disorder caused by pathogenic *KDM5C* variants has been known by multiple names (OMIM #300534), including 'Claes-Jensen syndrome,' 'Intellectual developmental disorder, X-linked, syndromic, Claes-Jensen Type (MRXSCJ)', and 'KDM5C-associated X-linked intellectual disability (KDM5C-associated XLID)'. Due to the variable expressivity of the traits observed, we have opted to use the more broadly inclusive term KDM5C-related neurodevelopmental disorder (KDM5C-related NDD, abbreviated as KDM5C-NDD). Like all KDM5 family proteins, the best-described role of KDM5C is its enzymatic function as a histone demethylase, which removes di- and tri-methyl marks from Histone H3 at lysine 4 (H3K4) through the joint action of its Jumonji N (JmjN) and Jumonji C (JmjC) domains [13]. H3K4me3 is a promoter-proximal chromatin mark associated with actively transcribed genes, indicating that variants affecting KDM5C activity likely impact brain function by altering gene expression. In addition to its canonical enzymatic activity, KDM5C and other KDM5 family proteins also impact gene expression through less characterized non-enzymatic functions mediated by other regions of the protein [14]. Both demethylase-dependent and independent functions of KDM5C are likely to be critical for its role in brain development and function [12].

Previous reports of individuals and families with *KDM5C* variants have noted a spectrum of neurological and other

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symptoms [7, 15–65]. The most prevalent features described include intellectual disability, developmental delay, challenges with fine and gross motor skills, speech and language issues, short stature, epilepsy, and behavioral traits like aggression, anxiety, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder (ASD). However, while the presence of some of these features, such as intellectual disability and short stature, is frequently reported in the literature, details related to traits such as seizures/epilepsy and impacts on organ systems outside the central nervous system are often omitted. It is also notable that the literature tends to focus on describing the features of hemizygous males. Many studies overlook the characteristics of heterozygous females, although it should be noted that several recent articles have specifically focused on this issue [21, 39]. Therefore, additional and more comprehensive characterization of individuals with *KDM5C* variants is crucial to better understand this disorder and determine whether genotype–phenotype correlations can be identified.

Of the clinical manifestations of KDM5C-NDD, seizures represent an important yet poorly understood feature, and their presence can profoundly affect the quality-of-life of both affected individuals and their caregivers. Reports using larger cohorts in which seizures were specifically assessed indicate that 13%–20% of females and 33%–53% of males with *KDM5C* variants experience seizures [21, 39, 66]. However, the prevalence of seizures/epilepsy, how seizures manifest (including the type, age of onset, and frequency of episodes), and effective management strategies remain insufficiently characterized. Emphasizing this, several reports describe instances of KDM5C-NDD-associated seizures that cannot be controlled with conventional antiseizure medication (treatment-resistant; intractable) [24, 39, 64]. The incidence of treatment-resistant epilepsy in KDM5C-NDD is not well understood due to a lack of standardized reporting, however, in the general population of individuals with epilepsy, it is estimated that 15% of children and 34% of adults fail to have their seizures well-controlled by medication [67]. Although the relationship between the NDDs caused by loss of KDM5A, KDM5B and KDM5C remains unknown, KDM5A- and KDM5B-related NDDs are also associated with increased seizure susceptibility [6, 9]. Investigations into the cellular and molecular mechanisms underlying KDM5C-related seizures are likely to broadly inform our understanding of shared traits caused by the loss of KDM5 family proteins.

To study the mechanisms underlying KDM5-related NDDs that are difficult to investigate in humans, research has been conducted in *Drosophila* [68–72]. Unlike mammals, which possess four paralogous *KDM5* genes, *Drosophila* encodes a single ortholog, KDM5, whose functions likely reflect those of all mammalian KDM5 proteins [12]. Analysis using this model is therefore relevant not only to KDM5C-NDD but also to KDM5A- and KDM5B-related NDDs. *Drosophila* are well-established to display seizure-like behavior (hereafter referred to as 'seizures') and have been used to study the cell types and signaling pathways involved in seizure susceptibility for many years [73, 74]. Consistent with *Drosophila* being able to provide key insights into seizures caused by loss of *KDM5*-gene activity, we demonstrated that fly strains modeling specific pathogenic *KDM5C* missense variants exhibit an increased frequency of seizure activity in response to mechanical stress [71].

In the current study, we extend our understanding of human KDM5C-NDD and expand our model of epilepsy in *Drosophila*. Using molecular and phenotypic data obtained from the rare-disease-centric data collection platform RARE-X, we identified 31 previously uncharacterized individuals from 29 families, which expands the number of *KDM5C* variants in the scientific literature to 122. Phenotypic data from 27 of these individuals enabled the characterization of symptoms associated with KDM5C-NDD in both males and females, and to compare the features of this cohort with those in previous reports. Meta-analysis of our data and published literature revealed that 82% of individuals present with intellectual disability and that 35% present with seizures, with both features displaying sex-related differences. From our *Drosophila* studies, a newly designed apparatus for holding fly vials during filming of behavioral trials enabled us to reliably quantify seizures using heat stress, mechanical stress, and unprovoked paradigms. Our tests revealed that reducing KDM5 levels across all neurons, but not in glia, leads to seizures. These seizures occur independently of KDM5 function in the mushroom body, a brain region previously implicated as a key locus for seizure activity in flies, suggesting that other areas or neuronal subtypes contribute to seizures in this context. Together, these findings bridge human genetic data and functional studies in *Drosophila*, providing insights into the basis for KDM5C-related epilepsy.

Results

General characteristics of 31 newly reported individuals with *KDM5C* variants

As a rare disorder, understanding the full spectrum of traits associated with KDM5C-NDD is an ongoing process. The classical description of KDM5C-NDD, first described by Stephan Claes and Lars Jensen, includes features such as short stature, intellectual disability, developmental delay, and motor challenges [36, 75]. While many reports focus primarily on these characteristics, more recent publications [21, 39, 66] and anecdotal reports by members of the KDM5C-NDD community suggest that the symptoms experienced by those with *KDM5C* variants extend beyond the brain. Building on our evolving understanding of the heterogeneous nature of NDDs, we aimed to expand our knowledge of KDM5C-NDD by characterizing individuals with previously undescribed *KDM5C* variants.

To gain insight into the phenotypic spectrum of KDM5C-NDD, we mined data obtained by the non-profit organization RARE-X, a program of Global Genes [76]. Through their KDM5C Data Collection Program, RARE-X recruited individuals with *KDM5C* variants or their caregivers (when applicable) to complete the Health and Development-Head-to-Toe and symptom-based surveys. Raw, de-identified data were accessed after approval from the Albert Einstein College of Medicine Institutional Review Board (IRB) and the RARE-X Data Access Committee, following the completion of a Data Use Agreement. Using data provided in June 2025, we obtained information for 31 individuals with *KDM5C* variants and curatable genetic reports (Fig. 1A). 28 unique variants were identified, with two unrelated individuals carrying the same variant and three individuals from the same family inheriting the same allele (Table S1).

Variants were detected by several means, including whole exome sequencing, whole genome sequencing, multi-gene panel sequencing, and targeted familial variant testing (See Supplemental Note 1: Case Reports). These alleles were distributed throughout KDM5C and were classified as pathogenic (12 variants), likely pathogenic (13 variants), variants of uncertain significance (2 variants), or unclassified (2 variants) (Fig. 1B). Of the 28 unique alleles, 13 were missense, 8 were nonsense, four were frame shift, two were intronic variants, and one was a microdeletion (Fig. 1A).

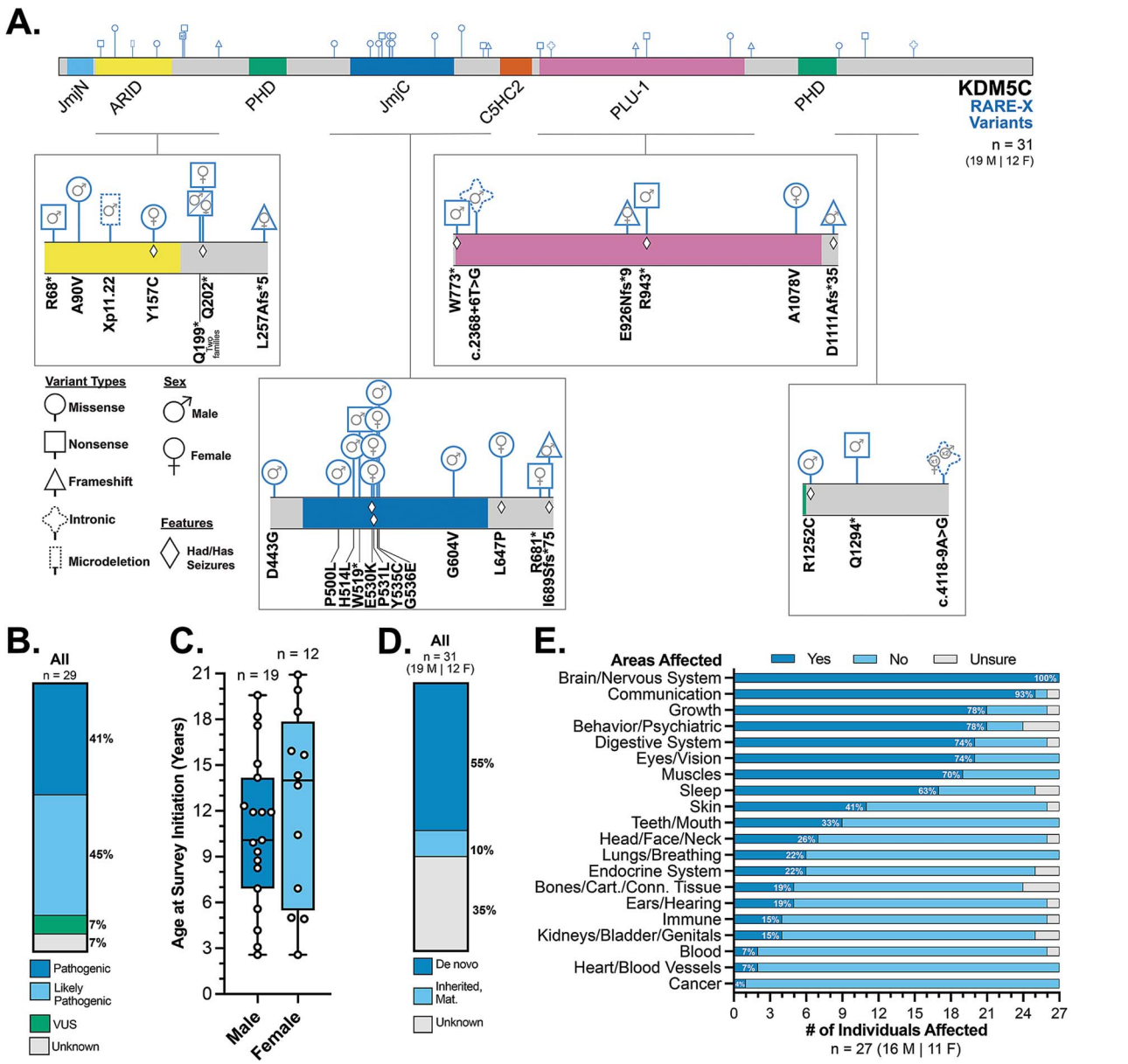

Figure 1. Demographic and phenotypic summary of 31 previously uncharacterized individuals with 28 unique *KDM5C* variants. (A) A schematic depicting the location, variant type, and sex of the individuals with variants in the protein KDM5C described in this study. Variants were reported to the non-profit organization RARE-X, a research program of Global Genes, and confirmed by the organization through assessment of uploaded genetic reports. Participants comprised of both males (M) and females (F) in this study. Variants in which seizures were reported in this study are annotated with a diamond. (B) Pathogenicity classification of *KDM5C* alleles revealed pathogenic, likely pathogenic, variant of uncertain significance (VUS), and unclassified variants within the 29 families. (C) Assessment of participants at the time of survey initiation showed that these individuals ranged in age from 2 years (31 months) old to 20 years (251 months) old. No significant difference was found in age between male and female participants (unpaired, two-tailed t-test, $t = 0.8889$, $df = 29$, $P = 0.3814$). (D) Review of available allele inheritance information revealed that the variants of this study's subjects are primarily the result of *de novo* events, maternally inherited, or of unknown origin. (E) Analysis of completed Health and Development-Head-to-Toe surveys demonstrated that while all participants reported issues with their nervous system, a range of body systems were affected in individuals with *KDM5C* variants.

A breakdown of participant allele information is provided in Table S1 and the Supplemental Note 1: Case Reports.

Both male (61%) and female (39%) KDM5C-NDD participants were represented in this study. At the time of survey initiation, affected individuals were between 2 years (31 months) and 20 years (251 months) old, with a mean age of 10.6 years for male participants and 12.4 years for female participants (Fig. 1C). For approximately one-third of reports, inheritance information was unavailable, possibly due to a lack of familial sequencing performed at the time of genetic report generation, adoption, or the submission of partial genetic reports (Fig. 1D). Of the 31 participating individuals, over half had *de novo* variants, while around 10% reported maternally inherited alleles.

The first survey that participants were asked to complete was a broad 'Health and Development' survey, which asked about issues with different body areas or functions. Among the surveys beyond the general information collection form, the Health and Development survey had the highest participation, with a completion rate of 87%. Notably, in the Head-to-Toe subsection of the Health and Development survey (hereafter referred to as the

Head-to-Toe survey), all participants reported brain or nervous system issues (100%), and most (93%) reported challenges related to communication (Fig. 1E). Other features that occurred in over half of the survey responders included phenotypes related to growth, behavior/psychiatric, the digestive system, eyes/vision, muscles, and sleep. For each of the other categories presented, at least one participant responded affirmatively to having had issues in that realm. Interestingly, despite reports linking KDM5C dysregulation to some cancers [77], only one participant (4%) reported a malignancy, which they noted in a later survey was an unspecified neoplasm/neoplasia/tumor of the central nervous system.

Comparing the frequency of features between sexes revealed no statistically significant difference using a Fisher's Exact test. This could, in part, be due to our limited sample sizes, which decreased as the surveys became more targeted. Therefore, we have chosen not to report additional inferential statistics for the remaining RARE-X data. Numerical differences existed between sexes (Supplemental Fig. 1A and B). For example, males never reported heart/blood vessel issues (0% of males, 18% of females).

As part of the overall Health and Development survey, participants were asked whether they had concerns about their development, when these concerns were first noted, and what specific issues were identified. Deviations from typical developmental milestones for males were noted between the ages of 3 and 30 months, with a median age of 8 months (Fig. 2A). For females, this occurred between the ages of 1 and 18 months, with a median age of 7.5 months (Fig. 2A). Areas of developmental concern showed similar themes to those seen in the Head-to-Toe survey, with over half of respondents having reported challenges with motor development, muscle tone, and coordination (77–96%), communication, babbling/speaking (73%–81%), and physical growth (65%) (Fig. 2B). Other common developmental features included unusually intense interests in certain topics (54%), vision problems (46%), pointing/gesturing/imitating (42%), unusual responses to stimuli (38%), issues related to eating (35%), and self-injurious behavior (35%). Few features were dramatically different between sexes, with the greatest difference being in the pointing/gesturing/imitating category (50% for males, 27% for females) (Supplemental Fig. 1C and D). In response to questions about bodily pain/discomfort, regression, and ambulation, most affected individuals could walk without assistance (96%). However, over a third (37%) reported experiencing regression, with similar rates among males and females. 27% of participants reported having bodily pain/discomfort (Fig. 2C). This differed by sex, with fewer males (13%) than females (50%) responding yes to this question.

Study participants frequently report growth, vision, digestive, and neurological features

Consistent with published descriptions of KDM5C-NDD, growth challenges were present in the majority of survey respondents (78% in the Head-to-Toe survey; Fig. 1E). Results from the level 2 (L2) Growth Survey revealed that 75% of KDM5C-NDD individuals exhibited short stature (Fig. 2D, Supplemental Fig. 1E and F). Interestingly, females reported higher rates of multiple growth-related features, with the greatest difference observed in obesity, which was not seen in males but was reported for 60% of females (Supplemental Fig. 1E and F). Somatropin, the synthetic form of human growth hormone, was taken by two individuals for delayed bone age or delayed growth.

74% of those in our cohort experienced eye or vision issues, which was also noted as an area of developmental concern (46%; Figs 1E and 2B). Responses from the L2 Vision survey highlighted that abnormal eye movement, such as strabismus or nystagmus, was present in the majority (91%) of responding individuals (100% of males, 80% of females) (Supplemental Fig. 2A-C). Several other vision/eye-related issues were noted, however, the only other feature seen in more than one male and female participant was farsightedness (36% for all participants).

Digestive system challenges were also common in our participants (Head-to-Toe-74%; Fig. 1E). Of the responders to the L2 Digestive System survey, the majority (83%) reported experiencing constipation, which was observed at the same rate among both sexes (83% of males and 83% of females) (Supplemental Fig. 2D-F). Other features observed in at least a third of participants included defecation problems (42%), esophageal issues (33%), and feeding difficulties (33%). Intestinal, liver, and pancreatic issues were not reported by any participant. Medications used for the management of constipation include osmotic laxatives, such as polyethylene glycol (1 individual) and lactulose (1 individual), and stimulant laxatives, such as sennosides (1 individual).

78% of respondents reported behavioral or psychiatric concerns in the Head-to-Toe survey, with the L2 Behavior survey underscoring the presence of several features within our cohort (Figs 1E and 2E, Supplemental Fig. 2G and H). Over half of participants reported short attention span (88%), impulsivity (88%), anxiety (71%), temper tantrums (65%), aggressive behavior (65%), autism spectrum disorder (65%), repetitive stereotypic behavior (59%), self-injurious behavior (53%), hyperactivity (53%), mood problems (53%), and difficulty making friends (53%). Several of these features differed between males and females, including repetitive stereotypic behavior (78% of males compared to 38% of females), depression (0% of males and 38% of females), and difficulty making friends (67% of males, 38% of females). Some individuals took medication for the management of behavioral/psychiatric concerns, which included sertraline (4 individuals) and risperidone (1 individual) for anxiety or depression, divalproex for aggression (1 individual), and methylphenidate (3 individuals), amantadine (1 individual), and guanfacine (1 individual) for ADHD or impulsivity.

From the Head-to-Toe survey, altered nervous system features were the most prevalent and best described of the features surveyed, with all respondents reporting changes (Fig. 1E). The L2 Brain and Nervous System survey noted coordination concerns (76%), cognitive impairment (65%), hypotonia (47%), and hypertonia (41%) (Fig. 2F, Supplemental Fig. 2I and J). Two of these traits showed sex differences: coordination, which was present in all females but only 56% of males, and unusual movements, which were not present in females but seen in 22% of males.

Analyses of seizure susceptibility through meta-analyses integrating RARE-X and published data

A key focus of our analysis of the RARE-X data was seizures, for which information was pooled from multiple survey sources to determine the prevalence of this trait in our cohort. For 32% of participants, no seizure information was available. For the remainder of KDM5C-NDD individuals with seizure information, 48% have (or have had) seizures (Fig. 2G). Seizures were observed in both male (42%) and female (56%) participants (Fig. 2H). Of the six who completed the Pediatric Epilepsy Learning Healthcare System (PELHS) survey, the age at first seizure ranged from 1 to 10 years, with a median age of 2 years (Fig. 2I). Several types of seizures were reported, including generalized tonic–clonic, motor, nonmotor, focal, and generalized seizures (Table S1, Supplemental Note 1:

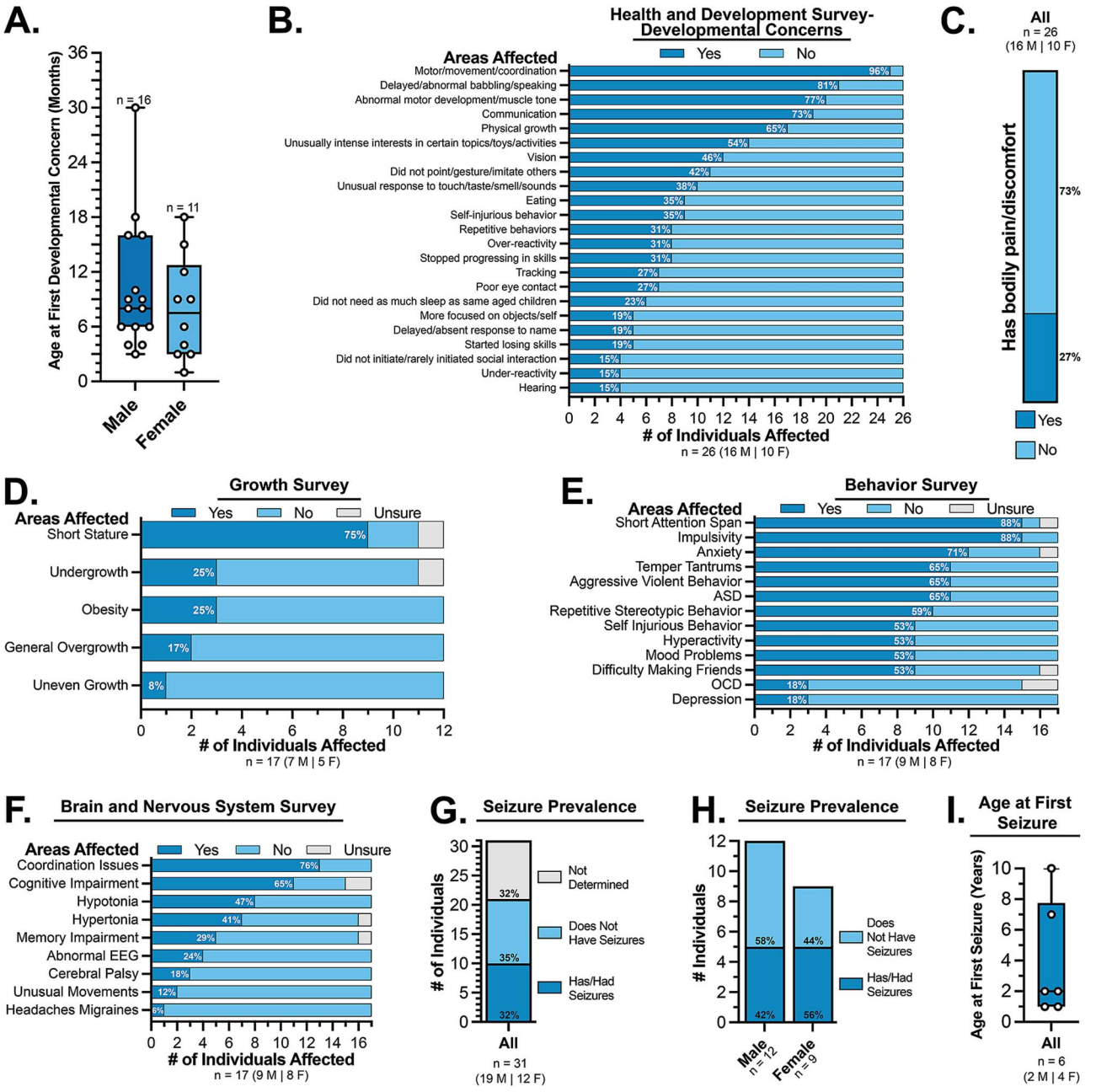

Figure 2. Individuals with *KDM5C* variants in the RARE-X cohort are frequently affected in the realms of development, growth, behavior, and nervous system function. (A) Assessment of completed Health and Development-Developmental Concerns surveys collected by RARE-X revealed that the individuals included in this study had their first developmental concern noted before the age of three, with some having concerns identified as early as one month in age. (B) Data from the Health and Development-Developmental Concerns surveys showed that motor and communication challenges were amongst the most prevalent developmental concerns reported by the individuals in this study. (C) Of those individuals with completed Health and Development surveys, more than a quarter reported to have bodily pain or discomfort. (D) Short stature is the most frequently reported issue of the features noted by those who completed the level 2 (L2) Growth survey. (E) Analysis of the L2 Behavioral/Psychiatric survey results indicated that short attention span, impulsive behavior, and anxiety were some of the most commonly reported issues. Other features, including temper tantrums, aggressive behavior, and autism spectrum disorder, were noted in over half of the participants. Abbreviations used: Autism spectrum disorder (ASD), obsessive compulsive disorder (OCD). (F) Evaluation of the features reported in the L2 Brain and Nervous System survey revealed that coordination issues and cognitive impairment were the most frequently reported features. Abbreviations used: Electroencephalography (EEG). (G) Similar percentages of individuals in our cohort have had a seizure before (32%) and have never had a seizure before (35%), while the remaining participants had no explicit seizure information available (32%). Seizure prevalence in our RARE-X cohort was calculated from data gathered from L2 Brain and Nervous System survey and information that RARE-X summarized from the genetic reports. (H) Seizures are a feature seen in many male and female participants. Seizure prevalence in our RARE-X cohort was calculated from data gathered from the L2 Brain and Nervous System Survey and information that RARE-X summarized from the genetic reports. (I) Pediatric Epilepsy Learning Healthcare System (PELHS) survey results showed the age at first seizure ranged between 1 and 10 years of age with a median age of 2.

Case Reports). Some individuals presented with different combinations of these seizure types, while others were diagnosed with a singular type. For example, one individual reported having Doose syndrome, also known as myoclonic atonic epilepsy, and another reported having Landau–Kleffner syndrome (Table S1, Supplemental Note 1: Case Reports). Several medications were used for seizure management, including oxcarbazepine (2 individuals), lamotrigine (brand name-Lamictal, 1 individual), levetiracetam (brand name-Keppra; 2 individuals), and midazolam (1 individual).

Although we have identified 31 previously unreported individuals with *KDM5C* variants and characterized some of their traits, we sought to integrate this information with the broader knowledge base of individuals with *KDM5C* variants. Through literature searches for papers relating to KDM5C-NDD, Claes-Jensen Syndrome, and *KDM5C* variants and neurodevelopment, we identified 101 families with *KDM5C* variants from 53 journal articles (Supplemental Fig. 3A and B). Combining these data with our RARE-X cohort yielded 122 unique variants from 130 families that were used for meta-analysis (Fig. 3A). Within these 130 families, there were 269 individuals with *KDM5C* variants, comprising 153 males, 112 females, and 4 individuals for whom sex was not reported. Inheritance information was available for 74% of families, with 17% possessing *de novo* variants, 47% inheriting their variant maternally, 1% possessing paternally inherited alleles (two individuals from one family and one individual from an unrelated family), and 9% stating the allele was inherited without specifying the parent of origin. A breakdown of the *KDM5C* variants that have been reported in the literature can be found in Table S2, and a simpler list of all of the KDM5C variants identified between this study and previous studies can be found in Table S3.

Variants were identified across the gene, with no obvious pattern in distribution (Fig. 3A). Of the 130 families in this meta-analysis, about half of the variants were missense variants, 23% were nonsense variants, 18% were frame shift variants, 5% were splice variants, and 3% were other types of variants, such as a microdeletion, a truncating variant, and two intronic variants (Fig. 3C). To categorize these variants, analyses were conducted at the family level, rather than the total pool of individuals, to avoid duplication of variants or inflation due to families with different numbers of individuals represented.

Of the 66 missense alleles identified, 33% were in interdomain regions of KDM5C, while the remainder were within characterized motifs such as the JmjC domain, which had the largest number of variants (36%) (Fig. 3D). This could point to a critical role for KDM5C's canonical demethylase function in pathogenesis, and/or that these alleles may be less likely to be categorized as a variant of uncertain significance (VUS) due to their location in the protein. Other domains with missense changes included the PLU-1 (5%), ARID (11%), C5HC2 (9%), JmjN (3%), and PHD2 (3%) domains. No missense variants were detected in the PHD1 domain. Nonsense variants were excluded from the domain location analysis due to their potential to affect multiple domains if a protein is produced and the likelihood that these variants result in nonsense-mediated decay of the affected mRNA, leading to no protein being produced. Likewise, microdeletions, frame-shift variants, intronic, and splice variants were excluded from the domain location analysis due to challenges in predicting their effects on overall protein structure.

Within the published literature, perhaps the most reported feature of KDM5C-NDD is intellectual disability (ID). Therefore, we sought to determine the prevalence of this feature in all 269 individuals and to investigate whether there was a sex-related difference, as previously reported [39, 66]. Of the 122 males and 79 females (201 annotated individuals) with ID information available, 82% were described as having ID (Supplemental Fig. 3C). There was a significant difference between sexes in terms of occurrence, with 98% of males and 56% of females having been diagnosed with ID (Fig. 3E). Of those with reported ID severity, 51% presented with severe ID, 26% with moderate ID, and 23% with mild ID, although it is unclear whether the same diagnostic criteria were used across all families (Supplemental Fig. 3D). ID severity varied significantly between sexes, with females generally reporting less severe cognitive impairment (Fig. 3F). While 70% of males had severe ID, only 8% of females fell into this category. This trend is reversed for mild ID, with 8% of males and 56% of females, while moderate ID was more similar across the sexes, with 22% of males and 36% of females.

As seizures were a major feature that we noted in our RARE-X cohort, we assessed overall seizure prevalence. For this analysis, seizures were assessed at both a family level and an individual level to gain insights into potential genotype–phenotype connections and to examine the penetrance of this trait. Any mention of epilepsy or seizures, even if the seizures were controlled or occurred once, was counted as the individual having had seizures. We identified 39 families with 39 unique variants associated with seizures, which were distributed across the gene (Fig. 3G; Table S4). At a family level, seizure information was not described or assessed for 46% of cases (Fig. 3H). Of the 70 families with noted seizure information, 56% had seizures, and 44% did not. On an individual level, of the 141 individuals for whom seizure presence could be determined, 35% of individuals had or have seizures (when sexes were pooled). When males and females were compared, a significant difference was noted between the sexes, with 47% of males having (or had) seizures, and 18% females having (or had) seizures (Fig. 3I).

Where available, we also gathered information from the published literature on seizure types and medications (Table S2). Reported seizure types included generalized, generalized tonic–clonic, multifocal, focal, and absence seizures. Several individuals from one family had self-limited epilepsy, and one individual from a different family had sleep-related frontal hypermotor seizures. Published cases reported taking (or had previously taken) several medications for seizure control. These included sodium valproate/valproic acid (5 individuals), levetiracetam (4 individuals), carbamazepine (3 individuals), phenytoin (1 individual), and topiramate (1 individual). Several individuals reported their epilepsy to be intractable, although a lack of standard reporting relating to seizure presence and management made it difficult to identify the true proportion of individuals for whom no effective treatment could be found. Of those with seizures, 91% also had ID, suggesting that individuals who present with seizures are likely to have ID (Fig. 3J, Supplemental Fig. 3E). However, for the reverse, the same does not hold true. Of those with ID, only 40% also had seizures, a rate similar to the overall population evaluated (37%) (Fig. 3K, Supplemental Fig. 3F). A clear genotype/phenotype pattern was not overtly obvious from the distribution of seizure-related alleles, which were found across KDM5C's domains. Variation was seen even within domains. For example, for the 16 families with JmjC missense variants and seizure information available (of 24 total families with JmjC variants), only 6 families presented with seizures, while 10 did not. For the domain with the next-highest number of total missense variants (the ARID domain, with 7 families), only 4 families had seizure information available, with 3 presenting with seizures and 1 without. However,

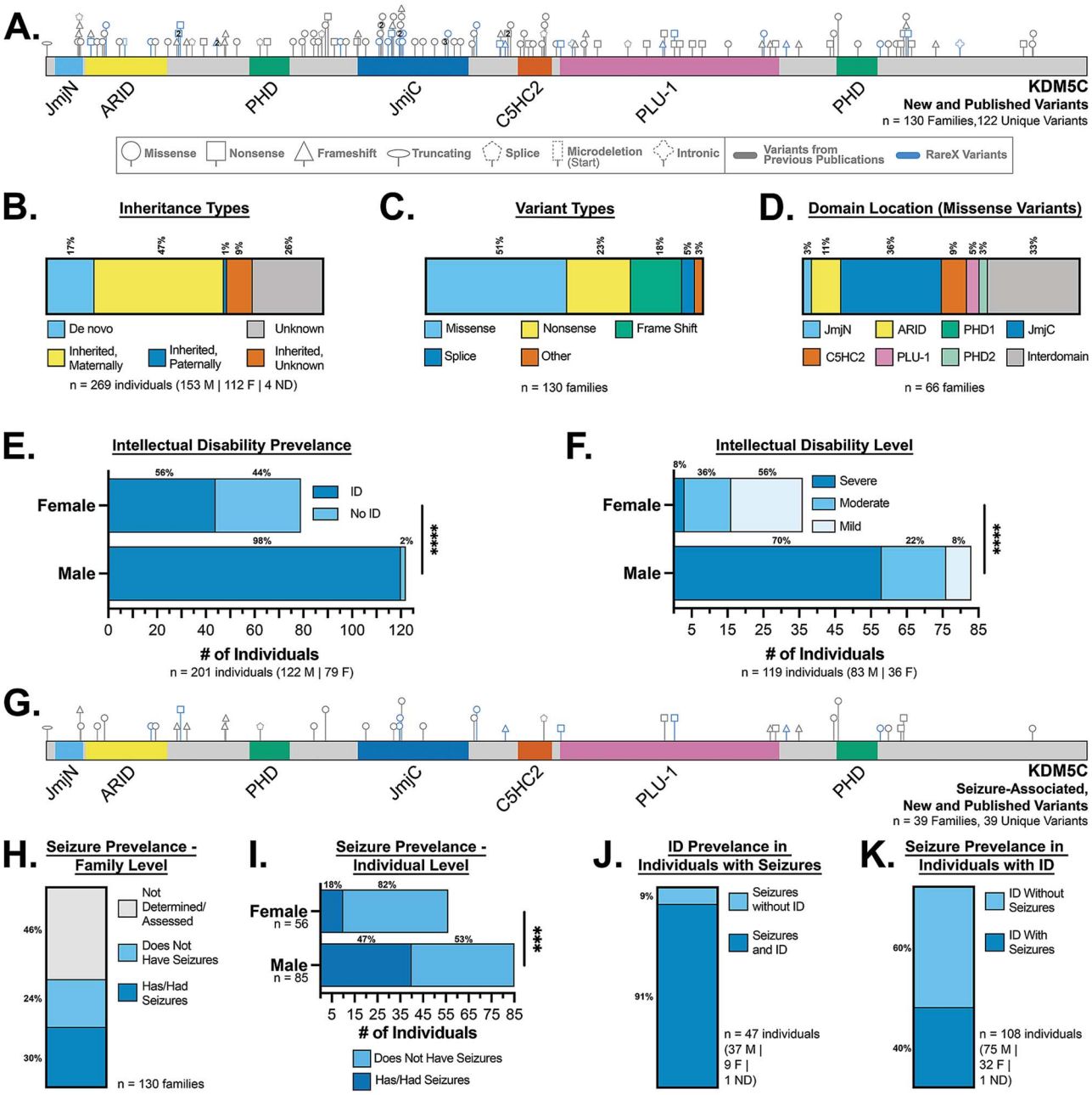

Figure 3. Assessment of the RARE-X cohort and previous reports reveal seizures and ID as common features in individuals with *KDM5C* variants. (A) A schematic illustrating the variant locations, variant types, and sexes of the individuals described in this study and reported in the literature. Variants in which there are more than one family with the same variant have a number in the center of the variant shape. (B) Analysis of the data collected by RARE-X and of information from previously published works indicates that maternal inheritance and *de novo* events are the most common means by which *KDM5C* variants are obtained. Paternal inheritance is a rare event in this population. Abbreviations used: Males (M), Females (F), Not Determined (ND). (C) Analysis of the data collected by RARE-X and of information from previously published works indicated that most reported variants are missense variants, followed by nonsense, frame shift, and splice variants. Other variants that occurred were intronic variants (2 instances), a microdeletion (1 instance), and a truncating (1 instance) variant. (D) Analysis of the data collected by RARE-X and of information from previously published works shows that *KDM5C* missense variants are found across the protein, within defined domains, and within interdomain regions. (E) Analysis of the data collected by RARE-X and of information from previously published works demonstrates that males with *KDM5C* variants are more likely to present with intellectual disability (ID) than females with *KDM5C* variants. (Fisher's exact test, two-sided, $P < 0.0001$). ****$P < 0.0001$. (F) Analysis of the data collected by RARE-X and of information from previously published works indicates that males and females with *KDM5C* variants of those that present with ID show differences in the severity of ID present (Fisher's exact test, $P < 0.0001$). ****$P < 0.0001$. (G) A schematic depicting the location, variant type, and sex of the individuals with *KDM5C* variants that have ever experienced seizures. Variants presented are from this study or previously published studies. (H) Analysis of the data collected by RARE-X and of information from previously published works indicates that while many reports do not explicitly mention seizures, 30% of families have at least one individual in the family that has had a seizure. Families and single individuals whose variant arose as a *de novo* event were counted as families. (I) Analysis of the data collected by RARE-X and of information from previously published works demonstrates that males with *KDM5C* variants are more likely to have seizures (or have had seizures) than females with *KDM5C* variants. (Fisher's exact test, two-sided, $P = 0.0006$). ***$P < 0.001$. (J) Analysis of the data collected by RARE-X and of information from previously published works shows that the majority of individuals who have experienced seizures also present with ID. (K) Analysis of the data collected by RARE-X and of information from previously published works shows that over a third of individuals who have ID also present (or have presented) with seizures.

the limited total number of reported cases make it challenging to assess genotype/phenotype patterns further.

Developing a robust system for quantifying seizures in *Drosophila*

To gain insight into how *KDM5C* variants might lead to seizure predisposition and epilepsy, we turned to *Drosophila* to develop a robust animal model. It should be noted that although the terms 'seizure-like activity' or 'seizure-like behavior' are the most accurate terms when describing this phenotype, we refer to this behavior as a 'seizure' for simplicity. Previous work in *Drosophila* has used various methods to induce seizures, including mechanical, heat, cold, strobe light, and electrical stimulation [78–81]. We used mechanical and heat stimulation to promote seizures, in addition to observing the behavior of flies without any stress induction method (spontaneous seizures) (Fig. 4A).

For mechanical induction, flies were vortexed at maximum speed for 10 seconds, as previously described [78]. For heat induction, flies in an empty vial were submerged in a 40°C water bath, ensuring that the fly could not climb past the heated zone, similar to prior studies [78]. For spontaneous seizure tests, flies were not given an exogenous stressor. For all conditions, flies were recorded, and videos were scored for the presence, duration, and frequency of seizures (Fig. 4A). To quantify seizures, flies were scored as seizing if they exhibited a loss of posture, characterized by lying on their side or back with their legs in the air for at least one second. Loss of posture is an established component of *Drosophila* seizure behavior and is accompanied by immobilization (paralysis) or movement that includes wing flapping, leg shaking, and abdominal muscle contractions (Fig. 4B and C) [73, 74, 82]. Seizure duration was calculated for each event instance from the initial loss of posture until the behavior ended and the fly self-righted, and summed across the total testing time. For heat-induced seizures, these behaviors tended to occur after 1 or more minutes of heat exposure. For mechanical induction, these behaviors could be present early in the recordings, appearing in the first frame of the video recording after vortexing, or at later time points within the one-minute observation window. Examples of the KDM5-associated seizure behavior are shown in Fig. 4C, Supplemental Videos 1 and 2.

While mechanical and heat stimulation have been successfully utilized for several years, there is no standardized method, with observation time varying and seizure behaviors often being scored in real time. To ensure that every seizure behavior was accurately identified, flies were recorded, and behaviors were quantified through detailed analyses of the videos. In addition to providing accurate seizure quantification, this system allowed us to simultaneously record multiple behavioral trials. However, after running several trials, we noted several challenges with our behavioral setup for all three assays. Key among these were 1) we needed to develop a sturdy apparatus to hold our vials in a consistent location to keep the vials in frame of the camera; 2) we needed our vials to be located in front of a solid, consistent background to allow for easy visual tracking of the fly; and 3) we needed an apparatus that could hold our fly vials, which are primarily filled with air, beneath the surface of the water bath when testing flies in our heat paradigm.

To address these challenges, we designed two custom 3D-printable models to hold multiple standard, narrow-sized *Drosophila* vials simultaneously (Fig. 4D and E). The first multiple-vial holder (MVH A) was designed for use in a water bath (Fig. 4D). For our heat seizure tests, we repurposed an immersion circulator designed for sous vide cooking to provide a consistent temperature throughout the water bath. This system additionally allows us to record the flies throughout their time exposed to heat by immersing the immersion circulator in a clear plastic container. Our MVH design A accommodates up to 16 vials simultaneously, while also providing space for the immersion circulator. To prevent the plastic apparatus from floating, space was left open in the center and at the bottom of the MVH for weights to be added (in our case, bricks). To prevent the vials themselves from floating out of the MVH, slots were added to the MVH to keep them underwater. All four sides of the apparatus can be filmed, depending on the number of recording devices available. To ensure that the fly was always in view, plugs were cut at an angle to limit recording blind spots. For non-heat-induced seizure analyses, a second design was created that can hold up to 18 vials at once (MVH B, Fig. 4E). The MVHs can be 3D-printed inexpensively using the supplied files. Recording in our new 3D-printed apparatus enabled flies to be seen clearly, even when recording multiple vials simultaneously (Fig. 4C, Supplemental Videos 1 and 2).

Drosophila KDM5 is required in neurons to prevent spontaneous and induced seizures

Both glial and neuronal dysfunction can contribute to seizures in humans and other mammals, as glial cells, such as astrocytes, microglia, and oligodendrocytes, play significant roles in regulating neuronal activity [56, 83–85]. Neuronal and glial dysfunction can similarly contribute to seizures in *Drosophila*, with perturbation of different proteins exclusively in either cell type having been linked to seizures [86–90]. Previous work from our lab showed that missense variants in *Drosophila* that model KDM5C-NDD alleles displayed increased susceptibility to seizures in response to mechanical stress [71]. However, since these strains are whole-body mutants, the particular cell types responsible for triggering these seizures remain unidentified.

To determine whether KDM5 was required in neurons, we used the UAS/GAL4 system to drive the expression of a short hairpin RNA targeting *Kdm5* only in neurons, using the Elav-GAL4 driver (*elav > shKdm5*). To confirm knockdown, we used immunohistochemistry to label neurons of the adult brain using an antibody against Elav and labeled KDM5 using an antibody against a C-terminal hemagglutinin (HA) tag that is part of the endogenous *Kdm5* gene [69]. In the brains of control animals in which Elav-GAL4 was crossed to the *Kdm5:HA* strain with no short-hairpin transgene (*elav > +*), KDM5 was broadly expressed in neurons, and this was significantly reduced in *elav > shKdm5* flies (Fig. 5A and B). Despite the reduced expression of KDM5 in neurons, the brains of knockdown animals appeared grossly indistinguishable from those of wild-type animals. Previous work demonstrated that knocking down KDM5 in precursor cells of the mushroom body, a key structure involved in learning and memory, can lead to axonal growth and guidance defects [69]. To determine if neuronal knockdown of *Kdm5* using Elav-GAL4 caused defects, we examined the neuromorphology of the mushroom body α/β lobes by detecting the cell adhesion molecule Fasciclin II (FasII). 49% of adult brains from *elav > shKdm5* showed growth and guidance defects in the α/β lobe, which were not observed in control animals (Fig. 5C and D). Of the morphological changes noted, 73% had a midline defect, 21% had a missing α or β lobe, and 6% had a midline defect and a missing lobe.

To determine whether mechanical stimulation could induce seizures, control *elav > +* and *elav > shKdm5* were subjected to vortexing. Significantly more knockdown flies exhibited seizures (53%) than controls (19%) in the one minute immediately after

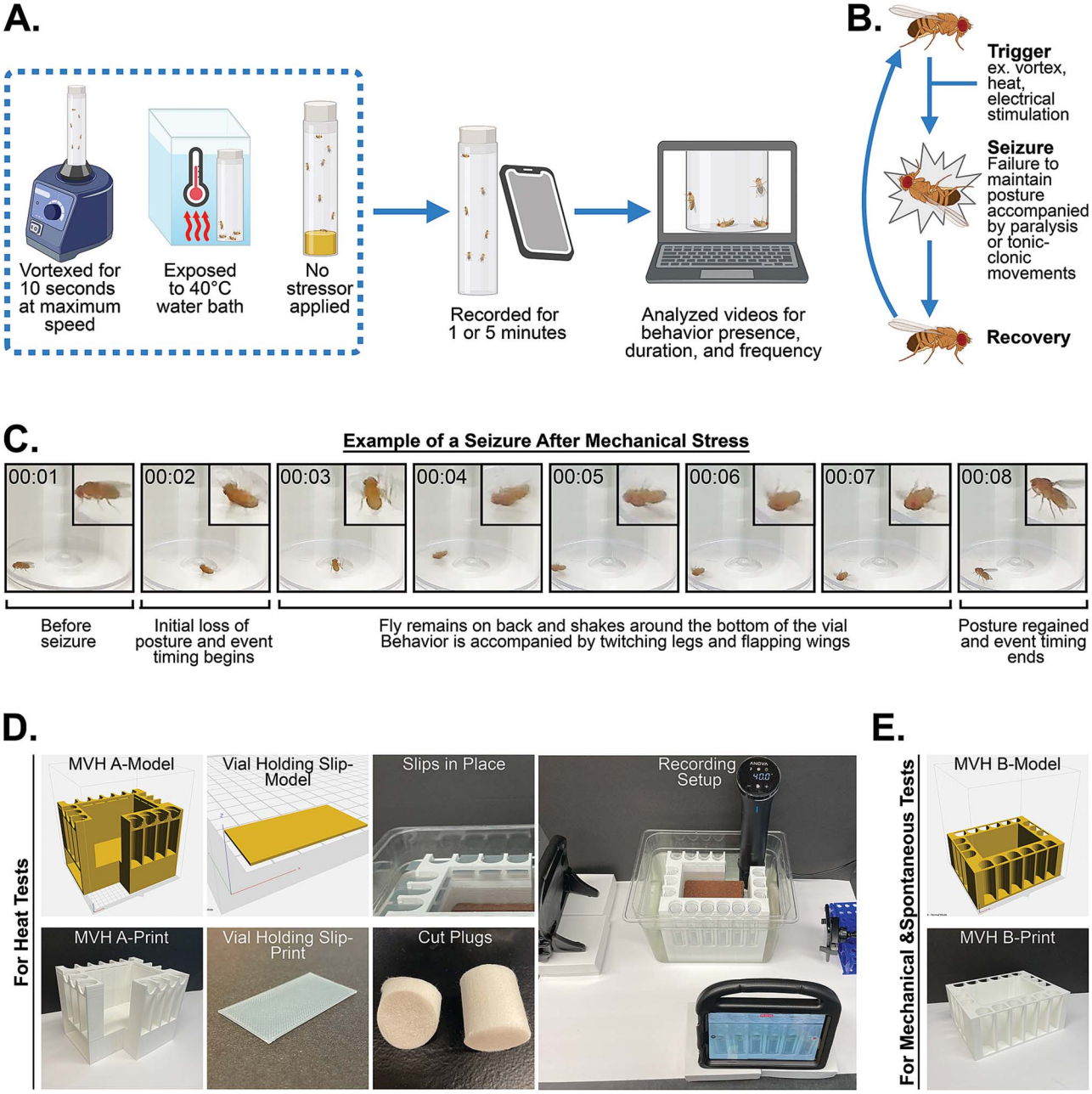

Figure 4. Experimental setup and presentation of seizure activity in *Drosophila*. (A) An overview of the seizure induction methods, recording, and analysis procedures used in this study. Seizure-like activity was unprovoked or stimulated using heat or mechanical stress. Flies were recorded throughout this process for subsequent tracking in Adobe Premiere. (B) An illustration depicting the progression of *Drosophila* seizure behavior. In this study we use loss of posture as an indicator of seizure behavior and measure parameters such as seizure duration, number of seizures, and time to first behavior. Loss of posture (a fly lying on their side or back) can take place with paralysis or can occur with shaking, wing flapping, or muscle contractions. (C) An example of loss of posture following mechanical stress. Times are depicted in the format of mm:ss (minutes:seconds) following stress induction. (D) Renderings and product images of the custom-designed multiple vial holder (MVH A) to allow the recording of multiple vials during heat seizure assays. (E) Rendering and product image of the custom-designed multiple vial holder (MVH B) for holding and recording vials during mechanical or spontaneous seizure assays.

vortexing (Fig. 5E). elav>*shKdm5* flies also exhibited longer seizure duration periods than controls, with means of 16.8 seconds and 2.5 seconds, respectively (Fig. 5F). Quantifying the cumulative proportion of seizure activity over one minute revealed that more *shKdm5* flies continued to have seizures past the first couple of seconds, unlike the controls, which seized early after vortexing (Fig. 5G). This behavior distinguishes our phenotype from that of the classical bang-sensitive lines that have been previously described, which seize immediately after vortexing. In this regard, our phenotype is more similar to the 'late-phase' seizures previously noted in *Klp3A* knockdown flies, which display freezing or normal behavior before seizing rather than losing posture and shaking immediately [91]. Given previous descriptions of multiple seizure bouts in other fly seizure models [91–93], we also sought to determine whether *Kdm5* knockdown flies were more likely to have multiple seizures in comparison to controls. The number of events per fly, however, was not different from controls, with most flies having only one seizure (Supplemental Fig. 4A-C).

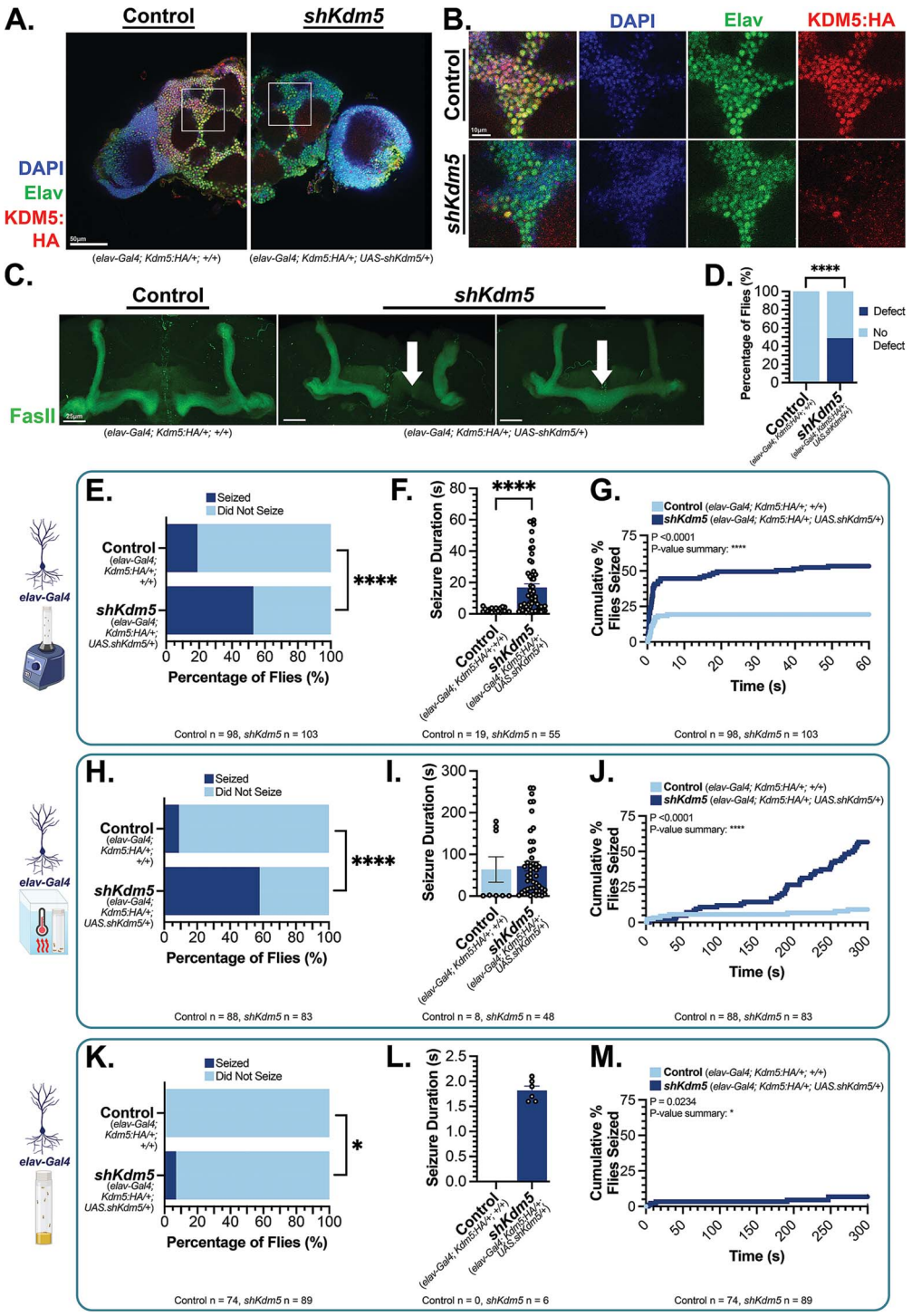

Figure 5. KDM5 is expressed in neurons and its loss causes seizure-like behavior and mushroom body defects. (A) Central brain region from adults from *elav* > + control (*elav-Gal4*; *Kdm5:HA/+*; +/+; left panel) and *elav* > *shKdm5* knockdown (*elav-Gal4*; *Kdm5:HA/+*; *UAS-shKdm5/+*; right panel) groups. These strains have endogenous KDM5 C-terminally tagged with a hemagglutinin (HA) epitope. Anti-HA detects endogenous KDM5:HA, which is observed in most neurons detected by anti-Elav. KDM5:HA is significantly reduced in Elav positive cells in *elav* > *shKdm5* animals. Maximum projection images were generated from immunostained adult fly brains (collected ≤7 days post-eclosion). Scale = 50 μm. (B) Magnified images from the boxed regions in panel A showing reduced KDM5:HA only in knockdown animals. Scale = 10 μm. (C) *elav* > *shKdm5* and not *elav* > + animals show growth and guidance defects (white arrows) in mushroom body neurons detected using anti-FasII. Scale = 25 μm. (D) Quantification of growth and guidance defects presented in panel E. Control n = 40, *shKdm5* n = 67. Statistical analysis was done using a Fisher's exact test (two-sided, $P < 0.0001$). ****$P < 0.0001$. (E-G) *elav* > *shKdm5* flies seized significantly more frequently and for longer durations than controls in response to mechanical stress. Statistical analysis for panel E was done using a Fisher's exact test (two-sided, $P < 0.0001$). Statistical analysis for panel F was done using a Mann–Whitney test (two-tailed, $P < 0.0001$). Statistical analysis for panel G was done using a log-rank (Mantel-Cox) test ($P < 0.0001$). ****$P < 0.0001$. (H-J) *elav* > *shKdm5* flies seized significantly more frequently and for longer durations than controls in response to heat stress. Statistical analysis for panel I was done using a Fisher's exact test (two-sided, $P < 0.0001$). Statistical analysis for panel J was done using a Mann–Whitney test (two-tailed, $P = 0.1667$). Statistical analysis for panel K was done using a log-rank (Mantel-Cox) test ($P < 0.0001$). ****$P < 0.0001$. (K-M) *elav* > *shKdm5* flies seized significantly more frequently, but no longer in duration, than controls in response to no stimulus. Statistical analysis for panel K was done using a Fisher's exact test (two-sided, $P = 0.0323$). Statistical analysis for panel L was not able to be undertaken, given that no control flies seized spontaneously. Statistical analysis for panel G was done using a log-rank (Mantel-Cox) test ($P = 0.0234$). *$P < 0.05$.

In addition to being sensitive to mechanical stress, *elav > shKdm5* flies were also susceptible to heat-induced seizures (Fig. 5H-J). During heat stress, *elav>shKdm5* flies showed a significantly increased overall seizure susceptibility (58%) compared to controls (9%) (Fig. 5H). Quantifying seizures over a five-minute window, differences between groups became apparent after the first minute of recording, with the total number of flies that seized continuing to increase over time (Fig. 5J). However, unlike the increased seizure duration observed after mechanical stress, there was no significant difference in duration between the control and *shKdm5* groups. (Fig. 5I). The number of seizure events also did not significantly differ between groups (Supplemental Fig. 4D-F).

In addition to characterizing seizure behavior following the application of a stressor, we also quantified this behavior at baseline, without the application of a stressor. This was done by placing vials into the fly holding apparatus described in Fig. 4 and filming. Significantly more *elav > shKdm5* flies seized (6.74%) than controls, none of which seized during these tests (Fig. 5K and M). Seizure duration and number of seizures, however, could not be compared between groups, as no flies exhibited seizures in the control group (Fig. 5L, Supplemental Fig. 4G-I).

Control *elav > +* flies were noted to respond to both heat and mechanical stress but did not display seizures when tested in the spontaneous seizure condition (Fig. 5). While these seizures occurred at much lower rates and durations than those associated with *Kdm5* knockdown, it was interesting to note that they occurred at all in flies with normal levels of KDM5. To investigate how genetic background differences may have contributed to this baseline phenotype in our controls, we characterized two additional control groups using our assays. The first control strain contains the attachment site P (attP2) site into which the *shKdm5* transgene is inserted, but no transgene, and the second has a short hairpin directed toward the non-*Drosophila* gene LexA (*shLexA*). When crossed to Elav-GAL4, both the attP2 and *shLexA* strains exhibited seizures in response to mechanical and heat stress (Supplemental Fig. 5A and C). Susceptibility to seize occurred at similar rates in both groups in response to mechanical (attP2–21%, *shLexA*-25%) and heat (attP2–7%, *shLexA*-11%) stress when compared to our original KDM5:HA control results (mechanical-19%, heat-9%). Event duration also occurred at similar lengths to controls, with the majority of events occurring for only several seconds in both assays (Supplemental Fig. 5B and D). Thus, in our hands, low-level seizure responses appear to be a general feature across genetic backgrounds. These data further emphasize that the increase in seizures seen in *Kdm5* neuronal knockdown animals represents a true, gene-specific effect.

Because individuals with *KDM5C* variants exhibit sex-related differences in seizure prevalence, we examined whether male and female flies showed similar increases in seizure susceptibility. For the two stress conditions, male and female flies showed similar rates of seizure susceptibility (58% of females and 49% of males for mechanical stress, 60% of females and 55% of males for heat stress) (Supplemental Fig. 6A, C, E, G). Likewise, seizure duration was similar between sexes for both conditions, with an average of 15.4 seconds for females and 18.5 seconds for males for mechanical stress and an average of 76.5 seconds for females and 65.8 seconds for males for heat stress (Supplemental Fig. 6B, D, F, H). For the spontaneous condition, both sexes seized (2% of females and 11% of males with an average duration of 2.1 seconds for females and 1.8 seconds for males; Supplemental Fig. 6I-L). Statistical analyses were conducted to compare the female and male groups across all three conditions, and no significant differences were detected in any analysis, indicating a lack of sex-related differences. Together, these results support the interpretation that the absence of sex-specific seizure phenotypes in *Drosophila* reflects the autosomal location of the sole *Kdm5* gene, distinguishing it from the X-linked *KDM5C* locus in humans and likely explaining the sex differences seen clinically.

KDM5 activity is not required in glia or mushroom body neurons to suppress seizures

To test whether glia contribute to the seizures in our model, we used the pan-glial driver Repo-GAL4 to knock down *Kdm5*. In control *repo > +* flies, KDM5 was expressed in the majority of glial cells examined, and *repo > shKdm5* efficiently reduced levels of KDM5 in Repo-positive cells of the adult brain (Fig. 6A and B). As glia can be a driver of mushroom body defects, we also probed brains from *repo > shKdm5* knockdown flies for morphological defects in the α/β lobes [94–96]. Anti-FasII staining revealed that only a small portion of the *repo > shKdm5* knockdown flies showed growth defects (6.5%) (Fig. 6C and D). While this was significantly higher than in control flies, which showed no defects, it was far lower than the defects observed in *elav > shKdm5* flies (49%). *repo > shKdm5* flies did not, however, exhibit changes to the number or severity of seizures in comparison to controls in response to mechanical or heat stress, or without an applied stressor (Fig. 6E-G; Supplemental Fig. 7A-F). Finally, separating flies into female and male-specific comparisons revealed no significant differences between groups, except for seizure duration for the female flies undergoing the vortex assay (Supplemental Fig. 8A-L). *shKdm5* flies in this condition, however, showed an overall lower overall susceptibility to seize. Reduced levels of KDM5 in glial cells is therefore not sufficient to drive seizures in flies.

Building on our finding that KDM5 plays a critical role in neurons to suppress seizure activity, we tested the role of the mushroom body, as this structure can be a source of seizures in *Drosophila* [92, 97]. Consistent with this possibility, Elav-GAL4, but not Repo-GAL4, driven knockdown of KDM5 caused both seizures and neuromorphological changes to the mushroom body. To test this, we knocked down *Kdm5* using the well-characterized OK107-GAL4 driver. As we have shown previously, OK107-GAL4 driven knockdown of *Kdm5* produces animals with striking mushroom body defects in the adult brain (Fig. 7A) [69]. Despite structural defects in the mushroom body, no statistically significant difference in mechanical stress or heat stress was observed in *OK107 > shKdm5* animals (Fig. 7B-J, Supplemental Fig. 9A-L). For the spontaneous seizure condition, the overall susceptibility trended towards significance ($P = 0.0577$), and the assessment of susceptibility over time using a survival curve revealed a significant difference between groups ($P = 0.0281$). None of the control flies in this condition displayed seizure behavior, whereas we observed seizures in our knockdown flies. Together, these data suggest that while mushroom body neurons may contribute to the development of this behavior, it is not an epicenter of seizure behavior in our KDM5-deficient flies.

Discussion

In this study, we characterized 31 individuals from 29 families with 28 unique variants. While several other reports have provided sizable cohorts of individuals with *KDM5C* variants (notably Leonardi et al., 2022; Carmignac et al., 2020; Jensen et al., 2005; and Tzschach et al., 2006), our study provides the largest cohort of families described to date. Analysis of body-wide symptoms revealed several features observed in the majority of participants,

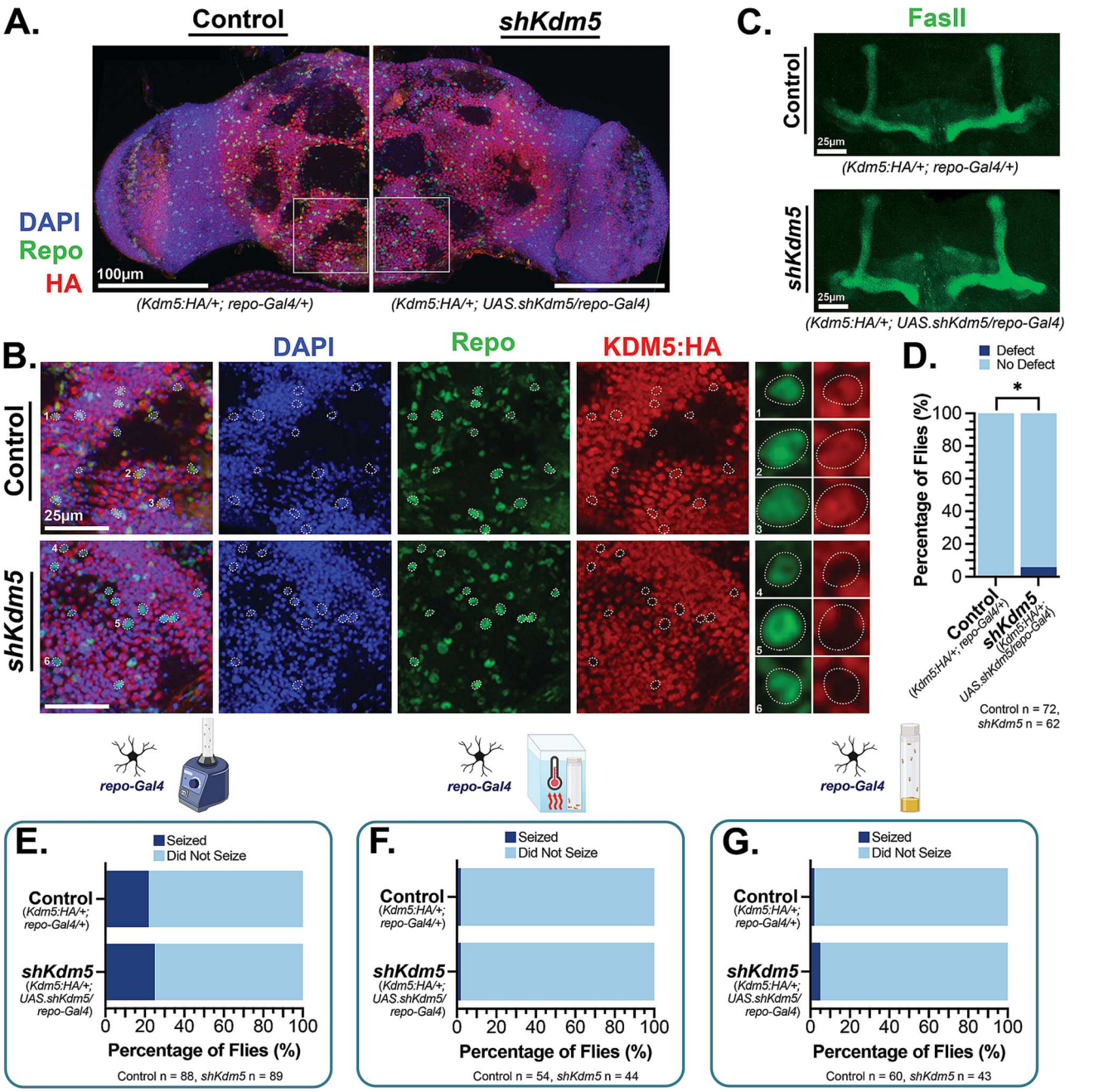

Figure 6. KDM5 is expressed in glia and its loss causes limited mushroom body defects, but not seizures. (A) Endogenously tagged KDM5:HA is found in many glia (Repo+) of the central brain in *repo > +* controls (*Kdm5:HA/+; repo-Gal4/+*) animals and is greatly reduced upon *Kdm5* knockdown in *repo > shKdm5* (*Kdm5:HA/+; UAS.shKdm5/repo-Gal4*) animals. Maximum projection images were generated from immunostained adult fly brains (collected ≤ 7 days post-eclosion). Scale = 100 μm. (B) Magnified images from the boxed regions in panel A. Examples of Repo positive cells are outlined with a white dashed line. Three of these cells per condition were enlarged for illustrative purposes. Scale = 25 μm. (C) FasII immunostaining shows no structural changes in alpha and beta lobes of the adult mushroom body in the majority of flies following *Kdm5* knockdown in glia in *repo > shKdm5* (*Kdm5:HA/+; UAS.shKdm5/repo-Gal4*) animals. (D) Reduction of KDM5 levels in glia affects mushroom body structure in 6% of animals studied. Statistical analysis was done using a Fisher's exact test (two-sided, $P = 0.0434$). *$P < 0.05$. (E) *repo > shKdm5* flies showed no difference in seizure susceptibility in response to mechanical stress. Statistical analysis was done using a Fisher's exact test (two-sided, $P = 0.7265$). (F) *repo > shKdm5* flies showed no difference in seizure susceptibility in response to heat stress. Statistical analysis was done using a Fisher's exact test (two-sided, $P > 0.9999$). (G) *repo > shKdm5* flies showed no difference in seizure susceptibility when observed without an induced trigger. Statistical analysis was done using a Fisher's exact test (two-sided, $P > 0.5696$).

including short stature, coordination issues, cognitive impairment, short attention span, impulsivity, anxiety, abnormal eye movements, and constipation. Seizures were also a major feature, occurring in 48% of individuals. Interestingly, in our cohort, although we observe differences between sexes, particularly in coordination, unusual movements, obesity, impulsivity, and repetitive stereotypic behavior, many features were present in both males and females at similar rates (Supplemental Figs 1 and 2). However, when considered in the broader context of all individuals described in the KDM5C literature, differences between sexes may become clearer. This was seen in our meta-analysis of seizures and intellectual disability, in which

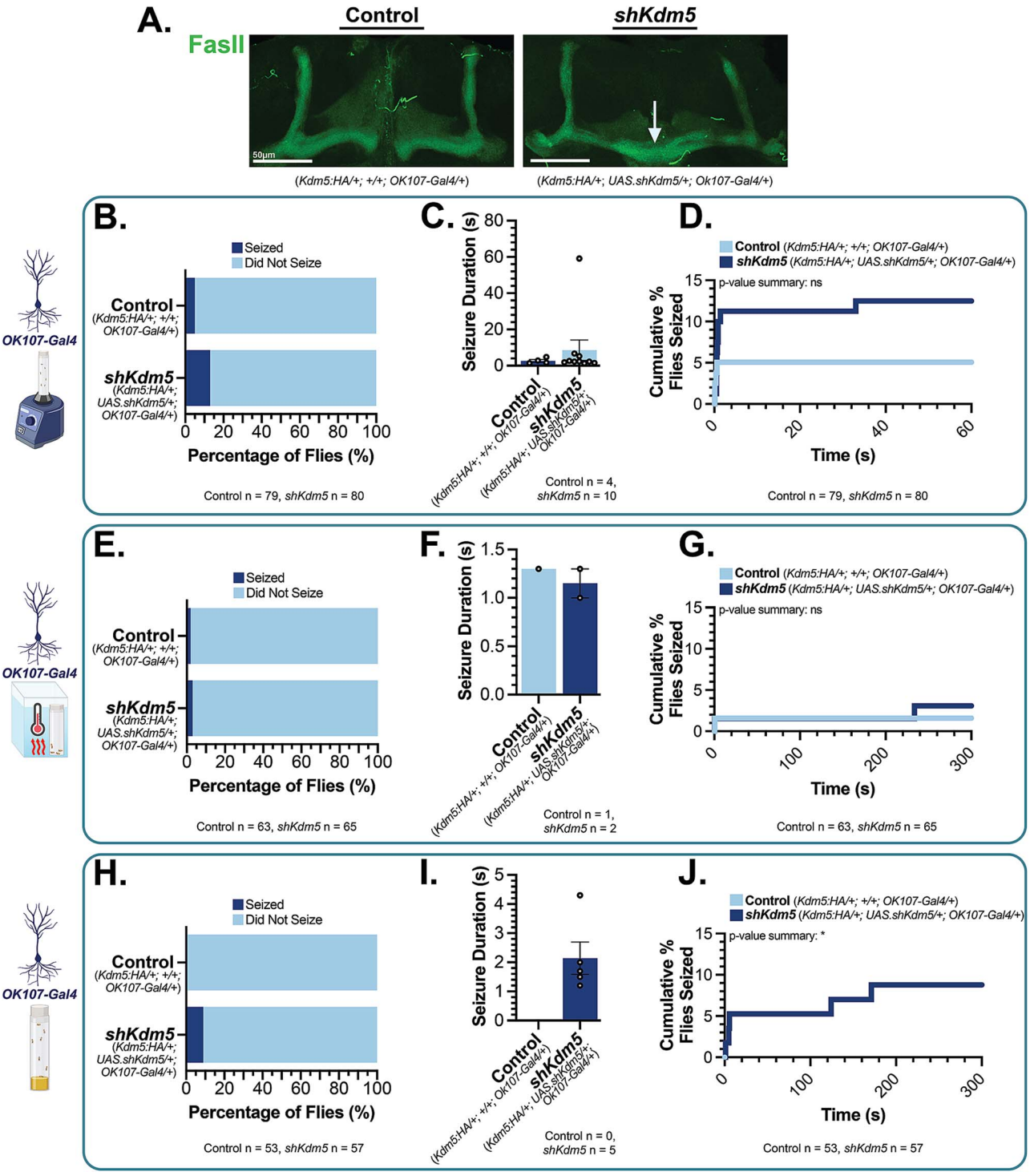

Figure 7. KDM5 reduction in mushroom body neurons promotes mushroom body defects, but not seizures. (A) Anti-FasII staining shows that reduction of KDM5 levels in mushroom body neurons promotes growth and guidance defects (white arrow), which are not seen in controls. Control genotype is *OK107 > +* (*Kdm5:HA/+; UAS.shKdm5/+; OK107-Gal4/+*) and knockdown *OK107 > shKdm5* genotype is (*Kdm5:HA/+; UAS.shKdm5/+; Ok107-Gal4/+*). Scale = 50 μm. (B-D) *OK107 > shKDM5* and *OK107 > +* flies showed similar seizure rates and durations following mechanical seizure induction. Statistical analysis for panel B was done using a Fisher's exact test (two-sided, $P = 0.1600$). Statistical analysis for panel C was done using a Mann–Whitney test (two-tailed, $P = 0.7103$). Statistical analysis for panel D was done using a log-rank (Mantel-Cox) test ($P = 0.1092$). (E-G) *OK107 > shKDM5* and *OK107 > +* flies seized at indistinguishable rates and durations following heat seizure induction. Statistical analysis for panel E was done using a Fisher's exact test (two-sided, $P > 0.9999$). Statistical analysis for panel F was not able to be completed because of the limited number of flies that seized. Statistical analysis for panel G was done using a log-rank (Mantel-Cox) test ($P = 0.5791$). (H-J) *OK107 > shKDM5* and *OK107 > +* flies seized at similar rates and durations when observed for spontaneous seizures. Statistical analysis for panel H was done using a Fisher's exact test (two-sided, $P = 0.0577$). Statistical analysis for panel I was not able to be completed because of the limited number of flies that seized. Statistical analysis for panel J was done using a log-rank (Mantel-Cox) test ($P = 0.0281$).

more males than females displayed seizures and intellectual disability when compared to the RARE-X data set (Figs 2 and 3, Supplemental Fig. 3).

The majority of the RARE-X variants described here are unique, further increasing the number of orphan alleles associated with KDM5C-NDD. We do, however, note several unrelated individuals/families with the same variant within our RARE-X study, as well as between our study and previous reports, and between previous reports. These include Q199* (2 families), V504M (2 families) [7, 31], P531L (2 families) [39], R599C (3 families) [7, 21, 39], R694* (2 families) [36, 43], and R943* (2 families) [55] (Table S1-S3). As additional participants with variants across the different domains of KDM5C are more thoroughly characterized and more families with overlapping variants are profiled, genotype–phenotype relationships may begin to be elucidated; however, a lack of standardized reporting and a limited number of individuals preclude this possibility at this time. Even among recurrent alleles, we do not observe consistent differences in clinical severity or feature profile. Continued identification of new alleles and better standardization of clinical reporting may help highlight these trends in the future. It is, however, possible that additional genetic and/or environmental factors contribute to the observed trait heterogeneity. Notably, even in *Drosophila*, where the genetic background is tightly controlled, not all individual flies display the same behavioral or neuromorphological phenotypes [68, 71, 72]. Across species, KDM5 dysfunction may therefore inherently yield a spectrum of outcomes.

Knowledge gained from describing the spectrum of KDM5C-NDD-associated symptoms and their impact on daily life is expected to guide healthcare professionals, direct service providers, and others with limited exposure to this rare disorder in recognizing the potential features that may be present or emerge. Phenotypic data provided here is also likely to assist in the development of screening and assessment tools to guide clinical management of KDM5C-NDD and identify areas for additional intervention or testing, similar to resources available for other genetic conditions and NDDs [98–102]. Given the age range of participants (2–20 years) and the overall communication challenges noted among participants, it is likely that our study does not provide a complete picture of the traits associated with KDM5C-NDD. Some symptoms may not be present yet or may not be able to be effectively conveyed to the caregiver completing the survey. Because RARE-X prompts participants to update their survey responses yearly, going forward, we expect to gain additional insights into how the changing needs and presentation of older adults may differ from those of the children and young adults studied thus far. In the future, increasing recruitment efforts aimed at older participants or other underrepresented groups, such as international participants, will enhance the representation of these groups in our data. The ongoing nature of the RARE-X data collection also enables us to increase the total number of participants in our study and to address the participant drop-off typically seen more often with the level 2 surveys that provide more detailed information about specific topics.

Based on the limited understanding of the association between pathogenic variants in *KDM5C* and epilepsy, we developed a *Drosophila* model of seizures to enable molecular investigations of this association. To enable robust investigations, we optimized three different paradigms to quantify seizures that utilize a new 3D-printed apparatus for holding vials (Fig. 4). More importantly, our study demonstrates that KDM5 is crucial for normal neuron function, as its loss in neurons but not glia leads to seizures (Figs 5 and 6). This feature was not seen when KDM5 was reduced in cells of the mushroom body, despite reduced KDM5 altering axonal growth and guidance of this structure (Figs 5-7). Future refinement of the types of neurons that contribute to this phenotype, as well as the brain regions that function as seizure epicenters, will provide important insights into KDM5-related seizures. Transcriptomic and cellular assays will also provide opportunities to define the signaling pathways that are perturbed and lead to seizure susceptibility. This, in turn, is likely to highlight pathways for the development of targeted therapeutics. The scalability of *Drosophila* studies provides a unique opportunity to conduct large-scale screens to test the efficacy of new and existing anti-epileptic medications using our knockdown animals, as has been done previously with other *Drosophila* epilepsy models [103, 104].

Extending our studies using *Drosophila*, we anticipate including future studies on age-related seizure susceptibility, as this animal model has a median lifespan of approximately 50 days. The studies described here focused on younger adult flies (0–7 days in age). Other seizure models show age-related changes, suggesting that older flies may exhibit more pronounced differences in seizure susceptibility than younger flies [105, 106]. *Drosophila* larvae have also been used to model seizures, and analyzing this developmental time point may help identify critical windows for KDM5 function in preventing seizures [78]. Like the intersection between age and seizures, we could also investigate the interactions between seizures and other traits, such as altered sleep or diet/microbiome, as has been studied in other fly models [107–111].

Taken together, our human and *Drosophila* findings underscore the critical role that KDM5 proteins play in nervous system functioning, where their loss or dysfunction predisposes to altered cognition and seizures. By capturing the seizure and other behavioral features of KDM5C-NDD, our *Drosophila* model provides a means to understand the basis of traits observed in humans. Studying more cases of individuals with variants in *KDM5A*, *KDM5B*, and *KDM5C*, along with modeling these alleles in *Drosophila*, will broaden our understanding of the mechanisms underlying KDM5 family-related neurodevelopmental conditions.

Materials and methods

Subjects surveyed through RARE-X

All research involving human participants was conducted with approval from the Albert Einstein College of Medicine Institutional Review Board (IRB) (protocol number #2024–15912). Access to data collected by RARE-X, a program of Global Genes, was obtained under a Data Use Agreement between Global Genes and the Albert Einstein College of Medicine. RARE-X is a non-profit organization that collects and stores health-related information about individuals with rare diseases. Informed consent was obtained by Global Genes/RARE-X for each participant/caregiver.

For simplicity, we refer to all individuals described in this study as 'participants' or 'respondents' when discussing information gathered through RARE-X-administered surveys, regardless of whether responses were provided directly by the individual with a *KDM5C* variant or by a proxy caregiver. Any individual with a *KDM5C* variant could enroll (directly or by proxy) in the KDM5C Data Collection Program by requesting access to the RARE-X platform. Inclusion criteria for this study included any person who has a confirmed variant in the *KDM5C* gene and has uploaded a genetic report for validation by RARE-X. Those without curatable genetic reports were excluded from the current analysis. For most

participants, the variants reported were described in reference to isoform 1, the longest isoform, of *KDM5C*. Two individuals (participants with the Xp11.22 (53242376_53247160) and H514L variants) did not have a RefSeq accession number provided but were assumed to be referencing isoform 1. One participant was originally described in reference to isoform 2 of KDM5C. For comparison purposes, this variant was realigned to isoform 1, changing the reported variant from R1185C (isoform 2) to R1252C (isoform 1).

For this study, the cohort of 31 participants was comprised of individuals who provided curatable genetic reports between March 2023 and June 2025. Participants represented five countries worldwide, with the majority from the United States (25), along with participants from the United Kingdom (3), Canada (1), Mexico (1), and Germany (1). Enrolled individuals (or their caregivers if applicable) were given several initial surveys to collect basic, demographic, and overall health information and were asked to upload their genetic reports for curation and de-identification by the RARE-X team. Of these initial surveys, one particularly important survey included the Health and Development-Head-to-Toe survey, where participants were asked to note whether a certain area or function of the body was affected with a 'Yes,' 'No,' or 'Unsure.' Based on responses to the Health and Development-Head-to-Toe survey, level 2 (L2) surveys were assigned by RARE-X to gain a more comprehensive picture of the symptoms observed in each participating individual. Fewer responses were noted as survey level increased, in part because non-affected individuals should not have been assigned L2 surveys to complete.

Descriptions of the presentation of each of the 31 participants are included in the Supplemental Note: Case Reports. However, several notes regarding specific participants will be made here. Firstly, three participants with the same confirmed genetic variant (c.4118-9A > G) from the same country were enrolled in this study. Given that two of the three report familial/targeted variant testing that was done the following year after the first participant's whole exome sequencing and without any features excluding the possibility that these three individuals are related, they were treated as one family. Two different individuals presented with the same variant (Q199*), however these were ruled as unrelated individuals as they were from different countries and the female participant is noted as having a *de novo* variant.

Three individuals report variants previously noted in the literature (L257Afs*5, P531L, and R943*). For L257Afs*5, due to age (with our participant being much younger), reporting that the female participant is unsure about a family history, and the female participant being from a different country on the same continent than where the literature study was conducted, this participant is being treated as a unique individual from a different family [112]. For P531L, the individuals were of different sexes and from different continents, so they were treated as unique individuals from different families [39]. For R943*, the individuals are from different continents and both report *de novo* inheritance and were thus treated as unique individuals from different families [55].

Literature review

A literature search was conducted using different combinations of a variety of search terms, some of which included 'Claes-Jensen Syndrome,' 'KDM5C,' 'JARID1C,' 'SCMX,' 'X-linked intellectual disability', 'neurodevelopmental,' and 'developmental.' Identified journal articles were reviewed for the mention of intellectual disability, intellectual disability level, seizures, seizure type, seizure-related medications taken, protein/genetic variant, sex, and inheritance type. If a variant was reported to be inherited, the parent from whom it was inherited from (if described) was included as a *KDM5C*-variant possessing individual in the literature review table, even if no additional information was included (Table S2). If sex or inheritance type was not explicitly mentioned, it was reported as 'Unknown.'

When assessing intellectual disability and seizure features at an individual level, if a feature (Seizures/Intellectual Disability) was explicitly noted to be present, the feature was marked as a 'Yes.' If a feature was explicitly noted to not be present, the feature was marked as a 'No.' If a feature was not explicitly mentioned, the feature was reported as 'Not Described/Not Assessed' or '(ND/NA).' When assessing intellectual disability and seizure features at a family level, families were marked as 'Yes' if any member of the family reported to have that feature. Family-level features were marked as 'No' if it was explicitly stated that none of the family members have that feature. Family-level features were marked as 'No/ND/NA' if there were no reports of that feature, but not every single family member was explicitly defined as to not have that feature.

Fly strains and care

Flies used in this study were kept at 25°C on a 12-h light:dark cycle. A standard cornmeal/molasses/yeast diet was provided [68]. Male and female flies were analyzed for each experiment, and data were pooled if no sex-based difference was detected. The Elav-GAL4 strain used also contained tub-GAL80^{ts}, but this was kept inactive by raising the animals at 25°C, allowing knockdown in neurons. For use in this study, the UAS-*shKdm5* and UAS-*shLexA* strains (both inserted into the attP2 site on chromosome III) were crossed into the w^{1118}; *Kdm5:HA* background that has an HA-tag within the endogenous *Kdm5* locus on chromosome II. The strain harboring the attP2 alone (no transgene inserted) was crossed into the w^{1118} genetic background.

The following fly strains were obtained for use in this study:

Source	Nomenclature used	Genotype
BDSC #602908	*repo-Gal4*	w[1118]; P[w[+mC] = repo-Gal4.L]Oatp30B[repoF3]
BDSC #458	*elav-Gal4*	P[GawB]elav^{C155}
BDSC #7017	*tub-Gal80^{ts}*	P[tubP-Gal80^{ts}]2
BDSC #854	*OK107-Gal4*	w[*]; P[w[+mW.hs] = GawB]OK107 ey[OK107]/In(4)ci[D], ci[D] pan[ciD] sv[spa-pol]
BDSC #35706	*UAS-shKdm5*	y [1] sc[*] v [1] sev [21]; P[y[+t7.7] v[+t1.8] = TRiP.GLV21071]attP2/TM3, Sb [1]
Hatch et al., 2021 [69]	*Kdm5:HA*	*Kdm5*:3xHA
BDSC #67945	*UAS-shLexA*	y [1] sc[*] v [1] sev [21]; P[y[+t7.7] v[+t1.8] = TRiP.HMS05773]attP2
BDSC #8622	*attP2*	Y [1] w[67c23]; P[y[+t7.7] = CaryP]attP2

Seizure induction techniques

For all seizure tests, fly vials were plugged with Charlie Plugs (Jaece CP-N730) that were cut at an angle to ensure that no flies were occluded from view during testing. Flies were tested at an age of ≤ 7 days post-eclosion, and the sex of the flies was noted

for each vial/trial. The day before intended testing, flies were separated into food-containing vials in groups of ≤7 flies and allowed to recover overnight after being anesthetized with carbon dioxide. On the day of testing, flies were transferred into empty vials and allowed to recover for at least an hour before testing. Vials were recorded with an iPhone or iPad mini camera, and all testing was conducted between 9 a.m. and noon.

Mechanical seizures were induced by individually shaking vials on a laboratory vortex (Fisher Scientific #02215365) at maximum speed for ten seconds at room temperature. Immediately post-vortexing, vials were moved to custom-designed vial holders (see 3D Printing methods section for more information). Vials were recorded for one minute after shaking. This protocol was adapted from Mituzaite et al., 2021 [78].

Heat seizures were induced by placing fly vials directly into a 40°C water bath for five minutes. A twelve-quart sous vide container (EVERIE Sous Vide Container Bundle from Amazon.com) was used as a water bath and a consistent water temperature was maintained by an immersion circulator originally meant for Sous Vide cooking (Anova Culinary Nano Sous Vide Precision Cooke, #AN400-US00). Plugs were pushed down to around the 75% of the height of the vial so that the vials could be submerged to a point where the area in which the flies could move would be completely covered by the water in the water bath, but not so much that water could enter in through the top. Vials were held in the water with a custom designed vial holder (see 3D Printing methods section for more information) that was weighed down by a brick (8″ x 4″ x 2″, Lowe's #817669) or a four-way modular tube rack (Cole-Parmer UX-06733-00). Flies were recorded for five minutes post water bath exposure for later analysis. This protocol was adapted from Mituzaite et al., 2021 [78].

Spontaneous seizures were defined as any seizure activity during a five-minute period of time without any stressor (heat or mechanical) given.

Behavioral analysis

Videos were assessed in Adobe Premiere Pro (v.24.5.0) by adding markers to video clips when a fly displayed seizure activity. The markers were added so that the 'In' time was the beginning of the behavior and the 'Out' time was the end of that behavior. This was done for each time that an individual fly seized, so that the following parameters could be measured: seizure presence (yes/no), total seizure duration, time to first seizure, and number of seizures. Seizure susceptibility and duration was based on the period during which a fly turned on its side or back and displayed shaking or immobilization (paralysis). This is a characteristic part of *Drosophila* seizure behavior and the clearest visual to determine if a fly demonstrates seizure behavior [74]. An event was defined as a seizure for all events lasting at least one second.

3D printing

For video recording purposes, flies were held in either a four-way modular tube rack (Cole-Parmer UX-06733-00) with a white backdrop or one of two custom-designed 3D-printed vial holder racks. The vial holders were designed in Tinkercad (Autodesk, https://www.tinkercad.com/) and printed in white Polylactic Acid (PLA, Flashforge) using an Adventurer 4 Pro 3D printer (Flashforge). The larger vial holder was custom designed with water bath heat-induced seizure assays in mind and was created to accommodate an immersion circulator and a brick or some other sort of weight. The smaller vial holder was designed with mechanical seizure induction in mind and created to be used out of water. STL files are available as supplemental files.

Antibodies

The following antibodies were used in this study:

Company	Product	Company Product Number	RRID	Concentration Used
Cell Signaling Technology	HA-Tag (C29F4) [Rabbit monoclonal]	3724	AB_1549585	1:200
Developmental Studies Hybridoma Bank	1D4 anti-Fasciclin II [Mouse monoclonal]	1D4	AB_528235	1:25
Developmental Studies Hybridoma Bank	Elav-9F8A9 [Mouse monoclonal]	9F8A9	AB_528217	1:10
Developmental Studies Hybridoma Bank	8D12 anti-Repo [Mouse monoclonal]	8D12	AB_528448	1:300
Thermo Fisher Scientific	Goat polyclonal anti-mouse Alexa-488	A32723	AB_2633275	1:500
Thermo Fisher Scientific	Goat polyclonal anti-mouse Alexa-568	A11004	AB_2534072	1:500
Thermo Fisher Scientific	Goat polyclonal anti-rabbit Alexa-488	A11034	AB_2576217	1:500
Thermo Fisher Scientific	Goat polyclonal anti-rabbit Alexa-568	A11004	AB_2534072	1:500

Immunohistochemistry

Adult *Drosophila* of ≤7 days of age were fixed in 4% paraformaldehyde (PFA, Thermo Scientific™ #28908) in phosphate buffered saline (PBS) + 0.2% Triton X-100 (Fisher Scientific #BP151–100) (PBS-T) for three hours. Fixed flies were washed three times for fifteen minutes each with PBS-T at room temperature. Whole brains were dissected out and then blocked for an hour in a solution of 4% normal donkey serum (NDS, LAMPIRE Biological Laboratory # 7332100) in PBS-T. Brains were then incubated overnight in primary antibody solution consisting of primary antibodies, 4% NDS, and PBS-T. The following day brains were washed three times for fifteen minutes with PBS-T before incubation overnight in secondary antibody solution consisting of secondary antibodies, 4% NDS, and PBS-T. After one more set of three fifteen minutes washes in PBS-T the next day, brains were mounted onto slides in DAPI Fluoromount-G (SouthernBiotech™ #010020).

Image acquisition

Representative images were taken on a Leica SP8 confocal microscope with a 40x oil immersion lens. Images were taken at either a size of 1024 × 1024 pixels with a speed of 400 hz or a size of 1024 × 1024 pixels with a speed of 100 hz. Image z-stacks taken

to assess mushroom body morphology were acquired with a step size of 1 μm. Image stacks were processed in the Leica Application Suite X software program (Leica 3.7.6.25997). Figure illustrations were created with BioRender.com and Adobe Illustrator (v.29.8.1). Figures were assembled in Adobe Photoshop (v. 25.9.1).

Statistical analysis

Statistical analyses (including Fisher's exact tests, unpaired two-tailed Mann–Whitney tests, unpaired two-tailed t-tests, and Log-rank (Mantel-Cox) tests) were conducted using GraphPad Prism (v.10.2.2). For the RARE-X related studies and the combined literature and participant meta-analyses, the participant number (broken down by sex) are reported in the figure legend and statistical analyses (if completed) were included in the figure and/or figure legend for each analysis conducted. For the *Drosophila* studies, animal number evaluated, statistical analyses, and p-values are noted in each figure or legend for each analysis conducted.

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Author contributions

Bethany K. Terry (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing), Amira Mahoney (Data curation, Methodology, Writing—original draft, Writing—review & editing), Brian I. Lee (Investigation), Julie Secombe (Conceptualization, Funding acquisition, Project administration, Writing—original draft, Writing—review & editing).

Supplementary data

Supplementary data is available at *Human Molecular Genetics* online.

Conflict of interest statement: Julie Secombe is the Chair of the Scientific Advisory Board for the KDM5C Advocacy, Research, Education, & Support (KARES) Foundation (volunteer position).

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