NEUROSCIENCE

Normal sleep requires the astrocyte brain-type fatty acid binding protein FABP7

Jason R. Gerstner,[1,2]* Isaac J. Perron,[3,4] Samantha M. Riedy,[1,2] Takeo Yoshikawa,[5] Hiroshi Kadotani,[6] Yuji Owada,[7] Hans P. A. Van Dongen,[1,2] Raymond J. Galante,[3] Kaitlin Dickinson,[8] Jerry C. P. Yin,[8] Allan I. Pack,[3†] Marcos G. Frank[1,2†]

Sleep is found widely in the animal kingdom. Despite this, few conserved molecular pathways that govern sleep across phyla have been described. The mammalian brain-type fatty acid binding protein (Fabp7) is expressed in astrocytes, and its mRNA oscillates in tandem with the sleep-wake cycle. However, the role of FABP7 in regulating sleep remains poorly understood. We found that the missense mutation FABP7.T61M is associated with fragmented sleep in humans. This phenotype was recapitulated in mice and fruitflies bearing similar mutations: *Fabp7*-deficient mice and transgenic flies that express the FABP7.T61M missense mutation in astrocytes also show fragmented sleep. These results provide novel evidence for a distinct molecular pathway linking lipid-signaling cascades within astrocytes in sleep regulation among phylogenetically disparate species.

INTRODUCTION

Sleep occurs throughout the animal kingdom, suggesting that it serves an important and conserved function (*1*). Phylogenetically conserved mechanisms governing sleep across species are known to include neurotransmitters, cytokines, adenosine 5′-triphosphate, and genetic factors (*2*–*4*). The influence of these factors on sleep regulation has traditionally been considered in the context of neuronal function. More recently, astrocytes, a type of glial cell in the brain, have been recognized as an integral player in sleep regulatory processes (*5*–*7*). However, the role of an astrocyte gene in regulating phylogenetically conserved sleep behavior across multiple species has not been reported.

Fatty acid binding proteins (FABPs) comprise a family of small (~15 kDa) hydrophobic ligand binding carriers with high affinity for long-chain fatty acids, which they transport within the cell. FABPs are associated with metabolic, inflammatory, and energy homeostasis pathways (*8*, *9*) and have been implicated in cognitive disorders (*10*). FABPs have a conserved fingerprint (PRINTS pattern FATTYACIDBP; PR00178) defined by three motifs that form β strands, along with functional domains, which include a nuclear localization signal (NLS), a nuclear export signal (NES), and a hormone-sensitive lipase (HSL) binding site (Fig. 1). Three FABPs are expressed in the adult mammalian central nervous system: Fabp3 (H-Fabp), Fabp5 (E-Fabp), and Fabp7 (B-Fabp). Fabp3 is predominantly expressed in neurons, Fabp5 is expressed in multiple cell types, including both neurons and glia, and Fabp7 is expressed in astrocytes and neural progenitors (*8*).

We previously characterized diurnal *Fabp7* mRNA expression throughout the mouse brain (*11*, *12*) and showed that transgenic flies overexpressing either murine Fabp7 or the *Drosophila melanogaster* homolog dFabp have increased total sleep time (*13*). Although these observations suggest that Fabp7 influences sleep, a specific role for Fabp7 in regulating sleep across phylogenetically disparate species has not been determined.

Here, we determined the effects of the mutated *FABP7* gene on sleep in humans, mice, and fruitflies. We identified a single-nucleotide polymorphism (SNP) of the *FABP7* gene (rs2279381) that is associated with fragmented sleep in humans. We also showed that the human fragmented sleep phenotype is recapitulated in *Fabp7*-deficient mice. Last, astrocyte-specific expression of the human *FABP7* mutant generated a similar fragmented sleep phenotype compared to the human *FABP7* wild type (WT) in transgenic fruitflies. These results provide the first documented evidence for an astrocyte-enriched gene regulating complex behavior across multiple species.

RESULTS

To determine whether allelic variants in *FABP7* are associated with sleep disruption in humans, we examined a group of 294 adult male Japanese subjects who underwent 7 days of actigraphy and analysis of DNA for polymorphisms. In 29 of the 294 subjects, we found the presence of the natural variant C to T in the DNA sequence of *FABP7* that encodes a missense threonine-to-methionine mutation at position 61 (T61M) of the FABP7 protein (Fig. 1). The threonine at position 61 of FABP7 (T61) is conserved in mammals and is a residue that interacts with the omega-3 fatty acid docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid known to have high affinity for Fabp7 (*14*). T61 is also in close proximity to a highly conserved region containing a phenylalanine site (F57) known to regulate the NLS in FABPs (*15*). The NLS is not in the primary sequence, but upon binding with activating ligands, the NLS is revealed in the three-dimensional (3D) structure of the protein, located in the helix-loop-helix region (Fig. 1).

Upon DHA binding, the NLS of WT FABP7 is normally formed following a 3D shift of the K21 site (HSL-interacting), which is affected by the T61M mutation (Fig. 1), and predicted to cause abnormal function by the PolyPhen-2 software. Total sleep was similar between carriers and noncarriers [339.3 ± 6.6 min (FABP7 T61M) versus 336.8 ± 2.9 min (FABP7 WT), not significant (n.s.)], whereas the average length of an episode (or "bout") of sleep (see Materials and Methods) in subjects carrying the FABP7 T61M mutation was shorter compared with normal

[1]Department of Biomedical Sciences, Elson S. Floyd College of Medicine, Washington State University, Spokane, WA 99202, USA. [2]Sleep and Performance Research Center, Washington State University, Spokane, WA 99210, USA. [3]Center for Sleep and Circadian Neurobiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. [4]Neuroscience Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. [5]RIKEN Brain Science Institute, Wako, Saitama 351-0198, Japan. [6]Department of Sleep and Behavioral Sciences, Shiga University of Medical Science, Otsu City, Shiga 520-2192, Japan. [7]Department of Organ Anatomy, Tohoku University Graduate School of Medicine, Sendai, Miyagi 980-8575, Japan. [8]Department of Genetics, University of Wisconsin-Madison, Madison, WI 53706, USA.
†These authors contributed equally to this work.
*Corresponding author. Email: j.gerstner@wsu.edu

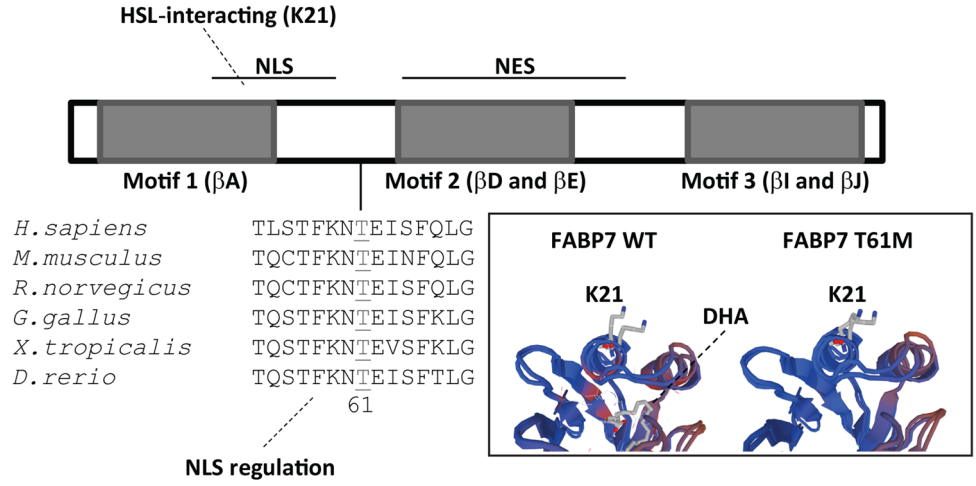

Fig. 1. Effects of a human *FABP7* point mutation on FABP7 protein structure. The FABP7 protein sequence contains three highly conserved motifs consisting of β sheets across FABP types. T61 is flanked by NLS and NES regions. T61 is located adjacent to F57, a site important for generating the NLS with the K21 domain that interacts with HSL. Upon ligand binding (that is, with DHA), a 3D conformational shift in the protein generates an NLS with K21 in WT FABP7, which is disrupted by the T61M variant, affecting nuclear localization and lipid-targeted transcriptional cascades.

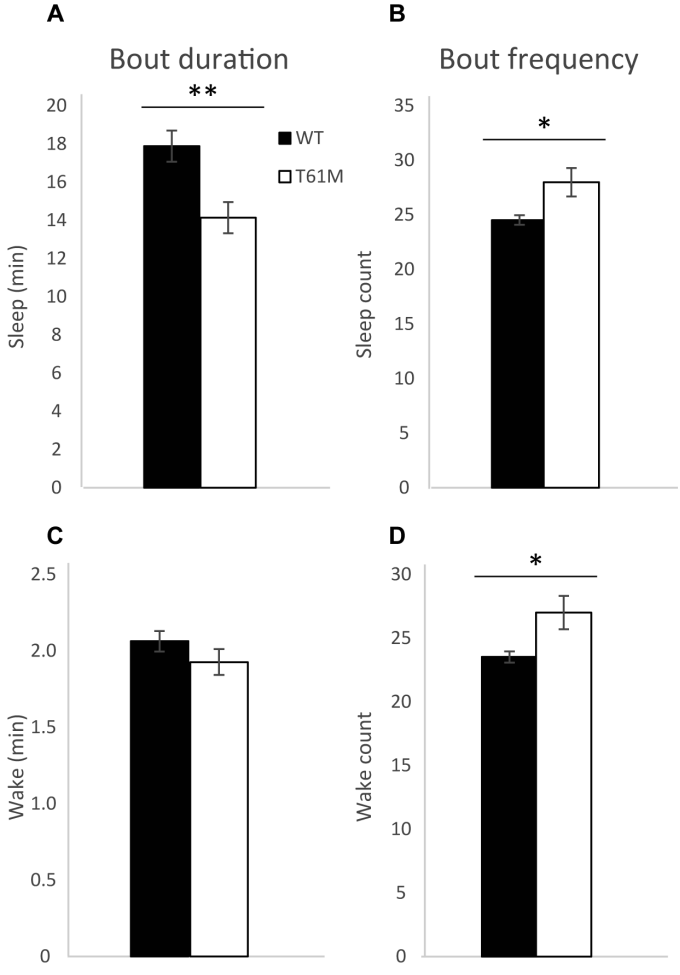

Fig. 2. The FABP7 T61M missense mutation is associated with fragmented sleep in humans. (**A** and **B**) Sleep bout duration (A) was significantly decreased and sleep bout frequency (B) was significantly increased in T61M carriers ($n = 29$) versus noncarriers ($n = 265$). (**C**) Wake bout duration was not affected by the T61M variant. (**D**) Wake bout frequency was significantly higher in T61M carriers versus noncarriers. *$P < 0.05$, **$P < 0.01$. Data are from the 7-day average of 24-hour bins. Error bars represent SEM.

subjects (Fig. 2A), and the frequency of sleep bouts was higher in mutation carriers compared with noncarriers (Fig. 2B). Wake after sleep onset [52.4 ± 2.9 min (FABP7 T61M) versus 48.2 ± 1.2 min (FABP7 WT), n.s.] and the average wake bout length (Fig. 2C) were not found to be different between carriers and noncarriers; however, the frequency of wake bouts was higher in carriers compared to noncarriers (Fig. 2D). Carriers did not significantly vary in age, body mass index, or sleepiness compared to noncarriers (table S1). Carriers also reported no significant differences in overall health (eight of the eight scaled scores from the Short Form 36 Health Survey; table S2). Compared to noncarriers, carriers showed a significant increase in Zung's Self-Rating Depression Scale (table S2) but were still within the normal range of nondepression (*16*). Collectively, these results indicated that the FABP7 T61M mutation resulted in abnormally fragmented sleep without any observed comorbidities.

We found a similar sleep phenotype in *Fabp7* knockout (KO) mice. *Fabp7* KO mice had shorter non–rapid eye movement (NREM) bout durations and more NREM bouts during their active phase (dark period) compared to control WT littermate mice (Fig. 3, A and B). There were no differences in REM bout duration during either the light or the dark period (Fig. 3C). However, REM bout frequency was increased in the dark period in *Fabp7* KO mice compared to WT (Fig. 3D). *Fabp7* KO mice also had shorter wake bout durations and more wake bouts during the dark period compared to control WT littermate mice (Fig. 3, E and F). Analysis of wake bout distribution across varying bout lengths showed that *Fabp7* KO mice had a significant increase in the number of shorter wake bouts compared to WT (Fig. 3G). *Fabp7* KO mice also had a significant increase in the number of state transitions compared to WT (Fig. 3H). Differences in remaining sleep architecture between *Fabp7* KO and WT mice were not observed (fig. S1). To exclude the possibility that the sleep fragmentation phenotype is due to hyperactivity, we also examined running wheel revolutions between *Fabp7* KO and WT mice and did not observe any differences (fig. S2). Collectively, these data suggest that, similar to carriers of the human *FABP7* missense mutation, *Fabp7*-deficient mice show increased sleep fragmentation compared to WT controls.

We then determined whether *Fabp7* KO also influences sleep need by examining sleep changes during both baseline and after sleep

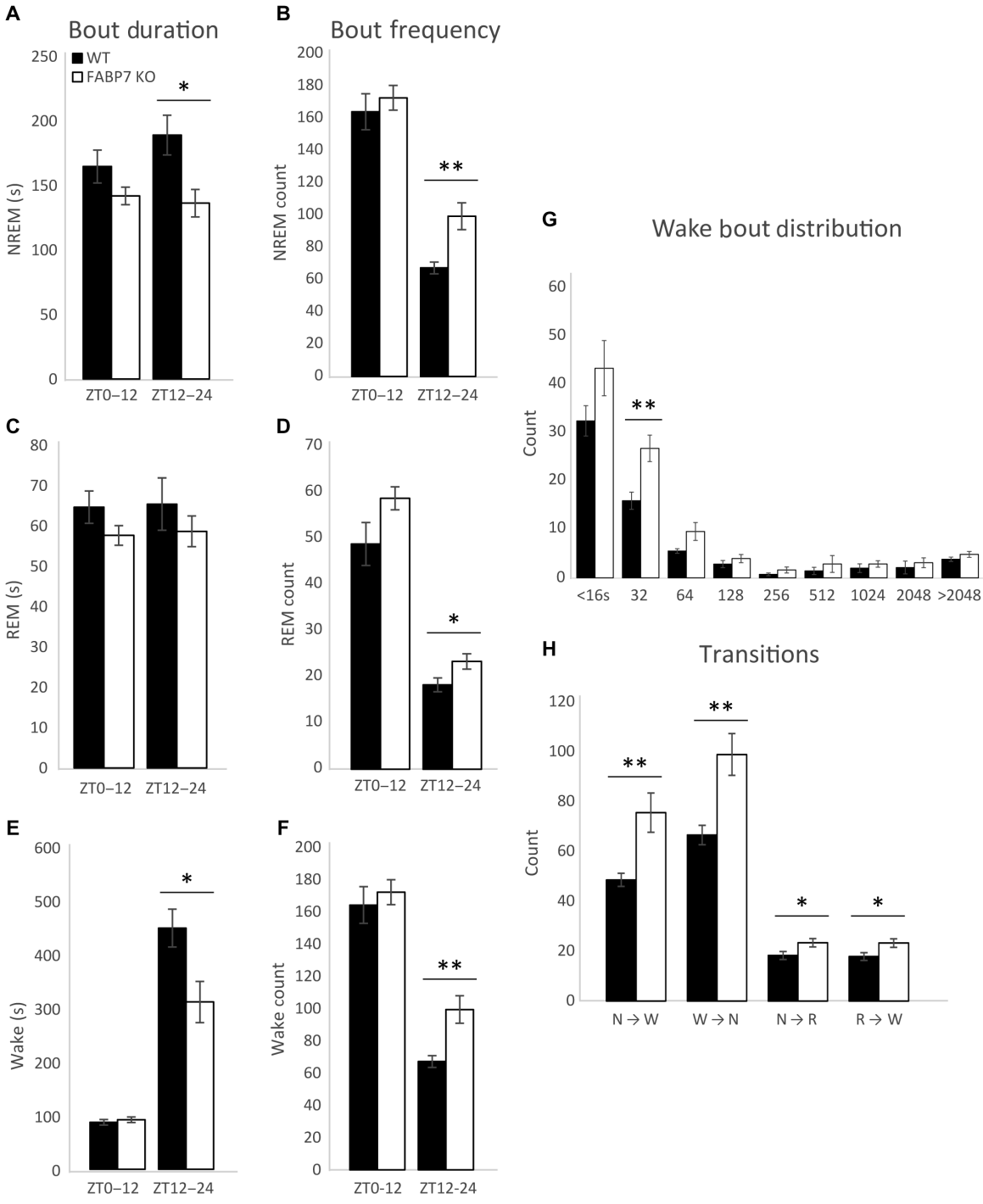

Fig. 3. The human *FABP7* point mutation phenotype is recapitulated in *Fabp7* KO mice, which also showed sleep fragmentation. (**A**) NREM bout duration was significantly lower in *Fabp7* KO mice ($n = 8$) compared to WT littermates ($n = 7$) during the dark phase [Zeitgeber time (ZT) 12 to ZT 24] but was unaffected during the light phase (ZT 0 to ZT 12). (**B**) NREM bout frequency was significantly higher in *Fabp7* KO mice compared to WT littermates during the dark phase (ZT 12 to ZT 24) but was unaffected during the light phase (ZT 0 to ZT 12). (**C**) REM bout duration was not affected in *Fabp7* KO mice compared to WT mice. (**D**) REM bout frequency was significantly higher in *Fabp7* KO mice compared to WT littermates during the dark phase (ZT 12 to ZT 24). (**E**) Wake bout duration was significantly lower in *Fabp7* KO mice compared to WT during the dark phase (ZT 12 to ZT 24) but was unaffected during the light phase (ZT 0 to ZT 12). (**F**) Wake bout frequency was significantly higher in *Fabp7* KO mice compared to WT during the dark phase (ZT 12 to ZT 24) but was unaffected during the light phase (ZT 0 to ZT 12). (**G**) The number of short wake bouts was increased in *Fabp7* KO mice compared to WT (ZT 12 to ZT 24). (**H**) The number of NREM to wake (N→W), wake to NREM (W→N), NREM to REM (N→R), and REM to wake (R→W) transitions was increased in *Fabp7* KO mice compared to WT mice (ZT 12 to ZT 24). *$P < 0.05$, **$P < 0.01$. Error bars represent SEM.

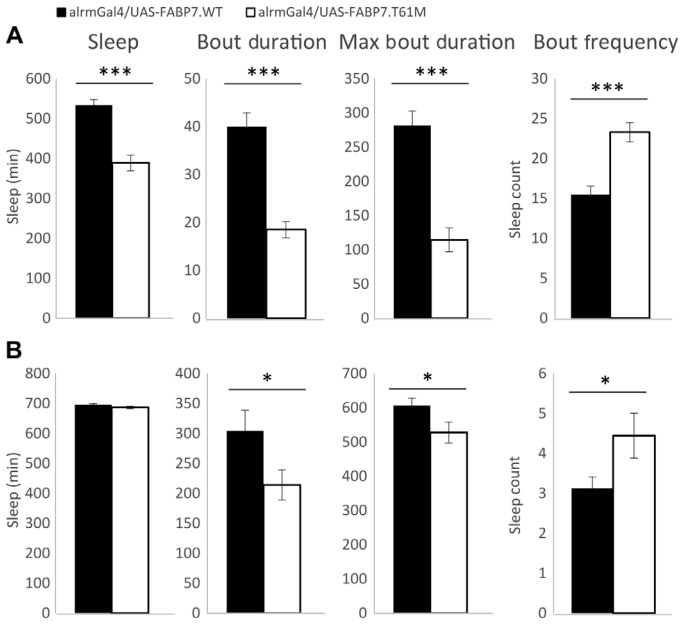

Fig. 4. Overexpression of *FABP7.T61M* mutation in astrocytes fragments sleep in *Drosophila*. Daytime (**A**) and nighttime (**B**) sleep was fragmented in flies overexpressing FABP7.T61M compared to FABP7.WT using an astrocyte-specific (Alrm-Gal4) driver. Total sleep, bout duration, maximum bout duration, and frequency of bouts are shown. *$P < 0.05$, ***$P < 0.001$, $n = 32$ flies (WT) and 27 flies (T61M). Error bars represent SEM.

deprivation conditions. These metrics included NREM electroencephalography (EEG) delta power (0.5 to 4.0 Hz) and REM sleep time. NREM delta power increases with sleep pressure, declines following subsequent sleep, and is under genetic control (*17*). In mice, REM sleep time also increases in a compensatory manner following total sleep deprivation (*17*). *Fabp7* deficiency did not affect the normal dissipation of NREM delta power during the baseline light period or following 6 hours of sleep deprivation [repeated-measures analysis of variance (ANOVA), factors for genotype, condition, and time; all genotype effects/interactions $P > 0.05$, data not shown]. However, there was a significant increase in REM sleep during the subsequent dark period (ZT 12 to ZT 24) in *Fabp7* KO versus WT mice (fig. S3F). The increase in REM rebound in the KO suggests that Fabp7 influences REM sleep regulation.

We then explored whether a role for Fabp7 in sleep was conserved across phyla by examining *D. melanogaster*. Previously, we showed that murine Fabp7 or dFabp pan-cellular overexpression in *Drosophila* increases sleep (*13*), suggesting that FABP influences on sleep are conserved across species. To test whether an astrocyte-specific functional Fabp7 is required for normal sleep, we generated transgenic flies that express either *FABP7* WT (FABP7.WT) or *FABP7* T61M (FABP7.T61M) using the UAS (upstream activation sequence)–GAL4 binary system (*18*). When crossed with flies that carry the Alrm-GAL4 driver (*19*), UAS-FABP7.WT or UAS-FABP7.T61M is expressed specifically in *Drosophila* astrocytes. Transgenic flies that express FABP7.T61M under astrocyte control show decreased total sleep time over 24 hours [1076.0 ± 21.9 min (FABP7.T61M) versus 1228.5 ± 15.1 min (FABP7.WT), $P < 0.001$], but this effect was restricted to differences in daytime, whereas no differences were observed during night (Fig. 4, A and B). Similar to human FABP7 T61M carriers, the effects of FABP7.T61M show increased sleep fragmentation compared to FABP7.WT flies. The FABP7.T61M flies had shorter bout durations, a reduction in the maximum sleep bout duration, and an increase in the frequency of sleep bouts. Analogous to human FABP7 T61M carriers and *Fabp7* KO mice, an increase in frequency of wake bouts was observed in FABP7.T61M flies compared to FABP7.WT flies (fig. S4). These effects were recapitulated in male flies (figs. S5 and S6). To control for potential developmental effects, we measured sleep in adult flies with conditionally expressed FABP7.T61M or FABP7.WT in glial cells using the GeneSwitch System. The glial-GeneSwitch works by expressing a progesterone receptor–fused Gal4 downstream of a glial driver (*20*). Upon RU486 treatment, conditional expression of UAS-FABP7.T61M or FABP7.WT is induced. We observed a significant reduction in nighttime sleep, night bout duration, and nighttime maximum bout duration and an increase in the number of night sleep bouts in UAS-FABP7.T61M flies when treated with RU486 compared to FABP7.WT flies (fig. S7). Together, these results indicate that the FABP7.T61M mutation expressed in astrocytes causes sleep fragmentation in fruitflies. In conjunction with our findings in humans and mice, they suggest that Fabp7 has a conserved role in sleep across diverse animal phyla.

DISCUSSION

The current study demonstrates that an astrocytic-associated gene influences sleep in humans, mice, and flies. Our findings are generally consistent with a previous study that found that SNP mutations in the human *Dec2* gene are associated with disrupted sleep (*21*, *22*) in humans, mice, and flies (*21*). The pan-neuronal driver *elav*-Gal4 was used to express the *Dec2* gene mutation in flies, but this driver does not rule out the potential effects of astrocytic Dec2. *Dec2* gene expression is quite modest in neurons compared to its abundant expression in astrocytes and microglia (*23*). Dec2 is a transcriptional repressor and negative regulator of the molecular clock (*24*). Although Fabp7 circadian transcription is regulated by Nr1d1, another clock repressor (*25*), it is possible that Dec2 may influence Fabp7 gene expression through downstream transcriptional regulation on molecular clock output genes, including Nr1d1. In addition, Fabp7 nuclear localization may provide feedback on the clock because FABPs are known to regulate peroxisome proliferator–activated receptor (PPAR) transcription (*8*), and PPARs, in turn, regulate clock genes to integrate circadian rhythms with energy metabolism (*25*, *26*).

Although it is conceivable that Fabp7 operates through shared pathways with Dec2, there are other possible mechanisms that link Fabp7 with sleep regulation. DHA supplementation has been shown to decrease the number of night awakenings and increase sleep in children (*27*), and therefore, DHA signaling may represent one possible mechanism linking FABP7 to sleep. For example, following DHA binding to FABP7, the NLS is normally formed with a 3D shift of the K21 site, which is affected by the T61M mutation (Fig. 1). We observe more sleep fragmentation in carriers of the T61M mutation compared to WT FABP7 (Fig. 2). This suggests that FABP7 nuclear localization mediated by DHA binding and downstream transcriptional events in astrocytes may facilitate consolidated sleep. *Fabp7* KO mice show aberrant dendritic morphology with a reduction in numbers of excitatory synapses, along with decreased synaptic transmission (*28*). Hippocampal neurons acutely dissociated from *Fabp7* KO mice also show a suppression of DHA-induced *N*-methyl-D-aspartate currents (*29*), suggesting a dysfunction in normal excitatory synaptic transmission. Although *Fabp7* KO mice show increased anxiety-like phenotype

(*29*), it is not obvious how this would affect changes in sleep architecture. We have shown that *Fabp7* mRNA and protein targeted to the fine perisynaptic astrocytic processes oscillate in tandem with the sleep-wake cycle in the mouse brain (*12*). Therefore, it is possible that the cycling of Fabp7 expression with sleep and wake acts to regulate synaptic events required for normal sleep-wake behavior.

MATERIALS AND METHODS

All studies followed relevant institutional review guidelines. Please see acknowledgments for details.

Human subjects

The subjects used in this study were male employees of a wholesale company in Osaka, Japan. Of 466 male subjects invited to participate, 322 took part in a 1-week survey, which included sleep diaries and actigraphy. A total of 310 subjects agreed to have their genomic DNA from blood analyzed, and data from subjects with validated DNA sequencing were used (n = 294). Sleep-wake schedules were obtained by 7-day sleep logs with coincident wrist actigraphy (Actiwatch AW-Light, Mini-Mitter) recorded in 1-min bins as previously described (*30*). Genomic DNA was extracted from leukocytes with the QIAamp DNA Blood Mini kit (Qiagen K.K.). Genome-wide genotyping was performed with Illumina HumanOmniExpress v1.0 (Illumina). Fabp7 SNP, rs2279381 data were used in this study.

Genotyping of subjects was confirmed by DNA sequencing (RIKEN Brain Science Institute). Genotyping of FABP7 Thr61Met (rs2279381) was carried out using the TaqMan SNP Genotyping Assays (Applied Biosystems) (assay ID: C__15967661_20) according to the manufacturer's recommendations. Analysis was performed by ABI 7900HT and SDS v2.4 software (Applied Biosystems). The accuracy of genotype based on sequencing can be seen in our previous work (*31*).

Human sleep analysis

Records were scored for in Actiware 6 software (Philips Respironics) with a wake threshold value of 40, immobile minutes for sleep onset of 10 min, and immobile minutes for sleep end of 10 min; these settings are conservative in the context of sleep fragmentation. Subjects' sleep diaries were used to distinguish time-in-bed intervals from off-wrist intervals. For each 24-hour period, the sleep interval with the longest duration was considered the main sleep period. Analyses were performed blind to genotype.

Animal subjects and handling

Generation of *Fabp7* null (*Fabp7* KO) mice has been previously described (*29*). Animals used in these studies were coisogenic males (C57BL/6J background) between 2 and 5 months of age, born and maintained from a breeding colony. Animals were entrained to a 12-hour light/12-hour dark schedule for a minimum of 2 weeks. ZT 0 is lights on and ZT 12 is lights off.

EEG/EMG monitoring and analysis

Surgery for EEG/EMG (electromyography) recording and data acquisition and analysis were performed as previously described (*32*, *33*). Briefly, EEG/EMG headmounts were manually constructed and implanted onto mice anesthetized with ketamine (100 mg/kg) and xylene (10 mg/kg). Silver ball electrodes were pushed into holes drilled above the frontal and parietal brain areas, and the headmount was secured with dental cement. After 1-week recovery, mice were tethered to recording cables and allowed an additional week of acclimation. Undisturbed sleep/wake (24 hours) was recorded, followed the next day by 6 hours of sleep deprivation (ZT 0 to ZT 6) and recovery (ZT 6 to ZT 24). Polysomnographic recordings were segmented into 4-s epochs and manually scored as one of three behavioral states: wake, NREM sleep, or REM sleep. Cumulative NREM delta power (*34*, *35*) was computed for each 1-hour bin and normalized to the average NREM delta power during ZT 8 to ZT 12 (*17*) of the undisturbed recording day. Analyses were repeated with normalization to NREM delta power during the whole 24-hour, undisturbed recording day (results were equivalent). Normalization factors were not significantly different between *Fabp7* KO and WT mice ($P > 0.05$).

Running wheel activity monitoring and analysis

Diurnal activity analysis was obtained using running wheels and running wheel analysis software (Med Associates). Briefly, wireless running wheels were placed in normal cages, and mice were allowed ad libitum access to running wheels under a 12-hour light/12-hour dark cycle for 10 days. On the 10th day, revolutions were counted and compared between *Fabp7* KO and WT mice in hourly and 12-hour bins.

Cloning *FABP7*

The *FABP7* WT open reading frame and *FABP7* c/t mutation (at position 182 to generate T61M missense mutation from ATG start site codon) complementary DNA were ordered from GeneART (Life Technologies); primers on the 5′ containing Eco RI and 3′ containing Bgl II restriction site (underlined, in yellow highlight, respectively) were as follows:

hFABP7 5′-full_EcoR1_24: accaccgaattccaacatggtggaggctttctgtgctacc

hFABP7 3′-full_BglII_30: accaccagatcttcttatgccttctcatagtggcgaacagcaac

These were polymerase chain reaction–amplified, TA-cloned, subcloned into pUAST vector, and confirmed by DNA sequencing (see sequences below). Transgenic flies were made by injecting *Drosophila* embryos in the *w* (*isoCJ1*) isogenic background (BestGene).

hFABP7: accaccgaattccaacatggtggaggctttctgtgctacctggaagctgaccaacagtcagaactttgatgagtacatgaaggctctaggcgtgggctttgccactaggcaggtgggaaatgtgaccaaaccaacggtaattatcagtcaagaaggagacaaagtggtcatcaggactctcagcacattcaagaacacggagattagtttccagctgggagaagagtttgatgaaaccactgcagatgatagaaactgtaagtctgttgttagcctggatggagacaaacttgttcacatacagaaatgggatggcaaagaaacaaattttgtaagagaaattaaggatggcaaaatggttatgacccttacttttggtgatgtggttgctgttcgccactatgagaaggcataaagaagatctggtggt

hFABP7 c/t mt (Thr61Met): accaccgaattccaacatggtggaggctttctgtgctacctggaagctgaccaacagtcagaactttgatgagtacatgaaggctctaggcgtgggctttgccactaggcaggtgggaaatgtgaccaaaccaacggtaattatcagtcaagaaggagacaaagtggtcatcaggactctcagcacattcaagaacactggagattagtttccagctgggagaagagtttgatgaaaccactgcagatgatagaaactgtaagtctgttgttagcctggatggagacaaacttgttcacatacagaaatgggatggcaaagaaacaaattttgtaagagaaattaaggatggcaaaatggttatgacccttacttttggtgatgtggttgctgttcgccactatgagaaggcataaagaagatctggtggt

Protein modeling

The FABP7 WT and T61M protein structure modeling was carried out using SWISS-MODEL (https://swissmodel.expasy.org/) (*36*).

Drosophila sleep analysis

Transgenic flies in the *w* (*isoCJ1*) isogenic background were maintained on standard yeast-cornmeal food at 25°C and entrained to a 12-hour light/12-hour dark cycle for 2 to 3 days before they were assayed for sleep. For analysis of sleep behavior, ~6-day-old female and male flies were assayed using the *Drosophila* Activity Monitoring System (TriKinetics), carried out as previously described (*37*). *Drosophila* glial-GeneSwitch Gal4 driver lines were obtained from the Bloomington Stock Center.

GeneSwitch experiments were carried out as previously described (*38*). Briefly, inducible expression of downstream UAS-transgenic lines was obtained by adding 500 μM RU486 (mifepristone, dissolved in 80% ethanol) in 2% agar and 5% sucrose minimal medium. Flies were first recorded in tubes containing only 2% agar and 5% sucrose minimal medium with vehicle and, after 4 days, were switched to minimal medium containing drug for the duration of the recording.

Statistical analyses

Group comparisons were made using Student's *t* test or, in the case of unequal sample sizes, Welch's *t* test. Where multiple comparisons were required, we used repeated-measures ANOVA with factors for comparison as indicated. Significant differences were $P < 0.05$, unless indicated otherwise.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/4/e1602663/DC1

table S1. Age, body mass index, and sleepiness comparison between FABP7.T61M carriers and noncarriers.

table S2. Health status comparison between FABP7.T61M carriers and noncarriers.

fig. S1. Baseline total sleep-wake time is not affected in *Fabp7* KO mice.

fig. S2. Locomotor running wheel activity is not affected in *Fabp7* KO mice.

fig. S3. REM sleep time is increased in *Fabp7* KO mice during the recovery period following sleep deprivation.

fig. S4. Overexpression of *FABP7.T61M* mutation in astrocytes fragments wake only during the daytime in *Drosophila*.

fig. S5. Overexpression of *FABP7.T61M* mutation in astrocytes in male flies also fragments sleep.

fig. S6. Overexpression of *FABP7.T61M* mutation in astrocytes in male flies also fragments wake.

fig. S7. Conditional overexpression of *FABP7.T61M* mutation in glial cells of adult male flies also fragments sleep in *Drosophila*.

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