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

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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 Fabp7s (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...
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 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...
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 during the dark phase (ZT 12 to ZT 24) but was unaffected during the light phase (ZT 0 to ZT 12). (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.
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 FABP7 influences sleep in conserved across species. To test whether an astrocyte-specific functional Fabp7 is required for normal sleep, we generated transgenic flies that overexpress 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.

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. Error bars represent SEM.

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, immobilie 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 byABI 7900HT and SDS v2.4 software (Applied Biosystems). The accuracy of genotype based on sequencing can be seen in our previous work (31).

Animal subjects and handling

Generation of Fabp7 null (Fabp7 KO) mice has been previously described (29). Animals used in these studies were cosogenic 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). Repeated measures 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 3′-full_EcoR1_24: acaccgaatctccacgctggtagttcagggacag
hFABP7 3′-full_BglII_30: accacgccagcccatcttggataacacac

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 (isoCI1) isogenic background (BestGene).

hFABP7: accaccgaatctcaccaggtggattcatatgaaccacctcaggctctgatttctggctctcat
hFABP7 c/t (Thr61Met): accaccgaatctcaccaggtggattcatatgacggctctgatttctggctctcat

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 (isoCI1) 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. Flies were then 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.


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 gial cells of adult male flies also fragments sleep in Drosophila.

**REFERENCES AND NOTES**


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