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Genome-wide identification and functional validation of the WW domain containing oxidoreductase gene associated with sleep duration

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Individual differences in sleep duration have been reported, and genetic components of sleep duration have been identified showing various heritability. To identify genetic variants that contribute to sleep duration, we conducted a human genome-wide identification on sleep duration and performed confirmatory experiments using a *Drosophila* model. Genome-wide association study in human was analyzed to determine the association of the genetic variants with self-aware sleep duration from two community-based cohort, Ansan (cohort 1, $n = 4635$) and Ansung (cohort 2, $n = 4205$), recruited from the Korean Genome and Epidemiology Study. Individual single nucleotide variants (rs16948804 and rs4887991) in the WW domain containing oxidoreductase (*WWOX*) gene were associated with self-aware sleep duration in human (p-values, 1.11×10^{-7} and 2.05×10^{-7}, retrospectively). To examine the functional relevance of the *WWOX* gene identified in the genome-wide association study, we analyzed the sleep duration of *Drosophila* loss-of-function mutants. The deletion of *Wwox* in flies reduced sleep duration and quality with average bout length during daytime and increased night-time sleep duration (all of p-values < 0.01). Our findings suggested that *WWOX* expression is associated with sleep duration in both humans and *Drosophila* and genetic factors play a role in inter-individual variability in sleep characteristics.

Keywords Genome-wide association study, Loss-of-function, Sleep duration, *WWOX*, *Drosophila*

Abbreviations

ABL	Average bout length
dTau	Drosophila Tau
FDR	False discovery rate
GWAS	Genome-wide association study
HWE	Hardy–Weinberg equilibrium
KoGES	Korea Genome and Epidemiology Study
MAF	Minor allele frequency

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QIAGEN	QIAzol reagent
qPCR	Quantitative real-time PCR
ROS	Reactive oxygen species
SDR	Short-chain dehydrogenase/reductase
SNV	Single nucleotide variant
TIB	Time in bed
WWOX	WW domain containing oxidoreductase

Sleep is controlled by homeostatic mechanisms and circadian clock function. Sufficient sleep duration is essential for maintaining body and brain health. It is known that optimal sleep duration for adults is approximately 7–8 h. The relation of insufficient sleep duration with various health states and diseases has been studied. Prospective epidemiologic studies suggest that both short (< 6 h) and long (> 9 h) sleep durations are linked to metabolic[1], cardiovascular[2], neurological[3], and immunological dysfunction[4], leading to obesity, diabetes, heart diseases, dementia, and cancer[5]. However, how sleep duration plays an important role in physiological health and disease remains to be established[6]. Studying sleep duration has helped to shed light on its important role in the development of diseases.

In human, individual differences in sleep duration have been reported, with a normal distribution ranging from 5 to 10 h[7], and genetic components of sleep duration have been identified in twin- and family-based studies, showing 9–45% heritability[8]. Genetic and molecular controls of sleep and circadian function are associated with the expression of core clock genes such as *CLOCK*, *BMAL*, *CRY*, and *PER*[9,10]. Recently, a large genome-wide association study (GWAS) has identified genetic loci associated with self-reported sleep duration with genome-wide significance levels[6,8]. However, there have been few consistent findings of common variants associated with sleep duration across GWASs. Further studies are warranted to identify the associated genes and confirm the relevance of candidate genes reported to date.

Since it was first demonstrated that fruit flies share most of the fundamental characteristics of mammalian sleep[11,12], numerous reports have shown that many genetic and molecular regulators of sleep are conserved between flies and mammals. Thus, *Drosophila* has become a useful experimental model in GWASs to verify candidate susceptibility genes involved in sleep regulation[13,14].

The present study aimed to identify genes associated with self-aware sleep duration in a human GWAS and integrate the results of the human GWAS with those of functional analyses in *Drosophila*. To begin, we performed a GWAS to identify genes associated with sleep duration from two community-based Korean cohorts. Furthermore, we confirmed the functional relevance of genes identified in human GWAS by knocking out its homolog in *Drosophila*, including sleep duration and quality.

Results

Participant characteristics in humans

Demographic information of the study participants from the two Korean population cohorts (Ansan and Ansung cohorts) is shown in Table 1. Of a total of 4,635 participants in discovery set (Cohort 1, Ansan cohort), 2,262 (48.8%) were women, but 2,396 (57.0%) were women in replication set (Cohort 2, Ansung cohort: $N = 4,205$). The mean ages of the participants were 49.1 ± 7.9 years in Ansan cohort and 55.7 ± 8.7 years in Ansung cohort (total set: 52.2 ± 8.9). Average self-aware sleep duration was 6.6 ± 1.3 h (Ansan) and 7.2 ± 1.4 h (Ansung), respectively (total set: 6.8 ± 1.4 h).

Associations of SNVs with sleep parameters in human GWAS

The most significant association was found between self-aware sleep duration and rs16948804 in human GWAS, which is located in the WW domain containing oxidoreductase (*WWOX*) intronic region (Ansan

	Ansan cohort (Cohort 1, discovery set) ($N = 4,635$, 52.4%)	Ansung cohort (Cohort 2, replication set) ($N = 4,205$, 47.6%)	Total ($N = 8,840$, 100.0%)
Age (years)	49.1 ± 7.9	55.7 ± 8.7	52.2 ± 8.9
Women, no. (%)	2,262 (48.8)	2,396 (57.0)	4,658 (52.7)
BMI (kg/m^2)	24.7 ± 3.0	24.5 ± 3.3	24.6 ± 3.1
Occupation, no. (%)			
White collar	1,658 (35.9)	461 (11.1)	2,119 (24.1)
Blue collar	338 (7.3)	2,431 (58.4)	2,769 (31.5)
Housework	1,535 (33.25)	991 (23.8)	2,526 (28.8)
Self-aware sleep duration (h)	6.6 ± 1.3	7.2 ± 1.4	6.8 ± 1.4
Time in bed (h)	6.7 ± 1.3	7.3 ± 1.4	7.0 ± 1.4
Habitual sleep efficiency (%)	98.6 ± 12.2	98.0 ± 12.9	98.3 ± 12.6
Sleep latency (min)	18.7 ± 19.2	21.4 ± 22.1	19.9 ± 20.6
Epworth sleepiness scale	6.1 ± 3.8	6.4 ± 4.2	6.2 ± 4.0

Table 1. Demographics information of the Ansan (cohort 1) and Ansung (cohort 2) cohorts. Values are expressed as the mean ± standard deviation (SD) for continuous data.

cohort: beta = -0.114, $p = 1.37 \times 10^{-4}$; Ansung cohort: beta = -0.121, $p = 3.74 \times 10^{-4}$; total set: beta = -0.125, $p = 1.11 \times 10^{-7}$) (Fig. 1, Tables 2 and Supplementary Fig. 1). Significant association was also identified between self-aware sleep duration and rs4887991 (total set: beta = -0.124, $p = 2.05 \times 10^{-7}$) after adjusting for $p < 0.05$ using false discovery rate (FDR) correction for multiple comparison (Supplementary Table 1). The small genomic control inflation factor (λ) of 1.0051 indicated a low possibility of false positive associations from population stratification (Supplementary Fig. 2).

The associations between *WWOX* expression and sleep parameters are presented in Table 2. The minor homozygous group in all two variants (rs16948804 and rs4887991 in *WWOX*, pFDR < 0.05) presented shorter self-aware sleep duration and time in bed (TIB) than the major homozygous group (all p-values ≤ 0.01). However, there were no significant associations with other sleep variables, including habitual sleep efficiency and the Epworth Sleepiness Scale scores (Table 3).

Deletion of *Wwox* reduced daytime sleep quality but increased night-time sleep duration in *Drosophila*

To investigate the functional relevance of *Wwox* in sleep duration, we analyzed sleep in *Wwox* loss-of-function mutant flies, $Wwox^{f04454}$. We first verified that the *Wwox* mRNA levels in $Wwox^{f04545}$ flies were markedly reduced compared with those in w^{1118} control flies (Fig. 2A). We measured sleep duration under 12-h:12-h light: dark (LD) conditions at 25 °C. The flies exhibited a typical bimodal pattern of sleep (Fig. 2B). In $Wwox^{f04545}$ flies, daytime sleep was reduced throughout the day (p-value < 0.0001), whereas night-time sleep was higher and lasted longer than that in control flies (p-value < 0.001) (Fig. 2B and C).

On the other hand, $Wwox^{f04545}$ fly activity was lower throughout the day, which implied that the sleep phenotype of $Wwox^{f04545}$ did not simply result from reduced locomotor activity (Fig. 2D). The alteration of sleep duration could not be attributed to a defective core circadian clock, as the free-running periods and rhythmicity were equivalent between control and $Wwox^{f04545}$ flies (Fig. 2E).

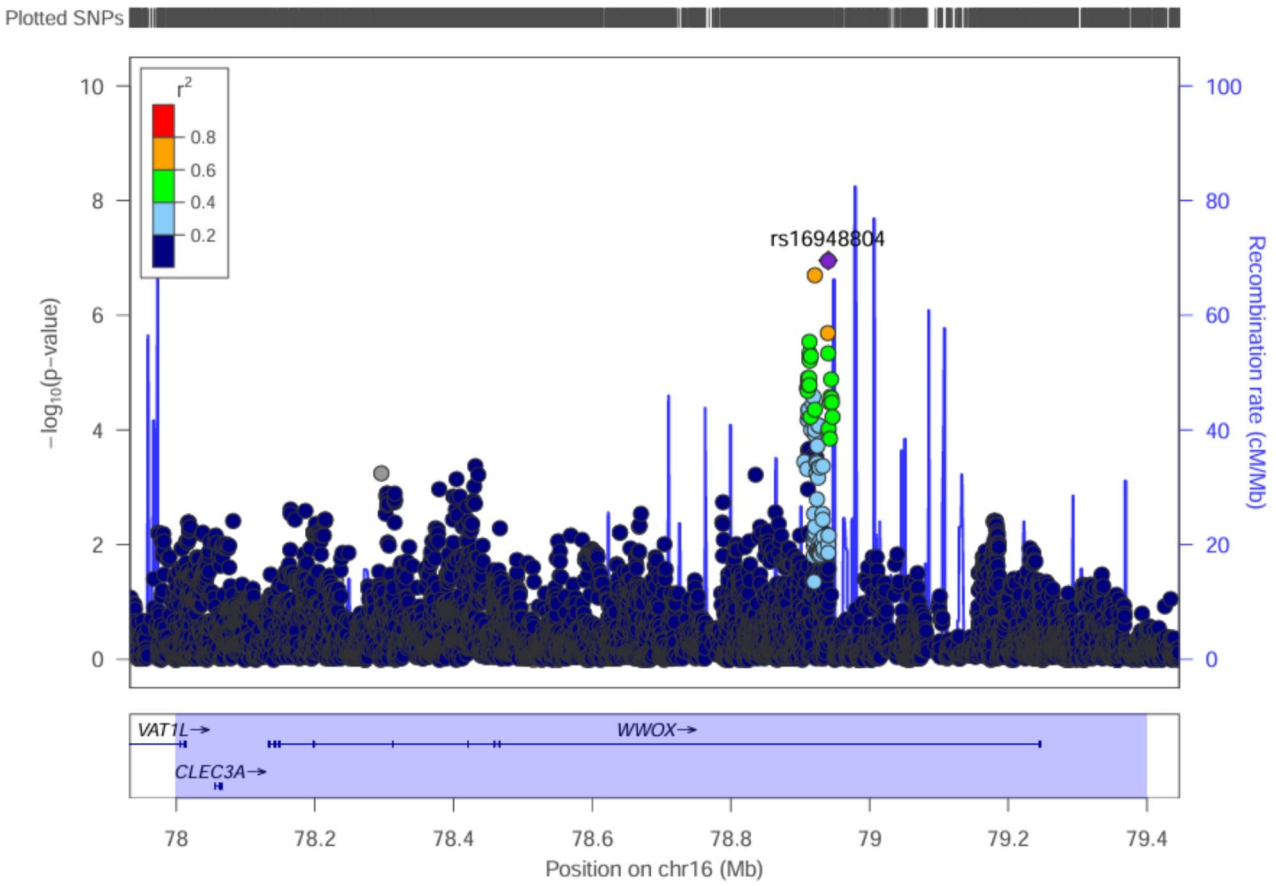


Fig. 1. Regional association signals between single-nucleotide variants (SNVs) at the *WWOX* locus on chromosome 16 and self-aware sleep duration. The association was drawn from multivariate linear regression analysis adjusted for age, sex, area, and occupation based on the additive model. A plot shows the most strongly associated SNV, rs16948804 (purple diamond) in meta-analysis, and circles represent the other SNVs in the region, with coloring from blue to red corresponding to r^2 values from 0 to 1 with the index SNVs. This plot was generated by LocusZoom. y axis (left), negative $\log_{10}$ (*p-value*) from GWAS analysis; y axis (right), genetic recombination rate (blue lines); x axis, genomic position.

SNV	Region	Allele (M/m)	Cohort 1 (Ansan) (*N* = 4,635)			Cohort 2 (Ansung) (*N* = 4,205)			Total (*N* = 8,840)		
			MAF	Beta (SE)	*P*-value[a]	MAF	Beta (SE)	*P*-value[a]	Beta (SE)	*P*-value[a]	pFDR
rs16948804	16q23.1-q23.2	G/C	0.26	-0.114 (0.030)	1.37×10^{-4}	0.27	-0.121 (0.034)	3.74×10^{-4}	-0.125 (0.024)	1.11×10^{-7}	0.0182
rs4887991	16q23.1-q23.2	A/G	0.23	-0.112 (0.031)	2.77×10^{-4}	0.25	-0.131 (0.034)	1.41×10^{-4}	-0.124 (0.024)	2.05×10^{-7}	0.0182
rs10431970	16q23.1-q23.2	C/T	0.32	-0.103 (0.028)	1.79×10^{-4}	0.34	-0.107 (0.032)	8.23×10^{-4}	-0.104 (0.022)	2.03×10^{-6}	0.1188
rs2656620	16q23.1-q23.2	C/A	0.23	-0.091 (0.031)	3.36×10^{-3}	0.24	-0.124 (0.035)	3.52×10^{-4}	-0.113 (0.024)	2.92×10^{-6}	0.1188
rs2656621	16q23.1-q23.2	G/A	0.22	-0.093 (0.031)	2.60×10^{-3}	0.24	-0.119 (0.035)	6.03×10^{-4}	-0.111 (0.024)	4.56×10^{-6}	0.1188
rs60554456	16q23.1-q23.2	G/T	0.16	-0.097 (0.035)	6.24×10^{-3}	0.17	-0.141 (0.040)	3.82×10^{-4}	-0.127 (0.028)	4.61×10^{-6}	0.1188
rs7203648	16q23.1-q23.2	T/C	0.24	-0.093 (0.030)	2.27×10^{-3}	0.25	-0.124 (0.034)	2.98×10^{-4}	-0.109 (0.024)	5.19×10^{-6}	0.1188
rs2656619	16q23.1-q23.2	G/A	0.22	-0.093 (0.031)	2.74×10^{-3}	0.24	-0.119 (0.035)	6.03×10^{-4}	-0.109 (0.024)	5.91×10^{-6}	0.1188
rs2656618	16q23.1-q23.2	G/T	0.23	-0.089 (0.031)	3.72×10^{-3}	0.25	-0.124 (0.034)	3.35×10^{-4}	-0.109 (0.024)	6.03×10^{-6}	0.1188

Table 2. Single-nucleotide variants (SNVs) in *WWOX* most strongly associated with self-aware sleep duration (h) (P-value$_{total}$ $<1.00\times10^{-5}$). M, major allele; m, minor allele; MAF, minor allele frequency; pFDR, P-value adjusted for false discovery rate. [a]P-values are based on linear regression analysis of sleep duration adjusted for age, sex, area, occupation, and principal components (PCs, PC1 to PC10). An additive genetic model was used in the present study.

SNV	Region	Allele (M/m)	Major homozygous group (MM)	Heterozygous group (Mm)		Minor homozygous group (mm)	
				Beta (SE)	*P*-value[a]	Beta (SE)	*P*-value[a]
Self-aware sleep duration (h)							
rs16948804	16q23.1-q23.2	G/C	Reference	-0.131 (0.030)	< 0.001	-0.209 (0.060)	< 0.001
rs4887991	16q23.1-q23.2	A/G	Reference	-0.078 (0.030)	0.009	-0.304 (0.063)	< 0.001
Time in bed (h)							
rs16948804	16q23.1-q23.2	G/C	Reference	-0.113 (0.030)	< 0.001	-0.149 (0.060)	0.01
rs4887991	16q23.1-q23.2	A/G	Reference	-0.071 (0.030)	0.02	-0.260 (0.063)	< 0.001
Habitual sleep efficiency (%)							
rs16948804	16q23.1-q23.2	G/C	Reference	-0.346 (0.280)	0.22	-0.840 (0.569)	0.14
rs4887991	16q23.1-q23.2	A/G	Reference	-0.280 (0.283)	0.32	-0.770 (0.594)	0.20
Epworth sleepiness scale score							
rs16948804	16q23.1-q23.2	G/C	Reference	0.138 (0.091)	0.13	-0.074 (0.184)	0.69
rs4887991	16q23.1-q23.2	A/G	Reference	0.167 (0.092)	0.07	0.107 (0.193)	0.58

Table 3. Association of sleep parameters with single-nucleotide variants (SNVs) in *WWOX* gene through meta-analysis (pFDR < 0.05). M, major allele; m, minor allele; pFDR, P-value adjusted for false discovery rate. [a] P-values are based on linear regression analysis of sleep parameters adjusted for age, sex, area, occupation, and principal components (PCs, PC1 to PC10).

Next, to analyze sleep quality, we measured the average bout length (ABL) of sleep. Daytime sleep ABL was lower in *Wwox*^{f04545} flies than in control flies, indicating that daytime sleep was fragmented (p-value < 0.01); however, night-time sleep ABL was not affected (Fig. 2F). Collectively, our results showed that the deletion of *Wwox* not only reduced the duration and quality of daytime sleep but also increased the duration of night-time sleep.

Discussion

In this study, we investigated the association between self-aware sleep duration and *WWOX* expression in human GWAS and confirmed WWOX's functional relevance in sleep regulation using a *Drosophila* model. We found that *WWOX* expression was most highly associated with self-aware sleep duration and time in bed but not with other sleep characteristics, including sleep efficiency or daytime sleepiness. In *Drosophila*, *Wwox* deletion reduced daytime sleep duration and quality, while increasing night-time sleep.

The most significant GWAS peak for sleep duration in humans was observed at rs16948804, located within the intronic region of *WWOX* (Fig. 1, Tables 2 and Supplementary Fig. 1). Previous GWA and Mendelian randomization studies have associated *WWOX* gene with neurological phenotypes like insomnia symptoms in bipolar disorder[15], late-onset Alzheimer's disease[16], and obstructive sleep apnea syndrome[17]. The major functions associated with the *WWOX* gene that have been elucidated in the previous literature are as follows. Firstly, the *WWOX* gene has been known to act as a tumor suppressor/transducer in many stress-related signaling pathways since its discovery in the past 20 years, and the *WWOX* controls the growth and progression of various cancers, including breast and prostate cancers[18–20]. Secondly, recent studies have also provided evidence that the *WWOX*

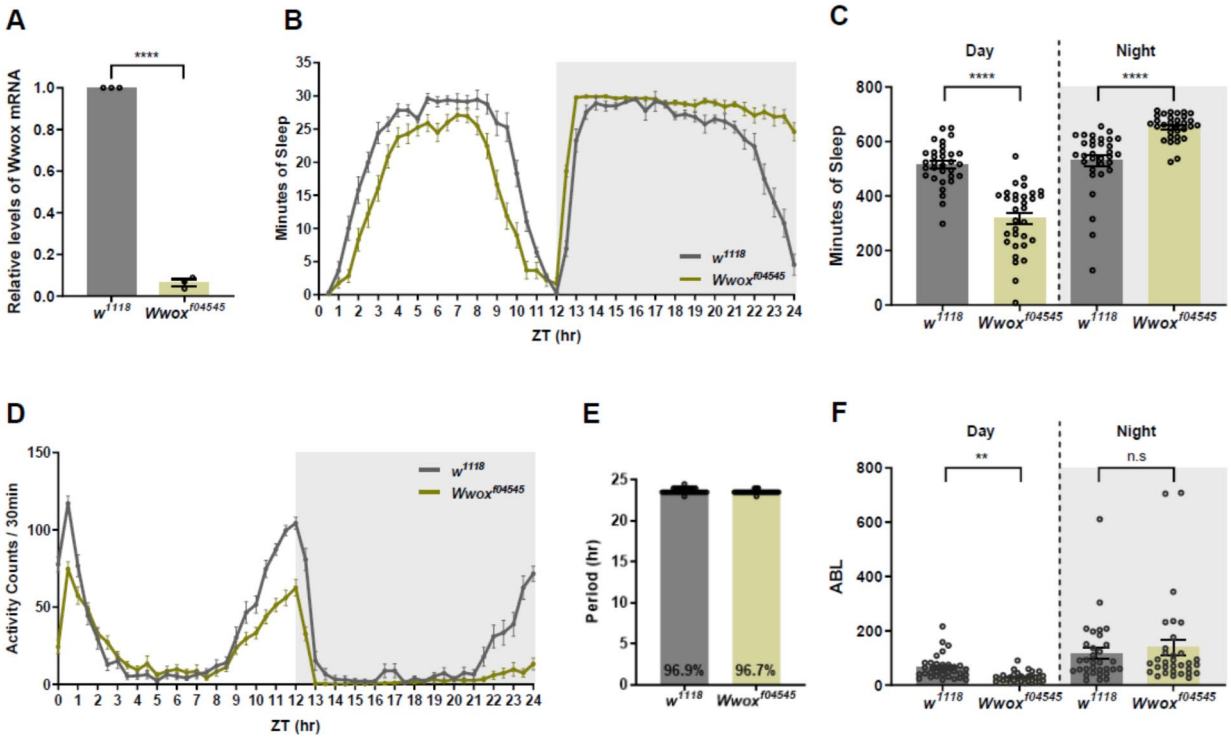


Fig. 2. Deletion of *Wwox* changed sleep duration without affecting the circadian rhythm in *Drosophila*. (**A**) Relative *Wwox* mRNA levels in control (w^{1118}) and *Wwox* loss-of-function mutant ($Wwox^{f04545}$) flies. Values indicate average ± SEM of three independent experiments. Student's *t*-test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. (**B**) Sleep profiles of w^{1118} and $Wwox^{f04545}$ male flies under a 12-h light:12-h dark cycle (ZT0: light on, ZT12: light off). Values indicate sleep duration per 30 min bins, mean ± SEM (w^{1118}, $n = 32$; $Wwox^{f04545}$, $n = 32$). (**C**, **F**) Minutes of sleep and average bout length (ABL) of sleep in individual flies were analyzed. Values represent average ± SEM (each genotype, $n = 32$). Mann-Whitney *U* test : $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ (**D**). Activity profiles of w^{1118} and $Wwox^{f04545}$ male flies under a 12-h light:12-h dark cycle. Values indicate beam crossing count per 30 min bins, mean ± SEM (w^{1118}, $n = 32$; $Wwox^{f04545}$, $n = 32$). (**E**) Free-running period and rhythmicity (inside each bar) of given genotype of flies are shown. Values are represented as mean ± SEM (w^{1118}, $n = 32$; $Wwox^{f04545}$, $n = 30$). SEM, standard error of the mean.

gene may be associated with metabolic diseases and homeostasis of lipid metabolism. *WWOX* gene alterations are associated with abnormal plasma high-density lipoprotein cholesterol and triglyceride levels in humans[21,22], and *Wwox* knockout mice revealed a significant role for *Wwox* in regulating HDL and lipid metabolism[23]. Therefore, *WWOX* may play an important role in pathways related to energy metabolism. Next, there are also studies suggesting that the *WWOX* gene may be associated with a role in the nervous system, including Alzheimer's disease. Sze et al. reported that significant downregulation of the protein level for *WWOX* has been shown in the hippocampus region of Alzheimer's disease patients, compared with age-matched controls[24], and Wang et al. suggested that *WWOX* affects the hyperphosphorylation of Alzheimer's Tau through regulating glycogen synthase kinase 3β (GSK3β) activity and subsequently promoting neuronal differentiation in response to retinoic acid[25]. It plays an important role in the development and degeneration of nerve cells. Finally, the *WWOX* gene may also affect the function of the immune system[26] and play an important role in regulating immune responses. Taken together, *WWOX* plays a crucial role in neurological disorders, including sleep regulations, metabolic disorders, and premature death occur in humans and animals.

WWOX gene encodes a 46-kDa protein with WW domains and short-chain dehydrogenase/reductase (SDR) domain[18]. The WW domain mediates interactions with several proteins such as p73[27], ErbB4[28], Ap2γ[29], RUNX2[30], and HIF1α[31], supporting WWOX's role in cellular processes including cell proliferation, differentiation, and metabolism. The function of SDR domain, however, remains unclear.

WWOX is conserved across species, with *Drosophila Wwox* sharing 49% amino acid identity with human *WWOX*[18,32]. In *Drosophila*, *Wwox* loss-of-function mutants ($Wwox^{f04545}$ flies) show altered gene expression in aerobic metabolic pathways[33]. The *Drosophila* Wwox protein also physically interacts with isocitrate dehydrogenase, a TCA cycle enzyme, implicating a role of *Drosophila* Wwox in metabolic homeostasis. Further supporting this, conditional *Wwox* knockout mice show a hypoglycemic phenotype[34]. In addition, Wwox reduction in *Drosophila* leads to mitochondrial-mediated cellular dysfunction likely mediated by alterations in ROS, AKT, and HIF1α pathways[35]. This mitochondrial phenotype is consistent with findings that muscle-specific ablation of Wwox results in reduced mitochondrial mass and reduced TCA cycle gene expression[36].

Overall, *Drosophila* Wwox appears to regulate cellular metabolic homeostasis through mechanisms shared with mammals.

Consistent with significant association of *Wwox* expression with sleep duration, *Drosophila Wwox* mutants showed sleep behavior defects (Fig. 2). Loss of *Wwox* function reduced daytime sleep duration while increasing nighttime sleep. Potential underlying mechanisms could include disrupted cellular metabolism, as nutritional status is known to impact sleep across species-starvation suppresses sleep in both mammals and flies, likely to support foraging for food[37,38]. Dysregulated metabolism from loss of *Wwox* function may similarly suppress sleep[33,35]. Furthermore, Wwox in *Drosophila* affects reactive oxygen species (ROS) levels with effects varying by context[35,39]. ROS and sleep have a bidirectional relationship, where oxidative stress promotes compensatory sleep[40,41]. Thus, perturbed ROS regulation may have increased sleep during the night in $Wwox^{f04545}$ flies. *WWOX* is also implicated in neurodegeneration, affecting Tau phosphorylation and aggregation in mammals[24,25,42,43]. Interestingly, *Drosophila* Tau (dTau) null flies showed decreased day-time sleep but not night-time sleep which is similar to the sleep phenotype of $Wwox^{f04545}$ flies[44], suggesting that disrupted dTau function could influence sleep duration in $Wwox^{f04545}$ flies.

Daytime sleep and night-time sleep differ in their quality and regulation in *Drosophila*[45]. Daytime sleep episodes are generally shorter, and the arousal threshold is higher during nighttime sleep[46]. Only a few genes are known to regulate sleep differently by day and night in flies[47]. Overall, our results indicate that Wwox influences cellular processes such as metabolic pathway, ROS regulation, and neurodegeneration, which differentially impact daytime and nighttime sleep. However, the precise mechanisms by which WWOX regulates sleep duration remain largely unexplored, warranting further investigation.

Based on previous reports, healthy sleep involves not only getting enough hours of sleep but also sleeping at the optimal time of day. For instance, natural short sleep is defined as a stable phenotype involving sleeping for 4–6.5 h/night without daytime sleepiness or sleep deprivation[48]. To understand the mechanism that drives this difference between natural short sleepers and regular sleepers, previous studies have identified genetic variants causing a short sleep phenotype, such as the *DEC2*, *ADRB1*, and *GRM1* genes[49–51]. Clearly, the mechanisms of sleep regulation are complex and involve many components, including genes expressed in coordination to maintain homeostasis. Therefore, additional genes, including *WWOX*, are expected to be involved in this complex mechanism.

There are several limitations in this study. Firstly, the *WWOX* gene did not reach the conventional genome-wide significance level in the human GWAS results. Secondly, the number of subjects in each cohort was not sufficient for GWAS. However, although many SNVs have been associated with various phenotypes reaching the genome-side significance level in many large-scale GWASs, only a few of these SNVs have been validated for functional experiment designs. Therefore, although SNVs in human GWAS did not reach the conventional genome-wide significance level, we performed both human GWAS and functional experiments in *Drosophila* to understand causal associations between genes and sleep characteristics in the present study. In addition, we analyzed two multiple comparison tests using FDR and Bonferroni corrections in human GWAS and both p-values are shown in Supplementary Table 1. In summary, the top two SNVs, rs16948804 and rs4887991, reached the level of $p < 0.05$ according to both tests.

In summary, genetic variants of *WWOX* that influenced sleep duration in the human GWAS were identified. In addition, *Wwox* deletion in flies reduced daytime sleep duration but increased night-time sleep duration compared with those in control flies. Our findings strongly suggested that genetic factors play a role in inter-individual variability in sleep duration in humans. Further studies are warranted to investigate the role of *WWOX* in sleep duration including external validation at other race/ethnicity groups.

Materials and methods

This retrospective cross-sectional study in humans was considered as minimal risk to participants and received an informed consent waiver from the institutional review board of Korea University Ansan Hospital (IRB no. 2020AS0118) and ethics committee of the Korean Center for Disease Control (no. NBK-2020-093). All methods were carried out in accordance with relevant guidelines and regulations. The experimental protocol of fly study was approved by the institutional animal care and use committee of the Ajou University.

Human studies

Study population and phenotypes

This human GWAS included a total of 8,840 participants from two community-based cohorts, Ansan (cohort 1, $n = 4{,}635$) and Ansung (cohort 2, $n = 4{,}205$), recruited from the Korean Genome and Epidemiology Study (KoGES) in South Korea. Detailed information on participant recruitment is available in previous studies[52,53]. Participants underwent a comprehensive health examination and questionnaire-based interview conducted by health professionals in a baseline study from 2001 to 2003. The questionnaire included demographic characteristics, lifestyle choices, medical history, and sleep habits. All participants responded to questions about sleep quantity, including self-aware sleep duration and sleep latency during the past month, and the Epworth sleepiness scale score to measure daytime sleepiness. Time in bed (TIB) was calculated as the time difference between bedtime and waking time and then used to calculate habitual sleep efficiency (habitual sleep efficiency = % sleep duration/TIB) (Supplementary Table 2).

Genotyping and quality control

For each participant, single nucleotide variant (SNV) genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix Inc., Santa Clara, CA, USA). The details of the GWAS of the KoGES have been described previously[49,50]. Quality control procedures were conducted to remove SNVs with missing genotyping rates > 5%, minor allele frequency (MAF) < 0.01, or Hardy–Weinberg equilibrium (HWE) $< 1 \times 10^{-4}$

using PLINK version 1.90. SNV imputation was performed using IMPUTE (v2.644) with the 1000 Genomes Phase I (version 3) in NCBI build 37 (hg19) as a reference panel. Of these, we dropped SNVs with a posterior probability score < 0.90, low genotype information content (info < 0.5), HWE ($P < 1.0 \times 10^{-7}$), MAF < 0.01, and SNV missing rate > 0.1. The final number of SNVs after imputation was 6.42 million for the Ansan and Ansung cohorts. Detailed information on the SNV imputation method is available in a previous report[56].

Fly studies

Drosophila strains and behavioral analysis

All flies were maintained in standard cornmeal–yeast–agar medium at 25 °C. Control w^{1118} (BL5905) flies and loss-of-function mutant $Wwox^{f04545}$ (BL18783) flies were obtained from the Bloomington Drosophila Stock Center. To ensure a similar genetic background, we backcrossed $Wwox^{f04545}$ with w^{1118} six times. Homozygous mutants were used in the present study.

Sleep and circadian rhythms were analyzed using the *Drosophila* activity monitoring system (Trikinetics, Waltham, MA, USA). Two to five days old adult male flies in glass tubes containing 2% agar and 5% sucrose were entrained for 3 days under a 12-h:12-h light (L): dark (D) cycle at 25 °C (lights-on at ZT0; lights-off at ZT12), and sleep was measured on day 4 of the LD cycle. A sleep bout was defined as a behavioral episode during which flies did not show any activity for 5 min or longer. Sleep parameters were accordingly analyzed using Excel macro[57]. Minutes of sleep were calculated as the average number of minutes of sleep/day. The duration of activity was calculated as the total number of beam crossings during the phase of interest. ABL was calculated as the sleep duration during one sleep bout.

For circadian rhythm analysis, young male flies were exposed to a 12 L:12D photoperiod for 4 days and then maintained in constant darkness for 7 days at 25 °C. Circadian rhythm analysis was performed using FaasX software (Fly Activity Analysis Suite for MacOSX), which was generously provided by Francois Rouyer (Centre National de la Recherche Scientifique, France). Periods were calculated for each fly using χ^2 periodogram analysis. Individual flies with a power ≥ 20 and width ≥ 2 were considered rhythmic. Power and width represent the height and width of the periodogram peak, respectively[58].

qRT-PCR

The total RNA was extracted from whole flies using QIAzol reagent (QIAGEN). The total RNA (1 µg) was reverse transcribed using an oligo(dT)20 primer and PrimeScript RTase (TaKaRa). Quantitative real-time PCR (qPCR) was performed using Rotor Gene 6000 (QIAGEN) with TB Green Premix Ex Taq (Tli RNaseH Plus, TaKaRa). The following primers were used: *Wwox* forward, 5′- CGCTCTCGACTTGAGCTCTT-3′; *Wwox* reverse, 5′- G CACAATGATCCGTGTTTTG −3′. *cbp20* mRNA was used to normalize gene expression with the following primers: *cbp20* forward, 5′-GTATAAGAAGACGCCCTGC-3′; and *cbp20* reverse, 5′-TTCACAAATCTCA TGGCCG-3′. The data were analyzed using Rotor Gene Q- Pure Detection software (version 2.2.3), and the relative mRNA levels were quantified using the $2^{-\Delta\Delta Ct}$ method in which ΔΔCt = [(Ct target − Ct cbp20) of the experimental group] − [(Ct target − Ct cbp20) of control group].

Statistical analysis

Statistical analysis of descriptive variables was performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R software version 4.2.3 (2023-03-15, R Foundation for Statistical Computing). Descriptive variables were summarized using mean and standard deviation for continuous variables and percentages for categorical variables. To explore loci associated with sleep parameters such as sleep duration as a quantitative feature, we used PLINK software (version 1.90, Free Software Foundation Inc., Boston, MA, USA) and performed multivariate linear regression including age, sex, occupation, and principal components (PCs, PC1 to PC10) as covariates. We conducted principal component analysis using PLINK software on GWAS data, to generate principal component scores and eigenvalues. Additive models were used for the analysis, false discovery rate (FDR) and Bonferroni adjustments were used for multiple testing corrections (Supplementary Table 1). Subsequently, the most significant SNVs identified in the GWAS were further investigated for function analysis in *Drosophila*.

Data availability

We provide the suggestive ($p \leq 1.00E\text{-}5$) signals in WWOX gene identified in the GWAS meta-analyses (Table 2). Individuals genotyping and other cohort data used for the GWAS are stored in the National Biobank of Korea Database and managed by the National Institute of Health, Republic of Korea (https://biobank.nih.go.kr/eng/cmm/main/mainPage.do). Further information and requests for data published including loss-of-function analysis in Drosophila would be contacted to leeh@ewha.ac.kr or chol-shin@korea.ac.kr. In response to reasonable requests of this data, we could share the summary of data and results after blinding personal information under the approval of the Distribution Review Board in NIH, Republic of Korea.

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Author contributions

Soriul Kim: contributed to the study concept and design, data curation, formal analysis, interpretation and drafted the manuscript. So Who Kang: contributed to the data curation, formal analysis, interpretation and drafted the manuscript. Song E Kim: contributed to interpretation of data and critical revision of the manuscript. Hyeon Jin Kim: contributed to interpretation of data and critical revision of the manuscript. Sol Ah Kim: contributed to data acquisition and interpretation of data. Yae Won Lee: contributed to data acquisition and interpretation of data. Eun Young Kim: contributed to the study concept and design, interpretation of data and critical revision of the manuscript. Chol Shin: contributed to the study concept and design, project administration, funding acquisition, interpretation of data and critical revision of the manuscript. Hyang Woon Lee: contributed to the study concept and design, project administration, funding acquisition, interpretation of data and critical revision of the manuscript. All authors approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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