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ARTICLE

A variant at 9q34.11 is associated with *HLA-DQB1*06:02* negative essential hypersomnia

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Abstract

Essential hypersomnia (EHS) is a lifelong disorder characterized by excessive daytime sleepiness without cataplexy. EHS is associated with human leukocyte antigen (*HLA*)-*DQB1*06:02*, similar to narcolepsy with cataplexy (narcolepsy). Previous studies suggest that *DQB1*06:02*-positive and -negative EHS are different in terms of their clinical features and follow different pathological pathways. *DQB1*06:02*-positive EHS and narcolepsy share the same susceptibility genes. In the present study, we report a genome-wide association study with replication for *DQB1*06:02*-negative EHS (408 patients and 2247 healthy controls, all Japanese). One single-nucleotide polymorphism, rs10988217, which is located 15-kb upstream of carnitine O-acetyltransferase (*CRAT*), was significantly associated with *DQB1*06:02*-negative EHS ($P = 7.5 \times 10^{-9}$, odds ratio = 2.63). The risk allele of the disease-associated SNP was correlated with higher expression levels of *CRAT* in various tissues and cell types, including brain tissue. In addition, the risk allele was associated with levels of succinylcarnitine ($P = 1.4 \times 10^{-18}$) in human blood. The leading SNP in this region was the same in associations with both *DQB1*06:02*-negative EHS and succinylcarnitine levels. The results suggest that *DQB1*06:02*-negative EHS may be associated with an underlying dysfunction in energy metabolic pathways.

Introduction

Essential hypersomnia (EHS) is characterized by excessive daytime sleepiness. The disorder is characterized by short episodes of daytime sleepiness and refreshing daytime naps [1–5]. Cataplexy, a core symptom of narcolepsy, is not present in EHS. Nocturnal sleep is basically normal in quantity in EHS. Patients with EHS are classified as narcolepsy without cataplexy or idiopathic hypersomnia without long sleep time based on the criteria in the International Classification of Sleep Disorders second edition (ICSD-2) [6].

Narcolepsy is strongly associated with human leukocyte antigen (*HLA*)-*DQB1*06:02* [7, 8]. Almost all patients with narcolepsy in various ethnic populations consistently carry *DQB1*06:02*. Approximately 12% of Japanese, 25% of Caucasian, and 38% of African-American healthy individuals are *DQB1*06:02* positive [7–10]. The positivity of

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*DQB1*06:02* in EHS patients is also significantly higher (30–40%) than that in the general population, but less than that in patients with narcolepsy [2, 3]. We consider that EHS can be divided into two classes based on the presence or absence of *DQB1*06:02* [1, 4, 11]. Several susceptibility genes are shared among *DQB1*06:02*-positive EHS and narcolepsy patients [12]. Single-nucleotide polymorphisms (SNPs) located in T-cell receptor alpha (*TCRA*) are strongly associated with narcolepsy [13], and a replication study for EHS indicated that the SNPs are associated with only *DQB1*06:02*-positive EHS, and not *DQB1*06:02*-negative EHS [4]. The orexin (hypocretin-1) level is reduced in the cerebrospinal fluid (CSF) [14], and postmortem examination shows a reduced number of orexin-producing neurons in the hypothalamus of patients with narcolepsy [15]. In contrast, only 10–20% of patients with EHS show a reduction in the CSF orexin level, and most such cases are observed in *DQB1*06:02*-positive EHS patients [16]. The clinical features in *DQB1*06:02*-positive EHS patients were also shown to be similar to those of narcolepsy [17]. These results suggest that *DQB1*06:02*-positive EHS and narcolepsy share the same etiology regarding a deficiency in orexin-producing neurons. On the other hand, *DQB1*06:02*-negative EHS has a different etiological pathway from that of *DQB1*06:02*-positive EHS and narcolepsy, and this pathway has not been clarified (Supplementary Fig. 1). We recently performed a genome-wide association study (GWAS) for *DQB1*06:02*-negative EHS and reported several loci as susceptibility candidate genes [18]. The associations did not reach a genome-wide significance level ($P < 5 \times 10^{-8}$), and further replication was not conducted. Therefore, to identify novel susceptibility genes for *DQB1*06:02*-negative EHS in the present study, we reanalyzed our GWAS and performing a replication study for validation. The effect of the variant identified through the genetic analysis was examined to lead to the discovery of the molecular mechanism of hypersomnia pathogenesis.

Materials and methods

Subjects for genetic studies

In the present study, 408 Japanese patients with *DQB1*06:02*-negative EHS and 2247 healthy Japanese controls were included. Among them, the sample for the GWAS consisted of 125 patients with *DQB1*06:02*-negative EHS and 1814 controls (replication study: 283 cases and 433 controls). Finally, we studied 640 Japanese patients with *DQB1*06:02*-positive narcolepsy, 100 Japanese patients with *DQB1*06:02*-positive EHS, and 1912 healthy Japanese controls. All subjects gave written informed consent. This study was approved by the local institutional review boards at participating institutions.

GWAS and replication study

In the discovery GWAS, SNPs were genotyped using Affymetrix Genome-Wide SNP Array 6.0 (Affymetrix, Inc.). Genotype calling was done using the Birdseed algorithm in Birdsuite (http://www.broadinstitute.org/) [19]. Quality control procedures were performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) [20]. Samples with overall call rates lower than 97% were excluded. Samples from individuals who had reported a family relationship with other participants or with a mean probability of identity-by-descent value >0.125, as calculated by PLINK, were excluded, until only one sample remained of reported or estimated family members. To eliminate population stratification, outliers in principal component analysis were also excluded using EIGENSOFT (http://www.hsph.harvard.edu/alkes-price/software/) [21]. In the principal component analysis, 91 JPT (Japanese in Tokyo, Japan) and 90 CHB (Han Chinese in Beijing, China) were used that were derived from the HapMap project (http://hapmap.ncbi.nlm.nih.gov/). The HapMap populations and our sample populations were combined after quality control steps as follows. SNPs were removed if they had a minor allele frequency (MAF) <0.05, deviated from the Hardy–Weinberg equilibrium (HWE) ($P < 0.001$), had SNP call rates <99%, or were located in sex chromosomes. In this study, the patients with EHS were *DQB1*06:02* negative, whereas some of the controls were predicted to carry *DQB1*06:02* because the positivity of Japanese individuals are approximately 12%. Therefore, SNPs in the *HLA* region (chromosome 6, physical position: 27,000,000–34,000,000 in NCBI build 36/hg18) were excluded to avoid confounding caused by secondary associations that resulted from linkage disequilibrium with *DQB1*06:02*. After the quality control procedures, 476,572 SNPs in 119 patients with *DQB1*06:02*-negative EHS and 1582 healthy controls were analyzed (Supplementary Fig. 2). The result of the principal component analysis of these data is shown in Supplementary Fig. 3. A replication study was conducted using 12 candidate SNPs with a threshold of $P < 10^{-5}$ in the allelic model in the discovery GWAS and based on the previous GWAS [18]. Additionally, a SNP in myelin basic protein (*MBP*) was genotyped in the replication sample set because our results suggested that demyelination may be associated with the development of EHS. A total of 13 SNPs were genotyped using Taqman genotyping assays (Thermo Fisher Scientific, Inc.). To perform the replication study, we included a total of 283 EHS patients who were *DQB1*06:02* negative and 433 healthy controls who were not analyzed in the GWAS. Finally, the replicated SNP was tested in 640 patients with

*DQB1*06:02*-positive narcolepsy, 100 patients with *DQB1*06:02*-positive EHS, and 1912 healthy controls who were not genotyped in the GWAS.

Statistical analysis of genetic data and imputation

Association analyses were performed using the chi-square test. Three models (allelic, recessive, and dominant models) were evaluated in the analyses. Significance levels were adjusted by the number of comparisons (Bonferroni correction) to correct for multiple testing. We set the significance level at $P < 1.3 \times 10^{-3}$ for the replication study (13 SNPs and three models: adjusted $\alpha = 0.05/39$). The genome-wide significance level was set as $P < 1.6 \times 10^{-8}$ (three models: adjusted $\alpha = 5 \times 10^{-8}/3$). An inflation factor was estimated based on the median chi-square value from the allelic model. IMPUTE2 was performed with the HapMap3 reference panel to impute untyped SNPs in the candidate regions (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) [22]. Low-quality imputed SNPs were filtered out by applying the following conditions: HWE $P <$ 0.0001, SNP call rates <0.95, and probability of imputation certainty < 0.9. Manhattan plots and regional plots of association studies were generated by using Haploview and LocusZoom [23], respectively.

Expression and metabolome analyses

An expression quantitative trait loci (eQTL) analysis was performed using GTEx-generated eQTL data according to the analysis methods of the GTEx project (http://www.gtexportal.org/home/) in order to evaluate whether SNPs were correlated with expression levels of nearby genes [24, 25]. To examine whether SNPs influenced the human metabolome in blood, we utilized the Metabolomics GWAS Server (http://mips.helmholtz-muenchen.de/proj/GWAS/gwas/index.php) [26] and data from previous study combining genetics and metabolome data [27]. Levels of succinylcarnitine in sera were measured using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (Sekisui Medical Co., Ltd) [28]. The sample of the succinylcarnitine measurement consisted of 48 patients with *DQB1*06:02*-negative EHS and 20 control subjects. All subjects gave written informed consent. The control subjects were assessed by the multiple sleep latency test after staying in the hospital for one night, and were diagnosed as not hypersomnia but insufficient sleep. A linear regression analysis was performed to assess the relationship between case–control status and levels of succinylcarnitine with adjustment for the effects of age, sex, body mass index (BMI), and rs10988217 genotype.

MBP measurement

Quantification of MBP was carried out in CSF samples from 23 patients with normal orexin hypersomnia (corresponding to EHS), 21 patients with low orexin hypersomnia (corresponding to narcolepsy), and 29 controls with a quantitative MBP enzyme-linked immunosorbent assay (ELISA) (Beckman Coulter Inc., DSL-10-58300), which is specialized for quantitative measurement of MBP in CSF. All subjects gave written informed consent. Experiments were carried out according to the manufacturer's instructions. All measurements were performed in duplicate including standards, positive controls with a known MBP concentration, and samples. Inter- and intra-assay coefficients of variation were calculated to ensure reproducibility of data. MBP in the CSF in normal individuals should be <4 ng/ml. MBP levels between 4 and 8 ng/ml may indicate a sign of a chronic breakdown of myelin or recovery from an acute episode of myelin breakdown [29]. When the MBP level is >9 ng/ml, myelin is actively breaking down.

Results

To identify novel loci associated with *DQB1*06:02*-negative EHS, we performed an association study of 119 patients with *DQB1*06:02*-negative EHS and 1582 controls using 476,572 SNPs after quality control procedures. When the SNPs were subjected to statistical analysis using a basic allele test, the inflation factor was estimated to be <1.01, suggesting that the effect of population stratification was negligible (Supplementary Fig. 4). No SNPs reached the genome-wide significance threshold in the GWAS (Supplementary Table 1 and Supplementary Fig. 5). For replication, we selected eight loci with SNPs with $P < 10^{-5}$ in this GWAS and three loci listed as candidates in a previous study [18]. The leading SNP (or a proxy in strong linkage disequilibrium, $r^2 > 0.8$) of each candidate locus was genotyped in 283 patients with *DQB1*06:02*-negative EHS and 433 controls as a new sample set (Supplementary Table 2). Of these SNPs, one SNP (rs10988217) was significantly associated with *DQB1*06:02*-negative EHS in the replication study after Bonferroni correction (Table 1 and Supplementary Table 2). After imputation of this region, rs10988217 genotyped by the SNP array had the most significant association as before (Fig. 1). The recessive model of the SNP was the best fitting model. The *P*-values for the SNP in the GWAS and replication study were 2.4×10^{-5} with an odds ratio (OR) of 3.00 and 1.3×10^{-4} with an OR of 2.83, respectively. The combined analysis of both studies gave $P = 7.5 \times 10^{-9}$ with an OR of 2.63 (Table 1 and Fig. 1). The association reached genome-wide

Table 1 Association of rs10988217 with *DQB1*06:02*-negative EHS

Study	Genotype freq. (case)			Genotype freq. (control)			MAF (case)	MAF (control)	Allelic model		GG+GA vs AA		GG vs GA+AA	
	GG	GA	AA	GG	GA	AA	G	G	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)
GWAS	16.2%	31.6%	52.1%	6.1%	37.8%	56.2%	32.1%	25.0%	1.6.E-02	1.42 (1.07–1.89)	4.0.E-01	1.18 (0.81–1.71)	2.4.E-05	3.00 (1.76–5.11)
Replication	13.2%	37.0%	49.8%	5.1%	35.0%	60.0%	31.7%	22.6%	1.3.E-04	1.59 (1.25–2.02)	7.7.E-03	1.51 (1.11–2.04)	1.3.E-04	2.83 (1.63–4.90)
Combine	14.1%	35.4%	50.5%	5.9%	37.2%	57.0%	31.8%	24.4%	1.5.E-05	1.44 (1.22–1.70)	1.7.E-02	1.30 (1.05–1.61)	7.5.E-09	2.63 (1.88–3.69)

The GWAS sample included 119 cases and 1582 controls. The replication sample contained 283 cases and 433 controls

MAF minor allele frequency, *OR* odds ratio, *CI* confidence interval

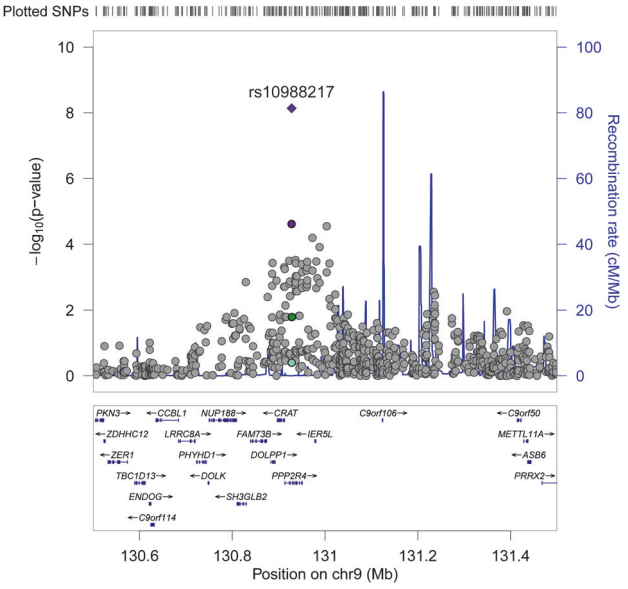

Fig. 1 Association plots within the *CRAT* region with *DQB1*06:02*-negative EHS. The negative logarithm of the association *P*-values of genotyped and imputed SNPs is plotted according to the physical position. *P*-values based on three models (allelic, recessive, and dominant models) in the GWAS are shown as circles. The *P*-value (7.3.E-09) of rs10988217 in the recessive model in both GWAS and replication samples is indicated by a purple diamond. Purple, green, and light green circles represent the *P*-values of rs10988217 based on the recessive, allelic, and dominant models, respectively, in the GWAS samples. Light blue in the background indicates the local recombination rate

significance ($P < 1.6 \times 10^{-8}$). Next, we examined whether rs10988217 is associated with narcolepsy or *DQB1*06:02*-positive EHS. This SNP did not show significant associations (Table 2).

The SNP rs10988217 is located 15-kb upstream of carnitine O-acetyltransferase (*CRAT*) and in the intronic region of protein phosphatase 2 A activator, regulatory subunit 4 (*PPP2R4*). We therefore evaluated whether the SNP affected transcription of *CRAT* and *PPP2R4* using the eQTL database. eQTL analyses revealed that rs10988217 was significantly correlated with expression levels of both *CRAT* and *PPP2R4* in several tissues and cell types including brain (the lowest *P*-value: *CRAT*, $P = 1.4 \times 10^{-15}$, *PPP2R4*, $P = 4.8 \times 10^{-13}$) (Supplementary Table 3 and Supplementary Fig. 6). Higher expression levels of both genes were observed in the risk allele group. We next examined whether SNPs in the *CRAT* region influenced human blood metabolites using the Metabolomics GWAS Server [26], because CRAT is a key enzyme in the metabolic pathway. This SNP was significantly associated with levels of succinylcarnitine (C4-DC), which is a short-chain acylcarnitine, and succinylcarnitine levels were increased in the risk allele group (G) compared with the non-risk allele group (A) ($P = 1.4 \times 10^{-18}$) (Table 3). When other SNPs of the *CRAT* region were tested for associations with levels of

Table 2 Association of rs10988217 with narcolepsy or *DQB1*06:02*-positive EHS

	Genotype freq.			MAF	Allelic model		GG+GA vs AA		GG vs GA+AA	
	GG	GA	AA	G	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)
Narcolepsy	8.1%	39.8%	52.0%	28.0%	2.2.E-02	1.18 (1.02–1.36)	5.0.E-02	1.20 (1.00–1.43)	7.7.E-02	1.36 (0.97–1.91)
*DQB1*06:02*- positive EHS	6.0%	37.0%	57.0%	24.5%	9.2.E-01	0.98 (0.71–1.37)	9.2.E-01	0.98 (0.65–1.47)	9.6.E-01	0.98 (0.42–2.28)
Control	6.1%	37.4%	56.5%	24.8%						

The sample in this analysis was comprised of 640 patients with *DQB1*06:02*-positive narcolepsy, 100 patients with *DQB1*06:02*-positive EHS and 1912 controls

MAF minor allele frequency, *OR* odds ratio, *CI* confidence interval

succinylcarnitine, rs10988217 showed the most significant association signal in SNPs located in this region after imputation (Supplementary Table 4). To assess the relationship between *DQB1*06:02*-negative EHS and succinylcarnitine, we measured levels of succinylcarnitine in sera of 48 patients with *DQB1*06:02*-negative EHS and 20 control subjects using a LC-MS/MS method. Unexpectedly, we found that succinylcarnitine levels were lower in the patients with *DQB1*06:02*-negative EHS than in the control subjects ($P = 0.031$) (Fig. 2).

In the GWAS stage, SNPs of QKI, KH domain containing, RNA binding (*QK1*) had the lowest *P*-value of all SNPs assessed (Supplementary Table 1). QKI protein directly regulates several myelin-specific genes including the expression of MBP. One SNP, rs4890893, located in *MBP*, also showed a suggestive association with *DQB1*06:02*-negative EHS in the GWAS stage (Supplementary Table 1). MBP is a major component of the myelin sheath, and MBP and QKI are involved in the formation and stabilization of the myelin sheath. Demyelination may be a pathological event that is involved in the development of EHS. However, these SNPs in *QKI* and *MBP* were not associated with *DQB1*06:02*-negative EHS in the replication study (Supplementary Table 2). ELISA was conducted to measure the MBP levels in the CSF in 23 patients with normal orexin hypersomnia. Levels of MBP in all hypersomnia subjects were <4 ng/ml, suggesting a low possibility of myelin breakdown.

Discussion

This study of patients with *DQB1*06:02*-negative EHS provides new information on a genetic factor for the rare disease. Our study revealed a genome-wide significant association between *DQB1*06:02*-negative EHS and rs10988217 in the *CRAT* and *PPP2R4* regions. *CRAT* encodes the carnitine acetyltransferase protein, which is a key enzyme in metabolic pathways involved in the control of the short-chain acyl-CoA/CoA ratio in mitochondria, peroxisomes, and endoplasmic reticulum [30, 31]. CRAT catalyzes the reversible transfer of short-chain acyl groups from a short-chain acyl-CoA thioester to carnitine, thus forming the corresponding short-chain acylcarnitine. The metabolomics GWAS showed that rs10988217 was the SNP in this region that was the most significantly associated with levels of succinylcarnitine, a short-chain acylcarnitine. Of note, the top associated SNP was the same in both the *DQB1*06:02*-negative EHS and succinylcarnitine analyses. In addition, we reasonably assume that levels of succinylcarnitine are elevated by higher expression levels of *CRAT* in the risk allele group of rs10988217. Therefore, we initially expected that higher levels of succinylcarnitine

Table 3 Association between higher succinylcarnitine levels and the risk allele of rs10988217

Metabolite	Effect allele/the other allele	Effect (beta)	Standard error	*P*-value
Succinylcarnitine	G/A	0.0164	0.0019	1.4E-18

Genetic association data for succinylcarnitine were obtained from earlier reports [26]

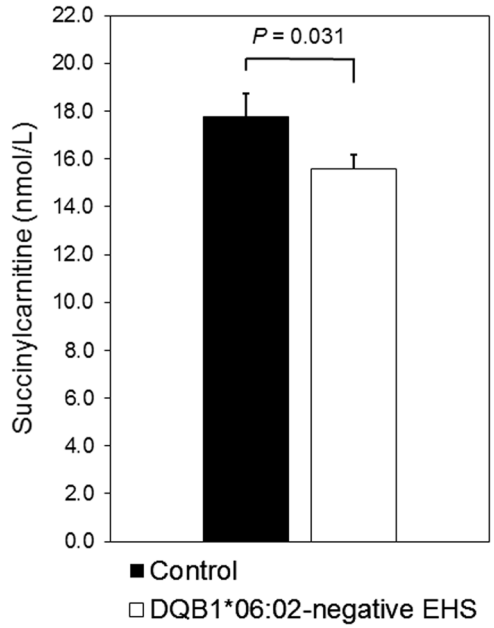

Fig. 2 Levels of succinylcarnitine in 48 patients with *DQB1*06:02*-negative EHS and 20 control subjects. The *P*-value was calculated by a linear regression analysis with adjustment for the effects of age, sex, BMI, and rs10988217 genotype to assess the relationship between case–control status and levels of succinylcarnitine. Error bars represent standard error

would be found in sera of patients with *DQB1*06:02*-negative EHS. Contrary to our expectations, lower levels of succinylcarnitine in patients with *DQB1*06:02*-negative EHS were observed compared with control subjects. Although the difference in succinylcarnitine levels between *DQB1*06:02*-negative EHS and control subjects showed statistically significance (Fig. 2), we cannot deny that the significance was caused by unmeasured potential confounding factors. However, in this measurement, we used blood samples after a certain time had passed from the disease onset. If a higher level of succinylcarnitine is a trigger of the disease onset, it is necessary to analyze samples from patients with recent-onset disease. In addition, CSF samples might be more appropriate because *DQB1*06:02*-negative EHS is a central disorder of hypersomnolence. However, it has been reported that the concentration values for acylcarnitines in CSF were significantly lower as compared with plasma [32]. The means of concentrations for acylcarnitines in CSF were about 10–20 times lower as in plasma [33, 34]. We estimated the mean of concentrations for succinylcarnitine in CSF to be 1.6–3.2 nmol/L, because that in sera was about 16 nmol/L (Fig. 2). As the lower limit of quantification of the LC-MS/MS method was 10 nmol/L for succinylcarnitine, measurement of succinylcarnitine in CSF was considered to be difficult in the present study. A more sensitive method is required to determine levels of succinylcarnitine in CSF in a future study.

*PPP2R4 e*ncodes a phosphotyrosyl phosphatase activator of protein phosphatase 2 A. PPP2R4 plays an important role in many aspects of cell growth and division [35, 36], and is reported to be a haploinsufficient tumor-suppressor gene [37]. A recent study identified 80 synaptic sleep need index phosphoproteins (SNIPPs) associated with sleep need using quantitative phosphoproteomic analysis [38]. The phosphorylation–dephosphorylation cycle of SNIPPs may be an important way that the brain regulates sleep–wake homeostasis. Phosphatase catalyzes dephosphorylation and rs10988217 was correlated with expression levels of *PPP2R4* in the eQTL analysis, suggesting that *PPP2R4* can be a potential susceptibility gene for *DQB1*06:02*-negative EHS.

Several reports have also implicated fatty acid beta-oxidation and the carnitine system in sleep regulation. First, a GWAS for narcolepsy in a Japanese population identified a significant association of a SNP adjacent to carnitine palmitoyltransferase 1B (*CPT1B*) [39]. CPT1B is the rate-controlling enzyme of long-chain fatty acid beta-oxidation. Second, fasted juvenile visceral steatosis ($jvs^{-/-}$) mice with systemic carnitine deficiency exhibit a higher frequency of fragmented wakefulness and rapid eye movement sleep (REM) sleep, and reduced locomotor activity [40]. Fasted $jvs^{-/-}$ mice are activated by modafinil, a drug used for improving wakefulness in patients with excessive sleepiness. Third, a mouse model deficient in short-chain acyl-CoA dehydrogenase (encoded by *Acads*), an enzyme catalyzing the first step of beta-oxidation, shows slow theta frequency during REM sleep [41]. Administration of acetyl-L-carnitine, which restores beta-oxidation of fatty acids in the mitochondria, significantly corrects the slow theta frequency in mice lacking *Acads*. Additionally, our group has reported a clinical trial of oral L-carnitine in narcolepsy. The results suggested that oral L-carnitine is a candidate treatment for narcolepsy [42]. Taken together, these results indicate that dysfunctional metabolism of short-chain fatty acids may lead to the development of the disease.

The recessive model of rs10988217 showed the most significant association with *DQB1*06:02*-negative EHS in the three models (Table 1). The recessive model was not statistically significant in narcolepsy ($P = 7.7 \times 10^{-2}$, OR = 1.36), although the allelic model was significant

($P = 2.2 \times 10^{-2}$, OR = 1.18) (Table 2). In a previous study, this SNP was significantly associated with European narcolepsy based on the allelic model (allelic model: $P = 3.9 \times 10^{-2}$, OR = 1.13; recessive model: $P = 2.0 \times 10^{-1}$, OR = 1.11) [18]. The ORs in narcolepsy were much lower than that of *DQB1*06:02*-negative EHS, suggesting that the effect size of rs10988217 might be small in narcolepsy. Therefore, a further replication study with a larger sample will be needed to evaluate the association between rs10988217 and narcolepsy.

Associations with SNPs of *QKI* and *MBP* in the GWAS stage and the functions of the encoded proteins suggested that demyelination may be involved in the development of EHS. However, the associations were not replicated in a new sample set, and levels of MBP were within the normal range in 23 patients with normal orexin hypersomnia. We also measured the levels of MBP in 21 patients with low orexin hypersomnia (narcolepsy) and 29 controls to test the possibility of mild demyelination in normal orexin hypersomnia. The MBP levels in patients with normal orexin hypersomnia were not higher than those with low orexin hypersomnia or controls. Thus, demyelination is unlikely to be involved in *DQB1*06:*02-negative EHS patients, although patients with normal orexin hypersomnia were assessed before diagnosis.

We report a significant association of rs10988217 with *CRAT* in *DQB1*06:02*-negative hypersomnia. We also found that not only *CRAT* expression levels but also levels of succinylcarnitine were increased in the risk allele group of rs10988217. Further analyses are necessary to elucidate the underlying factors associated with EHS.

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Compliance with ethical standards

Conflict of interest Dr. Yuichi Inoue has received grants and payment for lectures including service on speakers' bureaus, and has provided expert testimony for MSD K.K., Takeda Pharmaceutical Co. Ltd, and Eisai Co. Ltd. The remaining authors declare that they have no conflict of interest.

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