Journal of Translational Medicine

RESEARCH

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Mapping fatigue: discovering brain regions and genes linked to fatigue susceptibility

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Abstract

Background The relationship between the brain and fatigue is gaining increasing attention, with numerous studies indicating that certain specific brain regions may be closely linked to fatigue. Our study aimed to identify brain regions exhibiting significant causal relationships to fatigue and discover potential neurotherapeutic targets associated with fatigue, in the pursuit of seeking new approaches for fatigue treatment.

Methods A bidirectional two-sample Mendelian randomization (TSMR) method was employed to investigate causal relationships between cortical and subcortical gray matter volumes in 83 regions and fatigue. Then, we utilized frontal cortex expression Quantitative Trait Loci data, employing the methods of Summary-data-based Mendelian Randomization (SMR) and Bayesian colocalization to identify genes that exhibit significant association with fatigue. Finally, the transcription levels of candidate genes were assessed in a central fatigue rat model using RT-qPCR.

Results The results of the TSMR analysis revealed that an increased in the volume of the right lateral orbitofrontal, left caudal middle frontal, right caudal middle frontal, and right rostral middle frontal cortices may be correlated with a diminished susceptibility to fatigue. The SMR and Bayesian colocalization analysis identified ECE2, GPX1, METTL21EP, RP11-665J16.1, and SNF8 as candidate genes associated with fatigue. RT-qPCR results confirmed significantly elevated transcription levels of *Ece2*, *Gpx1*, and *Snf8* in the frontal cortex of central fatigue model rats compared to controls.

Conclusions Our findings afford substantial theoretical support for the connection between the brain and fatigue, while also providing novel insights into the genetic mechanisms and therapeutic targets for fatigue, particularly central fatigue.

Keywords Mendelian randomization, Summary-data-based Mendelian randomization, Bayesian colocalization, Brain, Fatigue

Introduction

Fatigue is characterized as a physiological state in which the body cannot maintain a particular level of performance or an organ is unable to support a specified intensity of activity [1]. Extended durations of fatigue are associated with a heightened risk of various disorders, including Parkinson's disease and depression, significantly impacting overall quality of life [2]. The pathophysiological basis of fatigue is complex, and the underlying pathophysiological mechanisms remain unclear [3]. Previous pharmacological studies on fatigue have primarily focused on alleviating physical fatigue, resulting in

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limited effectiveness in addressing the symptoms of fatigue feelings [4].

Recently, a growing body of studies have begun to emphasize the critical role of the brain in the fatigue mechanisms. The dysregulation of neurotransmitters in the brain, such as serotonin, dopamine, and prolactin, can also precipitate the onset of fatigue-related symptoms, a condition commonly referred to as central fatigue [5]. In contrast to peripheral fatigue or physical fatigue, there are currently no satisfactory clinical treatments available for central fatigue [6]. Consequently, identifying therapeutic targets in the brain related to fatigue is of critical importance.

In the past few years, studies have also revealed that various regions of the brain may play distinct roles in the mechanisms underlying fatigue. It has been shown that mental fatigue is associated with functional dysregulation across various brain regions, with the frontal cortex region exhibiting the most pronounced changes in brain electrical signals during states of mental fatigue [7]. For physical fatigue, a clinical study has observed variations in [18F]-FDG uptake across different brain regions. Specifically, participants with physical fatigue exhibited significantly increased FDG uptake in the occipital, frontal, and temporal cortices regions compared to other regions [8]. Although researchers have identified several brain regions that are closely associated with fatigue, there currently exists a paucity of systematic investigations into the relationship between specific brain areas and fatigue. Moreover, the majority of studies conducted in this field were observational studies or animal experiments, which were not able to establish causal relationships between different brain regions and fatigue. Mendelian randomization (MR) is a robust new method for investigating causal relationships [9]. MR studies use genetic variants as instrumental variables for exposure, a design akin to the random allocation of interventions in randomized clinical trials, which effectively overcome the issues of reverse causation and confounding in observational studies. Therefore, MR studies are well-suited for investigating potential causal relationships between different brain regions and fatigue [10].

Meanwhile, the specific molecular mechanisms by which different brain regions mediate fatigue also remain unclear. Currently, it has been only observed that activation of the brain lactate receptor GPR81 in animal models can exacerbate exercise-induced central fatigue [11]. However, this research did not focus on specific brain regions. There is still lack of research on brain gene markers associated with fatigue, and it is still unknown which genes within fatigue-related brain regions are closely linked to the experience of fatigue. In recent years, many studies have started to use expression Quantitative Trait Locis (eQTLs) as proxies for gene expression levels and apply a combination of Summary-data-based Mendelian Randomization (SMR) and Bayesian colocalization approaches to identify genes with colocalized associations with specific traits or diseases from large-scale eQTL data, aiming to discover genetic biomarkers related to those traits or diseases [12–16].

The total brain volume is a crucial metric of the human brain morphometry, encompassing the overall size of the brain as well as the quantification of specific regions [17]. Variations in the brain volume are associated with numerous neuropsychiatric disorders, cognitive abilities, and even susceptibility to certain physical health conditions, providing valuable insights into the neural underpinnings of various cognitive functions, behaviors, and disease processes [18, 19]. Currently, research has demonstrated that structural changes in the brain are closely associated with functions such as memory, emotional regulation, and motor control, and that abnormalities in these functions are important contributors to the fatigue [20–22]. In this study, we propose to identify specific brain regions that have significant causal relationship with fatigue using bidirectional two-sample Mendelian randomization (TSMR) methods based on Genome-Wide Association Studies (GWAS) data of brain volumes. Subsequently, For the brain regions identified as having significant relationships, we will apply SMR and Bayesian colocalization analyses to find genes closely associated with fatigue in the fatigue-related brain region. Furthermore, we will validate these findings by constructing a central fatigue animal model, thereby seeking to uncover potential brain targets for the fatigue treatment.

Methods

Study design

Figure 1 presents the detailed overview of our study's design. Initially, we employed GWAS data derived from brain magnetic resonance imaging, concentrating on the volumes of cortical and subcortical gray matter regions. Using the bidirectional TSMR method, we investigated the causal relationships between these brain structures and fatigue, with the objective of identifying the brain regions strongly linked to fatigue. Subsequently, we applied the SMR analysis and Bayesian colocalization analysis to examine the association between eQTLs located in the frontal cortex regions of the brain and fatigue, aiming to identify genes that are significantly correlated with fatigue. Meanwhile, Drug SIGnatures database (DSigDB) and DrugBank database were used to found the potential corresponding drugs for the identified genes. Finally, we validated our findings experimentally by establishing a central fatigue rat

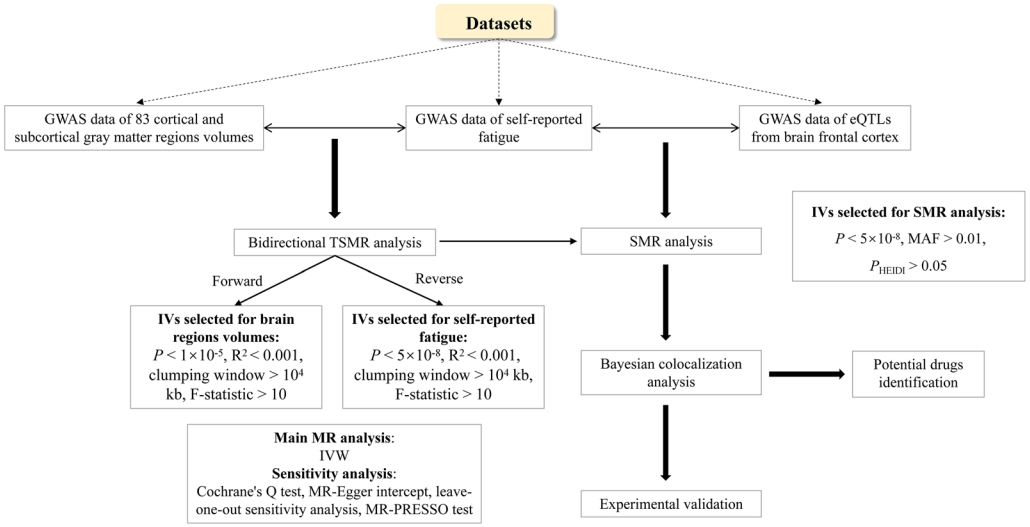

Fig. 1 The flow chart of our study. GWAS: Genome-Wide Association Studies. TSMR: two-sample Mendelian randomization. SMR: Summary-data-based Mendelian Randomization. IVs: instrumental variables. MAF: minor allele frequency. IVW: inverse variance weighted. eQTLs: Quantitative Trait Locis

model to assess the expression of the identified genes in the frontal cortex tissue.

Data sources

The brain morphometric MRI data were derived from a genome-wide association study involving 36,778 Europeans, focusing on the volumes of 83 cortical and subcortical gray matter regions (including 33 cortical regions as defined by the Desikan-Killiany atlas were analyzed in each hemisphere, along with 8 subcortical regions in each hemisphere and a brainstem) [23].

The genetic variants associated with fatigue were derived from a GWAS study which involved 449,019 participants and primarily aimed to investigate the genetic contributions to self-reported fatigue [24]. The diagnosis of self-reported fatigue in the GWAS study comes from a specific question in the authoritative Mental Health Questionnaire: "Over the past two weeks, how often have you felt tired or had little energy?".

The eQTL data for the normal human frontal cortex tissue were sourced from the Genotype-Tissue Expression (GTEx) Portal (https://gtexportal.org/home/) with a sample size of 209. The specific frontal cortex eQTL data can be downloaded from https://www.gtexportal.org/home/dataset. In the SMR analysis, cis-eQTLs were utilized as instrumental variables (IVs) for gene expression. cis-eQTLs in this study refer to genetic variations located within 1 Mb of the coding sequence, which are closely associated with gene expression [25].

TSMR analysis

TSMR is a novel extension of MR that extracts genetic effect estimates from two independent groups of individuals, effectively enhancing causal inference [26]. We investigated the causal relationships between the volumes of cortical and subcortical gray matter in 83 different regions and fatigue using a bidirectional TSMR method. When conducting TSMR analysis, it is essential to meet three critical assumptions: (i) a robust correlation must be established between IVs and the exposure factors; (ii) the IVs must remain independent of potential confounders; and (iii) the IVs should exert their influence on the outcome exclusively through the exposure factors, without any effects via alternative pathways [27]. To verify that the IVs were strongly associated with the risk factor of exposure, we identified single nucleotide polymorphisms (SNPs) as potential IVs based on their p-value. In this study, the threshold of p-value was selected to balance statistical significance with the retention of a sufficient number of candidate SNPs for subsequent analysis. While more stringent thresholds can reduce false positives, they may also miss potentially important signals. In the forward MR analysis, due to the limited number of SNPs in some of the exposure GWAS data related to brain morphometry, SNPs with p-value less than 1×10^{-5} were considered to be strongly associated with the trait and hence selected as instrumental variables for this study [28]. This criterion ensured that the MR analysis between each brain region volume and fatigue included sufficient number of SNPs for analysis (greater than three). If fewer than three SNPs were included in

the MR analysis, it becomes impossible to perform the MR-PRESSO sensitivity analysis, which may introduce substantial bias to the results [29]. In the reverse MR analysis, the large number of SNPs related to the exposure allows for the selection of SNPs strongly associated with the exposure based on a genome-wide significance threshold of $p < 5 \times 10^{-8}$, thereby enhancing the reliability of the statistical results [30]. To fulfill assumption (ii), it is crucial that the genetic variants employed in the MR analysis are independent of confounding factors. First, a linkage disequilibrium analysis was conducted to make sure that all selected SNPs must not be in the state of linkage disequilibrium. Linkage disequilibrium refers to the non-random association of alleles at two or more loci. MR analysis requires that the distribution of genetic variants is random and assumes that SNPs are independent of each other [31, 32]. This ensures that SNPs are independent of potential confounding factors. If the selected SNPs exhibit high correlation with the loci of other risk factors, it may introduce potential confounding effects [33]. In both the forward and reverse MR analyses, we established the threshold for linkage disequilibrium correlation coefficient at $r^2 = 0.001$, with a clumping window set to 10^4 kb. Typically, under the conditions of the clumping algorithm with $r^2 \leq 0.001$ and 10^4 kb clumping window, the influence of strong linkage disequilibrium can be effectively reduced, thereby ensuring the selection of independent SNPs across the genome [34, 35]. Second, the LDlink online tool (https://ldlink.nih.gov/) was used to identify SNPs associated with risk factors for the outcome and potential confounders, which were then excluded from our study to mitigate the influence of confounding factors [36]. Immediately afterwards, we extracted the corresponding SNPs from the outcome GWAS dataset. If a specific SNP was absent from the outcome dataset, it would be replaced by an appropriate proxy SNP [37]. If no suitable proxy SNP was available, the original SNP was excluded from our TSMR analysis. Meanwhile, IVs exhibiting incompatible alleles, as well as palindromic SNPs with intermediate allele frequencies, were also excluded from the analysis [38]. The ambiguous and replicated SNPs were excluded from the datasets, along with those exhibiting a minor allele frequency (MAF) less than 0.01 [39]. To assess the strength of the SNPs in our study, we calculated F-statistics using the formulas (1) and (2) [40]. IVs with F-statistics less than 10 were excluded to mitigate weak instrument bias [41].

$$R^2 = 2 \times MAF \times (1 - MAF) \times \beta^2 \quad (1)$$

$$F = R^2(n - k - 1) / k\left(1 - R^2\right) \quad (2)$$

"MAF" is the minor allele frequency of SNP used as IVs, "n" is the sample size, and "k" is the number of IVs used in each study.

The TSMR analysis was conducted by using the "TwoSampleMR" (version 0.5.6) package in R software (version 4.2.1). Several statistical methods were utilized to evaluate the causal relationships, including inverse variance weighted (IVW), weighted median (WM), MR-Egger regression, simple mode, and weighted mode. Among them, the IVW method was selected as the primary analytical method. The IVW method operates under the assumption that the genetic variants used as instrumental variables are valid, meaning they are associated with the exposure of interest and not directly with the outcome, except through the exposure [42]. Consequently, the IVW method is capable of providing the most precise effect estimates. The causal relationships were presented as odds ratios (ORs) with their 95% confidence intervals (CIs). Statistical significance was set as $p < 0.05$. Holm–Bonferroni method was employed to adjust statistical significance in multiple comparisons [43]. After Bonferroni correction, an adjusted p-value < 0.0006 (where $p = 0.05/83$) was considered statistically significant for TSMR analysis [44].

Sensitive analyses

Sensitivity analysis was primarily conducted to determine whether there were potential heterogeneity and horizontal pleiotropy present during the MR analysis [45]. Cochrane's Q test was utilized to assess the heterogeneity. The random-effects inverse variance weighted model was applied to yield a more conservative effect estimate when heterogeneity was identified ($p < 0.05$). Conversely, if no heterogeneity was found, the fixed-effect IVW model was employed. The MR-Egger intercept method was employed to detect and evaluate the potential horizontal pleiotropy, with a p-value greater than 0.05 indicating the absence of horizontal pleiotropy among the SNPs [46]. Leave-one-out sensitivity analysis was primarily conducted to determine whether any single SNP disproportionately influences the causal relationship in TSMR analysis, thereby investigating the source of heterogeneity [47]. If the analysis results showed substantial variation upon the exclusion of a particular SNP, that SNP was classified as an outlier and subsequently removed from the dataset [48]. Finally, the MR-PRESSO test was employed to identify potential pleiotropic outliers among the SNPs, and the adjusted correlation results were derived after excluding these outliers [37]. For the MR-PRESSO analysis, the number of permutations was set to 1000 [49].

SMR analysis

SMR is an innovative research method which is always employed to ascertain pleiotropic associations between the expression level of a specific gene and a complex trait of interest, utilizing aggregated data from GWAS and eQTL investigations [50]. The SMR analysis was conducted using the SMR software (version 1.3.1, https://yanglab.westlake.edu.cn/software/smr/#Download). SMR analysis also needs to satisfy the three core assumptions of MR analysis. To meet the three core assumptions, we included only SNPs with a strong correlation to cis-eQTLs ($P < 5 \times 10^{-8}$) in the SMR analysis, while also ensuring that the MAF for each SNP was greater than 0.01 [51]. The HEIDI test, utilized as a sensitivity analysis method in SMR analysis, is primarily designed to distinguish causal associations and exclude potential pleiotropy, with the threshold set at $P_{HEIDI} = 0.05$ [52]. SNPs meeting the above criteria were ultimately used as IVs, ensuring that the causal relationship between gene expression and the occurrence of fatigue was not confounded by other factors. The false discovery rate (FDR) method was employed to correct for multiple comparisons in this SMR analysis [53].

Bayesian colocalization analysis

Bayesian colocalization analysis quantifies the probability that two traits share a common causal genetic variant, thereby facilitating the identification of candidate genes that exhibit a stable association with the respective phenotypes [54]. Bayesian colocalization analysis is commonly used to evaluate the probability that two traits share a common causal variance [54]. It was performed using the coloc R package (version 5.2.2) in R software (version 4.2.2) with the default parameters provided in the software. The colocalization analysis carefully examined the genetic regions associated with the two phenotypes [55]. By meticulously mapping the causal variation between the two traits, this approach minimized the identification of spurious pleiotropy and indicated the presence of biological mechanisms linking the two traits through shared genetic predictors [51]. The colocalization analysis reported five different posterior probabilities corresponding to the following five hypotheses: (1) H0: Neither trait has causal variation; (2) H1: Only trait 1 has causal genetic variation; (3) H2: Only trait 2 has causal genetic variation; (4) H3: Both traits have causal genetic variation, but they do not share the same variation; (5) H4: Both traits share the same causal variation [56]. A posterior probability of H4 (PPH4) > 0.8 is considered strong evidence for the co-localization of fatigue GWAS and eQTL.

Identification of potential drugs

DSigDB is a comprehensive drug-gene interaction database that encompasses 22,527 genomes, 17,389 compounds, and 19,531 genes, providing detailed information on the associations between genes and known or potential drugs [57]. We identified potential corresponding drugs for the genes significantly associated with fatigue by utilizing protein-drug interaction data from the DSigDB database by Enrichr (https://maayanlab.cloud/Enrichr/). Moreover, we further integrated data from the DrugBank database (https://go.drugbank.com/) to establish the reliability of the candidate drugs. DrugBank is a comprehensive, freely accessible online database that includes extensive information on drugs and their targets, along with the current approval status of these drugs [58]. Screening through the DrugBank database allows us to identify drugs that have already received approval.

Experimental validation

Due to the limited number of SNPs associated with the cis-eQTL in the frontal cortex region, our predicted results may lead to inaccuracies. Therefore, we conducted in vivo experiments to further validate the predicted genes. We established a central fatigue rat model using a well-established modeling method from our previous study [59]. Following the initiation of the experiment, the model group rats were subjected to a stationary water environment platform (provided by the Neuroimmunology Laboratory of Beijing University of Chinese Medicine, Beijing, China) for 14 h each night, from 18:00 to 8:00 the following morning. On odd days, the model group rats were given adequate maintenance feed and water, while on even days, their food intake was deliberately restricted to only sufficient water, resulting in increased food intake compared to normal conditions on odd days. This modeling procedure was consistently implemented over a 21-day period. A series of behavioral tests were conducted for the evaluation of the model stability. The more specific procedures for central fatigue model construction and the behavioral tests can be found in the Supplementary File. Reverse transcription-quantitative PCR (RT-qPCR) method was employed to assess the transcription levels of the predicted genes. We collected frontal cortex tissue from both the control group and the model group rats and extracted the total RNA using a total RNA extraction kit (Magen Biotech, Guangzhou, China). According to the manufacturer's instructions, RNA was reverse transcribed using the RT first-strand cDNA synthesis kit (F0202, Lablead, China), followed by qPCR using SYBR Green PCR Master Mix (R0202, Lablead, China). Each reaction was performed in triplicate. Relative RNA transcription levels were

determined using the $2^{-\Delta\Delta CT}$ method by researchers who were blinded to the animals grouping. All the primers were synthesized by Sangon Biotech (Shanghai) Co.,Ltd. The primer sequences are shown in Table 1.

Statistical analyses

All statistical tests were conducted using SPSS 26.0 software (IBM, Armonk, NY, USA) and the GraphPad Prism (version 9.5.0, GraphPad, San Diego, CA, United States) was used to create relevant graphics based on the statistical results. Measurement data are presented as mean ± standard deviation (mean ± SD). If the data met normality, then a two-tailed independent *t*-test or Welch's *t*-test would be used; otherwise, the Mann–Whitney U-test would be employed. A p-value < 0.05 was considered statistically significant.

Results

The TSMR analysis

Basic information of IVs

The IVs were chosen according to the criteria outlined in the methods section. All IVs exhibited F-statistics exceeding 10, demonstrating that there were no weak IVs present.

Results of the forward TSMR

Through IVW method, we identified the volume of 24 regions in cortical or subcortical gray matter as potential risk factors for the occurrence of fatigue. Following Bonferroni correction, 4 regions exhibited a stable causal relationship with the onset of fatigue (Supplementary Table 1 and Fig. 2). We found that the right lateral orbitofrontal volume ($\beta = -4.01E^{-05}$, $se = 1.00E^{-05}$, $p = 6.01E^{-05}$), left caudal middle frontal volume ($\beta = -3.02E^{-05}$, $se = 8.08E^{-06}$, $p = 1.85E^{-04}$), right caudal middle frontal volume ($\beta = -3.02E^{-05}$, $se = 8.48E^{-06}$, $p = 3.70E^{-04}$), and right rostral middle frontal volume ($\beta = -1.43E^{-05}$, $se = 4.09E^{-06}$, $p = 4.63E^{-04}$) were significantly negatively correlated with the risk of developing fatigue, indicating that these volumes may mitigate the risk of fatigue occurrence.

Results of the reverse TSMR

Reverse MR analysis was conducted to investigate the potential reverse causal relationship between the volumes of the four abovementioned brain regions, which exhibit significant causal connections with fatigue, and the risk of fatigue. Utilizing the IVW method, our findings indicated that there was no reverse causal relationship between the volumes of the four brain regions and fatigue (Supplementary Table 4 and Fig. 3).

Sensitivity analyses

We conducted a comprehensive series of sensitivity analyses to examine potential biases within the MR analysis. The scatter plots were utilized to assess the robustness of the MR results (Supplementary Fig. 1 and 4) [60]. Detailed results regarding heterogeneity and pleiotropy assessments are provided in Supplementary Tables 2, 3, 5, 7. The p-values derived from Cochrane's Q test were all greater than 0.05, suggesting the absence of heterogeneity among our estimates. The MR-Egger intercept test provided no evidence of horizontal pleiotropy among the selected SNPs. Moreover, the results of the leave-one-out analysis revealed that the exclusion of any single SNP did not alter the significant results of the MR analysis, further supporting the absence of horizontal pleiotropy and underscoring the robustness of our study outcomes (Supplementary Fig. 3 and 6). The MR-PRESSO test yielded no outliers among the significant brain region volumes and fatigue. Finally, the symmetrical funnel plots served to bolster the dependability of our Mendelian randomization analysis, affirming the integrity of our statistical inferences (Supplementary Fig. 2 and 5).

SMR analyses of cis-eQTLs in the frontal cortex and the risk of fatigue

As shown in Fig. 4 and Supplementary Table 7, our SMR results indicated significant correlations between the cis-eQTLs of 12 genes (GPX1, BTN3A2, GMPPB, METTL21EP, RP11-665J16.1, NMUR2, ECE2, SNF8, CYP2D6, AFF3, RP11-166B2.1, and RP11-102M11.2) in the frontal cortex and the occurrence of fatigue following the FDR correction. The Manhattan plots illustrated the distribution of these 12 genes across the chromosomes (Fig. 5).

Table 1 The primer sequences used in our study

Gene name	Forward 5′–3′	Length (bp)	Reverse 5′–3′
Ece2	AGCGGCGTGATGAGGAGAAG	111	CAAGTGGCGACAACAAGAAAGAAAG
Gpx1	GCAATCAGTTCGGACATCAGGAG	120	TCACCTCGCACTTCTCAAACAATG
Snf8	TGGAGGAGCTACATCAGCAGGTG	112	AATCCAGTGCCCAGTGCTTTCAG
Gapdh	ACGGCAAGTTCAACGGCACAG	129	CGACATACTCAGCACCAGCATCAC

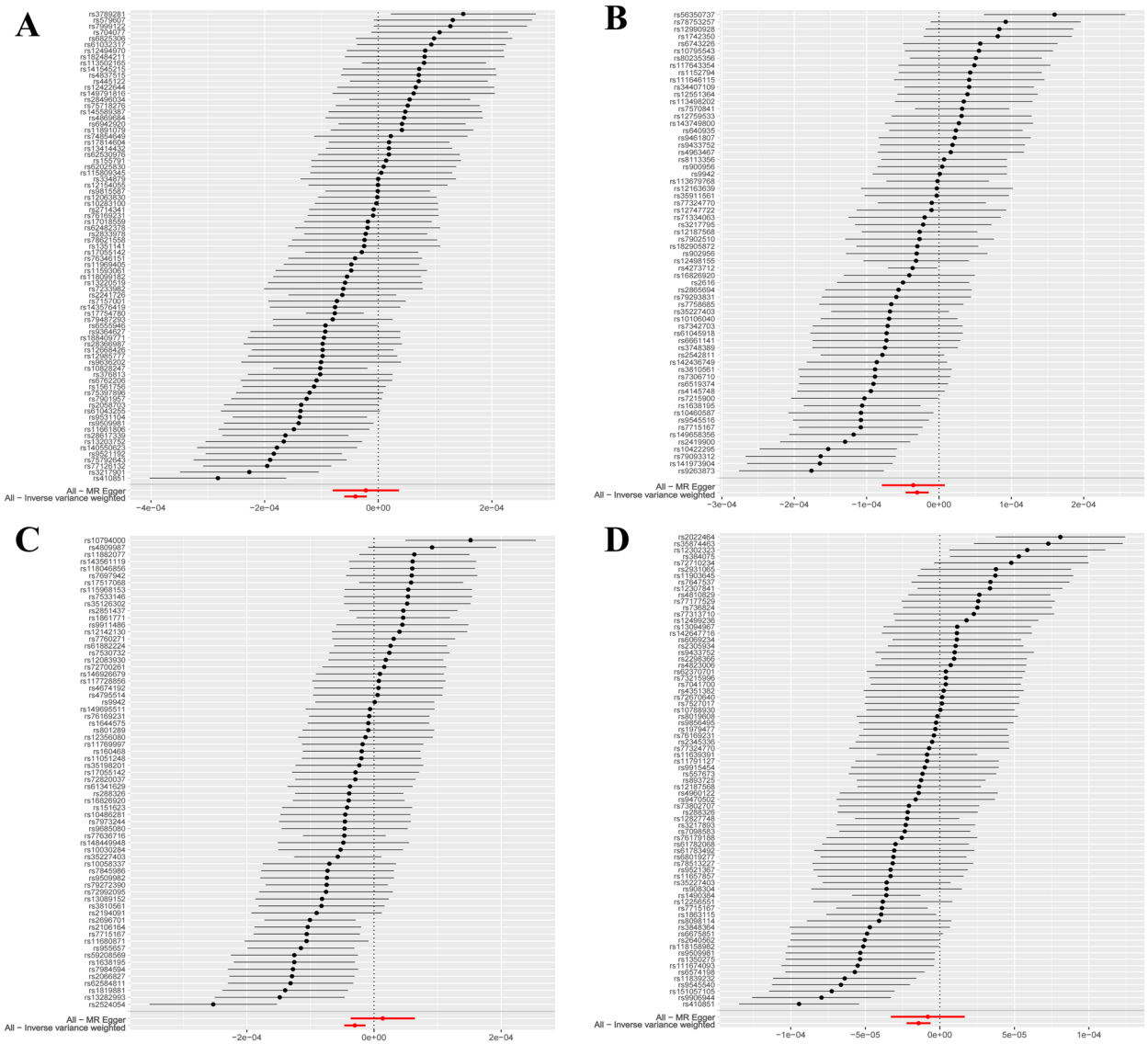

Fig. 2 The forest plots illustrated the forward MR results. **A** Right lateral orbitofrontal volume. **B** Left caudal middle frontal volume. **C** Right caudal middle frontal volume. **D** Right rostral middle frontal volume

Results of the Bayesian colocalization analysis

Bayesian colocalization analysis was further conducted to investigate the causal variants between the 12 identified genes and fatigue. The results revealed that 5 genes (ECE2, GPX1, METTL21EP, RP11-665J16.1, and SNF8) were capable of forming a stable colocalization association with fatigue, with PPH4 values of 95.1%, 93.7%, 97.1%, 91.7%, and 87.8%, respectively (Fig. 6). Among these, we found that SNF8 (OR = 1.023, 95% CI = 1.011–1.035), ECE2 (OR = 1.019, 95% CI = 1.009–1.028), GPX1 (OR = 1.040, 95% CI = 1.023–1.058), and METTL21EP (OR = 1.015, 95% CI = 1.008–1.022) were associated with an increased risk of fatigue. Conversely, RP11-665J16.1 was found to be inversely associated with the risk of fatigue (OR = 0.986, 95% CI = 0.979–0.993). The gene locus plots and effect plots were shown in Fig. 7.

Results of the potential drugs prediction

Through DSigDB database, 295 potential chemical drugs (p-value < 0.05) were predicted based on ECE2, GPX1, METTL21EP, RP11-665J16.1, and SNF8. Among these, 205 drugs were also retrievable in the DrugBank database: 202 for GPX1, 2 for both GPX1 and SNF8, 1 for both GPX1 and ECE2, and none for METTL21EP and RP11-665J16.1 (Supplementary Table 8). Of these, a total of 142 have been approved and are currently permitted

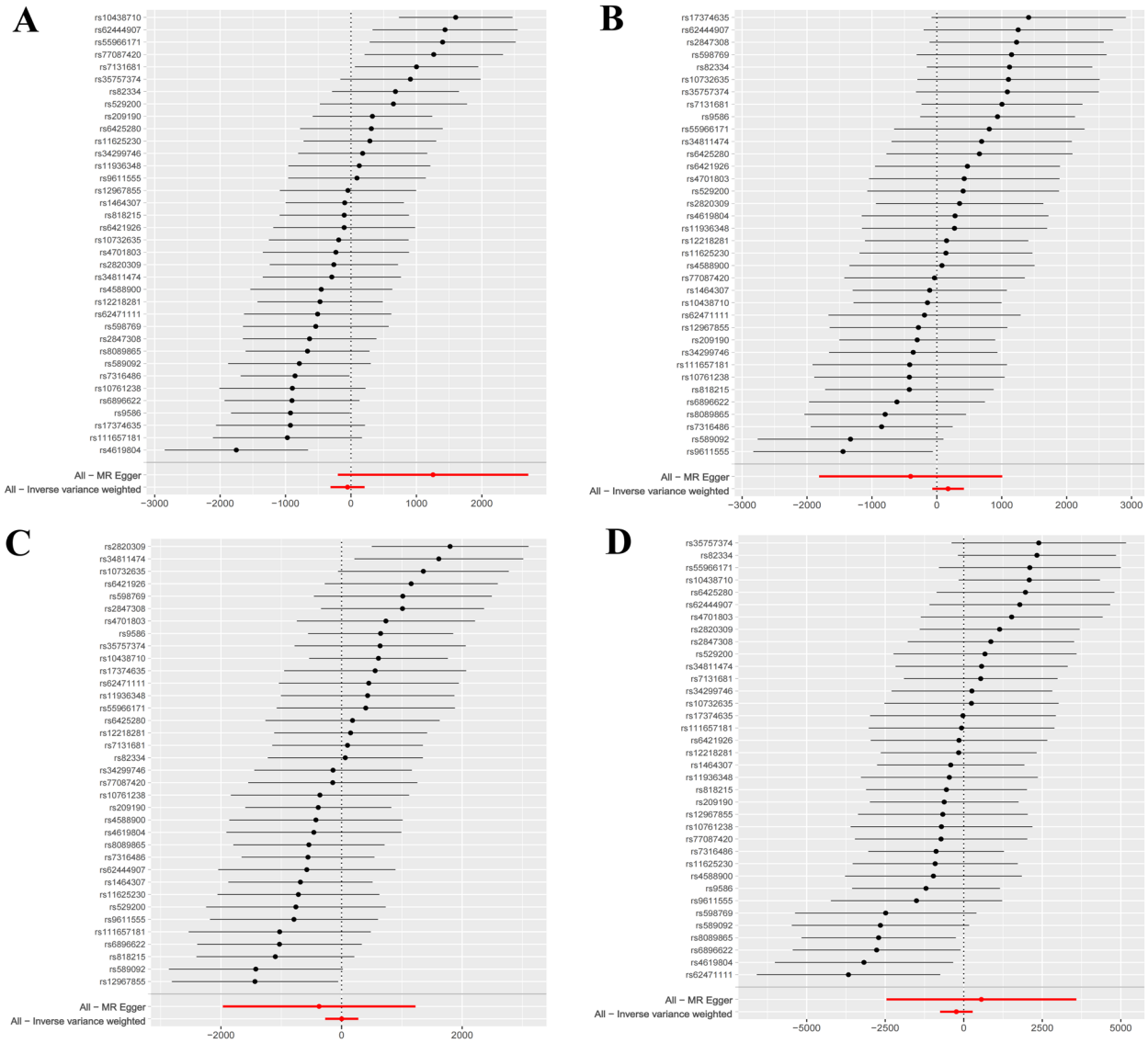

Fig. 3 The forest plots illustrated the reverse MR results. **A** Right lateral orbitofrontal volume. **B** Left caudal middle frontal volume. **C** Right caudal middle frontal volume. **D** Right rostral middle frontal volume

for use: 139 for GPX1, 2 for both GPX1 and SNF8, and 1 for both GPX1 and ECE2. The top 10 approved and currently permitted drugs are presented in Table 2.

The central fatigue rats exhibited significant behavioral abnormalities

Throughout the modeling process, we did not observe any deaths or injuries in the rats, indicating that our central fatigue model demonstrates reliable safety. The results of the behavioral test were shown in Fig. 8A–G and Supplementary Fig. 7–8. The results of the open field test demonstrated that, compared to the control group, the total distance traveled by the model rats was significantly reduced ($p=0.043$), as well as the number of crossings into the central zone ($p=0.01$). The tail suspension test showed that the total duration of immobility induced by tail suspension was significantly increased in the model rats ($p=0.02$). The Morris Water Maze test showed that rats in the model group exhibited a significantly reduced swimming distance in the target quadrant and percentage of time spent in the target quadrant compared to the control group ($p=0.019$, $p=0.007$). The grip strength test and the forced exhaustive swimming test indicated that the model group rats exhibited significantly reduced grip strength and exhaustive times compared to the control group rats ($p=0.000$, $p=0.005$).

Outcome	Gene	P.SMR	FDR	OR (95% CI)
Fatigue	GPX1	5.16e−06	0.005	1.040 (1.023 − 1.058)
Fatigue	SNF8	1.32e−04	0.027	1.023 (1.011 − 1.035)
Fatigue	GMPPB	4.89e−05	0.017	1.020 (1.010 − 1.029)
Fatigue	ECE2	1.11e−04	0.026	1.019 (1.009 − 1.028)
Fatigue	METTL21EP	5.64e−05	0.018	1.015 (1.008 − 1.022)
Fatigue	BTN3A2	4.22e−05	0.017	1.011 (1.005 − 1.016)
Fatigue	NMUR2	7.05e−05	0.018	1.010 (1.005 − 1.015)
Fatigue	RP11−166B2.1	2.04e−04	0.034	1.006 (1.003 − 1.009)
Fatigue	CYP2D6	1.36e−04	0.027	0.989 (0.984 − 0.995)
Fatigue	RP11−665J16.1	6.82e−05	0.018	0.986 (0.979 − 0.993)
Fatigue	RP11−102M11.2	2.37e−04	0.037	0.985 (0.977 − 0.993)
Fatigue	AFF3	1.44e−04	0.027	0.984 (0.976 − 0.992)

Fig. 4 SMR results demonstrated associations between genes expression in frontal cortex and fatigue risks. OR: Odds Ratio. 95% CI: 95% Confidence Interval. FDR: the false discovery rate

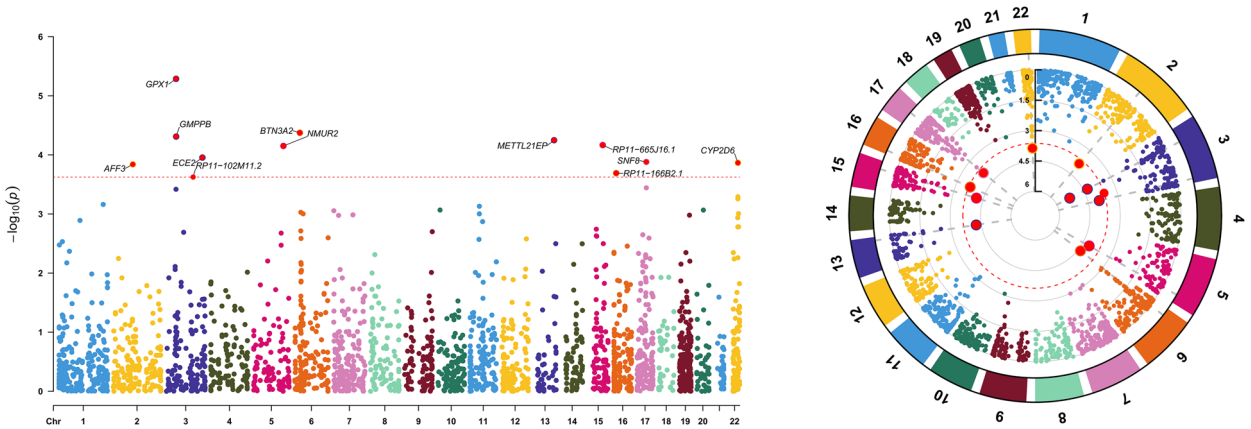

Fig. 5 The Manhattan plots illustrates the chromosomal locations of 12 significantly associated genes

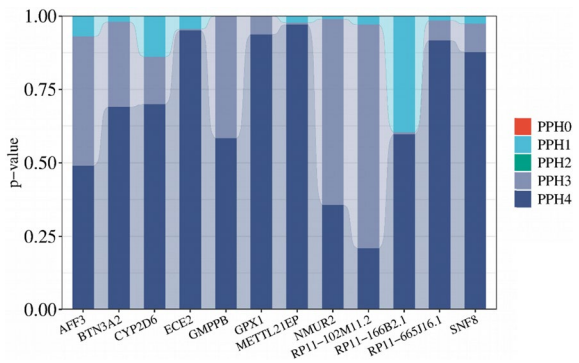

Fig. 6 The stacked bar chart showed the Bayesian colocalization results for the 12 genes. PP.H0: posterior probability of H0. PP.H1: posterior probability of H1. PP.H2: posterior probability of H2. PP.H3: posterior probability of H3. PP.H4: posterior probability of H4; A PP.H4 value greater than 0.8 is considered the threshold for evidence of colocalization between fatigue GWAS and eQTLs

In summary, it is manifest that the rats in our model group exhibit symptoms associated with central fatigue, including a decline in learning and memory, anxiety and depression-like behaviors, as well as physical fatigue manifestations. Thus, it serves as a reliable model for studying central fatigue.

The expression levels of *Ece2*, *Gpx1*, and *Snf8* in the frontal cortex of central fatigue rats

Due to the unavailability of corresponding gene sequences for METTL21EP and RP11-665J16.1 in rats, we only assessed the transcription levels of *Ece2*, *Gpx1*, and *Snf8* in the animal experiment. The results of the RT-qPCR experiment were shown in Fig. 8H–J. Compared to the control group, the transcription levels of *Ece2*, *Gpx1*, and *Snf8* in the frontal cortex of central fatigue model rats were significantly elevated

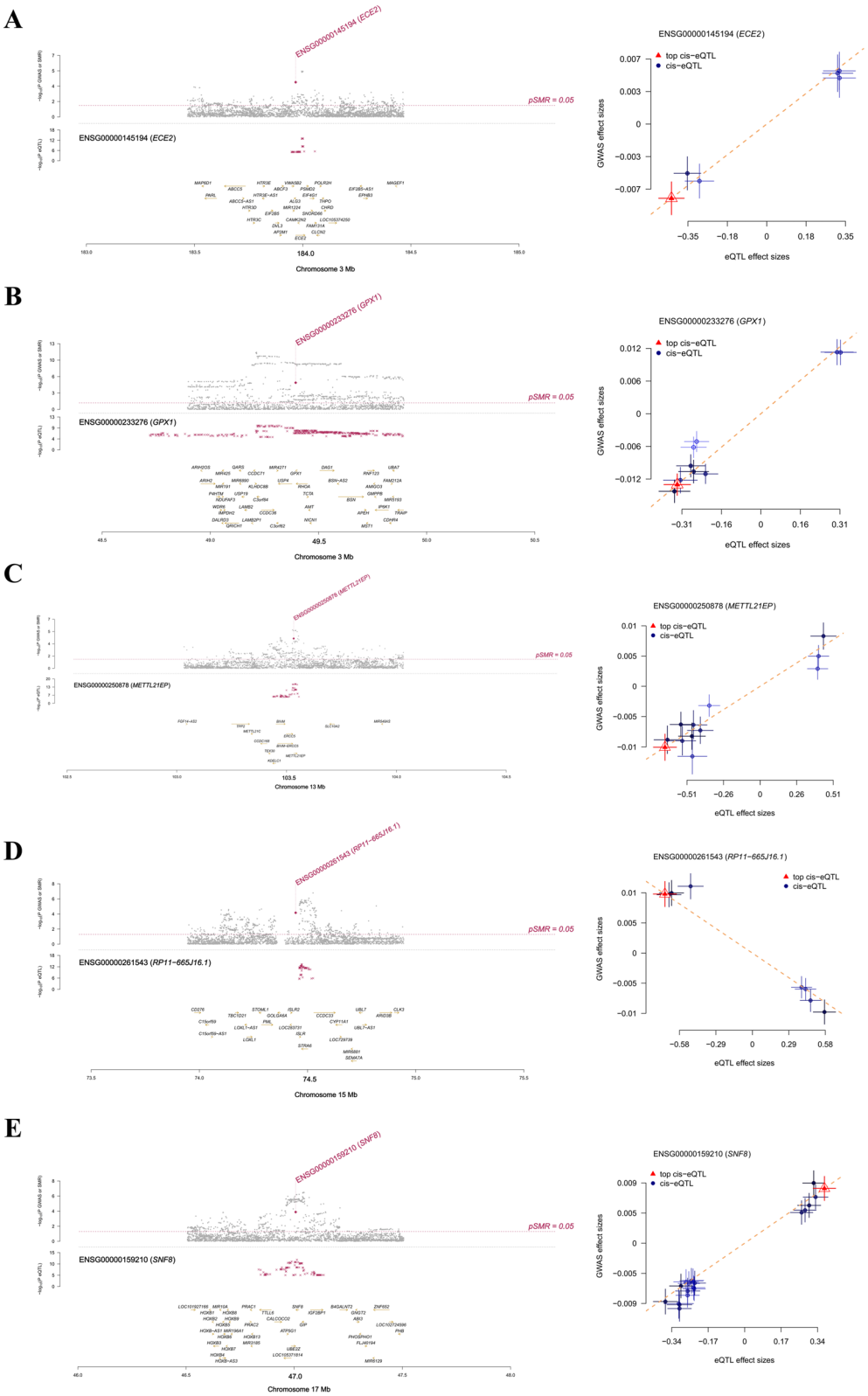

Fig. 7 The gene locus plots and effect plots of the 5 genes that exhibited stable colocalization with fatigue GWAS. **A** ECE2. **B** GPX1. **C** METTL21EP. **D** RP11-665J16.1. **E** SNF8

Table 2 Top 10 approved and currently permitted drugs

Drugs	Genes	*p*-value
Selenomethionine	GPX1; ECE2	1.69E-04
Hesperidin	GPX1	0.004990441
Apomorphine hydrochloride	GPX1	0.004990441
Methimazole	GPX1	0.004990441
Thiamine	GPX1	0.005488389
Magnesium sulfate	GPX1	0.005737289
Sitagliptin	GPX1	0.005986139
Benzthiazide	GPX1	0.006234939
Pyridoxine hydrochloride	GPX1	0.006483689
Sulfasalazine	GPX1	0.00673239

($p = 0.026$, $p = 0.029$, $p = 0.025$). These findings corroborated the predictions of the SMR and Bayesian colocalization analyses, suggesting that *Ece2*, *Gpx1*, and *Snf8* are reliable candidate genes. They may represent potential therapeutic targets within the brain for the alleviation of fatigue.

Discussion

In recent years, an increasing body of evidence has suggested that the brain serves as a central regulator of fatigue [61]. Increasingly, a proliferation of studies was turning their focus toward the pivotal role that the brain plays in the pathogenesis of fatigue [62]. Our study is the first to explore the relationship between the volumes of cortical and subcortical gray matter in 83 different brain regions and fatigue using MR method. At the same time, we identified potential therapeutic targets in the brain associated with fatigue through SMR and Bayesian colocalization methods. Then we conducted preliminary validation of these targets through animal experiments. The results indicated a significant causal relationship between the volumes of four specific frontal cortex regions and the occurrence of fatigue. Additionally, we identified 5 genes closely associated with fatigue from the cis-eQTL data of the frontal cortex, 3 of which were validated to have significantly elevated transcription levels in the frontal cortex tissue of central fatigue rats. These 3 genes may serve as potential brain intervention targets for fatigue.

Researchers have increasingly recognized that the volumetric properties of different regions within the human brain not only reflect individual variations in

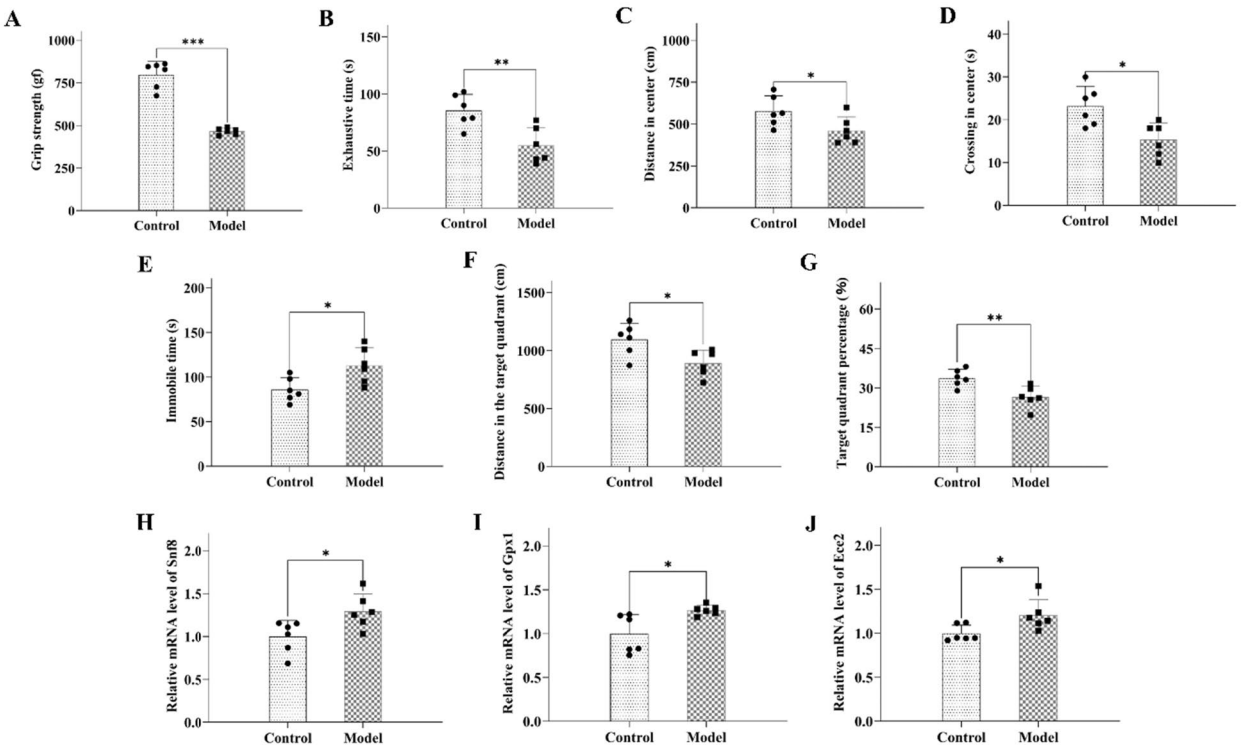

Fig. 8 The results of the experimental validation. **A** Grip strength. **B** Exhaustive time (time when the rat is completely submerged and cannot rise for 10 s) in FEST test. **C**, **D** The total distance traveled by rats in the central zone and the number of crossings into the central zone in the open field test. **E** Immobile time. **F**, **G** The swimming distance and percentage of time spent in the target quadrant in the Morris Water Maze test. **H**–**J** Relative mRNA levels of *Snf8*, *Gpx1*, and *Ece2* against *Gapdh*. All experiments were performed with $n = 6$

neuroanatomy but also provide valuable insights into the potential mechanisms underlying various diseases [63–66]. Currently, studies have emerged to explore the correlation between volumetric properties of various brain regions and fatigue; however, no consensus has yet been formed. A cross-sectional study involving over 800 young participants revealed that there was no significant correlation between the volumetric differences in regional gray matter volume and white matter based on the degree of fatigue [67]. Furthermore, a prospective cross-sectional study also found that the gray matter volume and whole-brain white matter volume in fatigue patients were comparable to those in non-fatigued individuals [68]. A morphometric study reported a reduction in gray matter volume in the bilateral prefrontal cortices of patients with chronic fatigue syndrome, whereas a comparable investigation revealed a decrease in gray matter volume specifically in the occipital and peri hippocampal regions, concurrent with a reduction in white matter volume within the occipital area [69, 70]. Another study has uncovered a significant reduction in white matter volume within the brain, pons, and right temporal lobe of individuals afflicted with chronic fatigue syndrome [71]. However, since the aforementioned studies were all cross-sectional, they were unable to determine whether there is a clear causal relationship between brain morphology measurements and fatigue.

In this study, we identified a potential causal relationship between fatigue and the volumes of four brain regions: right lateral orbitofrontal, left caudal middle frontal, right caudal middle frontal, and right rostral middle frontal, using MR method. An increase in the volume of these four brain regions may be associated with a reduced risk of fatigue. Studies have found a close association between the right lateral orbitofrontal cortex and working memory capacity [72, 73]. The left and right caudal middle frontal regions are primarily associated with mood disorders such as depression and cognitive functions [74–76]. The right rostral middle frontal region has also been found to be associated with cognitive functions and depressive disorders [74, 77]. It is commonly recognized that emotional fatigue and cognitive fatigue are the two primary components of central fatigue [22]. Consequently, it can be inferred that the variations in the volumes of the above four brain regions may alleviate fatigue by influencing cognitive functions and emotional regulation.

Upon a broader regional classification, we found that these four brain areas are all situated within the frontal cortex, indicating that functional abnormalities in the frontal cortex may be closely associated with fatigue. Building upon this foundation, we further investigated the genes that exhibit a significant correlation with the frontal cortex and fatigue, thereby providing an initial insight into the biological substrates underlying the perception of fatigue within the frontal cortex region. By integrating the findings from SMR, Bayesian colocalization, and experimental study, it has been discovered that ECE2, GPX1, and SNF8 are likely to be genes significantly associated with the occurrence of fatigue in the frontal cortex. ECE2 plays a pivotal role in brain development, participating in the processing of various neuroendocrine peptides and being closely related to neurogenesis and neuronal migration [78, 79]. Numerous studies have reported the important role of ECE2 in Alzheimer's disease (AD), considering it as a risk gene for the development of AD [80–82]. Fatigue is a common symptom in neurodegenerative diseases such as AD, and the potential therapeutic approaches for fatigue in AD patients are currently unclear [83]. Moreover, ECE2 can affect the degradation of Aβ, and Aβ in turn can upregulate the expression of ECE2 [84]. Studies have indicated that fatigue may be associated with an increase in Aβ in the hippocampus, particularly in individuals at higher risk for Alzheimer's disease [85]. Therefore, our finding highlights the potential applicative value of ECE2 in the treatment of fatigue associated with AD. SNF8 is a type of vacuolar sorting protein, which is also closely associated with neurodegenerative diseases [86]. Studies have found that mutations in SNF8 may associated with impaired autophagic flux related to a range of neurodegenerative phenotypes [87]. Consequently, SNF8 may also be considered a potential therapeutic target for mitigating fatigue associated with neurodegenerative diseases. The protein encoded by GPX1 acts as a significant endogenous antioxidant enzyme, playing a crucial role in the scavenging of peroxides [88]. A previous study has reported a significant relationship between the activity of the enzyme encoded by GPX1 in high-grade serous tumor tissues of ovarian cancer patients and symptoms of fatigue, suggesting a potential link between GPX1 and the occurrence of cancer-related fatigue [89]. Fatigue is closely related to oxidative stress [90]. Fatigue symptoms can be triggered when the human body enters a state of oxidative stress [91]. Our previous research has also confirmed the presence of significant oxidative stress damage in rats with central fatigue [92]. The frontal cortex is also one of the primary brain regions susceptible to oxidative stress-induced damage. When oxidative stress arises, the expression of the GPX1 gene is compensatory elevated significantly, thereby exerting its antioxidant function to preserve homeostasis [93, 94]. Consequently, the increased transcription level of the GPX1 gene in the frontal cortex under a state of fatigue may signify that the brain is under oxidative stress. Our study elucidated that

the regulation of the GPX1 gene in the frontal cortex may afford considerable assistance in the treatment of fatigue.

We identified potential drugs for the significant genes using the DSigDB and DrugBank databases. The results revealed that GPX1 has the most approved drugs currently available, whereas ECE2 and SNF8 have significantly fewer. This suggests that GPX1 is the most promising potential target for developing treatments for fatigue. The limited number of potential drugs for ECE2 and SNF8 may be due to the relatively sparse research on these targets. Therefore, further in-depth studies are needed to explore whether ECE2 and SNF8 can be viable targets for drug development in the future. Among the top 10 approved and currently permitted drugs, we discovered that selenomethionine [95], hesperidin [96], methimazole [97], thiamine [98], magnesium sulfate [99], sitagliptin [100], pyridoxine hydrochloride [101], and sulfasalazine [102] have been studied or applied in the treatment of brain diseases. This suggests that GPX1 has significant potential as a brain intervention target for fatigue, especially central fatigue.

Our study boasts several notable advantages. First, our study is primarily based on MR and SMR methodology, and we conducted rigorous sensitivity analyses to exclude the influence of confounding factors. This enables us to establish a more stable causal relationship between exposure factors and outcomes compared to observational studies. Second, the data used in our study is exclusively derived from the European population, minimizing the potential confounding factors associated with ethnic diversity. Third, our study further validated the predicted genes by establishing a central fatigue animal model, thereby enhancing the reliability of our results.

However, our study still exhibited some limitations. First, the GWAS data utilized in our analysis were solely obtained from European populations, which may limit the generalizability of our findings to other ethnic groups and populations. Individuals from different genetic ancestries exhibit varying allele frequencies and linkage disequilibrium patterns [103]. Consequently, genetic variants that show significant associations with traits in European populations may not have the same associations in other populations. This means that the effect sizes of these associations could vary, potentially leading to different conclusions if the study were replicated in non-European populations. Therefore, future research could include more diverse populations to enhance the generalizability and robustness of our findings. Second, although we carefully selected IVs based on stringent criteria, there remain numerous unmeasured confounders that could potentially bias our causal estimates. Third, although changes in brain volume are often associated with fatigue in various studies, functional changes in the brain may play a crucial role in the experience of fatigue, independent of structural changes. Current GWAS data on brain volume provide valuable insights into structural associations, but they may not fully capture the complexity of functional changes that could lead to fatigue. Therefore, as GWAS data continue to evolve, future research should integrate GWAS data with relevant indicators that objectively reflect the functions of different brain regions to draw more robust conclusions. Meanwhile, due to the limitations of current GWAS studies, we selected self-reported fatigue-related GWAS data for our research. This method of assessing fatigue may lack objectivity, potentially introducing some bias into the study results. Therefore, when feasible, future research should incorporate more objective and reliable fatigue measurement indicators to enhance the validity and reliability of the findings. Furthermore, there is approximately 8% or less sample overlap between the GWAS data for brain volume and fatigue. Although this has a limited impact on our results, such a potential confounding factor may still exert some influence. Last, although we have preliminary validated the predicted genes through in vivo experiment, the limited sample size and the number of SNPs utilized in our study for the cis-eQTL data may still introduce a certain degree of interference into our results. The limited sample size and the number of SNPs may reduce the statistical power of our analyses, increasing the likelihood of both type I (false positive) and type II (false negative) errors [104]. This means that we might incorrectly identify genetic variants as being associated with gene expression or miss true associations that exist, thereby affecting the reliability of our conclusions. Further studies are needed to elucidate the findings of this study.

Conclusion

Through this study, we have preliminarily identified 4 brain regions that may have a strong causal relationship with fatigue, along with 3 genes in the frontal cortex that exhibit a robust association with fatigue. These findings may provide novel and potentially effective therapeutic targets for the treatment of fatigue, particularly central fatigue.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06284-x.

Additional file 1

Additional file 2

Additional file 3

Acknowledgements
We acknowledge UK Biobank and GTEx Portal for providing us with a lot of data support and the program developers who developed the "TwoSampleMR""SMR" and "coloc" packages.

Author contributions
Yifei Zhang: Conceptualization, Investigation, Data Curation, Writing—Original Draft. Zehan Zhang: Conceptualization, Writing—Original Draft, Formal analysis, Investigation. Qingqian Yu: Investigation, Formal analysis, Data Curation, Writing—Original Draft. Yutong Jiang: Investigation. Chenyu Fei: Investigation. Fengzhi Wu: Methodology, Investigation, Formal analysis. Feng Li: Methodology, Writing—Review & Editing, Supervision, Funding acquisition.

Funding
This research was supported by Grants from the National Natural Science Foundation of China (Grant number: 81874428).

Data availability
Detailed statistics can be found in the supplementary materials**.** Additional data that support the findings of this study are available from the corresponding author on request. Source data are provided with this paper.

Declarations

Ethics approval and consent to participate
Our animal experiment procedures were approved by the Animal Experimentation Ethics Committee of Beijing University of Chinese Medicine (No. BUCM-2023090410-3777).

Competing interests
The authors declare that they possess no discernible financial conflicts of interest or personal affiliations.

Received: 2 January 2025 Accepted: 20 February 2025
Published online: 07 March 2025

References

1. Yang M, et al. Antifatigue effect of panax notoginseng leaves fermented with microorganisms: in-vitro and in-vivo evaluation. Front Nutr. 2022;9: 824525.
2. Peng Y, et al. Anti-fatigue effects of *Lycium barbarum* polysaccharide and effervescent tablets by regulating oxidative stress and energy metabolism in rats. Int J Mol Sci. 2022;23(18):10920.
3. Chen JQ, et al. The relationship between mindfulness, fatigue, and perceived symptoms among frontline nurses who performed nucleic acid sample collection during the COVID-19 in China: a cross-sectional study. Psychol Res Behav Manag. 2023;16:1165–80.
4. Kaplan Serin E, Ovayolu N, Ovayolu Ö. The effect of progressive relaxation exercises on pain, fatigue, and quality of life in dialysis patients. Holist Nurs Pract. 2020;34(2):121–8.
5. Kwak JJ, Yook JS, Ha MS. Potential biomarkers of peripheral and central fatigue in high-intensity trained athletes at high-temperature: a pilot study with Momordica charantia (Bitter Melon). J Immunol Res. 2020;2020:4768390.
6. Lin YP, et al. Effects of lower limb cycling training on different components of force and fatigue in individuals with Parkinson's disease. Front Bioeng Biotechnol. 2022;10: 829772.
7. Li J, et al. Mid-task break improves global integration of functional connectivity in lower alpha band. Front Hum Neurosci. 2016;10:304.
8. Kindred JH, et al. Regional differences of [(18)F]-FDG uptake within the brain during fatiguing muscle contractions. Brain Behav. 2015;5(4): e00319.
9. Yu X, et al. Exploring the causal role of the immune response to varicella-zoster virus on multiple traits: a phenome-wide Mendelian randomization study. BMC Med. 2023;21(1):143.
10. Nazarzadeh M, et al. Systolic blood pressure and risk of valvular heart disease: a Mendelian randomization study. JAMA Cardiol. 2019;4(8):788–95.
11. Li J, et al. Activation of brain lactate receptor GPR81 aggravates exercise-induced central fatigue. Am J Physiol Regul Integr Comp Physiol. 2022;323(5):R822-r831.
12. Ruan X, et al. In vivo functional analysis of non-conserved human lncRNAs associated with cardiometabolic traits. Nat Commun. 2020;11(1):45.
13. Manuel AM, et al. An integrative study of genetic variants with brain tissue expression identifies viral etiology and potential drug targets of multiple sclerosis. Mol Cell Neurosci. 2021;115: 103656.
14. Wang Z, et al. Mitochondria-related genome-wide Mendelian randomization identifies putatively causal genes for neurodegenerative diseases. Mov Disord. 2025.
15. Fang C, et al. Association of genetically proxied cancer-targeted drugs with cardiovascular diseases through Mendelian randomization analysis. J Transl Med. 2025;23(1):14.
16. Wang F, et al. Systematic druggable genome-wide Mendelian randomization identifies therapeutic targets for hyperemesis gravidarum. BMC Pregnancy Childbirth. 2024;24(1):848.
17. Provenzi L, et al. Is Brain-derived neurotropic factor methylation involved in the association between prenatal stress and maternal postnatal anxiety during the COVID-19 pandemic? Front Psychiatry. 2022;13: 950455.
18. Jansen PR, et al. Genome-wide meta-analysis of brain volume identifies genomic loci and genes shared with intelligence. Nat Commun. 2020;11(1):5606.
19. Migliore S, et al. Cognitive reserve in early manifest Huntington disease patients: leisure time is associated with lower cognitive and functional impairment. J Pers Med. 2022;12(1):36.
20. Fermin ASR, Friston K, Yamawaki S. An insula hierarchical network architecture for active interoceptive inference. R Soc Open Sci. 2022;9(6): 220226.
21. Romaus-Sanjurjo D, et al. Alzheimer's disease seen through the eye: ocular alterations and neurodegeneration. Int J Mol Sci. 2022;23(5):2486.
22. Lin ZG, et al. Effects of cognitive behavior therapy combined with Baduanjin in patients with colorectal cancer. World J Gastrointest Oncol. 2022;14(1):319–33.
23. Fürtjes AE, et al. General dimensions of human brain morphometry inferred from genome-wide association data. Hum Brain Mapp. 2023;44(8):3311–23.
24. Deary V, et al. Genetic contributions to self-reported tiredness. Mol Psychiatry. 2018;23(3):789–90.
25. Qi T, et al. Genetic control of RNA splicing and its distinct role in complex trait variation. Nat Genet. 2022;54(9):1355–63.
26. Pierce BL, Burgess S. Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. Am J Epidemiol. 2013;178(7):1177–84.
27. Li Q, et al. Causal association between cardiovascular diseases and erectile dysfunction, a Mendelian randomization study. Front Cardiovasc Med. 2023;10:1094330.
28. Xu Y, et al. Integrated analysis of large-scale omics data revealed relationship between tissue specificity and evolutionary dynamics of small RNAs in Maize (Zea mays). Front Genet. 2020;11:51.
29. Xia K, et al. Dietary-derived essential nutrients and amyotrophic lateral sclerosis: a two-sample Mendelian randomization study. Nutrients. 2022;14(5):920.
30. Li H, et al. Can intelligence affect alcohol-, smoking-, and physical activity-related behaviors? A Mendelian randomization study. J Intell. 2023;11(2):29.
31. Brix N, et al. Childhood overweight and obesity and timing of puberty in boys and girls: cohort and sibling-matched analyses. Int J Epidemiol. 2020;49(3):834–44.
32. Cerani A, et al. Genetic predisposition to increased serum calcium, bone mineral density, and fracture risk in individuals with normal calcium levels: Mendelian randomisation study. BMJ. 2019;366: l4410.
33. Mokry LE, et al. Vitamin D and risk of multiple sclerosis: a Mendelian randomization study. PLoS Med. 2015;12(8): e1001866.

34. Li Y, et al. The causal association of polyunsaturated fatty acids with allergic disease: a two-sample Mendelian randomization study. Front Nutr. 2022;9: 962787.
35. Liang Y, et al. The causal impact of childhood obesity on bone mineral density and fracture in adulthood: a two-sample Mendelian randomization study. Front Nutr. 2022;9: 945125.
36. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics. 2015;31(21):3555–7.
37. Shi Q, et al. Systemic inflammatory regulators and proliferative diabetic retinopathy: a bidirectional Mendelian randomization study. Front Immunol. 2023;14:1088778.
38. Wan X, et al. Does having rheumatoid arthritis increase the dose of depression medications? A Mendelian randomization study. J Clin Med. 2023;12(4):1405.
39. Cui Z, et al. Type 2 diabetes and glycemic traits are not causal factors of osteoarthritis: a two-sample Mendelian randomization analysis. Front Genet. 2020;11: 597876.
40. Gao Y, et al. Causal effect of central obesity on left ventricular structure and function in preserved EF population: a Mendelian randomization study. Front Cardiovasc Med. 2022;9:1103011.
41. Li G, et al. Causal effect of psychiatric disorders on epilepsy: a two-sample Mendelian randomization study. Brain Behav. 2023;13(4): e2939.
42. Yang G, Schooling CM. Investigating sex-specific associations of lipid traits with type 2 diabetes, glycemic traits and sex hormones using Mendelian randomization. Cardiovasc Diabetol. 2023;22(1):3.
43. Llano DA, et al. A novel dynamic network imaging analysis method reveals aging-related fragmentation of cortical networks in mouse. Netw Neurosci. 2021;5(2):569–90.
44. Yang K, et al. The effectiveness of postoperative chemotherapy on pT1bN0 and pT2N0 gastric cancer patients with risk factors: an international dual-center analysis. Yonsei Med J. 2021;62(2):109–17.
45. Yun Z, et al. Genetically predicted 486 blood metabolites in relation to risk of colorectal cancer: a Mendelian randomization study. Cancer Med. 2023;12(12):13784–99.
46. Yang M, et al. No evidence of a genetic causal relationship between ankylosing spondylitis and gut microbiota: a two-sample Mendelian randomization study. Nutrients. 2023;15(4):1057.
47. Wang C, et al. Efficacy and safety of pulsed radiofrequency in herpes zoster related trigeminal neuralgia: a systematic review and meta-analysis. J Pain Res. 2023;16:341–55.
48. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 2014;23(R1):R89-98.
49. Dovrou A, et al. Synergies of radiomics and transcriptomics in lung cancer diagnosis: a pilot study. Diagnostics (Basel). 2023;13(4):738.
50. Zhu Z, et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat Genet. 2016;48(5):481–7.
51. Qiu X, et al. Mendelian randomization reveals potential causal relationships between cellular senescence-related genes and multiple cancer risks. Commun Biol. 2024;7(1):1069.
52. Pei YF, et al. The genetic architecture of appendicular lean mass characterized by association analysis in the UK Biobank study. Commun Biol. 2020;3(1):608.
53. Chen J, et al. Therapeutic targets for inflammatory bowel disease: proteome-wide Mendelian randomization and colocalization analyses. EBioMedicine. 2023;89: 104494.
54. Wu BS, et al. Identifying causal genes for stroke via integrating the proteome and transcriptome from brain and blood. J Transl Med. 2022;20(1):181.
55. Nousias O, et al. Linkage mapping, comparative genome analysis, and QTL detection for growth in a non-model teleost, the meagre Argyrosomus regius, using ddRAD sequencing. Sci Rep. 2022;12(1):5301.
56. Chen S, et al. Integrative multi-omics summary-based mendelian randomization identifies key oxidative stress-related genes as therapeutic targets for atrial fibrillation and flutter. Front Genet. 2024;15:1447872.
57. Yoo M, et al. DSigDB: drug signatures database for gene set analysis. Bioinformatics. 2015;31(18):3069–71.
58. Knox C, et al. DrugBank 6.0: the DrugBank Knowledgebase for 2024. Nucleic Acids Res. 2024;52(D1):D1265-d1275.
59. Zhang Y, et al. Replicating human characteristics: a promising animal model of central fatigue. Brain Res Bull. 2024;212: 110951.
60. Lu H, et al. Growth factors and their roles in multiple sclerosis risk. Front Immunol. 2021;12: 768682.
61. Seguro CK, Demory Beckler M, Kesselman MM. Targeting the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome in psoriasis and fatigue. Cureus. 2022;14(5):e24704.
62. Boyas S, Guével A. Neuromuscular fatigue in healthy muscle: underlying factors and adaptation mechanisms. Ann Phys Rehabil Med. 2011;54(2):88–108.
63. Varghese M, et al. Autism spectrum disorder: neuropathology and animal models. Acta Neuropathol. 2017;134(4):537–66.
64. He Q, Zhang W, Li Q. Failure of crizotinib based systemic treatment in ALK positive histiocytosis involving the central nervous system: a case report and literature review. BMC Pediatr. 2022;22(1):308.
65. Statsenko Y, et al. Brain morphometry and cognitive performance in normal brain aging: age- and sex-related structural and functional changes. Front Aging Neurosci. 2021;13: 713680.
66. Díez-Cirarda M, et al. Multimodal neuroimaging in post-COVID syndrome and correlation with cognition. Brain. 2023;146(5):2142–52.
67. Nakagawa S, et al. Basal ganglia correlates of fatigue in young adults. Sci Rep. 2016;6:21386.
68. Pan J, et al. Hypoxemia, sleep disturbances, and depression correlated with fatigue in neuromyelitis optica spectrum disorder. CNS Neurosci Ther. 2015;21(7):599–606.
69. Okada T, et al. Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. BMC Neurol. 2004;4(1):14.
70. Zeineh MM, et al. Right arcuate fasciculus abnormality in chronic fatigue syndrome. Radiology. 2015;274(2):517–26.
71. de Lange FP, et al. Gray matter volume reduction in the chronic fatigue syndrome. Neuroimage. 2005;26(3):777–81.
72. Ross RS, et al. Role of the hippocampus and orbitofrontal cortex during the disambiguation of social cues in working memory. Cogn Affect Behav Neurosci. 2013;13(4):900–15.
73. Elward RL, Vilberg KL, Rugg MD. Motivated memories: effects of reward and recollection in the core recollection network and beyond. Cereb Cortex. 2015;25(9):3159–66.
74. Zhang HF, Mellor D, Peng DH. Neuroimaging genomic studies in major depressive disorder: a systematic review. CNS Neurosci Ther. 2018;24(11):1020–36.
75. Harika-Germaneau G, et al. Baseline clinical and neuroimaging biomarkers of treatment response to high-frequency rTMS over the left DLPFC for resistant depression. Front Psychiatry. 2022;13: 894473.
76. Hanford LC, et al. Cortical thickness correlates of cognitive performance in cognitively-matched individuals with and without schizophrenia. Brain Cogn. 2019;132:129–37.
77. Zhu X, et al. The effect of perceived stress on cognition is mediated by personality and the underlying neural mechanism. Transl Psychiatry. 2022;12(1):199.
78. Buchsbaum IY, et al. ECE2 regulates neurogenesis and neuronal migration during human cortical development. EMBO Rep. 2020;21(5): e48204.
79. Li Y, et al. SNHG15 enhances cisplatin resistance in lung adenocarcinoma by affecting the DNA repair capacity of cancer cells. Diagn Pathol. 2023;18(1):33.
80. Liao X, et al. Identification of Alzheimer's disease-associated rare coding variants in the ECE2 gene. JCI Insight. 2020; 5(4).
81. Palmer J, Love S. Endothelin receptor antagonists: potential in Alzheimer's disease. Pharmacol Res. 2011;63(6):525–31.
82. Weeraratna AT, et al. Alterations in immunological and neurological gene expression patterns in Alzheimer's disease tissues. Exp Cell Res. 2007;313(3):450–61.
83. Angioni D, et al. Fatigue in Alzheimer's disease: biological basis and clinical management-a narrative review. Aging Clin Exp Res. 2023;35(10):1981–9.
84. Palmer JC, Kehoe PG, Love S. Endothelin-converting enzyme-1 in Alzheimer's disease and vascular dementia. Neuropathol Appl Neurobiol. 2010;36(6):487–97.

85. Hooper C, et al. Cross-sectional associations of fatigue with cerebral β-amyloid in older adults at risk of dementia. Front Med (Lausanne). 2017;4:173.
86. Manian V, et al. Detection of target genes for drug repurposing to treat skeletal muscle atrophy in mice flown in spaceflight. Genes (Basel). 2022;13(3):473.
87. Brugger M, et al. Bi-allelic variants in SNF8 cause a disease spectrum ranging from severe developmental and epileptic encephalopathy to syndromic optic atrophy. Am J Hum Genet. 2024;111(3):594–613.
88. Buraczynska M, et al. Pro198Leu polymorphism in the glutathione peroxidase 1 gene contributes to diabetic peripheral neuropathy in type 2 diabetes patients. Neuromolecular Med. 2017;19(1):147–53.
89. Bayer JL, et al. Biobehavioral and neuroendocrine correlates of antioxidant enzyme activity in ovarian carcinoma. Brain Behav Immun. 2015;50:58–62.
90. Tian L, et al. A comparative study on relieving exercise-induced fatigue by inhalation of different citrus essential oils. Molecules. 2022;27(10):3239.
91. Huang G, et al. The prebiotic and anti-fatigue effects of hyaluronan. Front Nutr. 2022;9: 977556.
92. Zhang Y, et al. Dual-factor model of sleep and diet: a new approach to understanding central fatigue. Front Neurosci. 2024;18:1465568.
93. Zhao Q, et al. biological functions of selenoprotein glutathione peroxidases (GPXs) and their expression in osteoarthritis. J Inflamm Res. 2023;16:183–96.
94. Letsiou S, et al. Marine fungus aspergillus chevalieri TM2-S6 extract protects skin fibroblasts from oxidative stress. Mar Drugs. 2020;18(9):460.
95. Alasmari F, et al. Neuroinflammatory cytokines induce amyloid beta neurotoxicity through modulating amyloid precursor protein levels/metabolism. Biomed Res Int. 2018;2018:3087475.
96. Raza SS, et al. Hesperidin ameliorates functional and histological outcome and reduces neuroinflammation in experimental stroke. Brain Res. 2011;1420:93–105.
97. Liu B, et al. Dysregulation within the salience network and default mode network in hyperthyroid patients: a follow-up resting-state functional MRI study. Brain Imaging Behav. 2020;14(1):30–41.
98. Bacq Y. Liver diseases unique to pregnancy: a 2010 update. Clin Res Hepatol Gastroenterol. 2011;35(3):182–93.
99. Campbell K, Meloni BP, Knuckey NW. Combined magnesium and mild hypothermia (35 degrees C) treatment reduces infarct volumes after permanent middle cerebral artery occlusion in the rat at 2 and 4, but not 6 h. Brain Res. 2008;1230:258–64.
100. Davies DA, Adlimoghaddam A, Albensi BC. Role of Nrf2 in synaptic plasticity and memory in Alzheimer's disease. Cells. 2021;10(8):1884.
101. Lee HF, Chi CS, Tsai CR. Electroclinical variability of pyridoxine-dependent epilepsy caused by ALDH7A1 gene mutations in four Taiwanese children. Brain Dev. 2020;42(5):393–401.
102. Sontheimer H, Bridges RJ. Sulfasalazine for brain cancer fits. Expert Opin Investig Drugs. 2012;21(5):575–8.
103. Shadyab AH, et al. Replication of genome-wide association study findings of longevity in white, African American, and Hispanic women: the Women's health initiative. J Gerontol A Biol Sci Med Sci. 2017;72(10):1401–6.
104. Macmadu A, et al. Comparison of characteristics of deaths from drug overdose before vs during the COVID-19 pandemic in Rhode Island. JAMA Netw Open. 2021;4(9): e2125538.

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