

Daytime sleepiness, night sleep changes and ALS: a Mendelian randomization study

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Research

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Abstract

Background

Observational studies have indicated that there is a high prevalence of daytime sleepiness and night sleep changes in amyotrophic lateral sclerosis (ALS). However, the actual relation between these symptoms and ALS remains unclear. We aimed to determine whether daytime sleepiness and night sleep changes have an effect on ALS.

Methods

We used 2-sample mendelian randomization to estimate the effects of daytime sleepiness, sleep efficiency, number of sleep episodes and sleep duration on ALS. Summary statistics we used was from recent and large genome-wide association studies on the traits we chosen ($n = 85,670-452,071$) and ALS (cases $n = 20,806$, controls $n = 59,804$). Inverse variance weighted method was used as the main method for assessing causality.

Results

A genetically predicted 1-point increase in the assessment of daytime sleepiness was significantly associated with an increased risk of ALS (inverse-variance-weighted (IVW) odds ratio = 2.70, 95% confidence interval (CI): 1.27–5.76; $P = 0.010$). ALS was not associated with a genetically predicted 1-SD increase in sleep efficiency (IVW 1.01, 0.64–1.58; $P = 0.973$), Number of sleep episodes (IVW 1.02, 0.80–1.30; $P = 0.859$) or sleep duration (IVW 1.00, 1.00–1.01; $P = 0.250$).

Conclusions

Our results provide novel evidence that daytime sleepiness causes an increase in the risk of ALS and indicate that daytime sleepiness may be inherent in preclinical and clinical ALS patients, rather than simply affected by potential influencing factors.

Background

Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease characterized by progressive motor nerve damage; patients typically die within 3–5 years after diagnosis [1]. The synergy of preexisting genetic risk and long-lasting environment/lifestyle exposure is currently considered an important cause of ALS [2]. As there is no effective treatment for ALS, attention has been paid to mutable exposure factors to predict and guide the prevention of ALS.

Recent studies found sleep changes which are highly prevalent in many neurodegenerative diseases are not only just simple symptoms but also increase their incidence. In Parkinson's disease (PD), excessive daytime sleepiness was found correlated with more than 3 times increased risk of its developing [3]. Longitudinal studies also showed higher risk among participants with daytime sleepiness than for those without in many kinds of dementia [4–6]. As the other part of sleep problem, night sleep changes have been reported have same influence for neurodegenerative diseases [7, 8]. Although sleep-related complaints have shown a high prevalence in patients with ALS [9–11], these symptoms have usually been attributed to sleep-disordered breathing (SDB), movement disorders and psychological distress, which are main and common manifestation of ALS that correlation with sleep [12]. There is still scant evidence showing causality between these changes and incident ALS. Thus, whether these problems are as important in ALS as they are in other neurodegenerative diseases still needs to be clarified.

However, due to limited sample sizes and significant confounding factors in ALS, it is difficult to design an observational experiment that can directly verify the relationship between daytime sleepiness, poor night sleep and ALS. In this study, we chose existing sleep changes shown in ALS patients as potential risk factors for ALS; we then used Mendelian randomization (MR), a method that uses genetic variation associated with variable risk factors as instrumental variables (IVs) to assess the unbiased association of target exposure with diseases. We aimed to test whether the chosen sleep problems we mentioned are risk factors for ALS. MR analysis applies the Mendelian principle that genetic alleles are randomly assorted during conception and are unlikely to be affected by confounders; this method can reduce observational bias and avoid reverse causality of exposures to disease [13]. MR of determining the causal relationship between risk factors and diseases is increasingly used in a wide range of diseases. The results of this method may also provide new directions for disease interventions.

Methods

Identification of sleep changes as potential risk factors for ALS

We reviewed published articles on sleep problems in ALS and identified changes in sleep: daytime sleepiness, long sleep latency, frequent awakenings and low sleep efficiency that revealed in self-reported questionnaires and polysomnography as potential exposures for ALS [9–11]. Another potential exposure that we considered was abnormal sleep duration, which is reported to increase the risk of developing of other neurodegenerative diseases [14, 15].

Genome-wide Association Studies (GWASs) Summary Statistics For Sleep Traits

We searched PubMed for the summaries of most recent and largest available GWASs (up to November 2019) of the sleep issues identified above. The traits we chose were daytime sleepiness, sleep latency,

sleep efficiency and sleep duration. We also used the number of sleep episodes to represent frequent awakenings. The four largest and most recent GWASs of each trait in adult participants were chosen to provide genetic variation for the downstream association analyses. The characteristics of the GWASs we chose are described in the following text and **Additional Table 1**. Data on daytime sleepiness, sleep efficiency, number of sleep episodes and sleep duration were gathered from GWASs based on the UK Biobank, which gathered information from volunteers of European ancestry between 40 and 69 years of age in the years 2006–2010 [16].

IVs for daytime sleepiness were identified from a GWAS that included up to 452,071 individuals and assessed the severity of daytime sleepiness using the question “How likely are you to doze off or fall asleep during the daytime when you do not mean to?”, answered as a continuous variable on a scale from 1 to 4 points [17].

IVs for sleep efficiency and number of sleep episodes were extracted from one GWAS that included accelerometer recordings from 85,670 participants for up to 7 days [18]. Sleep efficiency was calculated by dividing actual sleep time by the time between the start of the first inactivity session and the end of the last inactivity session; the result was expressed as a percentage (%). The number of sleep episodes was determined by counting sleep episodes within the time window defined as the sleep period; the result was stated as a count value. Individuals who had an average of more than 30 or fewer than 5 episodes were excluded.

IVs for sleep duration were found in a GWAS including up to 446,118 individuals. Sleep duration, reported as a continuous variable, was assessed on the basis of self-reported questionnaires and activity monitoring [19]. The unit of sleep duration was hours.

IVs for sleep latency were drawn from a meta-analysis of GWASs involving 4,242 individuals of European ancestry from seven cohorts [20]. In all GWASs included in this meta-analysis, sleep latency was measured using the Munich Chronotype Questionnaire. Sleep latency on days off was used in the analyses for those cohorts. The unit of sleep latency was minutes.

The process of trait identification and the choice of IVs for each trait are shown in Fig. 1.

GWAS Summary Statistics For ALS

Genetic association data for ALS were obtained from a publicly available GWAS that included 20,806 ALS patients and 59,804 controls of European ancestry [21]. All individuals classified as ALS patients met the standard for a probable or definite diagnosis of ALS according to the El Escorial criteria (Brooks, 1994). The implementation of the assessment was performed by a neurologist specializing in ALS. The characteristics of the GWAS of ALS is described in **Additional Table 1**.

Selection Of Instrumental Variants

For each potential risk factor, independent genetic variants (single nucleotide polymorphisms (SNPs)) were chosen as IVs according to the following principles: 1. The SNPs are not in linkage disequilibrium, defined as $r^2 < 0.001$; 2. The genome-wide significance of the SNPs met the threshold ($P < 5 \times 10^{-8}$) for the corresponding risk factors. SNPs that were not available for ALS data were replaced with proxies ($r^2 > 0.9$) from the online website SNiPA. If an SNP was not available for ALS data and had no proxy that was, that SNP was excluded from downstream association analyses. Because there were no SNPs for sleep latency that met the inclusion criteria, this trait was excluded from the subsequent analysis.

MR Analysis

The MR approach was based on 3 assumptions: 1. The genetic variants used as IVs for the potential risk factors are associated with the target disease. 2. The genetic variants are not associated with any confounders. 3. The genetic variants are associated with the target disease only through the risk factor and not through any alternative causal pathway (Fig. 2). We used 2-sample MR, a method of MR analysis, to analyze the causal effect of daytime sleepiness and night sleep changes on ALS [22].

In the main analyses, we summarized the ratio estimates for individual genetic variants using the conventional fixed-effect inverse-variance-weighted (IVW) method [23]. Simple medians and weighted medians were used as sensitivity analyses to confirm the main findings [24].

Pleiotropy was evaluated based on the intercept calculated by MR-Egger regression [25]. To investigate the influence of outlying and/or pleiotropic genetic variants, we performed a leave-one-out analysis in which each genetic variant was omitted in turn.

Four sleep issues suspected to be risk factors were calculated in our study. We used Bonferroni correction to test for multiple comparisons. The corrected significance threshold was 0.0125 (0.05 divided by 4). However, $0.0125 < P < 0.05$ was also considered suggestive evidence for a potential association.

The results are expressed as the odds ratio (95% confidence interval) for a 1-point or 1-SD increase per genetic prediction in each risk factor. All analyses were performed in R Studio (Version 1.2.1335).

Results

Summary statistics for the assessment of daytime sleepiness, sleep efficiency, number of sleep episodes, sleep duration and quality control of SNPs in each GWAS are listed in **Additional Table 1**. According to the criteria we established, 10 SNPs for daytime sleepiness, 53 SNPs for sleep duration and 3 SNPs for sleep latency were excluded. The numbers of SNPs included in downstream association analyses for daytime sleepiness, sleep efficiency, number of sleep episodes and sleep duration were 32, 5, 21 and 25, respectively. Basic information on the SNPs along with their beta effects, standard errors and P-values for each exposure factor and ALS are listed in **Additional Table 2**.

The result showed a significant association between daytime sleepiness and ALS according to the P-value of IVW. Each genetically predicted 1-point increase in daytime sleepiness was associated with an increased risk of ALS (odds ratio = 2.70, 95% confidence interval (CI): 1.27–5.76; P = 0.010) (Fig. 3). The outcome was confirmed by two less precise types of sensitivity analysis, the median and weighted median methods (Fig. 3 and **Additional Table 3**).

In leave-one-out analysis, no single genetic variant showed an influence on the association we found (**Additional Fig. 1**). We did not find evidence of directional pleiotropy from the intercept of daytime sleepiness (Beta \pm SE: -0.001 \pm 0.014; P = 0.956) in MR-Egger analyses (Fig. 3 and **Additional Table 3**).

Regarding night traits, the results of the IVW, simple median, and weighted median analyses did not show any effect of sleep efficiency, number of sleep episodes or sleep duration on ALS (Fig. 3 and **Additional Table 3**). The leave-one-out analyses showed that no single genetic variant influenced the results for these traits (**Additional Fig. 1**).

The associations between genetically predicted SNPs for daytime sleepiness, sleep efficiency, number of sleep episodes and sleep duration, including their serial numbers, corresponding traits, OR, 95% CI and P-value, are listed in **Additional Table 4**. The effect sizes of SNPs on each trait versus the effect sizes on ALS are shown in Fig. 4.

Discussion

Building on previously discovered phenomena, our study focused on an unknown point, we conducted 2-sample MR using genetic variants found in GWASs as proxies for sleep traits. The current study is the first to assess causality between daytime sleepiness, night sleep changes and ALS directly. Although there was no evidence suggesting that night sleep traits are associated with the risk of ALS, our results suggest that daytime sleepiness not only appears as one of the symptoms of ALS but is also an independent risk factor that increases the incidence of ALS.

The inter-relationship between daytime sleepiness and ALS is clearly complex, and the issue of why daytime sleepiness occurs in ALS cannot be discerned with confidence based on previous data. According to research addressing sleep issue, insufficient sleep and mood disturbances, which are closely related to the occurrence of daytime sleepiness, accompany ALS throughout most of the ALS process [12]. However, in ALS, current evidence about whether these potential influencing factors are responsible for daytime sleepiness is contradictory. In 2012, *Lo coco et al* reported significant differences in daytime sleepiness and depressive degree between good sleepers and poor sleepers with ALS [9]. However, *Liu et al* reported contrasting evidence in 2018; they found no difference in sleep quality and mood disorders between patients with excessive daytime sleepiness and those without [11]. According to our findings, daytime sleepiness may inherent in preclinical and clinical ALS patients rather than entirely due to potential influencing factors. Our findings may explain the inconsistent results about the occurrence of daytime sleepiness and the symptoms that may cause daytime sleepiness in previous studies.

It is essential to seek a possible explanation for why the genetically predicted increase in daytime sleepiness was associated with an increased risk of ALS. There have been studies focused on the role of daytime sleepiness in PD, in addition to a risk factor [3], daytime sleepiness has also been observed to be associated with pathological changes in hypothalamus and thalamus [26, 27], which are the key positions of the center of circadian rhythms in the ascending reticular activating system [28]. Therefore, the pathophysiological process of daytime sleepiness seems to be involved in the process of neurodegenerative diseases. At present, ALS is recognized as a disease that not only affects the motor system but also includes significant extramotor involvement. Imaging observations have found atrophy in multiple cortex and subcortex structures in patients with ALS [29–32]. In addition to hypothalamic atrophy in both ALS patients and presymptomatic carriers of ALS risk genes, *Gorges et al* reported that anterior hypothalamic atrophy advances the age of onset of ALS [29]. Thus, although lesion of sleep-related regions is not known to be associated with daytime sleepiness in ALS, our findings support the hypothesis that daytime sleepiness in ALS may involve the same pathophysiological processes that lead to clinical ALS and its motor symptoms. If this assumption holds, treatment for sleepiness may also work for ALS. However, research in this direction is still scarce, and further studies are needed to seek the potential mechanism underlying the damaging effect of daytime sleepiness on ALS.

Night sleep changes have been observed as exposure factors of many neurodegenerative diseases [7, 8]. The glymphatic system, a recently discovered fluid-clearance system for removing abnormal products from the brain that is activated primarily during sleep may explain the connection between sleep changes and the occurrence of these diseases [33]. It has been proved disrupted sleep decrease the clearance of aggregated proteins that may cause neurodegenerative disease [34, 35]. Increasing evidence from both animal models and patients suggests that night sleep changes may also appear in the early stages of ALS [36, 37]. Our previous study even found sleep disturbances preceding motor symptom onset in SOD1-G93A mice [38]. As ALS is a disease featuring protein aggregates [1], preclinical sleep alternation might also increase the risk of ALS by reducing the clearance of aberrant protein deposits before ALS onset. However, in contrast to daytime sleepiness, the night sleep features analyzed in this report were not associated with the risk of ALS. Thus, night sleep changes may simply be early symptoms of ALS.

Our study has several strengths. This study used GWAS-derived genetic risk factors and an MR design to assess daytime sleepiness, night sleep problems associated with ALS. First, because genotype is lifelong, our results represent the impact of stable long-term status that avoid misleading results with temporary changes. Second, the IVs in this study are from the general population and thus avoid aspects of the disease that might affect the quality of the collected data. Consequently, our results are more reliable than those of traditional observational studies. Third, the MR design avoids bias from reverse causation by confounding factors to identify factors affecting ALS. Finally, the summarized GWAS data we used in this study contained 20,806 ALS cases and included genetic variants of each trait from large GWASs to avoid the problem of small sample size that has long plagued ALS research. However, our study also has limitations. According to the three assumptions described above, we used genetic variants derived from a relatively large sample size study that were strongly associated with exposure to avoid weak instrument problems [39], but our findings may still be affected by weak instrument bias [22]. Furthermore, we are

unable to verify that the relationship between the traits we chosen and ALS risk is not U-shaped. Therefore, our study cannot address the effects of extremely slight or serious disturbances on ALS risk. Finally, the datasets we used were composed entirely of participants with European ancestry to avoid population stratification, but there may have been some participant overlap, which would reduce the quality of data on the four sleep-wake traits and ALS. The degree of sample overlap is difficult to estimate, but this overlap is allowed in 2-sample MR analyses.

Conclusions

In this study, we used 2-sample MR to detect the effect of daytime sleepiness and night sleep changes on ALS, providing novel evidence that daytime sleepiness causes an increase in the risk of ALS. Our findings could guide further research to deepen the current understanding of daytime sleepiness in ALS, identify feasible interventions and even indicate new therapeutic targets for ALS patients.

Abbreviations

ALS: Amyotrophic lateral sclerosis; IVW: Inverse-variance-weighted; CI: Confidence interval; PD: Parkinson's disease; SDB: Sleep-disordered breathing; MR: Mendelian randomization; IVs: Instrumental variables; GWASs: Genome-wide association studies; SNPs: Single nucleotide polymorphisms

Declarations

Ethics approval and consent to participate

There were no patients directly involved in the overall process of our study. Our study is based on publicly available data only. All human studies included in this analysis were conducted according to the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

DF and TH conceived and designed the study, reviewed and edited the manuscript. GZ and LZ contributed to the acquisition and analysis of data. GZ wrote the manuscript. All authors read and approved the manuscript.

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Figures

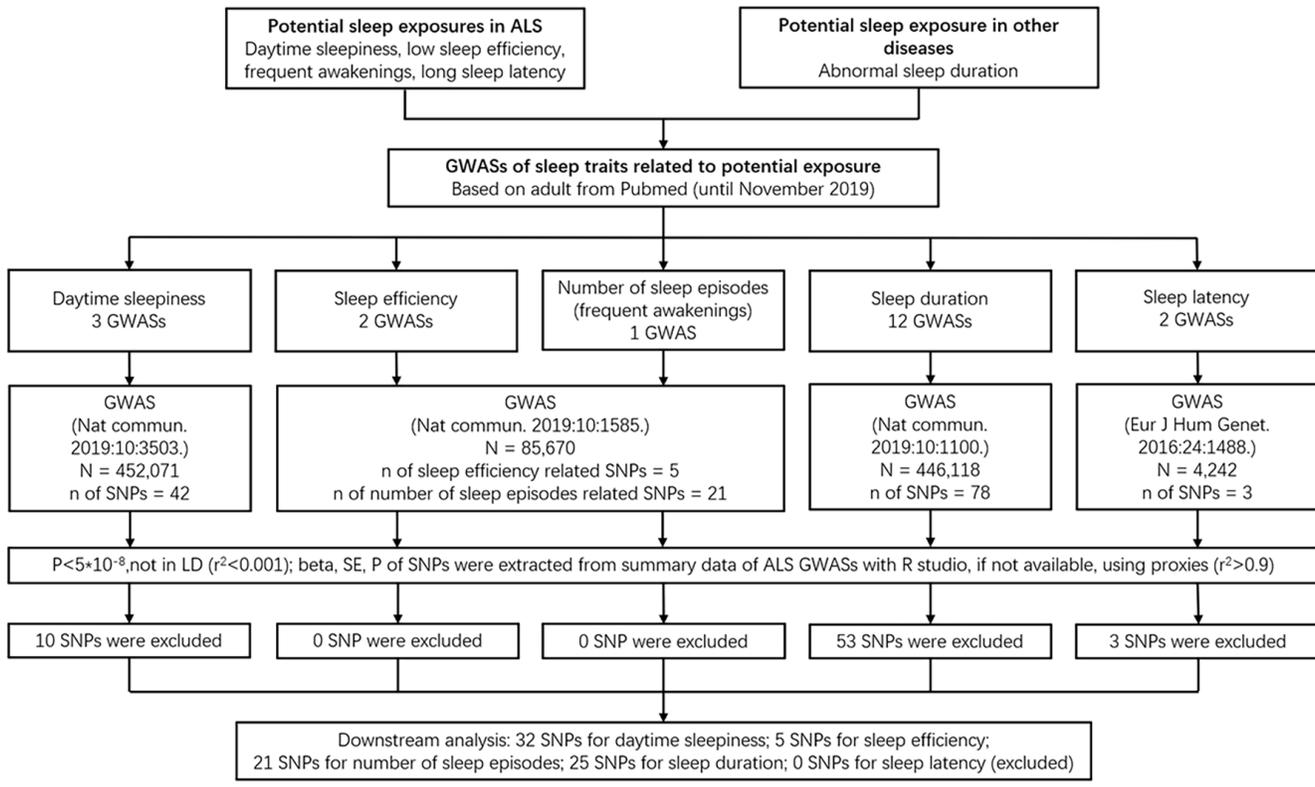


Figure 1

Flow chart for the process for identification of potential risk factors GWASs and instrumental variables to be included in this MR analysis. Summary data for ALS GWASs were obtained from the ALS Variant Server. ALS: amyotrophic lateral sclerosis; GWAS: genome-wide association study; N: sample size of populations; SNP: single nucleotide polymorphism; LD: linkage disequilibrium; SE: standard error.

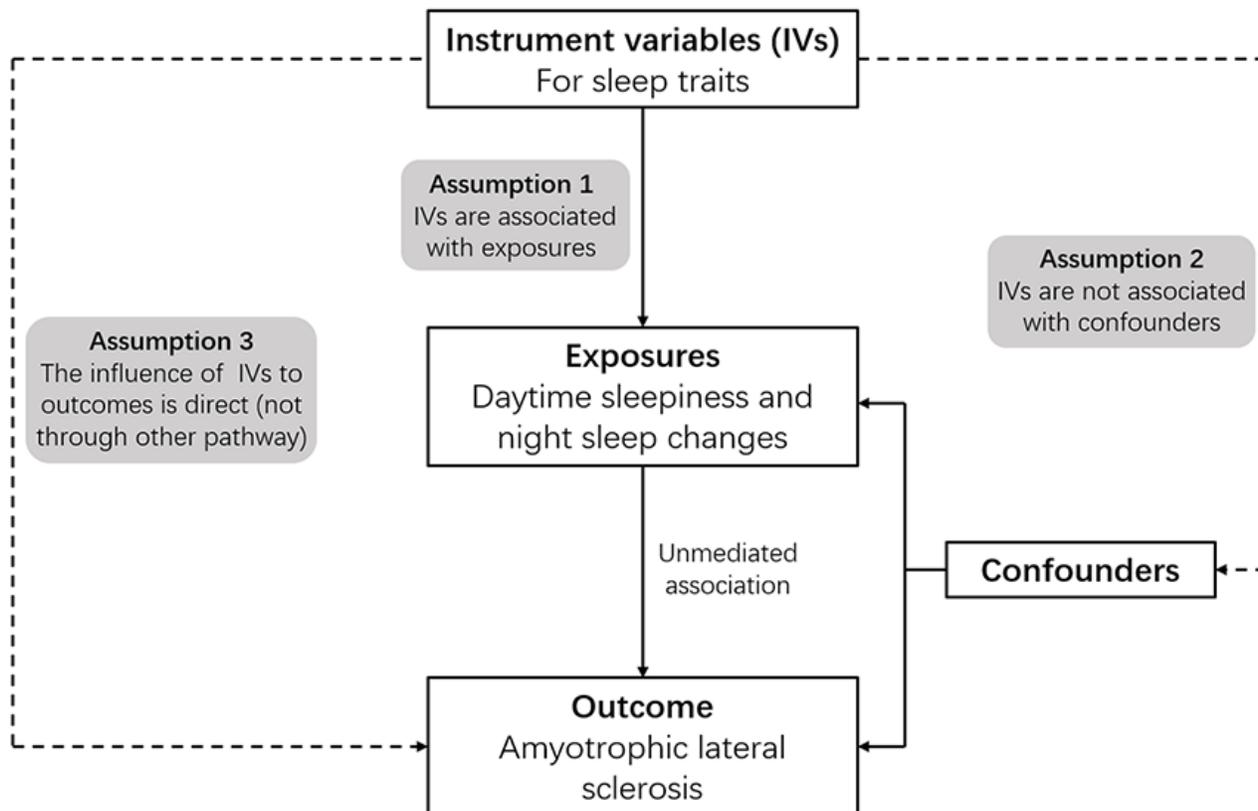


Figure 2

Assumptions of Mendelian randomization analysis of sleep traits as risk factors for ALS. Dashed lines represent potential pleiotropic or direct causality between variables that may violate the assumptions of Mendelian randomization. IVs: instrumental variables.

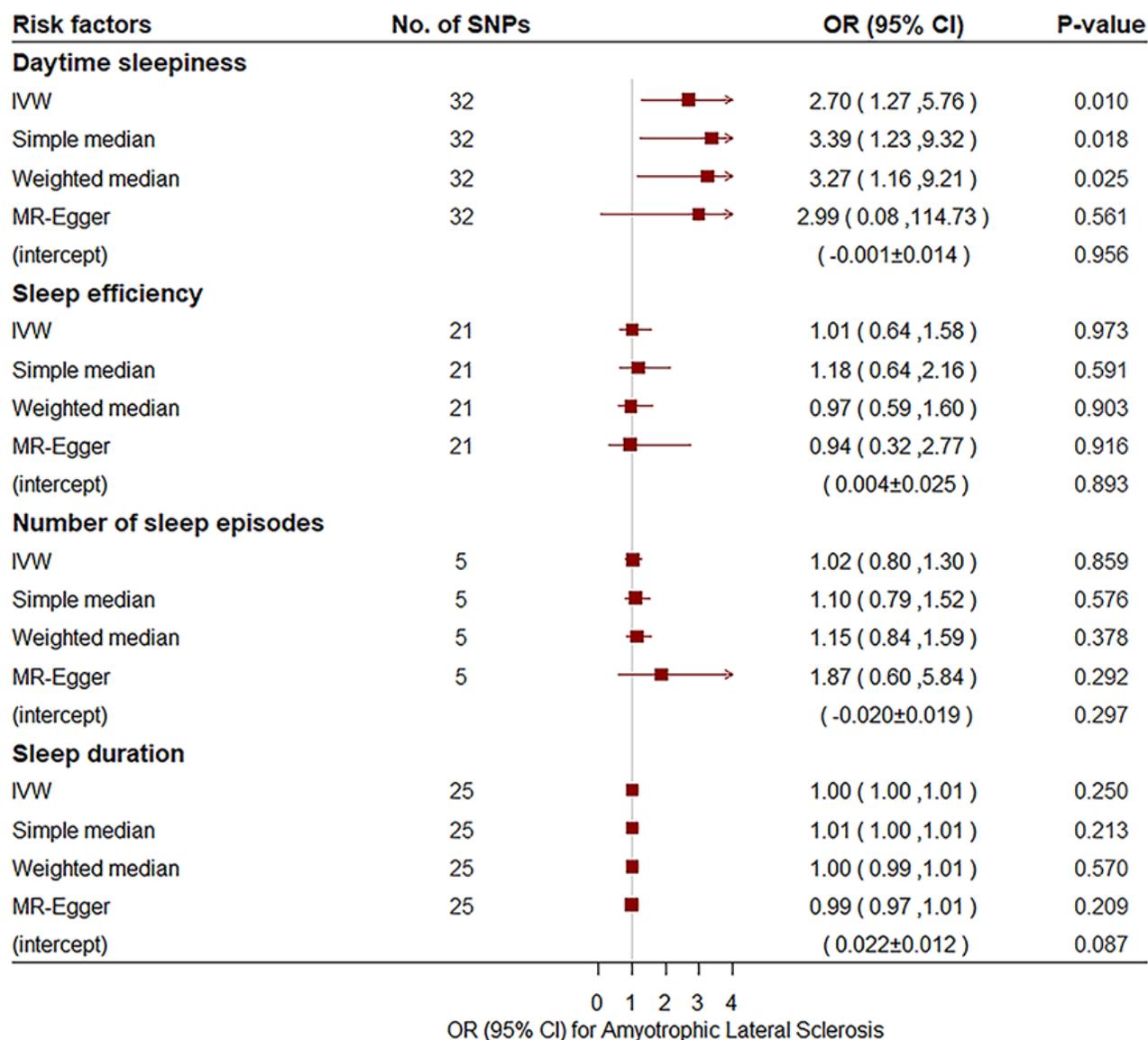


Figure 3

Odds ratio for the association between genetically predicted daytime sleepiness, night sleep changes and ALS. Estimates are per 1-point increase in daytime sleepiness and per approximately 1-SD increase in number of night sleep episodes (count), sleep efficiency (%) and sleep duration (hours). Intercept is expressed as (beta ± standard error). OR: odds ratio; 95% CI: 95% confidence interval.

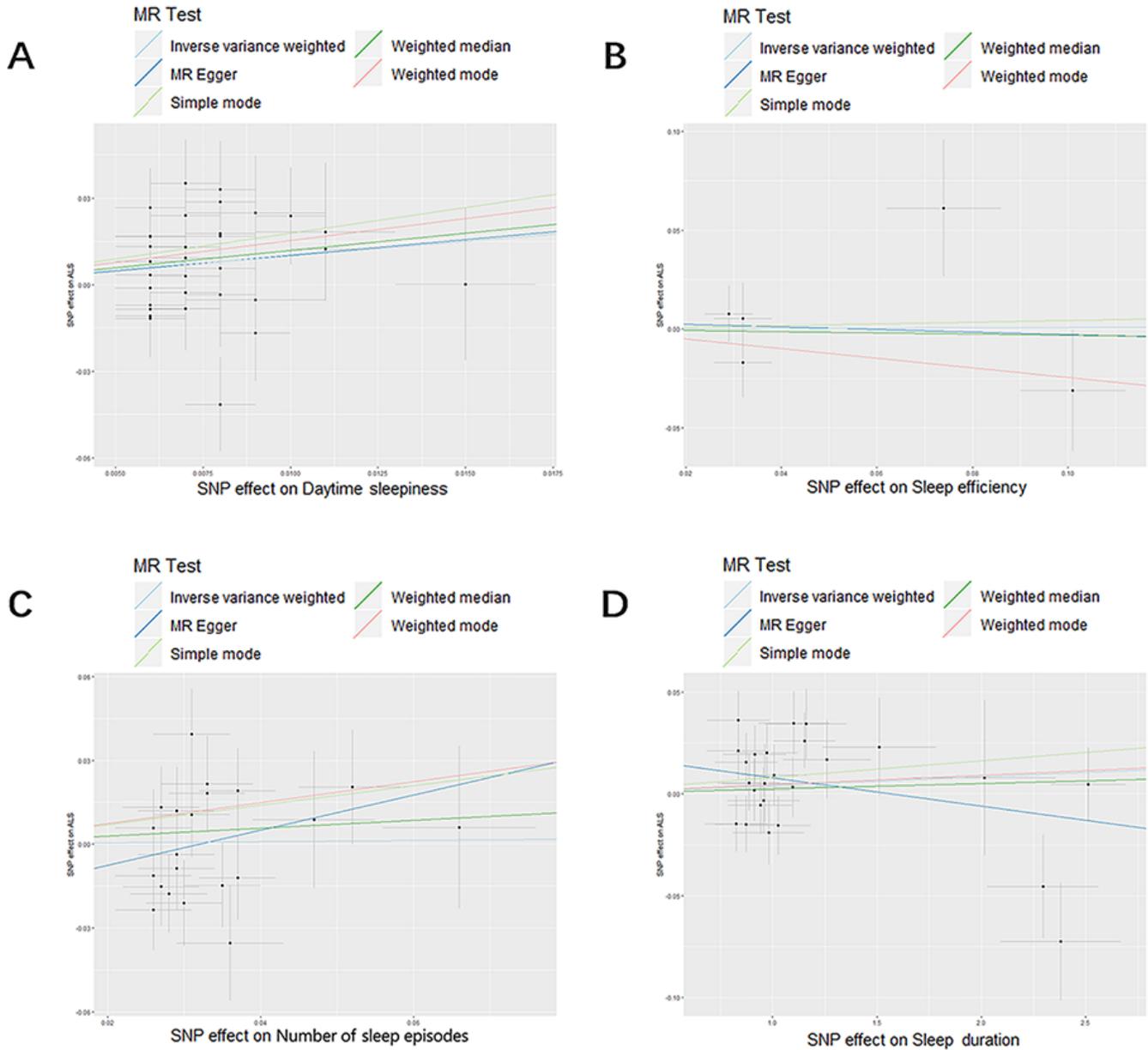


Figure 4

Scatterplot of potential effects of SNPs on sleepiness and night sleep changes vs ALS. The slope of each line corresponds to the estimated MR effect of each method. The examined sleep-wake disturbances included (A) daytime sleepiness, (B) sleep efficiency, (C) number of sleep episodes and (D) sleep duration. SNPs: single nucleotide polymorphisms; IVW: inverse-variance weighted; MR-Egger: Mendelian randomization-Egger; BMI: body mass index.

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