Lack of Structural Brain Alterations associated with Insomnia: Findings from the ENIGMA-Sleep working group

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Summary

Existing neuroimaging studies have reported divergent structural alterations in insomnia disorder (ID). Here, we performed a large-scale coordinated meta-analysis by pooling structural brain measures from 1,085 subjects (age 50.5±13.9 years, 50.2% female, 17.4% suffer from insomnia) across three international ENIGMA-Sleep cohorts (two sites recruited ID patients and controls: Freiburg 42/43 and KUMS n=42/49, while SHIP-Trend recruited population-based individuals with and without insomnia symptoms n=75/662). The influence of insomnia on MRI-based brain morphometry using an insomnia brain score was then assessed. Within each cohort, we used an ordinary least-squares linear regression to investigate the link between the individual regional cortical and subcortical volumes and the presence of insomnia symptoms. Then, we performed a fixed-effects meta-analysis across cohorts based on the first-level results. For the insomnia brain score, weighted logistic ridge regression was performed on one sample (Freiburg), which separated ID patients from controls to train a model based on the segmentation measurements. Afterward, the insomnia brain scores were validated using the other two samples. The model was used to predict the log-odds of the subjects with insomnia given individual insomnia-related brain atrophy. After adjusting for multiple comparisons, we did not detect any significant associations between insomnia symptoms and cortical or subcortical volumes, nor could we identify a global insomnia-related brain atrophy pattern. Thus, we observed inconsistent brain morphology differences between individuals with and without insomnia across three independent cohorts. Further large-scale cross-sectional/longitudinal studies using both structural and functional neuroimaging are warranted to decipher the neurobiology of insomnia.

1. INTRODUCTION

Insomnia is characterized by dissatisfaction regarding sleep quality or quantity, not attributable to sleep-disrupting external conditions. In particular, insomnia is defined by difficulties in falling asleep, difficulties maintaining sleep, early-morning awakenings with an inability to return to sleep, as well as subjective daily dysfunction (Morin et al., 2015; Sateia, 2014). Chronic insomnia disorder (ID) is the most common sleep disorder and is defined by insomnia symptoms occurring at least three times per week over a period of three months (Sateia, 2014), and is more prevalent in older individuals and in women (Morin et al., 2015; Riemann et al., 2022). Chronic ID is a persistent medical condition that is linked to adverse long-term physical and mental outcomes, including low quality of life, poor educational or work performance, memory impairment, higher risk of motor vehicle accidents, cardiovascular and metabolic diseases, anxiety, perinatal depression, major depressive disorder (MDD), bipolar disorder, posttraumatic stress disorder (PTSD), and neurodegenerative disorders (Ahmadi et al., 2022; de Almondes, Costa, Malloy-Diniz, & Diniz, 2016; Emamian, Khazaie, Okun, Tahmasian, & Sepehry, 2019; Garbarino et al., 2017; Grandner, Jackson, Pak, & Gehrman, 2012; Riemann et al., 2022). Insomnia, rather than sleep duration, is an independent risk factor for suicide in patients with MDD across all age ranges (Simmons, Erickson, Hedges, & Kay, 2020). The worldwide prevalence of insomnia symptoms is approximately 30-35% and for ID is around 10% (Morin et al., 2015). Recently, the rate of insomnia has drastically increased during the COVID-19 pandemic in the general population across different countries. For example, an international, multicenter study (n=22,330) of adults across 13 countries demonstrated that the rates of insomnia symptoms (36.7%), ID (17.4%), and anxiety (25.6%) are remarkable during the first wave of the COVID-19 pandemic - around twice the levels measured during non-pandemic periods (Morin et al., 2021). The annual economic cost of insomnia has been estimated at $6.6 billion in Canada in 2007-2008 and $45.2 billion in Australia in 2016-2017, mainly due to insomnia-related work absences, reduced productivity of affected individuals, and treatment of insomnia (Daley, Morin, LeBlanc, Grégoire, & Savard, 2009; Hillman et al., 2018). Despite the high prevalence, as well as the resulting socioeconomic and medical burden, the underlying pathophysiological mechanisms of insomnia are still poorly understood.

Recently, various neuroimaging studies have been conducted to identify underlying neurobiological mechanisms of insomnia. Several structural MRI studies have reported gray matter alterations in ID in widespread cortical regions, including the orbitofrontal cortex, temporal cortex, precuneus, dorsomedial prefrontal cortex, and anterior cingulate cortex (ACC) (Altena, Vrenken, Van Der Werf, van den Heuvel, & Van Someren, 2010; Winkelman et al., 2013), as well as in subcortical regions, including the hippocampus, amygdala, thalamus, caudate, putamen, and nucleus accumbens (Emamian et al., 2021; Joo et al., 2013; Koo, Shin, Lim, Seong, & Joo, 2017). However, their results are divergent and often conflicting. Interestingly, even

in a coordinate-based meta-analysis (CBMA) using the activation likelihood estimation method, no consistent structural and functional regional alteration was identified across 19 neuroimaging studies (Tahmasian et al., 2018). The observed inconsistencies may be due to heterogeneity in clinical populations, small samples, diversity in image acquisition protocols and experimental designs, and flexible statistical approaches used in individual studies. However, one major limitation of the CBMA approach is relying on stereotaxic coordinates reported in existing publications that used different analytic protocols, which makes it susceptible to publication bias and well as methodological variation across these existing publications. Due to these issues, the important question is whether the lack of consistent findings in prior CBMA is due to the limited sample available for the meta-analysis or if there is no regional convergence of brain abnormalities in insomnia.

The Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA)-Sleep consortium (https://enigma.ini.usc.edu/ongoing/enigma-sleep) aims to increase the number of subjects analyzed with sleep disturbances and harmonize the methods for preprocessing and analysis. The consortium uses unified protocols and combines data across various countries to better understand the neurobiology of sleep disorders at the international level (Tahmasian et al., 2021). Thus, to overcome the limitations of individual structural MRI studies and even CBMAs, we performed a coordinated meta-analysis of structural MRI in 1,085 subjects with and without insomnia symptoms. Specifically, we analyzed data from three cohorts participating in the ENIGMA-Sleep group to measure cortical and subcortical brain differences between subjects with and without insomnia symptoms. Furthermore, to investigate the effect of insomnia on brain structure, we measured an insomnia brain score, which (similar to the brain age score) quantifies subtle but widespread deviations in regional brain structures (Frenzel et al., 2019; Weihs et al., 2021).

2. METHODS

2.1. Study Populations

We included data from three cohorts within the ENIGMA-Sleep group (Tahmasian et al., 2021). In particular, we analyzed two case-control samples collected by the Kermanshah University of Medical Sciences (KUMS, Iran) and the University of Freiburg Medical Center (Freiburg, Germany), as well as a general population sample from the Study of Health in Pomerania (SHIP-Trend, Germany) (Volzke et al., 2011; Volzke et al., 2022). Cohort details are provided below and in **Table 1**.

The KUMS sample: The KUMS data is a case-control sample including 55 chronic ID patients and 49 healthy subjects. Chronic ID patients were recruited from Sleep Disorders Research Center, KUMS. All patients met the diagnostic criteria of chronic ID according to the third edition of the International Classification of Sleep Disorders (ICSD-3) criteria (Sateia, 2014) and psychiatric interview and overnight polysomnography (PSG) (to exclude other sleep disorders) before brain MRI acquisition. Healthy subjects were recruited through local advertisement and were defined as those with no present or past neurological or psychiatric illness, and all controls had a total PSQI score < 5. The exclusion criteria included taking any neuropsychiatric medications, pregnancy, any other medical, neurological, psychiatric, or other sleep disorders, as well as contraindications to MR imaging. The study was approved by the ethics committee of KUMS, and written informed consent was obtained from all participants. The details of this data are described elsewhere (Emamian et al., 2021).

The Freiburg sample: The Freiburg sample is a case-control sample including 42 patients fulfilling the criteria for chronic ID (originally recruited as patients with primary insomnia) and 43 healthy participants. ID patients were recruited through the sleep disorders clinic of the University of Freiburg Medical Center. Healthy controls were recruited through local advertisements. For screening purposes, all participants underwent a semi-standardized psychiatric and sleep-related interview. All participants were required to be free of any psychoactive medication for at least two weeks before and during study participation. The Institutional Review Board of the University of Freiburg Medical Center approved the original study protocols. Details of the data collection are described elsewhere (Regen et al., 2016; Spiegelhalder et al., 2013).

The SHIP-Trend sample: SHIP-Trend is a general population cohort comprising 4,420 individuals randomly drawn from local registries in Western Pomerania in Germany, who were assessed between 2008 and 2012 (Volzke et al., 2011; Volzke et al., 2022). After excluding all subjects who refused participation in the whole-body MRI or who fulfilled an exclusion criterion (e.g., the presence of a cardiac pacemaker), MRI scans were performed on a sub-sample of 2,159 individuals. Of these, 1,264 subjects underwent an in-depth sleep assessment including various questionnaires such as the insomnia severity scale (ISI) (Morin, Belleville, Belanger, & Ivers, 2011), assessing the severity of various night- and day-time components of insomnia, and the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989), assessing subjective sleep quality, as well as overnight PSG, during which the apnoea-hypopnea index (AHI) was assessed according to American Academy of Sleep Medicine (AASM) 2007 criteria (Iber, 2007; Ruehland et al., 2009). To account for the population-based nature of SHIP-Trend, more stringent selection criteria were used to exclude subjects due to missing variables, the presence of gross structural abnormalities in the brain or the presence of multiple sclerosis, Parkinson's disease, epilepsy, or stroke.

2.2. Insomnia Definitions

In the KUMS sample, chronic ID was defined according to ICSD-3 criteria 2, while in the Freiburg cohort, the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) criteria was used (Association, 2013). In the SHIP-Trend sample, the presence of insomnia symptoms was defined based on an ISI score of ≥15 (Gerber et al., 2016; Morin et al., 2011).

2.3. MRI acquisition and image processing

In both the SHIP and KUMS samples, a 1.5T Siemens Magnetom Avanto scanner was used to obtain the 3D brain MRI scans. Cortical reconstruction and volumetric segmentation were performed using FreeSurfer 7.2, using the Desikan-Kiliany atlas for the cortical volumes (68 regions of interest) and the ASEG-atlas for subcortical volumes (34 regions of interest) (Desikan et al., 2006). In the Freiburg sample, a 3T MRI (Magnetom TIMTrio, Siemens) was used, and the segmentation was performed with FreeSurfer 5.1 using a similar protocol. At this stage, FreeSurfer also assessed the intracranial brain volume. Due to the skewed distribution of the ventricle volumes (i.e., left/right lateral ventricle, left/right inferior lateral ventricle, 3rd ventricle and 4th ventricle), a log transformation was performed on the corresponding regional measures. To correct for potential segmentation failures, outliers (i.e., values lying above 75th percentile + 3xIQR or below the 25th percentile – 3xIQR) were removed and later imputed using the "missForest" R-package (Stekhoven & Bühlmann, 2012).

2.4. Statistical analyses

All statistical analyses were performed with R Version 4.2.

Meta-analysis across three sites: Within each cohort, we used ordinary least squares regression with robust standard errors to investigate the relationship of the individual cortical and subcortical brain regions (outcome) to the presence of insomnia (exposure) (Long & Ervin, 2000). All models were adjusted for age, sex, age×sex and intracranial volume (ICV). To account for the non-linear dependency between the various outcomes and age, restricted cubic splines with four knots were used using the "rms"-package (https://cran.r-project.org/web/packages/rms/). Additionally, as the SHIP-Trend sample is not a case-control sample, the models were also adjusted for AHI, as well as for the self-reported intake of sleep medication. To pool the effects from the different cohorts, a fixed-effects meta-analysis was performed using the "meta"-Package (Balduzzi,

Rücker, & Schwarzer, 2019). Effects were considered significant at $p < 0.05$ using false discovery rate (FDR) correction for multiple testing (Benjamini & Hochberg, 1995).

Insomnia brain score: The insomnia brain score was based on the cortical and subcortical volumes from the Freiburg sample. This cohort was used as it contains clinical ID patients and has a similar population as the SHIP-Trend validation sample. This reduces the ethnic/genetic bias, that might be present if the KUMS sample were to be used. As applied previously (Frenzel et al., 2019), using the "glmnet" R-package (Friedman, Hastie, & Tibshirani, 2010), logistic ridge regression was used to train a binary classifier, which optimally separated subjects with insomnia from subjects without, given the subject's cortical and subcortical volumes. The optimal shrinkage parameter, λ^*, was defined as the λ with the smallest mean classification error estimated using a 5-fold cross-validation with 40 repetitions. All features were standardized to a zero mean and unit variance, and age, sex, and intracranial volume were also added to the logistic models as covariates of no interest to account for potential imbalances between the case and control group. The resulting model was then used to predict the insomnia brain score in the KUMS and the SHIP-Trend cohorts after standardizing all features based on the mean and standard deviation assessed in the Freiburg sample. The insomnia brain score was computed to represent the log-odds of having insomnia in new individuals whose data was not used to train the model. Put differently, a higher insomnia score represents a higher similarity of the subject's brain to the brain of someone with ID, as defined in the Freiburg sample. To validate the score, the relationship between the score (outcome) and the presence of ID (exposure) was investigated in KUMS and SHIP-Trend using the same covariates as in the meta-analysis. To further understand potential underlying mechanisms, using SHIP-Trend only, we additionally investigated the associations between the score and the continuous ISI score, as well as with the PSQI and its sub-scores.

3. RESULTS

3.1. Baseline characteristics of the sample population

In the KUMS sample, after excluding OSA (N=1) and with missing covariate data (N=14), 91 subjects remained. For the Freiburg sample, 85 subjects were considered. For the SHIP-Trend sample, 737 subjects were considered after excluding subjects due to missing variables (N=3,659), structural abnormalities (N=14), the presence of neurological disorders (N=3) and reported strokes (N=7). The baseline characteristics of the included sample may be found in **Table 1**.

Regarding the segmentation outliers, in the KUMS sample, 2 outliers were identified. The (locally) optimal imputation was reached after 4 iterations with an estimated normalized root mean square error (NRMSE)

of 0.00023 (see **Supplementary Figure 1**). Similarly, in the Freiburg sample, 2 outliers were identified with an NRMSE of 0.14 reached after 4 iterations (see **Supplementary Figure 2**). Finally, in the SHIP-Trend sample, 17 outliers were identified with an NRMSE of 0.0014 reached after 3 iterations (**see Supplementary Figure 3**).

3.2. Insomnia and morphometric brain measurements

After correcting for multiple testing (p-value < 0.05, FDR-corrected), no significant association of insomnia with morphometry of subcortical or cortical brain regions was observed in either the individual samples or on the meta-analysis level (see **Figure 1**). Results of the subcortical volume analyses can be found in **Supplementary Table 1** and **Supplementary Figure 4**, while the results of the cortical GM volume analyses can be found in **Supplementary Table 2** and **Supplementary Figure 5**.

3.3. Insomnia-related brain atrophy patterns

The optimal shrinkage parameter was estimated at λ=3.88, with a mean classification error of 43.6% (see Supplementary Figure 6). A list of the resulting weights of the individual regions can be found in **Supplementary Table 3** and **Figure 2**. However, no association was identified between the insomnia brain score and the presence of insomnia symptoms in the independent KUMS (estimate [95%-CI]: -0.012 [-0.067; 0.044], p=0.68) or SHIP-Trend cohorts (estimate [95%-CI]: 0.016 [-0.020; 0.052], p=0.37). Similarly, no effect could be observed concerning the SHIP-Trend ISI sum-score (estimate [95%-CI]: 0.0015 [-0.00075; 0.0038], p=0.19). Regarding the PSQI, on the other hand, a significant positive association was detected between the insomnia brain score and the PSQI sum-score (estimate [95%-CI]: 0.0040 [0.00062; 0.0073], p= 0.020) as well as with the 1st component of the PSQI (estimate [95%-CI]: 0.020 [0.0043; 0.035], p=0.012) assessing subjective sleep quality.

4. DISCUSSION

To assess the potential structural brain abnormality in subjects with insomnia and to overcome several of the limitations of individual neuroimaging studies of insomnia (e.g., small sample sizes, inconsistent analysis methods), we performed a fixed-effect meta-analysis across three cohorts with a harmonized analytic protocol. We did not detect any significant association between insomnia symptoms and cortical or subcortical volumes. Afterward, we calculated the insomnia brain score in one sample, and we tested the model in the other two

samples to predict individual insomnia-related brain atrophy. Again, while identifying some signals regarding subjective sleep quality, we did not observe a robust insomnia brain atrophy pattern across three cohorts.

The results of the current study support previous neuroimaging meta-analysis, in which no consistent regional brain abnormality in ID was observed (Tahmasian et al., 2018). Thus, the current and previous neuroimaging meta-analyses did not reveal a strong link between brain regions and insomnia, and the previous reports of structural alterations associated with insomnia may not be replicable due to their small samples (usually <100 participants) or other variabilities in the included participants (e.g., recruiting a particular ID subtype). While the insomnia brain score reduces the issues caused by multiple testing, it is dependent on a global signal being present in the data. As no clear signal was identified in the meta-analysis, it is not surprising that the insomnia brain score did not demonstrate any robust results across three sites. To our knowledge, this preliminary study is the first neuroimaging collaborative work in the field of insomnia to provide better insights into structural alterations in insomnia. Even so, we only had three samples, including two small samples with ID patients and controls (N ~ 90) and one larger population-based sample (N = 737), in which only 11% of subjects had insomnia symptoms. Thus, we clearly need more data to address the question of whether the observed absence of significant findings in the current study is due to the limited available samples and statistical power or depends on the imaging modality chosen and the type of structural analysis such as cortical thickness and surface area analysis.

Another potential reason for the observed weak structural findings in insomnia might be the clinical heterogeneity of insomnia. Insomnia is considered a heterogeneous disorder, which has different subtypes with noticeable inconsistencies in terms of pathophysiology, the clinical presentation of sleep-related and non-sleep-related symptoms, as well as treatment response (Benjamins et al., 2017). Blanken and colleagues performed data-driven subtyping of subjects with insomnia using high-dimensional multivariate phenotypic data of subjects with insomnia symptoms and found five ID subtypes, including highly distressed, moderately distressed but reward sensitive, moderately distressed and reward insensitive, slightly distressed with high reactivity, and slightly distressed with low reactivity (Blanken et al., 2019). Similarly, other studies identified discrepancies between insomnia subtypes in terms of demographics, daily symptoms, and polysomnographic findings (Bjorøy, Jørgensen, Pallesen, & Bjorvatn, 2020; Kao et al., 2021). For example, Bjorøy and colleagues divided insomnia into seven subtypes and observed that subjects with a combination of sleep onset insomnia, sleep maintenance insomnia, and early morning awakening insomnia have a higher rate of anxiety, depression, alcohol consumption, and use more hypnotics than participants with other insomnia subtypes (Bjorøy et al., 2020). A data-driven classification of objective PSG sleep duration and EEG spectral power revealed three insomnia subtypes including short-sleep delta-deficient, normal-sleep delta-deficient, and normal neurophysiological sleep (Kao et al., 2021). Another study based on event-related potential measures demonstrated that patients with psychophysiological insomnia could not inhibit information processing during sleep onset, while patients

with paradoxical insomnia had higher attentional processing for inhibition (Turcotte, St-Jean, & Bastien, 2011). A previous structural brain analysis demonstrated distinct alterations between two subtypes of ID including paradoxical and psychophysiological insomnia (Emamian et al., 2021). In particular, there was a shape abnormality in the caudate, putamen, and nucleus accumbens in paradoxical insomnia, but shrinkage in the thalamus, amygdala, and hippocampus was observed in psychophysiological insomnia (Emamian et al., 2021). Using a machine-learning-based classification via a support vector machine algorithm, a preliminary study has observed that multimodal (structural and functional) brain data can distinguish paradoxical insomnia from psychophysiological insomnia (Afshani M, 2022). These findings suggest a distinct role of cortical and subcortical brain regions in the pathophysiology of paradoxical and psychophysiological subtypes. Interestingly, Miller and colleagues applied cognitive-behavioural therapy for insomnia (CBT-I) protocols to manage different insomnia subtypes derived from polysomnography (Miller et al., 2018). The authors found that acceptability and tolerability to CBT-I differ between ID subtypes i.e., ID patients with normal sleep duration responded better to CBT-I than ID patients with short sleep duration (Miller et al., 2018). However, the literature on this issue is not consistent. For example, another study found that CBT-I is equally effective in both insomnia groups with objective (based on PSG) short and normal sleep duration (Crönlein, Wetter, Rupprecht, & Spiegelhalder, 2020).

These findings together suggest that ID may not be a unified diagnostic entity but rather comprises various subtypes with their own particular multivariate profile of personality characteristics, sleep microstructure, cognitions, mood, and neuroimaging biomarkers (Benjamins et al., 2017; Blanken et al., 2019). Using sub-clinical criteria such as the ISI, which are unable to assess the complexity of ID, thereby ignoring the heterogeneity and subtypes of insomnia, as well as not considering the length of the illness, which was unfortunately not available, might have led to the failure to identify and replicate structural findings across our different cohorts.

5. CONCLUSION

In the present study, we detected no structural brain differences between subjects with and without insomnia across three independent cohorts of ENIGMA-Sleep, neither in the meta-analysis nor in insomnia-related multivariate brain morphometry. Evidently, more cross-sectional samples are needed to address the question of whether the observed lack of structural findings in the current study is due to the limited available samples or other factors. Furthermore, upcoming collaborative works should assess the potential functional disruptions across intrinsic brain networks to elucidate the neural correlates of insomnia and its subtypes at the functional level. A recent study highlighted that thousands of subjects are needed to identify reproducible brain-wide

association (Marek et al., 2022). Besides cross-sectional studies, longitudinal and interventional studies using both structural and functional neuroimaging data may aid in detecting the long-term effects of insomnia on the brain and evaluate the effect of insomnia treatments such as CBT-I on the brain activity/connectivity of patients with ID. The core aim of this initiative is to invite sleep clinicians/scientists across different countries to join us, as we have no choice but to work together to decipher the neurobiological mechanisms of insomnia (Tahmasian et al., 2021). We hope that the upcoming studies from the ENIGMA-Sleep consortium provide robust and replicable results using various datasets worldwide.

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CONFLICT OF INTEREST

The authors declare no competing interests directly related to this work. HJG has received travel grants and speakers' honoraria from Fresenius Medical Care, Neuraxpharm, Servier, and Janssen Cilag and research funding from Fresenius Medical Care. RE has received speaker honoraria from Berlin Chemie, AstraZeneca, and Janssen, as well as consulting fees from LungPacer and OMT, and is a member of the LungPacer and Janssen advisory board. PMT and NJ have received research funding from Biogen, Inc. for work unrelated to this manuscript. PMT, NJ, and SIT are funded partly by the U.S. National Institutes of Health under grants

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AUTHOR CONTRIBUTIONS

AW, SF, MT, KRP, and KS designed the study. AW and SF performed the statistical analyses. RB, RE, HK, MR, MA, BS, HV, HG, KS, and DR collected data. AW and MT wrote the first draft of the manuscript. HB, JES, FH, NJ, DR, ST, PMT, SLV, MZ, SBE, HJG, KRP, and KS revised the paper.

Table 1. Baseline characteristics of the three cohorts.

	KUMS (ID patients / healthy controls)	**Freiburg (ID patients / healthy controls)**	**SHIP-Trend (general population)**
N	42/49	42/43	75/662
Age, mean ± SD	43.5±10.8 / 39.9±12.4	41.9±14.0 / 39.0±8.9	54.2±10.9 / 52.6±13.7
Gender, female, N (%)	27 (64.3) / 27 (55.1)	26 (61.9) / 25 (58.1)	52 (69.3) / 301 (45.5)
ICV, cm^3, mean ± SD	1549.6±182.8/ 1581.9±142.3	1187.6±244.0 / 1132.0±180.4	1533.6±147.0 / 1594.8±167.8
ISI, mean ± SD		14.9±3.6 / 2.1±2.3	17.2±2.3 / 5.9±3.9
PSQI, mean ± SD			11.9±3.1 / 5.4±3.3
AHI, mean ± SD			7.5±9.2 / 8.9±13.4
Sleep medication, yes, N (%)			25 (33.3) / 26 (3.9)
Abbreviations: AHI: Apnoea-Hypopnea Index, PSQI: Pittsburgh Sleep Quality Index, ICV: Intracranial Volume, ISI: Insomnia Severity Index			

Figures

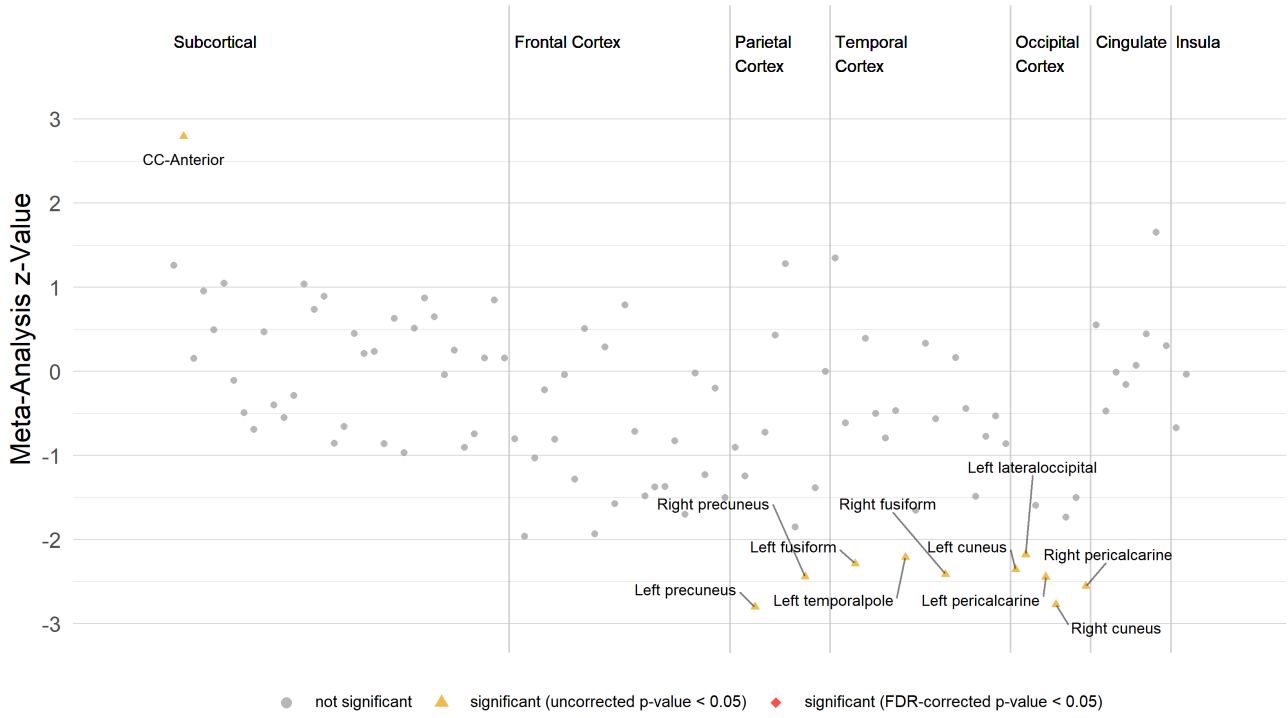

Figure 1. Morphometry of subcortical or cortical brain regions across all cohorts. Z-values from the fixed-effect meta-analysis aggregating the results from the KUMS, Freiburg and SHIP-Trend cohorts. Ten areas were nominally significant, while no region was significant after correcting for multiple testing (via the false discovery rate (FDR)).

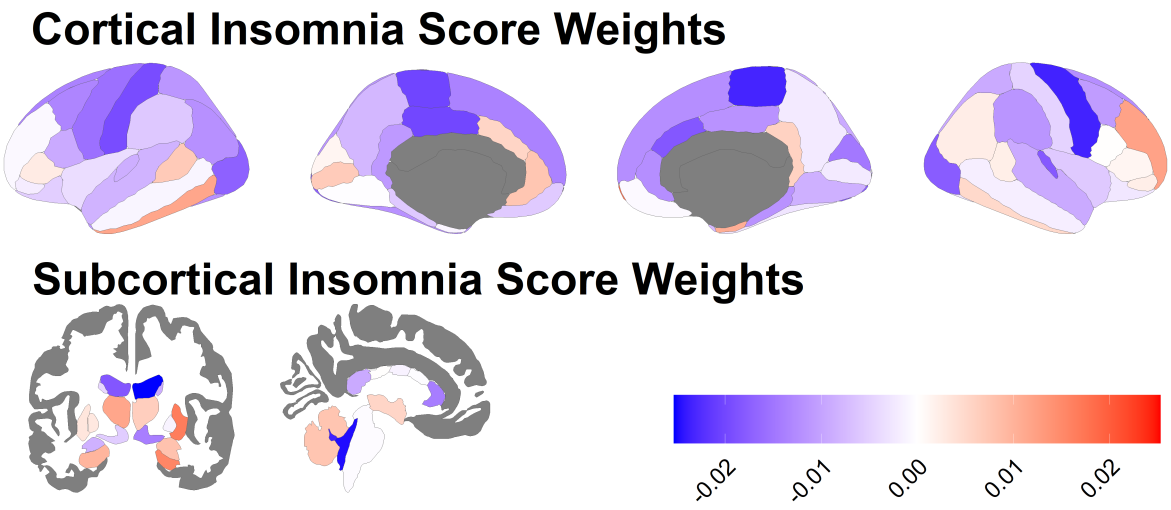

Figure 2. Weights assessed in the Freiburg cohort of the individual brain regions of the insomnia score using cortical and subcortical regions.

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