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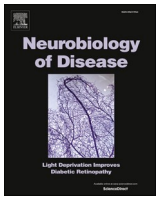

Genetic risk for insomnia is associated with objective sleep measures in young and healthy good sleepers

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ABSTRACT

Insomnia disorder (ID) is the second most common neuropsychiatric disorder. Its socioeconomic burden is enormous while diagnosis and treatment are difficult. A novel approach that reveals associations between insomnia genetic propensity and sleep phenotypes in youth may help understand the core of the disease isolated from comorbidities and pave the way for new treatments. We obtained quantitative nocturnal sleep electroencephalogram (EEG) features in 456 participants (18-31y, 49 women). Sleep EEG was recorded during a baseline night following at least 7 days of regular sleep times. We then assessed daytime sleep onset latency in a subsample of $N = 359$ men exposed to manipulations affecting sleep pressure. We sampled saliva or blood for polygenic risk score (PRS) determination. The PRS for ID was computed based on genome-wide common single nucleotide polymorphism assessments. Participants also completed a battery of behavioral and cognitive tests. The analyses revealed that the PRS for ID was negatively associated with cumulated EEG power in the delta (0.5-4 Hz) and theta (4-8 Hz) bands across rapid eye movement (REM) and non-REM sleep ($p \leq .0026$; $\beta \geq -0.13$) controlling for age, sex and BMI. The PRS for ID was also negatively associated with daytime likelihood of falling asleep ($\beta = -0.19$, $p = .0009$). Other explorations for associations with non-baseline-nights, cognitive measures, and mood did not yield significant results. These results propose that the need or the ability to fall asleep and to generate slow brain activity during sleep may constitute the core sleep-related risk factors for developing ID.

1. Introduction

Insomnia disorder (ID) is the most common sleep disorder and the second most common neuropsychiatric disorder(American Psychiatric Association, 2013). ID prevalence estimates range from 6 to 18% in the general population (Ohayon, 2002), but since it is an age-related disorder, it may be as high as 50% in the elderly (Patel et al., 2018). ID is associated with reduced life expectancy (Robbins et al., 2021) and increased risks for diabetes, cardiovascular diseases and psychiatric disorders (Anothaisintawee et al., 2016; Gangwisch et al., 2010), implying that ID constitutes a huge socioeconomic burden – tens of billion for the U.S. alone (Kessler et al., 2011). Yet, ID diagnosis and treatment remain difficult. The diagnosis is exclusively based on self-report. Traditional polysomnographic sleep scoring often fails to find a reduction in total sleep time matching subjective experience (Harvey and Tang, 2012). ID is also much more heterogeneous than initially apprehended (Benjamins et al., 2017) and shows high comorbidity with other diseases, particularly anxiety and depression (Wittchen et al.,

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2011), which are often preceded by ID. In our aging society, the need for in- depth understanding of ID leading to novel prevention and treatment targets has never been so high.

ID involves a chronic hyperarousal state of increased somatic, cognitive and cortical activation, and negatively impinges on sleep (Riemann et al., 2010). Beyond difficulties falling or remaining asleep, signatures of hyperarousal are even found during sleep, which contains more arousals and fast electroencephalogram (EEG) oscillations. This restless sleep would interfere with the downregulation of emotion and arousal on the short term (Wassing et al., 2019a) and long term (Wassing et al., 2019b). The course of ID is commonly described within the '3P' model which posits predisposing, precipitating and perpetuating factors (Spielman et al., 1987). While several perpetuating factors have been recognized, including poor sleep hygiene and negative beliefs about sleep, much less is known about how predisposing, genetic factors promote the development of insomnia.

Insomnia heritability is substantial, with twin study estimates ranging from 0.28 to 0.59 (Barclay et al., 2021). Genome wide association studies (GWAS) have established that ID is highly polygenic with 202 loci identified using stringent statistical criteria and up to 956 different genes reported in the literature (Jansen et al., 2019). Genetic variants revealed by GWAS explain only a small proportion of phenotypic variation in complex diseases like ID. This phenomenon, known as the missing heritability problem, arises from the infinitesimal contribution of multiple genetic variations. A common way to aggregate the additive contributions of multiple genetic variants is calculating an individual's polygenic risk score (PRS). The PRS is a weighted sum of the number of risk alleles and provides an estimate of the individual's genetic risk. PRS have been previously used to link the genetic risk for Alzheimer's disease with the cognition (Coors et al., 2022) and with cerebrovascular function (Chandler et al., 2019); schizophrenia with cognitive and neural plasticity (Zhao et al., 2022); other psychiatric disorders with cognition, behaviour and brain imaging (Gui et al., 2022). How a set of risk allele variants contributes to predisposing, precipitating and perpetuating factors of insomnia is however unknown. Revealing how genetic variants affect sleep biology is arguably fundamental to a better understanding of insomnia disorder.

Since individuals devoid of sleep disorders may differ with regards to genetic liability for ID, investigating how the PRS for ID impacts sleep EEG in unaffected young healthy adults may be the best way to obtain clues about the underlying biology of ID. This type of approach would assess whether inherited liability for ID may already manifest in objectively detectable sleep phenotypes in people without disorders, relatively uncontaminated by the variance related to aging, comorbidities and lifetime experiences that would be present in people that have actually developed chronic ID. In a cross-sectional study, we therefore assessed the PRS for ID and sleep EEG in 456 young individuals devoid of any sleep complaints and psychiatric symptoms. We hypothesized that the PRS for ID would be associated with EEG markers of insomnia vulnerability assessed during normal nocturnal sleep (e.g. lower and higher power in slower and faster EEG oscillations, respectively) and under conditions of altered sleep pressure. We then also explored whether the PRS would be associated with subjective metrics of sleep, anxiety and mood, as well as with cognitive consequences of altered sleep pressure.

2. Material and methods

2.1. Standard protocol approvals, registrations, and patient consents

All the participants signed an informed consent and experiments were approved by the Ethics Committee of the Faculty of Medicine of the University of Liège.

2.2. Participants and protocols

We retrospectively analyzed DNA and in-lab EEG recordings of sleep of 456 young and healthy individuals aged 18 to 31y (22 ± 2.7y; 49 women) collected across 6 different studies conducted at University of Liège, Belgium (Supplementary Table S1). All protocols (Gaggioni et al., 2019; Ly et al., 2016; Mascetti et al., 2013; Muto et al., 2021; Muto et al., 2016; Vandewalle et al., 2009) included baseline EEG recordings of night-time sleep at habitual sleep times following at least one week of regular sleep-wake schedules monitored by actigraphy.

Exclusion criteria aimed at constituting a sample of very healthy men devoid of any chronic disease, including sleep disorders and were as follows: body mass index (BMI, kg/m^2) <18 or >29, diabetes, excessive alcohol (>14 units/week) and caffeine (>3 cups/day) consumption, addiction, diagnosed psychiatric disorders, including insomnia, depression and anxiety, shift work during the past year, transmeridian travel in the last 3 months, use of psychoactive drugs, sleep medication. A screening night of sleep under full polysomnography excluded sleep disorders (apnea-hypopnea index $\geq$15/h; periodic limb movement, $\geq$15/h; REM sleep behaviour disorder, sleep walking). Participants were requested to refrain from caffeine at least 3 days prior to the study. Anxiety and depression symptoms were assessed with the Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) (Beck et al., 1988a; Beck et al., 1988b). Sleep quality and sleepiness were assessed with the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) and Epworth sleepiness scale (ESS) (Johns, 1991), respectively.

Some additional analyses were performed in a sub-sample of 359 young healthy men, which is described in details in (Muto et al., 2021).

2.3. Sleep EEG metrics

Sleep EEG was recorded using either a V-Amp 16 (Ly et al., 2016; Muto et al., 2021; Muto et al., 2016; Vandewalle et al., 2009), a QuickAmp-72 (Mascetti et al., 2013) (Brain Products GmbH, Germany) or a N7000 (Gaggioni et al., 2019) (Natus, Planegg, Germany) amplifier. EEG montage varied across studies but included at least Fz, Cz, Pz, Oz and mastoid leads, as well as bipolar electrooculogram, electromyogram and electrocardiogram channels. EEG data were digitized at 200 or 500 Hz sampling rate. EEG data were re-referenced off-line to the average of both mastoids using Matlab (Mathworks Inc., Sherbom, MA).

Sleep data were scored in 30-s epochs using a validated automatic sleep scoring algorithm (ASEEGA, PHYSIP, Paris, France) (Berthomier et al., 2007). Arousals and artefacts were detected automatically as previously described (Wallant et al., 2016) and excluded from power spectral density analyses (pwelch matlab function; 4 s epochs without artefact or arousal; 2 s overlap). Only frontal electrodes were considered because the frontal region is most sensitive to sleep pressure manipulations (Cajochen et al., 1999) as well as to facilitate interpretation of future large-scale studies using headband EEG, often restricted to frontal electrodes. Averaged power was computed per 30 min bins, adjusting for the proportion of rejected data (containing artefact/arousal), and subsequently aggregated in a sum separately for REM and NREM sleep. Thus we computed slow wave energy (SWE) - cumulated power in the delta frequency band during NREM sleep, an accepted measure of sleep need (Plante et al., 2016), and similar to that we computed the cumulated theta (4-8 Hz) power in REM sleep. We then computed the cumulated power over the remaining EEG bands, separately for NREM and REM sleep: alpha (8-12 Hz), sigma (12-16 Hz) and beta (16-25 Hz) bands. The cumulated power score would increase with time spent in REM and NREM sleep, so we included total sleep time (TST) as a common covariate in all analyses, as well we then controlled for REM and NREM sleep duration for REM and NREM sleep power respectively.

Primary analyses focused on six sleep metrics to limit issues of multiple comparisons while spanning the most important aspects of sleep EEG previously associated with ID: 1) sleep onset latency (SOL) and 2) wake after sleep onset (WASO), to assess overall sleep quality and

continuity(Perlis et al., 2010); 3) SWE during NREM sleep to assess slow wave generation; 4) cumulated overnight beta power during NREM sleep to quantify high frequency activity (Merica, 1998); 5) the number of arousals during REM sleep to reflect its instability (Riemann et al., 2012); and 6) cumulated theta power during REM sleep to assess its most typical oscillatory activity (Benz et al., 2020).

2.4. Quality control of genetic data and imputation

Genotyping was performed at Genomics platform of ULiège GIGA institute using blood samples or buccal swabs and Illumina Infinium BeadChip arrays based on Human Build 37 (GRCh37). The samples were frozen (−20 °C) within a few hours following collection and until DNA extraction. Quality control (QC) was performed using PLINK (http://zzz.bwh.harvard.edu/plink/), (Purcell et al., 2007). One participant was excluded from subsequent analyses due to mismatch between actual and imputed sex. No sample presented >10% missing genotypes. We removed SNPs as follows: <95% call rate, <0.01 minor allele frequency (MAF), out of Hardy-Weinberg equilibrium (p-value $<10^{-4}$ for the Hardy-Weinberg test), on 23rd chromosome, ambiguous SNPs (A-T, T-A, C-G, G-C). For one pair of individuals, the composite pi-hat score was 0.57 suggesting that they are first-degree relatives. We did not exclude these individuals, but removing one subject of this pair did not affect the statistical significance of any of the tests reported below. As a part of QC, we merged our data with 1000 Genomes Project (1KGP, https://www.internationalgenome.org), and applied principal component analysis on the merged data to verify that our cohort was located in the European cluster (Supplementary Fig. S1A). We then compared the allele frequencies in our cohort with those of the European subset of the 1KGP (Supplementary Fig. S1B). We removed SNPs which minor allele frequencies >0.2 compared with European subset (Supplementary Fig. S1C—D).

Imputation was performed using the Sanger imputation server (https://imputation.sanger.ac.uk/) by choosing "Haplotype Reference Consortium (r1.1)" as reference panel and EAGLE2 pre-phasing algorithm. We applied the same QC including allele frequency check with the European cohort of 1KGP. We also excluded SNPs with quality of imputation <0.3. After QC, 7300849 SNPs remained for statistical analyses.

2.5. Predicting height as part of quality control

As part of quality control, we computed a polygenic score for the height of the participants based on the summary statistics for height which includes 3290 genome-wide significant loci that explain approximately 25% of the phenotypic variation in height in European ancestry individuals (Yengo et al., 2018). Correlation between polygenic scores based upon these SNPs with actual height in our cohort is 0.4, $p = 1.5*10^{-18}$ (Supplementary Fig. S2), similar to previously reported (Yengo et al., 2018).

2.6. Polygenic risk score computation

The individual polygenic risk score (PRS) in our ID-free sample was calculated as the sum of SNPs associated with ID, weighted by estimated effect sizes of the summary statistics of a large ID case-control GWAS (Jansen et al., 2019). We used the results of the 23andMe sample used in the GWAS ($n = 944{,}477$), which is based on case-controls online surveys completion. PRS was computed in PLINK2 using standard approach (Privé et al., 2019): clumping, i.e. markers pruning based on linkage disequilibrium (LD) ($r^2 = 0.2$; window size = 1000 Kb), keeping the most significant markers of the GWAS, followed by applying a p-value threshold on GWAS summary statistics to select SNPs of interest (for more details see Supplementary materials).

It has been suggested that the inclusion of a larger number of single nucleotide polymorphisms (SNPs) in the PRS could increase the predictive accuracy and explained variance of diseases (Escott-Price et al., 2015). Yet, the best p-value threshold for inclusion of a SNP in ID case-control summary statistics to compute PRS is not established. We therefore computed 11 PRSs for insomnia based on increasing p-value thresholds to generate a range of outcomes from including only the SNPs reaching stringent GWAS significance, up to the most liberal threshold (p-value $<5*10^{-8}$, $5*10^{-6}$, $5*10^{-4}$, 0.001, 0.01, 0.05, 0.1, 0.3, 0.5, and 1). We also computed a PRS using all the SNPs, without clumping. Supplementary Table S2 provides the number of SNPs included in the computation of each PRS.

2.7. Statistical analysis

Associations of the PRS with sleep metrics as dependent variables, were evaluated using general linear models (GLM) implemented in Python, with age, BMI, and TST as covariates. Sleep metrics were standardized using a linear Z-transformation. Individual values were considered outliers if >4SD from the mean and removed from analyses: the number of individuals included in each model is reported below each dependent variable in the supplementary tables. For skewed data, generalized linear mixed models (GLMM) were computed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) to verify significance adjusting for data distribution.

Since the 11 PRS are correlated, we used the singular value decomposition (SVD) of the correlation matrix to estimate the number of independent tests. We ordered the eigenvalues after SVD and calculated the sum of all eigenvalues. We then calculated the minimum number of linear combination of the PRSs that explained 99% of the variance, which resulted in three. Hence, for any of our 6 EEG sleep metric-PRS combination to be statistically significant when taken multiple testing into account, the p-value threshold was set to 0.05 divided by 3*6, i.e. $p = 0.0028$. We computed the minimum detectable effect size given our sample size. According to G*Power 3 (version 3.1.9.4) (Faul et al., 2007), taking into account a power of 0.8, an error rate α of 0.0028, a sample size of 456 allowed us to detect medium effect sizes $r > 0.21$ (2-sided; absolute values; confidence interval: 0.12–0.29) within a linear multiple regression framework including 5 predictors.

3. Results

All 11 PRS were normally distributed in our cohort. Statistical analysis controlling for age, sex, BMI and TST first revealed significant negative associations between the PRS for ID and SWE ($p \leq 0.045$, $\beta \geq -0.09$) using the PRS at $p < 10^{-4}$ threshold up to including all SNPs (Supplementary Table S3, Fig. 1A). The association reached stringent experiment-wise corrected significance (i.e. $p < 0.0028$) for three p-value thresholds: $p = 0.1$, 0.5, 1 ($p \leq 0.002$; $\beta \geq -0.14$). Statistical analysis also revealed significant negative associations between the PRS for ID and cumulated theta power during REM sleep ($p \leq 0.027$, $\beta \geq -0.10$) using the PRS at $p < 10^{-3}$ threshold up to including all SNPs. Association reached stringent experiment-wise corrected significance for four p-value thresholds: p =0.1, 0.3, 0.5, 1 ($p \leq 0.0026$; $\beta \geq -0.13$). The negative sign of the associations (Fig. 1B, C) indicates that people with a higher PRS for ID tend to have less, or less intense, slow waves and theta oscillations, during NREM and REM sleep, respectively. Importantly, associations between the PRS for ID and NREM sleep SWE or REM sleep theta are not driven by the duration of NREM or REM as controlling respectively for NREM and REM sleep duration did not affect statistical outcomes (Supplementary Table S4).We further tested for potential associations of PRS for ID with REM and NREM percentage and ratio, and found no significant associations. (Supplementary Fig. S3). Note that computing the analyses on men only, as well as adding the first two principal components of the genetic data to our models led to the similar statistical outputs (data not shown).

In line with our assumptions, the PRSs for ID computed with p-value thresholds of 10^{-3} and 10^{-2}, were also positively associated with the

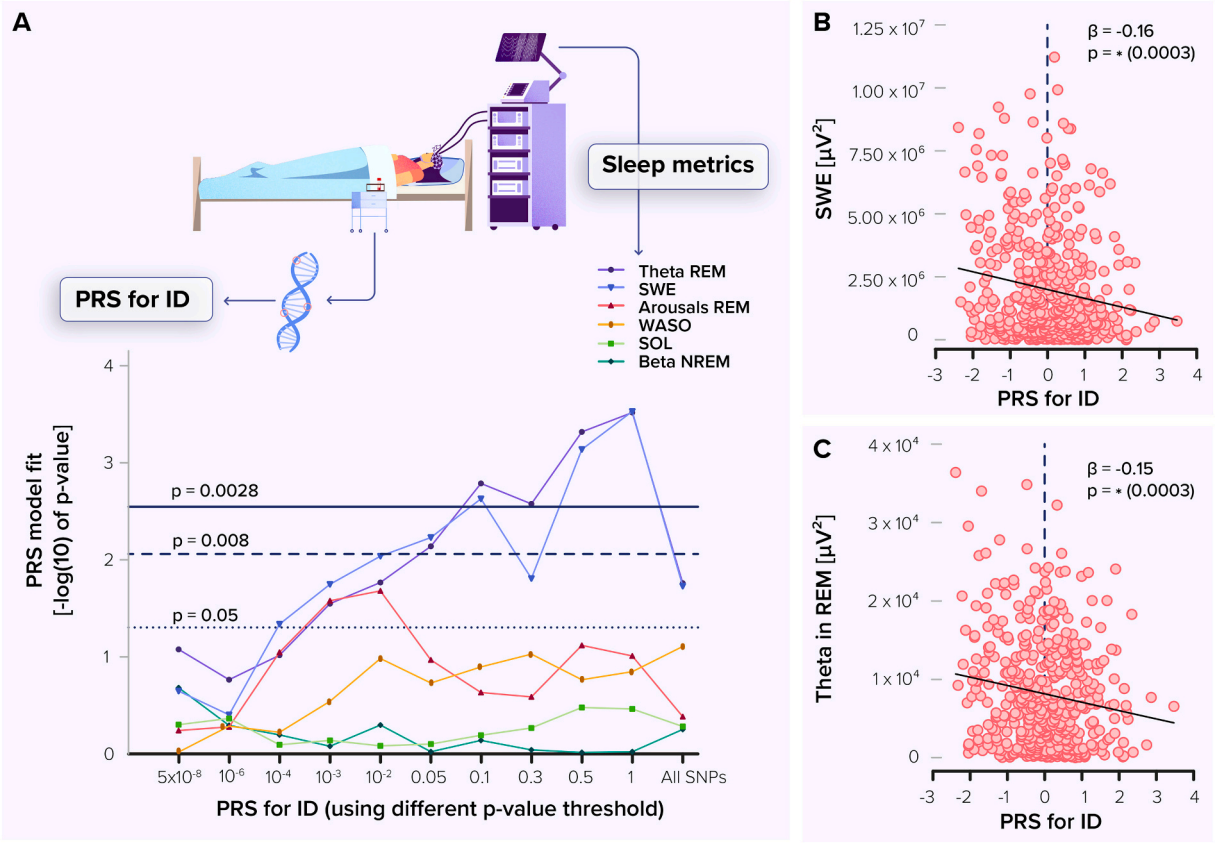

Fig. 1. Associations between PRS for ID and baseline night sleep metrics.
(A) Statistical outcomes of GLMs with six sleep metrics of interest versus PRS for ID from conservative ($p < 5 \times 10^{-8}$) p value threshold to using all SNPs ($N = 456$). GLMs are corrected for age, sex, BMI, and TST. Negative log transformation of p values of the associations is presented on the vertical axis. Horizontal lines in A indicate different p values thresholds: dotted = 0.05 (uncorrected); dashed = 0.008 (corrected for six sleep metrics); solid = 0.0028 (experiment-wise correction; see Material and methods).
(B) Negative association between SWE (overnight cumulated power in delta - 0.5-4 Hz – band) during baseline night and ID PRS including SNPs with p-value threshold = 1 (Spearman $r = -0.13$, $p = .0075$*).
(C) Negative association between overnight cumulated power in REMS theta (4-8 Hz) during baseline night and ID PRS including SNPs with p-value threshold = 1 (Spearman $r = -0.11$, $p = .014$*).
PRS for ID are expressed in arbitrary units which were z-scored. Spearman's correlations r are reported for completeness and do not substitute the statistical outputs of GLMs which are reported on the graphs and in the Supplementary Table S3. [* GLM significant association].
WASO, wake time after sleep onset; SOL, sleep onset latency; Arousals REM, number of arousals during REM sleep; SWE, slow-wave energy in NREM sleep (0.5–4 Hz); Beta NREM, cumulated power in beta (16-25 Hz) in NREM sleep; Theta REM, cumulated power in theta (4-8 Hz) in REM sleep.

number of arousals during REM sleep ($p \leq 0.026$, $\beta \geq 0.09$) (Supplementary Table S3, Fig. 1A), but this association did not reach experiment-wise stringent p-value correction for multiple comparisons. Contrary to our expectations, the PRSs for ID were not associated with SOL, WASO, and beta power during NREM sleep for any of the PRS p-value thresholds. Interestingly and in line with the literature (Rosinvil et al., 2021), NREM sleep SWE was negatively associated with the age covariate (Supplementary Table S3), in spite of the overall young age and limited age range of our sample. NREM sleep SWE was also associated with the sex covariate, with women generating less slow waves than men (Supplementary Table S3). This finding is discrepant with the literature (Rosinvil et al., 2021) and should be taken with caution given the underrepresentation of women in our sample.

Larger effects were found using PRSs with a threshold of $p = 1$ for SNPs in our primary analyses. Therefore, secondary analyses only used this PRS threshold. As a first secondary analysis, we assessed the specificity of our findings for the EEG frequency bands included in the primary analysis. We tested the associations for other slow rhythms in NREM and REM than in our primary analysis and considered theta power in NREM (rather than NREM SWE) and delta power in REM (rather than REM theta). We found that the PRS for ID was strongly associated with cumulated theta power during NREM sleep ($p = 0.002$, $\beta = -0.13$) and cumulated delta power during REM sleep ($p = 0.003$, $\beta = -0.13$), with p-values below correction threshold for 10 comparisons (i.e. $p < 0.005$; Fig. 2A, B). It appears therefore that higher PRS for ID is associated with lower power across a lower oscillatory mode ranging from 0.5 to 8 Hz both during NREM and REM sleep. The PRS for ID was also negatively associated with cumulated overnight sigma power (12-16 Hz) during NREM and REM sleep, but effects did not reach significance following correction for multiple comparisons (Fig. 2C, D) ($p = 0.01$, $\beta = -0.11$). In contrast, the PRS for ID was not associated with alpha and beta power during either NREM or REM sleep ($p > 0.25$; $\beta < 0.03$; Fig. 2E, F, G, H, Supplementary Table S5).

A second ancillary analysis assessed associations between the PRS and non-EEG sleep metrics, including actigraphy-assessed sleep quality, subjective sleep quality and daytime sleepiness, as well as scores on anxiety and depression questionnaires. The PRS for ID was not significantly associated with any of these metrics even at nominal $p < 0.05$ significance level (Supplementary Fig. S4A-E).

Subsequent exploratory analyses focussed on a large subsample ($N = 359$) of our dataset – only composed of men that participated in a 7-day long in-lab protocol including not only baseline sleep but also recordings

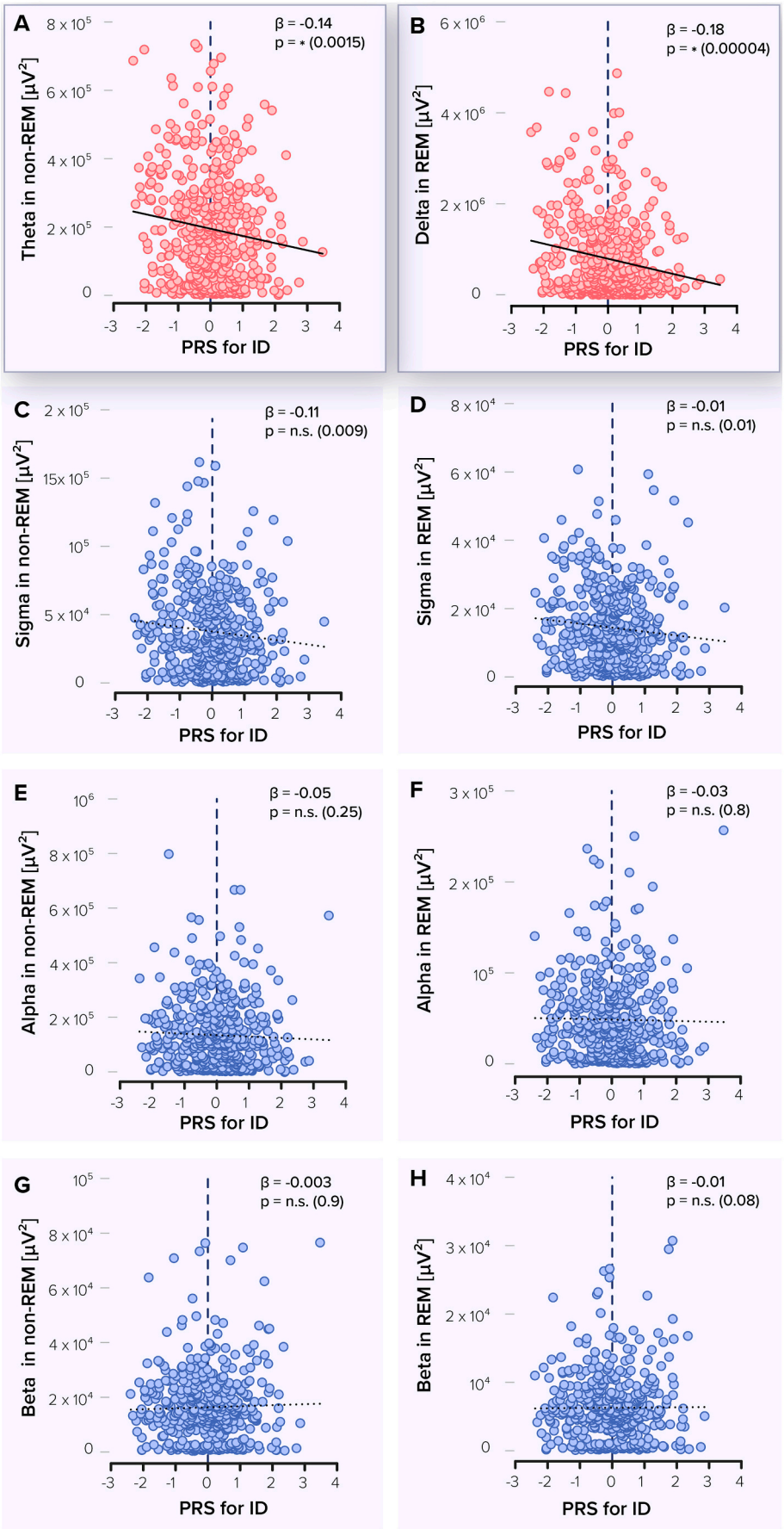

Fig. 2. Associations between PRS for ID and power in different frequency bands during baseline NREM sleep and REM sleep (N = 456).
GLM significant associations between PRS for ID and overnight cumulated power in (A) NREM sleep theta (4–8 Hz) (Spearman r = −0.11, p = .019*), (B) REM sleep delta (Spearman r = −0.13; p = .0045*).
GLM non-significant associations between PRS for ID and overnight cumulated power in (C) NREM sleep alpha (8-12 Hz; Spearman r = −0.069, p = .14), (D) REM sleep alpha (Spearman r = −0.031, p = .51), (E) NREM sleep sigma (12-16 Hz; Spearman r = −0.01; p = .026), (F) REM sleep sigma (Spearman r = −0.12; p = .013), (G) NREM sleep beta (16-25 Hz; Spearman r = −0.074, p = .13), and (H) REM sleep beta (Spearman r = −0.041, p = .38). Fitted trend lines are added for visualization purpose, and do not imply that the associations are significant.
PRS for ID (arbitrary units) was computed including SNPs with p-value threshold = 1 and z-scored. Spearman's correlations r are reported for completeness and do not substitute the statistical outputs of GLMs which are reported on the graphs and in the Supplementary Table S6 [* GLM significant association].

under three other sleep pressure conditions (sleep extension, recovery following total sleep deprivation, sleep following sleep satiation; as described in (Muto et al., 2021)). The PRS for ID was not significantly associated with either NREM sleep SWE or REM sleep theta assessed during altered sleep pressure conditions, even at nominal $p < 0.05$ significance level. Importantly however, data of the multiple sleep latency tests(Arand and Bonnet, 2019) that followed baseline sleep in this same 7-day protocol showed that the PRS for ID was negatively associated with the likelihood of falling asleep during these daytime sleep opportunities ($p = 0.0009$, $\beta = -0.19$; Fig. 3). Finally, the PRS for ID was not significantly associated with any of the cognitive measures spanning attentional, memory and executive function domains ($p > 0.05$) (Supplementary Table S6).

4. Discussion

In order to reveal sleep markers of insomnia vulnerability, we investigated whether genetic liability for ID is related to sleep metrics in a relatively large sample of polysomnographically assessed young individuals without ID complaints or comorbidities. In line with our hypothesis, we found that higher PRS for ID is associated with poorer sleep as indexed by reduced NREM sleep slow wave energy, i.e. the overnight cumulated power in the EEG delta band during NREM sleep (0.5-4 Hz), which reflects sleep intensity. Moreover, the association with slow EEG activity includes both delta and theta range in NREM as well as in REM sleep. The PRS for ID was specifically associated with these frequency bands, and not with cumulated power in the other frequency bands (i.e. 8-25 Hz) or sleep metrics not related to EEG power (WASO, SOL, arousal during REM). Overall, our findings indicate that the genetic vulnerability to insomnia involves either a reduced need or reduced ability to generate slow brain activity during sleep. Our study does not indicate, at least not in healthy young adults, a genetic contribution to the short sleep that has also been identified as a risk factor for developing ID (Fernandez-Mendoza et al., 2012).

The hyperarousal that is characteristic of ID is considered to be reflected by delta and theta power deficiency in NREM and REM sleep (Feige et al., 2013; Merica, 1998), which is reminiscent of the associations we found with the PRS for ID. Yet, ID hyperarousal has also been

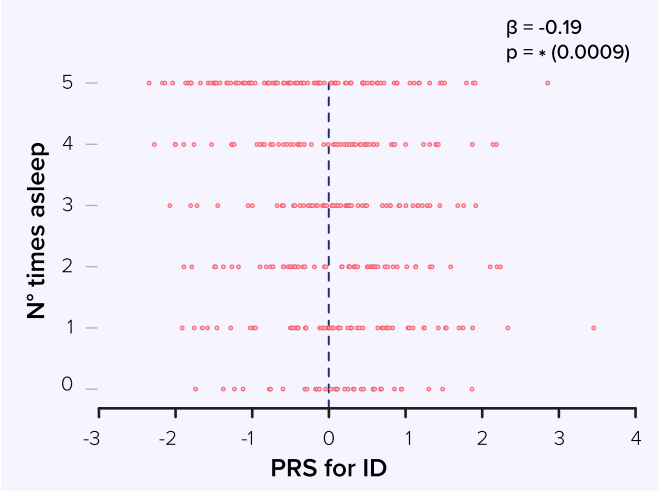

Fig. 3. Association between PRS for ID and multiple sleep latency test in a subsample ($N = 359$).
GLM significant negative association between PRS for ID and the number of times the individual fell asleep during daytime multiple sleep latency test (Spearman $r = -0.21$, $p = .00037$*).
PRS for ID (arbitrary units) was computed including SNPs with p-value threshold = 1 and z-scored. Spearman's correlation r is reported for completeness but does not substitute the statistical output of GLM which is reported on the graph and in the main text.

associated with enhanced beta frequency activity during sleep (Perlis et al., 2001), while we did not observe such an association with the PRS for ID. Based on our findings, we hypothesize that the cascade of developing ID primarily involves a genetic predisposition to reduced slow EEG activity during sleep and only secondarily the development of increased beta activity, for example emerging after experiencing precipitating factors (Spielman et al., 1987). The reduced intensity of slower brain activity during NREM and REM sleep may therefore mark a genetic contribution to the vulnerability of developing ID. How this genetic contribution relates to transcription, translation and epigenetic variations cannot be assessed as part of this study but would be of great interest.

It was suggested that hyperarousal may be especially problematic for REM sleep, as stability of REM sleep requires a delicate balance of arousing and de-arousing CNS mechanisms (Riemann et al., 2012). It has been specifically hypothesized that consolidated REM sleep is required to attain a prolonged state of locus coeruleus silencing and, consequentially, low norepinephrine, which facilitates appropriate memory trace adaptation (Swift et al., 2018). Meanwhile, the high limbic reactivation characteristic of REM sleep suggests replay of emotional activity. Synaptic plasticity taking place during low noradrenalin may thus facilitate favourable adaptation of emotional memory traces. In contrast, restless REM sleep may alter these synaptic processes and even result in sensitisation of emotional memory traces and consequentially hyperarousal (Wassing et al., 2019a, 2019b). The reduced REM theta power we observed in participants with a high PRS for ID may mark the vulnerability to restless REM sleep and its adverse emotional consequences. Indeed, lower REM theta (4-7 Hz) power spectral density has been observed in trauma-exposed persons who developed posttraumatic stress disorder compared with those who did not (Gazecki et al., 2018). Theta oscillations during REM sleep involve hippocampus, amygdala and neocortical activity and signal adaptation of emotional memories, including fear memory (Popa et al., 2010).

EEG slow waves, typically found in the delta frequency range during NREM sleep, are also involved in memory consolidation, and moreover provide a readout of homeostatic sleep pressure, which depends in part on the locus coeruleus (González et al., 1996). Slow waves are more prevalent at the beginning of the sleep episode and their intensity depends on the duration of prior wakefulness. The reduced overnight delta activity we found in participants with a high PRS for ID may reflect altered sleep homeostasis.

We also found that it was more difficult for participants with a higher PRS to fall asleep during daytime. This finding argues against the idea that the weaker expression of delta and theta during sleep in participants with a higher PRS for ID would signify an insufficient capacity to dissipate homeostatic sleep pressure, since this would result in a higher daytime sleep propensity. Rather, the findings converge to suggest a relatively specific contribution of the PRS for ID to the expression of slower EEG activity (delta and theta) and the ease of transitioning from wake to sleep. There was no association of the PRS for ID with subjective sleep quality or daytime sleepiness, nor with any of the cognitive tests we administered, which is in line with the fact that cognitive deficits are not that characteristic of ID (Goldman-Mellor et al., 2015).

5. Limitations of the study design and methodology

We acknowledge that our study bears some limitations. Exclusion criteria were rigorous and not common for large genetic case-control studies. The age range of our sample is limited to young individuals, while insomnia is more prevalent in older people. We also excluded ID patients during screening and thus we could not estimate the predicted value of our sample when in (Jansen et al., 2019) the PRS explained up to 2.6% of the variance in ID cases. This guarantees, however, that comorbidities related to aging or to ID do not bias our findings. It further provides argument in saying that we may have isolated the core associations between sleep electrophysiology variability and ID genetic risk.

A future study could include ID patients and focus on a larger sample. In addition, our sample mainly consisted of men, and the sub-group analyses were performed on men only, so potential sex differences could not be studied here. Furthermore, the reported associations may be not specific to ID and may relate to other psychiatric dimensions such as anxiety or depression, as their genetics highly correlate with ID (Zheng et al., 2022). We stress, however, that we excluded any diagnosed or treated depression or anxiety disorder from our sample of healthy individuals, while the sub-clinical variability in depression and anxiety scales we administered was not related to PRS for ID. Finally, while the method used here for PRS calculation that consists of clumping and *p*-value thresholding remains widely used, it tends to be superseded by new methods that model LD instead of filtering on it (e.g. LD pred (Vilhjalmsson et al., 2015)). Future studies should consider the LD modeling approach and also more recent GWAS on ID which identified more significant loci (e.g. (Watanabe et al., 2022)).

6. Conclusions

Using a PRS for ID computed from a large of GWAS (Jansen et al., 2019), we found the genetic risk to involve reduced abilities to express slow EEG activity during nocturnal sleep and to transition to sleep during daytime, which may be common to all ID subtypes. Our use of young and healthy individuals implies that our findings are not biased by comorbidities that are common in later life ID. Although current GWAS and therefore for PRSs derived from them only explain a limited part of the phenotypic variance of complex diseases (Dudbridge, 2013), our findings show that reduced abilities to express slow EEG activity during sleep and to transition to sleep may be at the core of ID and predisposition for ID.

Author contributions

Mrs. Koshmanova and Dr. Vandewalle had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Mrs. Koshmanova, Drs Muto, Collette, Georges, Maquet and Vandewalle.

Acquisition, analysis, or interpretation of data: all authors.

Drafting of the manuscript: Mrs. Koshmanova, Dr. Van Someren and Dr. Vandewalle.

Critical revision of the manuscript for important intellectual content: all authors.

Statistical analysis: Mrs. Koshmanova, Dr. Muto and Dr. Vandewalle.

Obtained funding: Drs Salmon, Luxen, Collette, Georges, Maquet, and Vandewalle.

Administrative, technical, or material support: Dr. Chylinski, Mrs. Reyt, M. Grignard, P.Talwar, M. Lambot, Drs Berthomier, Brandewinder, and Degueldre provided technical support; Dr. Salmon and Luxen provided administrative support.

Supervision: Dr. Vandewalle is the supervisor of Mrs. Koshmanova and Chylinski.

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Declaration of Competing Interest

C. Berthomier and M. Brandewinder have ownership and directorship of Physip and are employees of Physip who owns ASEEGA. This had no impact on study design and data analyses and interpretations. E. Koshmanova, V. Muto, D. Chylinski, C. Mouraux, M. Reyt, M. Grinard, P. Talwar, E. Lambot, N. Mortazavi, C. Degueldre, A. Luxen, E. Salmon, M. Georges, F. Collette, P. Maquet, E.Van Someren, and G. Vandewalle declare no conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2022.105924.

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