Title: Genome-wide Analysis of Insomnia (N=1,331,010) Identifies Novel Loci and Functional Pathways

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1 **Abstract**

2 Insomnia is the second-most prevalent mental disorder, with no sufficient treatment available.

3 Despite a substantial role of genetic factors, only a handful of genes have been implicated and

4 insight into the associated neurobiological pathways remains limited. Here, we use an

5 unprecedented large genetic association sample (N=1,331,010) to allow detection of a

6 substantial number of genetic variants and gain insight into biological functions, cell types

7 and tissues involved in insomnia complaints. We identify 202 genome-wide significant loci

8 implicating 956 genes through positional, eQTL and chromatin interaction mapping. We

9 show involvement of the axonal part of neurons, of specific cortical and subcortical tissues,

10 and of two specific cell-types in insomnia: striatal medium spiny neurons and hypothalamic

11 neurons. These cell-types have been implicated previously in the regulation of reward

12 processing, sleep and arousal in animal studies, but have never been genetically linked to

13 insomnia in humans. We found weak genetic correlations with other sleep-related traits, but

14 strong genetic correlations with psychiatric and metabolic traits. Mendelian randomization

15 identified causal effects of insomnia on specific psychiatric and metabolic traits. Our findings

16 reveal key brain areas and cells implicated in the neurobiology of insomnia and its related

17 disorders, and provide novel targets for treatment.

18 Insomnia is the second-most prevalent mental disorder¹. One third of the general population
19 reports insomnia complaints. The diagnostic criteria for Insomnia Disorder² (i.e. difficulties
20 with initiating or maintaining sleep with accompanying daytime complaints at least three
21 times a week for at least three months, which cannot be attributed to inadequate
22 circumstances for sleep³) are met by 10%, up to one third in samples of older age⁴. Insomnia
23 contributes significantly to the risk and severity of cardiovascular, metabolic, mood, and
24 neurodegenerative disorders². Despite evidence of a considerable genetic component
25 (heritability 38-59%⁵), only a small number of genetic loci moderating the risk of insomnia
26 have thus far been identified^{6,7}. Recent genome-wide association studies^{6,7} (GWAS) for
27 insomnia complaints (N=113,006) demonstrated its polygenic architecture and implicated
28 three genome-wide significant (GWS) loci and seven genes. A prominent role was reported
29 for *MEIS1*, which showed pleiotropic effects for insomnia complaints and restless legs
30 syndrome (RLS)⁷, yet the role of other genes was not unambiguously shown. We set out to
31 substantially increase the sample size to allow the detection of more genetic risk variants for
32 insomnia complaints, which may aid in understanding its neurobiological mechanisms. By
33 combining data collected in the UK Biobank v2⁸ (UKB; N=386,533) and 23andMe, Inc., a
34 personal genetics company^{9,10} (N=944,477), we obtained an unprecedented sample size of
35 1,331,010 individuals. Insomnia complaints were measured using questionnaire data, and the
36 specific questions were validated to be good proxies of insomnia disorder, using an
37 independent sample (The Netherlands Sleep Register, NSR)¹¹ in which we had access to
38 similar question data, as well as clinical interviews assessing insomnia disorder (see
39 **Supplementary Methods 1.1-1.3**). We find 202 risk loci for insomnia, and extensive
40 functional in silico analyses reveal the involvement of specific tissue and cell types, whereas
41 secondary statistical analyses reveal causal effects of insomnia on metabolic and psychiatric
42 traits.

43 **Meta-analysis yields 202 risk loci**

44 UKB assessed insomnia complaints (hereafter referred to as ‘insomnia’) using a touchscreen
 45 device while 23andMe research participants completed online surveys. Assessment of
 46 insomnia in both samples shows high accuracy (sensitivity=84-98%; specificity=80-96%) for
 47 Insomnia Disorder (see **Supplementary Methods 1.3**). The prevalence of insomnia in the
 48 UKBv2 sample was 28.3%, 30.5% in the 23andMe sample, and 29.3% in the combined
 49 sample, in keeping with previous estimates for people with advanced age in the UK⁴ and
 50 elsewhere^{12,13}. Older people dominate the UKB sample (mean age=56.7, SD=8.0) and the
 51 23andMe sample (two-thirds of the sample older than 45, one-third even older than 60 years
 52 of age). Prevalence was higher in females (34.6%) than males (24.5%), yielding an odds ratio
 53 (OR) of 1.6, close to the OR of 1.4 reported in a meta-analysis¹⁴.

54 Quality control was conducted separately per sample, following standardized, stringent
 55 protocols (see **Methods**). GWAS was run separately per sample (UKB; N=386,533,
 56 23andMe, Inc.; N=944,477) (**Extended Data Fig. 1**), and then meta-analyzed using
 57 METAL¹⁵ by weighing SNP effects by sample size (see **Methods**). We first analyzed males
 58 and females separately (**Extended Data Fig. 2, 3**), and observed a high genetic correlation
 59 between the sexes ($r_g=0.92$, SE=0.02, **Extended Data Table 1**), indicating strong overlap of
 60 genetic effects. Owing to the large sample size, the r_g of 0.92 was significantly different from
 61 1 (one-sided Wald test, $P=2.54 \times 10^{-6}$) suggesting a small role for sex-specific genetic risk
 62 factors, consistent with our previous report⁷. However, since sex-specific effects were
 63 relatively small, we here focus on identifying genetic effects important in both sexes and
 64 continued with the combined sample (**Supplementary Table 1, 2** and **Supplementary**
 65 **Discussion 2.1** provide sex-specific results).

66 We observe significant polygenic signal in the GWAS (lambda inflation factor=1.808) which
 67 could not be ascribed to spurious association (LD Score intercept=1.075)¹⁶ (**Extended Data**

68 **Fig. 4a**). Meta-analysis identified 11,990 genome-wide significant (GWS) SNPs ($P < 5 \times 10^{-8}$),
69 represented by 248 independent lead SNPs ($r^2 < 0.1$), located in 202 genomic risk loci (**Fig.**
70 **1a**, **Supplementary Fig. 1** and **Supplementary Table 3, 4**). All lead SNPs showed
71 concordant signs of effect in both samples (**Extended Data Fig. 4b**). We confirm two
72 (chr2:66,785,180 and chr5:135,393,752) out of six previously reported loci for insomnia ^{6,7}
73 (**Supplementary Table 5**). Polygenic score (PGS) prediction in three randomly selected
74 hold-out samples ($N = 3 \times 3,000$) estimated the current results to explain up to 2.6% of the
75 variance in insomnia (**Fig. 1b**, **Extended Data Fig. 5** and **Supplementary Table 6**).
76 The SNP-based heritability (h^2_{SNP}) was estimated at 7.0% ($SE = 0.002$). Partitioning the
77 heritability by functional categories of SNPs (see **Methods**) showed the strongest enrichment
78 of h^2_{SNP} in conserved regions (enrichment = 15.8, $P = 1.57 \times 10^{-14}$). In addition, h^2_{SNP} was
79 enriched in common SNPs ($MAF > 0.3$) and depleted in more rare SNPs ($MAF < 0.01$; **Fig. 1c**
80 and **Supplementary Table 7**).
81 We used FUMA¹⁷ to functionally annotate all 22,068 SNPs in the risk loci that were in LD
82 ($r^2 \geq 0.6$) with one of the independent significant SNPs (see **Methods**). The majority of these
83 SNPs (76.8%) were in open chromatin regions¹⁸ as indicated by a minimum chromatin state
84 of 1-7 (**Fig. 1d** and **Supplementary Table 8**). In line with findings for other traits^{7,19}, about
85 half of these SNPs were in intergenic (35.5%) or non-coding RNA (13.0%) regions (**Fig. 1e**),
86 and of these, 0.72% were highly likely to have a regulatory function as indicated by a
87 RegulomeDB Score < 2 (see **Methods**). However, of these 51.5% were located inside a
88 protein coding gene and 0.81% were exonic. Of the 177 exonic SNPs, 71 were exonic non-
89 synonymous (ExNS, **Supplementary Table 9**). *WDR90* included four ExNS (rs7190775,
90 rs4984906, rs3752493, and rs3803697) all in high LD with the same independent significant
91 SNP (rs3184470). There were two ExNS SNPs with extremely high Combined Annotation
92 Dependent Depletion (CADD) scores²⁰ suggesting a strong deleterious effect on protein

function: rs13107325 in *SLC39A8* (locus 56, $P=8.31 \times 10^{-16}$) with the derived allele T (MAF=0.03) associated with an increased risk of insomnia, and rs35713889 in *LAMB2* (locus 42, $P=1.77 \times 10^{-7}$), where the derived allele T of rs35713889 (MAF=0.11) was also associated with an increased risk of insomnia complaints. **Supplementary Table 10** and **Supplementary Discussion 2.2** provide a detailed overview of the functional impact of all variants in the genomic risk loci.

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100 Genes implicated in insomnia

To obtain insight into (functional) consequences of individual GWS SNPs we used FUMA¹⁷ to apply three strategies to map associated variants to genes (see **Methods**). Positional gene-mapping aligned SNPs to 412 genes by location. Expression Quantitative Trait Loci (eQTL) gene-mapping matched cis-eQTL SNPs to 594 genes whose expression levels they influence. Chromatin interaction mapping annotated SNPs to 159 genes based on three-dimensional DNA-DNA interactions between genomic regions of the GWS SNPs and nearby or distant genes (**Supplementary Fig. 2, Supplementary Table 11** and **Supplementary Discussion 2.3**). 91 genes were mapped by all three strategies (**Supplementary Table 12**) and 336 genes were physically located outside of the risk loci but were implicated by eQTL associations (306 genes), chromatin interactions (16 genes) or both (14 genes). Several genes were implicated by GWS SNPs originating from two distinct risk loci on the same chromosome (**Fig. 2a** and **2b**): *MEIS1*, located on chromosome 2 in the strongest associated locus (locus 20), was positionally mapped by 51 SNPs and mapped by chromatin interactions in 10 tissue types including cross-loci interactions from locus 21, and is a known gene involved in insomnia⁷. *LRGUK*, located on chromosome 7 in locus 106, was positionally mapped by 22 SNPs and chromatin interactions in 3 tissue types including cross-loci interactions from locus 105. *LRGUK* was also implicated by eQTLs associations of 125 SNPs in 14 general tissue

types. *LRGUK* was previously implicated in type 2 diabetes²¹ and autism spectrum disorder²² - disorders with prominent insomnia - but not yet directly implicated in sleep-related phenotypes, and is the most likely candidate to explain the observed association in loci 105 and 106.

Apart from linking individual associated genetic variants to genes, we conducted a genome-wide gene-based association analysis (GWGAS) using MAGMA²³. GWGAS provides aggregate association *P*-values based on all variants located in a gene, and complements the three FUMA mapping strategies (see **Methods**). GWGAS identified 517 associated genes (**Fig. 2c** and **Supplementary Table 13**). The top gene *BTBD9* ($P=8.51 \times 10^{-23}$) on chromosome 6 in locus 81 was also mapped by positional and eQTL mapping (tissue type: left ventricle of the heart), and is part of a pathway regulating circadian rhythms. *BTBD9* has been associated with RLS, periodic limb movement disorder^{24,25} and Tourette Syndrome²⁶. Involvement in sleep regulation was shown in *Drosophila*²⁷, and mouse mutants show fragmented sleep²⁸ and increased levels of dynamin 1²⁹, a protein that mediates the increased sleep onset latency following pre-sleep arousal³⁰.

Of the 517 MAGMA-based associated genes, 222 were outside of the GWAS risk loci, and 309 were also mapped by FUMA. In total, 956 unique genes were mapped by at least one of the three FUMA gene mapping strategies or by MAGMA (**Extended Data Fig. 6**). Of these, *MEIS1*, *MED27*, *IPO7* and *ACBD4* confirmed previous results^{6,7} (**Supplementary Table 14**). Sixty-two genes were implicated by all four mapping strategies indicating that apart from a GWS gene-based *P*-value, there were (i) GWS SNPs located inside these genes, (ii) GWS SNPs associated with differential expression of these genes and (iii) GWS SNPs that were involved in genomic regions interacting with these genes. We note that genes that were indicated by positional mapping and GWS gene-based *P*-values, but not via eQTL or chromatin interaction mapping (N=54 genes), may be of equal importance, yet are more

likely to exert their influence on insomnia via structural changes in the gene products (i.e. at the protein level) and not via quantitative changes in the availability of the gene products.

Implicated pathways, tissues and cell-types

To test whether GWS genes converged in functional gene-sets and pathways, we conducted gene-set analyses using MAGMA (see **Methods**). We tested associations of 7,473 gene-sets: 7,246 sets derived from the MsigDB³¹, gene expression values from 54 tissues from the GTEx database³², and cell-specific gene expression in 173 types of brain cells (**Fig. 2d**, **Supplementary Table 15**). Competitive testing was used and a Bonferroni corrected threshold of $P < 6.7 \times 10^{-6}$ ($0.05/7,473$) to correct for multiple testing. Of the MsigDB gene-sets, three Gene Ontology (GO) gene-sets survived multiple testing: GO:*locomotory behavior* ($P = 8.95 \times 10^{-7}$), GO:*behavior* ($P = 5.23 \times 10^{-6}$), and GO:*axon part* ($P = 4.25 \times 10^{-6}$). Twelve genes (*LRRK2*, *CRH*, *DLG4*, *DNM1*, *DRD1*, *DRD2*, *DRD4*, *GRIN1*, *NTSRI*, *SNCA*, *CNTN2*, and *CALBI*) were included in all of these gene-sets and two of these (*SNCA*, *DNM1*) had a GWS gene-based *P*-value (**Supplementary Table 16**). *SNCA* encodes alpha-synuclein and has been implicated in REM sleep behavior disorder³³ and Parkinson's disease³⁴. Altered expression in mice changes sleep and wake EEG spectra³⁵ along the same dimensions that have been implicated in insomnia disorder³⁶. *DNM1* encodes the synaptic neuronal protein dynamin 1, which is increased in *BTBD9* mutant mice²⁹ and mediates the sleep-disruptive effect of pre-sleep arousal (see above; *BTBD9* is the top associated gene). Conditional gene-set analyses suggested that the association with the gene-set *behavior* is almost completely explained by the association of *locomotory behavior*, and that the effect of *axon part* is independent of this (**Supplementary Discussion 2.4**). GO:*locomotory behavior* includes 175 genes involved in stimulus-evoked movement³⁷. This set included 16 GWS genes: *BTBD9*, *MEIS1*, *DABI*, *SNCA*, *GNAO1*, *ATP2B2*, *NEGR1*, *SLC4A10*, *GIP*, *DNM1*, *GPRC5B*, *GRM5*,

NRG1, *PARK2*, *TAL1*, and *OXR1*). GO:axon part reflects a very general cellular component representing 219 genes, of which 14 were GWS (*KIF3B*, *SNCA*, *GRIA1*, *CDH8*, *ROBO2*, *DNM1*, *RANGAP1*, *GABBR1*, *P2RX3*, *NRG1*, *POLG*, *DAG1*, *NFASC*, and *CALB2*).

Tissue specific gene-set analyses showed strong enrichment of genetic signal in genes expressed in the brain. Correcting for overall expression, four specific brain tissues reached the threshold for significance: overall cerebral cortex ($P=3.68 \times 10^{-6}$), Brodmann area 9 (BA9) of frontal cortex ($P=5.04 \times 10^{-7}$), BA24 of the anterior cingulate cortex ($P=3.25 \times 10^{-6}$), and cerebellar hemisphere ($P=5.93 \times 10^{-6}$)¹. Several other brain tissues also showed strong enrichment just below threshold, including three striatal basal ganglia (BG) structures (nucleus accumbens, caudate nucleus, putamen). To test whether genes expressed in all three BG structures together would show significant enrichment of low P -values, we used the first principal component (BG_{PC}) of these BG structures and found significant enrichment ($P=8.33 \times 10^{-8}$). When conditioning the three top cortical structures on the BG_{PC}, they were no longer significantly associated after multiple testing correction (minimum $P=0.03$), which was expected given that the BG_{PC} correlated strongly with gene-expression in cortical (and other) areas ($r>0.96$). Similar results were obtained vice versa, i.e. using the first principal component of all cortical areas and conditioning the three BG structures on this resulted in no evidence of enrichment of low P -values for BG structures (minimum $P=0.53$). These results show that (i) genes expressed in brain are important in insomnia, (ii) genes expressed in cortical areas are more strongly associated than genes expressed in BG, (iii) there is a strong correlation between gene expression patterns across brain tissues, which suggests involvement of general cellular signatures more than specific brain tissue structures.

¹ We caution that only a limited set of brain tissues were included and thus we cannot rule out associations with many important areas such as pons, midbrain or thalamus based on this analysis.

Brain cell type-specific gene-set analyses was first carried out on 24 broad cell-type categories. Cell type-specific gene expression was quantified using single cell RNA-sequencing of dissociated cells from somatosensory cortex, hippocampus, hypothalamus, striatum and midbrain from mouse (see **Methods**), which closely resembles gene-expression in humans³⁸. Results indicated that genes expressed specifically in the medium spiny neurons (MSN, $P=4.83 \times 10^{-7}$) were associated with insomnia, and no other broad cell-types specific gene-set survived our strict threshold of $P < 6.7 \times 10^{-6}$. MSNs represent 95% of neurons within the human striatum, which is one of the four major nuclei of the subcortical BG. Specifically, the striatum consists of the ventral (nucleus accumbens and olfactory tubercle) and dorsal (caudate nucleus and putamen) subdivisions. The association with MSNs thus likely explains the observed association of the BG striatal structures (nucleus accumbens, caudate nucleus, putamen).

Using broad cell classes risks not detecting associations that are specific to distinctive yet rare cell types; to account for this we then tested 149 specific brain cell-type categories, and found significant associations with 7 specific cell types: medio-lateral neuroblasts type 3, 4 and 5 ($P=2.36 \times 10^{-6}$, $P=1.88 \times 10^{-6}$, and $P=1.87 \times 10^{-6}$), D2 type medium spiny neurons ($P=2.12 \times 10^{-6}$), claustrum pyramidal neurons ($P=3.09 \times 10^{-6}$), hypothalamic Vglut2 Morn4 Prrc2a neurons ($P=4.36 \times 10^{-6}$), and hypothalamic Vglut2 Hcn16430411 K18 Rik neurons ($P=4.98 \times 10^{-6}$), known to have the densest number of melatonin receptors. These results suggest a role of distinct mature and developing cell types in the midbrain and hypothalamus. The hypothalamus contains multiple nuclei that are key to the control of sleep and arousal, including the suprachiasmatic nucleus (SCN) that accommodates the biological clock of the brain³⁹.

Low genetic overlap with sleep traits

Other sleep-related traits may easily be confounded with specific symptoms of insomnia, like early morning awakening, difficulties maintaining sleep, and daytime sleepiness. The most recent genome-wide studies for other sleep-related traits included 59,128 to 128,266 individuals, and assessed genetic effects on morningness^{6,40,41} (i.e. being a morning person), sleep duration^{6,41}, and daytime sleepiness/dozing⁴¹. Using increased sample sizes for each of these sleep-related traits (max N=434,835), we here investigated to what extent insomnia and other sleep-related traits are genetically distinct or overlapping. We performed GWAS analyses for the following six sleep-related traits: morningness, sleep duration, ease of getting up in the morning, naps during the day, daytime dozing, and snoring (**Supplementary Methods 1.1-1.2, Extended Data Fig. 7, 9**). Of the 202 risk loci for insomnia, 39 were also GWS in at least one of the other sleep-related traits (**Fig. 3, Supplementary Table 17**). The strongest overlap in loci was found with sleep duration, with 14 out of 49 sleep duration loci overlapping with insomnia. Insomnia showed the highest genetic correlation with sleep duration (-0.47 , $SE=0.02$; **Supplementary Table 18**) compared to other sleep-related traits, which was not surprising given that insomnia also shared the most risk loci with sleep duration (See further discussion sleep phenotypes in **Supplementary Discussion 2.5**).

Gene-mapping of SNP associations of sleep-related traits resulted in 973 unique genes (**Extended Data Fig. 9, Supplementary Table 19-23**). Gene-based analysis showed that of the 517 GWS genes for insomnia, 120 were GWS in at least one of the other sleep-related traits, and one gene (*RBFOX1*) was GWS in all except napping and dozing (**Supplementary Table 24**). The largest proportion of overlap in GWS genes for insomnia was again with sleep duration, with 37 of the 135 (27%) GWS genes for sleep duration also GWS for insomnia. There was overlap in tissue enrichment in cortical structures and basal ganglia between insomnia and both morningness and sleep duration. On the single cell level, the

medium spiny neurons were also implicated for morningness and sleep duration, but not for the other sleep-related traits (**Supplementary Table 25**). Taken together, these results suggest that at a genetic level, insomnia shows partial overlap with sleep duration, but minimal overlap with other sleep-related traits. Consistent short sleep across nights occurs only in a minor part of insomnia patients, even in a clinical sample⁴².

Strong overlap between insomnia and psychiatric and metabolic traits

We confirm previously reported genetic correlations between insomnia and neuropsychiatric and metabolic traits^{6,7} (**Supplementary Table 26**), and also identify several GWS SNPs for insomnia that have previously been associated with these traits (**Supplementary Table 27**).

The strongest correlations were with depressive symptoms ($r_g=0.64$, $SE=0.04$ $P=1.21\times 10^{-71}$), followed by anxiety disorder ($r_g=0.56$, $SE=0.11$ $P=1.40\times 10^{-7}$), subjective well-being ($r_g=-0.51$, $SE=0.03$ $P=4.93\times 10^{-52}$), major depression ($r_g=0.50$, $SE=0.07$ $P=8.08\times 10^{-12}$) and neuroticism ($r_g=0.48$, $SE=0.02$ $P=8.72\times 10^{-80}$). Genetic correlations with metabolic traits ranged between 0.09-0.20. The genetic correlations between insomnia and psychiatric traits were also stronger than the correlations between insomnia and the other sleep-related traits. Since a similar high reliability has been reported for both sleep and psychiatric phenotypes, the findings suggest that genetically insomnia more closely resembles neuropsychiatric traits than it resembles other sleep-related traits (**Fig. 4**). To infer directional associations between insomnia and these correlated traits, we performed bidirectional Multi-SNP Mendelian Randomization (MR) analysis⁴³ (see **Methods**). Results support a direct risk effect of insomnia on metabolic syndrome phenotypes including BMI ($b_{xy}=0.36$, $SE=0.05$, $P=1.25\times 10^{-12}$) type 2 diabetes ($b_{xy}=0.62$, $SE=0.11$, $P=2.29\times 10^{-8}$), and coronary artery disease ($b_{xy}=0.61$, $SE=0.09$, $P=2.88\times 10^{-12}$). In addition, insomnia was bidirectionally associated with educational attainment, with a stronger effect from insomnia on educational attainment

($b_{xy}=-0.32$, $SE=0.02$, $P=1.68\times 10^{-77}$) (i.e. a higher risk for insomnia leads to lower educational attainment) than vice versa ($b_{xy}=-0.10$, $SE=0.01$, $P=2.27\times 10^{-23}$), the same pattern was observed for intelligence. We also found risk effects of insomnia on several psychiatric traits, including schizophrenia ($b_{xy}=0.68$, $SE=0.10$, $P=5.12\times 10^{-11}$), ADHD ($b_{xy}=0.77$, $SE=0.06$, $P=2.50\times 10^{-45}$), neuroticism ($b_{xy}=0.46$, $SE=0.03$, $P=3.92\times 10^{-53}$) and anxiety disorder ($b_{xy}=0.47$, $SE=0.10$, $P=4.11\times 10^{-6}$), with no evidence of large reverse effects, except for a small risk effect of neuroticism on insomnia ($b_{xy}=0.09$, $SE=0.02$, $P=1.24\times 10^{-6}$) and depressive symptoms ($b_{xy}=0.09$, $SE=0.02$, $P=1.24\times 10^{-6}$)². Overall, there was only a small proportion of SNPs showing pleiotropy between insomnia and other traits (**Supplementary Table 28** and **Supplementary Discussion 2.6**).

Discussion

In the largest GWAS study to date of 1,331,010 participants we identified 202 genomic risk loci for insomnia. Using extensive functional annotation of associated genetic variants, we demonstrated that the genetic component of insomnia points towards a role of genes involved in locomotory behavior, and genes expressed in specific cell types from the claustrum, hypothalamus and striatum, and specifically in MSNs (**Fig. 5**). MSNs are GABAergic inhibitory cells and represent 95% of neurons in the human striatum, one of the four major nuclei of the BG (for reviews, see ⁴⁴⁻⁴⁶). MSNs receive massive excitatory glutamatergic input from the cerebral cortex and the thalamus, and are targets of dopamine neurons in substantia nigra and the ventral tegmental area. In addition, they receive inhibitory inputs from striatal GABAergic interneurons. MSNs themselves are GABAergic output neurons with exceptionally long projections to globus pallidus (GP), substantia nigra and ventral

² We do note that for major depression the reverse MR could not be carried out due to an insufficient number of SNPs with a low P -value

pallidum, and control the activity of thalamocortical neurons. Previous studies during the natural sleep-wake cycle, *in vitro*, and from anesthetized *in vivo* preparations have shown that MSNs show fast, synchronized cyclic firing, i.e. the so-called Up and Down states, during slow-wave sleep and irregular pattern of action potentials during wakefulness. In fact, MSNs were the first neurons in which the Up and Down states characteristic of slow wave sleep were described⁴⁷. Cell body-specific striatal lesions of the rostral striatum induce a profound sleep fragmentation, which is most characteristic of insomnia. A role for BG in sleep regulation is also suggested by the high prevalence of insomnia in neurodegenerative disorders, such as Parkinson's Disease and Huntington's disease in which the BG are affected. Vetrivelan et al.⁴⁴ proposes a cortex-striatum-GP_{external}-cortex network involved in the control of sleep-wake behavior and cortical activation, in which midbrain dopamine disinhibits the GP_{external} and promotes sleep through activation of D2 receptors in this network. Furthermore, brain imaging studies have suggested the caudate nucleus of the striatum as a key node in the neuronal network imbalance of insomnia⁴⁸, and also reported abnormal function in the cortical areas we found to be most enriched (BA9⁴⁹, BA24⁵⁰). Our results support the involvement of the striato-cortical network in insomnia, by showing enrichment of risk genes for insomnia in cortical areas as well as the striatum, and specifically in MSNs. We recently showed that, along with several other cell types, MSNs also mediate the risk for mood disorders⁵¹ and schizophrenia³⁸. MSNs are strongly implicated in reward processing and future work could address whether the genetic overlap between insomnia and mood disorders is mediated by gene function in MSNs.

Our results also showed enrichment of insomnia genes in pyramidal neurons of the claustrum. This subcortical brain region is structurally closely associated with the amygdala and has been implicated in salience coding of incoming stimuli and binding of multisensory information into conscious percepts⁵². These functions are highly relevant to insomnia,

because the disorder is characterized by increased processing of incoming stimuli⁵³ and by ongoing consciousness even during sleep, a phenomenon known as sleep state misperception⁵⁴. We also found enrichment of insomnia genes in mediolateral neuroblasts from the embryonic midbrain and in two hypothalamic cell types. The role of the mediolateral neuroblasts is less clear; although they were obtained from the embryonic midbrain, it is at present unknown what type of mature neurons they differentiate into. We note that the midbrain is similar on a bulk transcriptomic level to the pons⁵⁵, and lacking cells from that region we cannot conclusively say that midbrain cell-types are enriched.

The current findings provide novel insight into the causal mechanism of insomnia, implicating specific cell types, brain areas and biological functions. These findings are starting points for the development of new therapeutic targets for insomnia and may also provide valuable insights for other, genetically related disorders.

Methods:

Meta-analysis

A meta-analysis on the GWAS results of insomnia and morningness in UKB and 23andMe cohorts was performed using fixed-effects meta-analysis METAL¹⁵, using SNP *P*-values weighted by sample size. To investigate sex-specific genetic effects, we ran the meta-analysis between UKB and 23andMe datasets for males and females separately.

Genomic risk loci definition

We used FUMA¹⁷ (<http://fuma.ctglab.nl/>), an online platform for functional mapping and annotation of genetic variants, to define genomic risk loci and obtain functional information of relevant SNPs in these loci. FUMA provides comprehensive annotation information by combining several external data sources. We first identified *independent significant SNPs* that have a genome-wide significant *P*-value ($<5 \times 10^{-8}$) and are independent from each other at $r^2 < 0.6$. These SNPs were further represented by *lead SNPs*, which are a subset of the independent significant SNPs that are in approximate linkage equilibrium with each other at $r^2 < 0.1$. We then defined associated *genomic risk loci* by merging any physically overlapping lead SNPs (linkage disequilibrium [LD] blocks $< 250\text{kb}$ apart). Borders of the genomic risk loci were defined by identifying all SNPs in LD ($r^2 \geq 0.6$) with one of the independent significant SNPs in the locus, and the region containing all these *candidate SNPs* was considered to be a single independent genomic risk locus. LD information was calculated using the UK Biobank genotype data as a reference. Risk loci were defined based on evidence from independent significant SNPs that were available in both 23andMe and UKB. We note that SNPs that were GWS but only available in the 23andMe dataset were not included when defining genomic risk loci and were not included in any follow-up annotations or analyses, because there was no external replication in the UKB sample. If such SNPs were

located in a risk locus, they are displayed in Locuszoom plots (grey, as there is no LD information in UKB). When risk loci contained GWS SNPs based solely on 23andMe, we did not count that risk locus, as there were no other SNPs available in both samples that supported these GWS SNPs.

Gene-based analysis

SNP-based *P*-values from the meta-analysis were used as input for the gene-based genome-wide association analysis (GWGAS). 18,182 to 18,185 protein-coding genes (each containing at least one SNP in the GWAS, the total number of tested genes can thus be slightly different across phenotypes) from the NCBI 37.3 gene definitions were used as basis for GWGAS in MAGMA²³. Bonferroni correction was applied to correct for multiple testing ($P < 2.73 \times 10^{-6}$).

Gene-set analysis

Results from the GWGAS analyses were used to test for association in three types of 7,473 predefined gene-sets:

1. 7,246 curated gene-sets representing known biological and metabolic pathways derived from 9 data resources, catalogued by and obtained from the MsigDB version 6.0⁵⁶ (<http://software.broadinstitute.org/gsea/msigdb/collections.jsp>)
2. Gene expression values from 54 (53 + 1 calculated 1st PC of three tissue subtypes) tissues obtained from GTEx³², log2 transformed with pseudocount 1 after winsorization at 50 and averaged per tissue
3. Cell-type specific expression in 173 types of brain cells (24 broad categories of cell types, 'level 1' and 129 specific categories of cell types 'level 2'), which were calculated following the method described in³⁸. Briefly, brain cell-type expression data was drawn from single-cell RNA sequencing data from mouse brains. For each

gene, the value for each cell-type was calculated by dividing the mean Unique Molecular Identifier (UMI) counts for the given cell type by the summed mean UMI counts across all cell types. Single-cell gene-sets were derived by grouping genes into 40 equal bins based on specificity of expression. Mouse cell gene-expression was shown to closely approximate gene-expression in post-mortem human tissue³⁸. These gene-sets were tested using MAGMA. We computed competitive *P*-values, which represent the test of association for a specific gene-set compared with genes not in the gene-set to correct for baseline level of genetic association in the data⁵⁷. The Bonferroni-corrected significance threshold was $0.05/7,473 \text{ gene-sets} = 6.7 \times 10^{-6}$. Conditional analyses were performed as a follow-up using MAGMA to test whether each significant association observed was independent of all others. The association between each gene-set in each of the three categories was tested conditional on the most strongly associated set, and then, if any substantial ($P < 0.05/\text{number of gene-sets}$) associations remained, by conditioning on the first and second most strongly associated set, and so on until no associations remained. Gene-sets that retained their association after correcting for other sets were considered to represent independent signals. We note that this is not a test of association per se, but rather a strategy to identify, among gene-sets with known significant associations and overlap in genes, which set (s) are responsible for driving the observed association.

SNP-based heritability and genetic correlation

LD Score regression¹⁶ was used to estimate genomic inflation and SNP-based heritability of the phenotypes, and to estimate the cross-cohort genetic correlations. Pre-calculated LD scores from the 1000 Genomes European reference population were obtained from <https://data.broadinstitute.org/alkesgroup/LDSCORE/>.

Genetic correlations

Genetic correlations between sleep-related traits, and between sleep-related traits and previously published GWAS studies of sufficient sample size were calculated using LD Score regression on HapMap3 SNPs only. Genetic correlations were corrected for multiple testing based on the total number of correlations (between 6 sleep-related phenotypes and 27 previous GWAS studies) by applying a Bonferroni corrected threshold of ($P < 0.05/33 = 1.51 \times 10^{-3}$).

Stratified heritability

To test whether specific categories of SNP annotations were enriched for heritability, we partitioned SNP heritability for binary annotations using stratified LD score regression⁵⁸. Heritability enrichment was calculated as the proportion of heritability explained by a SNP category divided by the proportion of SNPs that are in that category. Partitioned heritability was computed by 28 functional annotation categories, by minor allele frequency (MAF) in six percentile bins and by 22 chromosomes. Annotations for binary categories of functional genomic characteristics (e.g. coding or regulatory regions) were obtained from the LD score website (<https://github.com/bulik/ldsc>). The Bonferroni-corrected significance threshold for 56 annotations was set at: $P < 0.05/56 = 8.93 \times 10^{-4}$.

Functional annotation of SNPs

Functional annotation of SNPs implicated in the meta-analysis was performed using FUMA¹⁷. We selected all candidate SNPs in genomic risk loci having an $r^2 \geq 0.6$ with one of the independent significant SNPs (see above), a P -value ($P < 1 \times 10^{-5}$), a $MAF > 0.0001$ for annotations, and availability in both UKB and 23andMe datasets. Functional consequences

for these SNPs were obtained by matching SNPs' chromosome, base-pair position, and reference and alternate alleles to databases containing known functional annotations, including ANNOVAR⁵⁹ categories, Combined Annotation Dependent Depletion (CADD) scores, RegulomeDB²⁰ (RDB) scores, and chromatin states⁶⁰. ANNOVAR categories identify the SNP's genic position (e.g. intron, exon, intergenic) and associated function. CADD scores predict how deleterious the effect of a SNP is likely to be for a protein structure/function, with higher scores referring to higher deleteriousness. A CADD score above 12.37 is considered to be potentially pathogenic²⁰. The RegulomeDB score is a categorical score based on information from expression quantitative trait loci (eQTLs) and chromatin marks, ranging from 1a to 7 with lower scores indicating an increased likelihood of having a regulatory function. Scores are as follows: 1a=eQTL + Transcription Factor (TF) binding + matched TF motif + matched DNase Footprint + DNase peak; 1b=eQTL + TF binding + any motif + DNase Footprint + DNase peak; 1c=eQTL + TF binding + matched TF motif + DNase peak; 1d=eQTL + TF binding + any motif + DNase peak; 1e=eQTL + TF binding + matched TF motif; 1f=eQTL + TF binding / DNase peak; 2a=TF binding + matched TF motif + matched DNase Footprint + DNase peak; 2b=TF binding + any motif + DNase Footprint + DNase peak; 2c=TF binding + matched TF motif + DNase peak; 3a=TF binding + any motif + DNase peak; 3b=TF binding + matched TF motif; 4=TF binding + DNase peak; 5=TF binding or DNase peak; 6=other; 7=Not available. The chromatin state represents the accessibility of genomic regions (every 200bp) with 15 categorical states predicted by a hidden Markov model based on 5 chromatin marks for 127 epigenomes in the Roadmap Epigenomics Project⁶¹. A lower state indicates higher accessibility, with states 1-7 referring to open chromatin states. We annotated the minimum chromatin state across tissues to SNPs. The 15-core chromatin states as suggested by Roadmap are as follows: 1=Active Transcription Start Site (TSS); 2=Flanking Active TSS; 3=Transcription at gene 5' and 3';

4=Strong transcription; 5= Weak Transcription; 6=Genic enhancers; 7=Enhancers; 8=Zinc finger genes & repeats; 9=Heterochromatic; 10=Bivalent/Poised TSS; 11=Flanking Bivalent/Poised TSS/Enh; 12=Bivalent Enhancer; 13=Repressed PolyComb; 14=Weak Repressed PolyComb; 15=Quiescent/Low.

Gene-mapping

Genome-wide significant loci obtained by GWAS were mapped to genes in FUMA¹⁷ using three strategies:

1. Positional mapping maps SNPs to genes based on physical distance (within a 10kb window) from known protein coding genes in the human reference assembly (GRCh37/hg19).

2. eQTL mapping maps SNPs to genes with which they show a significant eQTL association (i.e. allelic variation at the SNP is associated with the expression level of that gene). eQTL mapping uses information from 45 tissue types in 3 data repositories (GTEx³², Blood eQTL browser⁶⁰, BIOS QTL browser⁶²), and is based on cis-eQTLs which can map SNPs to genes up to 1Mb apart. We used a false discovery rate (FDR) of 0.05 to define significant eQTL associations.

3. Chromatin interaction mapping was performed to map SNPs to genes when there is a three-dimensional DNA-DNA interaction between the SNP region and another gene region. Chromatin interaction mapping can involve long-range interactions as it does not have a distance boundary. FUMA currently contains Hi-C data of 14 tissue types from the study of Schmitt et al⁶³. Since chromatin interactions are often defined in a certain resolution, such as 40kb, an interacting region can span multiple genes. If a SNP is located in a region that interacts with a region containing multiple genes, it will be mapped to each of those genes. To further prioritize candidate genes, we selected only interaction-mapped genes in which

one region involved in the interaction overlaps with a predicted enhancer region in any of the 111 tissue/cell types from the Roadmap Epigenomics Project⁶¹, and the other region is located in a gene promoter region (250bp up and 500bp downstream of the transcription start site and also predicted by Roadmap to be a promoter region). This method reduces the number of genes mapped but increases the likelihood that those identified will indeed have a plausible biological function. We used a $P\text{-FDR} < 1 \times 10^{-5}$ to define significant interactions, based on previous recommendations⁶³, modified to account for the differences in cell lines used here.

GWAS catalog lookup

We used FUMA to identify SNPs with previously reported ($P < 5 \times 10^{-5}$) phenotypic associations in published GWAS listed in the NHGRI-EBI catalog⁶⁴, which matched with SNPs in LD with one of the independent significant SNPs identified in the meta-analysis.

Polygenic risk scoring

To calculate the explained variance in insomnia by our GWAS results, we calculated polygenic scores (PGS) based on the SNP effect sizes in the meta-analysis. The PGS were calculated using two methods: LDpred⁶⁵ and PRSice⁶⁶, a script for calculating P -value thresholded PGS in PLINK. PGS were calculated using a leave-one-out method, where summary statistics were recalculated each time with one sample of $N=3,000$ from UKB excluded from the analysis. This sample was then used as a target sample for estimating the explained variance in insomnia by the PGS.

Mendelian Randomization

To investigate causal associations between insomnia and genetically correlated traits, we analyzed direction of effects using Generalized Summary-data based Mendelian Randomization (GSMR⁴³; <http://cnsgenomics.com/software/gsmr/>). This method uses effect sizes from GWAS summary statistics (standardized betas or log-transformed odds ratios) to infer causality of effects between two traits based on genome-wide significant SNPs. Built-in HEIDI outlier detection was applied to remove SNPs with pleiotropic effects on both traits, as these may bias the results. We tested for causal associations between insomnia and traits that were significantly genetically correlated with insomnia (b_{zx}). In addition, we tested for bi-directional associations by using other traits as the determinant and insomnia as the outcome (b_{zy}). We selected independent ($r^2 < 0.1$) lead SNPs with a GWS P -value ($< 5 \times 10^{-8}$) as instrumental variables in the analyses. For traits with less than 10 lead SNPs (i.e. the minimum number of SNPs on which GSMR can perform a reliable analysis) we selected independent SNPs ($r^2 < 0.1$), with a P -value $< 1 \times 10^{-5}$. If the outcome trait is binary, the estimated b_{zx} and b_{zy} are approximately equal to the natural log of the odds ratio (OR). An OR of 2 can be interpreted as a doubled risk compared to the population prevalence of a binary trait for every SD increase in the exposure trait. For quantitative traits, the b_{zx} and b_{zy} can be interpreted as a one standard deviation increase explained in the outcome trait for every SD increase in the exposure trait.

References:

1. Wittchen, H.-U. *et al.* The size and burden of mental disorders and other disorders of the brain in europe 2010. *Eur. Neuropsychopharmacol.* **21**, 655-679 (2011).
2. Morin, C.M. *et al.* Insomnia disorder. *Nat. Rev. Dis. Primers* **1**, 15026-15026 (2015).
3. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders (DSM-5®)*, (American Psychiatric Pub, 2013).
4. Morphy, H., Dunn, K.M., Lewis, M., Boardman, H.F. & Croft, P.R. Epidemiology of insomnia: A longitudinal study in a uk population. *Sleep* **30**, 274-280 (2007).
5. Lind, M.J., Aggen, S.H., Kirkpatrick, R.M., Kendler, K.S. & Amstadter, A.B. A longitudinal twin study of insomnia symptoms in adults. *Sleep* **38**, 1423-1430 (2015).
6. Lane, J.M. *et al.* Genome-wide association analysis identifies novel loci for chronotype in 100,420 individuals from the uk biobank. *Nat. Comm.* **7**, 10889 (2016).
7. Hammerschlag, A. *et al.* Genome-wide association analysis of insomnia identifies novel risk genes and genetic overlap with psychiatric and metabolic traits. *Nat. Genet.* **49**, 1584 (2017).
8. Sudlow, C. *et al.* Uk biobank: An open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Medicine* **12**, e1001779 (2015).
9. Eriksson, N. *et al.* Web-based, participant-driven studies yield novel genetic associations for common traits. *PLoS Genetics* **6**, e1000993 (2010).
10. Tung, J.Y. *et al.* Efficient replication of over 180 genetic associations with self-reported medical data. *PLoS One* **6**, e23473 (2011).
11. Benjamins, J.S. *et al.* Insomnia heterogeneity: Characteristics to consider for data-driven multivariate subtyping. *Sleep Med. Rev.* **36**, 71-81 (2017).
12. Paparrigopoulos, T. *et al.* Insomnia and its correlates in a representative sample of the greek population. *BMC Public Health* **10**, 531 (2010).
13. Cho, Y.W. *et al.* Epidemiology of insomnia in korean adults: Prevalence and associated factors. *Jour. Clin. Neurology* **5**, 20-23 (2009).
14. Zhang, B. & Wing, Y.-K. Sex differences in insomnia: A meta-analysis. *Sleep* **29**, 85-93 (2006).
15. Willer, C.J., Li, Y. & Abecasis, G.R. Metal: Fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26**, 2190-2191 (2010).
16. Bulik-Sullivan, B.K. *et al.* Ld score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat. Genet.* **47**, 291-295 (2015).
17. Watanabe, K., Taskesen, E., Bochoven, A. & Posthuma, D. Functional mapping and annotation of genetic associations with fuma. *Nat. Comm.* **8**, 1826 (2017).
18. Ernst, J. & Kellis, M. Chromhmm: Automating chromatin-state discovery and characterization. *Nat. Methods* **9**, 215-216 (2012).
19. Sniekers, S. *et al.* Genome-wide association meta-analysis of 78,308 individuals identifies new loci and genes influencing human intelligence. *Nat. Genet.* **49**, 1107-1112 (2017).
20. Kircher, M. *et al.* A general framework for estimating the relative pathogenicity of human genetic variants. *Nat. Genet.* **46**, 310-315 (2014).
21. Laramie, J.M. *et al.* Polymorphisms near exoc4 and lrguk on chromosome 7q32 are associated with type 2 diabetes and fasting glucose; the nhlbi family heart study. *BMC Med. Genet.* **9**, 46 (2008).
22. Butler, M.G., Rafi, S.K. & Manzardo, A.M. High-resolution chromosome ideogram representation of currently recognized genes for autism spectrum disorders. *Int. J. Mol. Sci.* **16**, 6464-6495 (2015).

- 567 23. de Leeuw, C.A., Mooij, J.M., Heskes, T. & Posthuma, D. Magma: Generalized gene-
568 set analysis of gwas data. *PLoS Computational Biology* **11**, e1004219 (2015).
- 569 24. Kripke, D.F. *et al.* Genetic variants associated with sleep disorders. *Sleep Med.* **16**,
570 217-224 (2015).
- 571 25. Stefansson, H. *et al.* A genetic risk factor for periodic limb movements in sleep. *N.*
572 *Engl. J. Med.* **357**, 639-647 (2007).
- 573 26. Janik, P., Berdyński, M., Safranow, K. & Żekanowski, C. The btbd9 gene
574 polymorphisms in polish patients with gilles de la tourette syndrome. *Acta Neurobiol.*
575 *Exp. (Wars.)* **74**, 218-226 (2014).
- 576 27. Freeman, A. *et al.* Sleep fragmentation and motor restlessness in a drosophila model
577 of restless legs syndrome. *Curr. Biol.* **22**, 1142-1148 (2012).
- 578 28. DeAndrade, M.P. *et al.* Motor restlessness, sleep disturbances, thermal sensory
579 alterations and elevated serum iron levels in btbd9 mutant mice. *Hum. Mol. Genet.* **21**,
580 3984-3992 (2012).
- 581 29. DeAndrade, M.P. *et al.* Enhanced hippocampal long-term potentiation and fear
582 memory in btbd9 mutant mice. *PLoS One* **7**, e35518 (2012).
- 583 30. Suzuki, A., Sinton, C.M., Greene, R.W. & Yanagisawa, M. Behavioral and
584 biochemical dissociation of arousal and homeostatic sleep need influenced by prior
585 wakeful experience in mice. *Proceedings of the National Academy of Sciences* **110**,
586 10288-10293 (2013).
- 587 31. Liberzon, A. *et al.* The molecular signatures database hallmark gene set collection.
588 *Cell systems* **1**, 417-425 (2015).
- 589 32. Consortium, G.T. The genotype-tissue expression (gtex) pilot analysis: Multitissue
590 gene regulation in humans. *Science* **348**, 648-660 (2015).
- 591 33. Toffoli, M. *et al.* Snca 3' utr genetic variants in patients with parkinson's disease and
592 rem sleep behavior disorder. *Neurol. Sci.* **38**, 1233-1240 (2017).
- 593 34. Edwards, T.L. *et al.* Genome-wide association study confirms snps in snca and the
594 mapt region as common risk factors for parkinson disease. *Ann. Hum. Genet.* **74**, 97-
595 109 (2010).
- 596 35. McDowell, K.A., Shin, D., Roos, K.P. & Chesselet, M.-F. Sleep dysfunction and eeg
597 alterations in mice overexpressing alpha-synuclein. *J. Parkinsons Dis.* **4**, 531-539
598 (2014).
- 599 36. Colombo, M.A. *et al.* Wake high-density electroencephalographic spatio-spectral
600 signatures of insomnia. *Sleep* **39**, 1015-1027 (2016).
- 601 37. Gene Ontology. The gene ontology (go) database and informatics resource. *Nucleic*
602 *Acids Res.* **32**, D258-D261 (2004).
- 603 38. Skene, N.G. *et al.* Genetic identification of brain cell types underlying schizophrenia.
604 *bioRxiv*, 145466 (2017).
- 605 39. Saper, C.B., Scammell, T.E. & Lu, J. Hypothalamic regulation of sleep and circadian
606 rhythms. *Nature* **437**, 1257-1263 (2005).
- 607 40. Hu, Y. *et al.* Gwas of 89,283 individuals identifies genetic variants associated with
608 self-reporting of being a morning person. *Nat. Comm.* **7**, 10448 (2016).
- 609 41. Jones, S.E. *et al.* Genome-wide association analyses in 128,266 individuals identifies
610 new morningness and sleep duration loci. *PLoS Genetics* **12**, e1006125 (2016).
- 611 42. Johann, A.F. *et al.* Insomnia with objective short sleep duration is associated with
612 longer duration of insomnia in the freiburg insomnia cohort compared to insomnia
613 with normal sleep duration, but not with hypertension. *PLoS One* **12**, e0180339
614 (2017).
- 615 43. Zhu, Z. *et al.* Causal associations between risk factors and common diseases inferred
616 from gwas summary data. *Nat. Comm.* **9**, 224 (2018).

- 617 44. Vettrivelan, R., Qiu, M.-H., Chang, C. & Lu, J. Role of basal ganglia in sleep–wake
618 regulation: Neural circuitry and clinical significance. *Front. Neuroanat.* **4**(2010).
- 619 45. Lazarus, M., Huang, Z.-L., Lu, J., Urade, Y. & Chen, J.-F. How do the basal ganglia
620 regulate sleep–wake behavior? *Trends Neurosci.* **35**, 723-732 (2012).
- 621 46. Swardfager, W., Rosenblat, J.D., Benlamri, M. & McIntyre, R.S. Mapping
622 inflammation onto mood: Inflammatory mediators of anhedonia. *Neurosci. Biobehav.*
623 *Rev.* **64**, 148-166 (2016).
- 624 47. Wilson, C.J. & Groves, P.M. Spontaneous firing patterns of identified spiny neurons
625 in the rat neostriatum. *Brain Res.* **220**, 67-80 (1981).
- 626 48. Stoffers, D. *et al.* The caudate: A key node in the neuronal network imbalance of
627 insomnia? *Brain* **137**, 610-620 (2013).
- 628 49. Altena, E. *et al.* Prefrontal hypoactivation and recovery in insomnia. *Sleep* **31**, 1271-
629 1276 (2008).
- 630 50. Dai, X.-J. *et al.* Altered intrinsic regional brain spontaneous activity and subjective
631 sleep quality in patients with chronic primary insomnia: A resting-state fmri study.
632 *Neuropsychiatr. Dis. Treat.* **10**, 2163 (2014).
- 633 51. Nagel, M. *et al.* Gwas meta-analysis of neuroticism (n= 449,484) identifies novel
634 genetic loci and pathways. *bioRxiv*, 184820 (2017).
- 635 52. Mathur, B.N. The claustrum in review. *Front. Syst. Neurosci.* **8**, 48 (2014).
- 636 53. Wei, Y. *et al.* I keep a close watch on this heart of mine: Increased interoception in
637 insomnia. *Sleep* **39**, 2113-2124 (2016).
- 638 54. Krystal, A.D., Edinger, J.D., Wohlgemuth, W.K. & Marsh, G.R. Nrem sleep eeg
639 frequency spectral correlates of sleep complaints in primary insomnia subtypes. *Sleep*
640 **25**, 630-40 (2002).
- 641 55. Hawrylycz, M. *et al.* Canonical genetic signatures of the adult human brain. *Nat.*
642 *Neurosci.* **18**, 1832-1844 (2015).
- 643 56. Liberzon, A. *et al.* Molecular signatures database (msigdb) 3.0. *Bioinformatics* **27**,
644 1739-1740 (2011).
- 645 57. de Leeuw, C.A., Neale, B.M., Heskes, T. & Posthuma, D. The statistical properties of
646 gene-set analysis. *Nature Reviews Genetics* **17**, 353-364 (2016).
- 647 58. Finucane, H.K. *et al.* Partitioning heritability by functional annotation using genome-
648 wide association summary statistics. *Nat. Genet.* **47**, 1228-1235 (2015).
- 649 59. Wang, K., Li, M. & Hakonarson, H. Annovar: Functional annotation of genetic
650 variants from high-throughput sequencing data. *Nucleic Acids Res.* **38**, e164-e164
651 (2010).
- 652 60. Westra, H.J. *et al.* Systematic identification of trans-eqtls as putative drivers of known
653 disease associations. *Nat. Genet.* **45**, 1238 (2013).
- 654 61. Kundaje, A. *et al.* Integrative analysis of 111 reference human epigenomes. *Nature*
655 **518**, 317 (2015).
- 656 62. Zhernakova, D.V. *et al.* Identification of context-dependent expression quantitative
657 trait loci in whole blood. *Nat. Genet.* **49**, 139-145 (2017).
- 658 63. Schmitt, A.D. *et al.* A compendium of chromatin contact maps reveals spatially active
659 regions in the human genome. *Cell Reports* **17**, 2042-2059 (2016).
- 660 64. MacArthur, J. *et al.* The new nhgri-ebi catalog of published genome-wide association
661 studies (gwas catalog). *Nucleic Acids Res.* **45**, D896-D901 (2017).
- 662 65. Vilhjalmsón, B.J. *et al.* Modeling linkage disequilibrium increases accuracy of
663 polygenic risk scores. *Am. J. Hum. Genet.* **97**, 576-592 (2015).
- 664 66. Euesden, J., Lewis, C.M. & O'Reilly, P.F. Prsice: Polygenic risk score software.
665 *Bioinformatics* **31**, 1466-1468 (2015).
- 666

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Author contributions: D.P. and E.J.W.V.S. conceived the idea of the study. D.P. supervised the pre- and post gwas analysis pipeline. P.R.J. and K.W. performed the analyses. S.St. performed quality control on the UK Biobank data and wrote the analysis pipeline. K.W. wrote the online platform (FUMA) that was used for follow-up analyses. C.d.L. conducted conditional gene-set analyses. J.B., N.S., A.M.M. and J.H.L. contributed scRNA information. J.T., D.H., V.V. and the 23andMe Research Team contributed and analyzed the 23andMe

cohort data. D.P., E.J.W.V.S. and P.R.J. wrote the paper. All authors discussed the results, and approved the final version of the paper.

Materials & Correspondence: The data analyzed in the current study was partly provided by the UK Biobank Study (www.ukbiobank.ac.uk), received under the UK Biobank application number 16406. Our policy is to make genome-wide summary statistics (sumstats) publicly available. Sumstats from the GWAS's conducted are available for download at <https://ctg.cncr.nl/>. Note that our freely available meta-analytic sumstats (insomnia and morningness) concern results excluding the 23andMe sample. This is a non-negotiable clause in the 23andMe data transfer agreement, intended to protect the privacy of the 23andMe research participants. To fully recreate our meta-analytic results for insomnia and morningness: (a) obtain insomnia and morningness sumstats from 23andMe (see below); (b) conduct a meta-analysis of our sumstats with the 23andMe sumstats. 23andMe participant data are shared according to community standards that have been developed to protect against breaches of privacy. Currently, these standards allow for the sharing of summary statistics for at most 10,000 SNPs. The full set of summary statistics can be made available to qualified investigators who enter into an agreement with 23andMe that protects participant confidentiality. Interested investigators should email dataset-request@23andme.com for more information.

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FIGURES

Fig. 1a-e. SNP-based results from the GWAS meta-analysis on insomnia (N=1,331,010). (a) Manhattan plot of the GWAS of insomnia, showing the $-\log_{10}$ -transformed P -value for each SNP (b) Heritability enrichment for functional SNP categories and minor allele frequency bins (MAF). Enrichment was calculated by dividing the proportion of heritability for each category by the proportion of SNPs in that category, significant enrichments after Bonferroni correction (28 functional categories + 6 MAF bins + 22 chromosomes) are indicated by an asterisk ($P < 0.05/56 = 8.93 \times 10^{-4}$) (c) Polygenic score (PGS) prediction in three hold-out samples (N=3,000), showing the increase in explained variance in insomnia (Nagelkerke's pseudo R^2) and 95% confidence interval for each P -value threshold. All P -value thresholds were statistically significant. (d) Distribution of CADD scores and RegulomeDB category of all annotated SNPs in LD ($r^2 \geq 0.6$) with one of the GWS SNPs ($n=22,068$) and (e) functional consequences of these SNPs.

Fig. 2a-d. Gene-based and gene-set analyses of insomnia. Zoomed-in circos plots showing genes implicated by two genomic risk loci on chromosome 2 (**a**) and chromosome 7 (**b**), genomic risk loci indicated as blue areas, eQTL associations in green, chromatin interactions in orange. Genes mapped by both eQTL and chromatin interactions are red. The outer layer shows a Manhattan plot containing the negative log₁₀-transformed *P*-value of each SNP in the GWAS meta-analysis of insomnia. Full circos plots of all autosomal chromosomes are provided in **Supplementary Fig. 2**. (**c**) Genome-wide gene-based analysis (GWAS) of 18,185 genes that were tested for association with insomnia in MAGMA. The y-axis shows the negative log₁₀-transformed *P*-value of the gene-based test, the x-axis shows the starting position on the chromosome. The red line indicates the Bonferroni corrected threshold for genome-wide significance ($P=0.05/18,185=2.75\times10^{-6}$). The top 15 most significant genes are highlighted. (**d**) Gene-set analysis of top 20 for each of the MsigDB pathways, tissue expression of GTEx tissue types, and cell types from single-cell RNA sequencing. Gene-set analyses were performed using MAGMA. The red line shows the Bonferroni significance threshold ($P<0.05/7,473=6.7\times10^{-6}$), correcting for the total number of tested gene-sets. Red bars indicated significant gene-sets.

Fig. 3a-f. Genome-wide analyses of six sleep-related traits. Manhattan plots of the genome-wide association analyses of (a) Morningness (N=434,835), (b) Sleep duration (N=384,317), (c) Ease of getting up (N=385,949), (d) Napping (N=386,577), (e) Daytime dozing (N=385,333) and (f) Snoring (N=359,916). The y-axis shows the negative log₁₀-transformed SNP *P*-value, the x-axis the base pair position of the SNPs on each chromosome. The red line indicates the Bonferroni corrected significance threshold ($P < 5 \times 10^{-8}$).

755 **Fig. 4. Genetic overlap of insomnia with other sleep-related traits and psychiatric and metabolic**
756 **traits.** Heatmap of genetic correlations between insomnia, sleep-related phenotypes and
757 neuropsychiatric and metabolic traits studies that were calculated using LD Score regression. Red
758 color indicates a positive r_g while green indicates negative r_g . Correlations that were significant after
759 Bonferroni correction ($P < 0.05/33 = 1.5110^{-3}$) are indicated with an asterisk (see also **Supplementary**
760 **Table 18, 26**).

Fig. 5. Overview of brain tissues and cell types associated with insomnia based on GWAS results from 1,331,010 individuals. For each associated gene-set, the top 5 genes driving the association are reported for each brain area and cell-type. Results for GTEx brain tissue type gene-sets are shown on the left side of the figure, while results from the level 2 single-cell gene expression are shown on the right.

EXTENDED DATA

Extended Data Table 1. LD Score regression estimates of the sex-specific GWAS of insomnia. Results are shown for UK Biobank, 23andMe and the sex-specific meta-analyzed sample. H²=estimated SNP-heritability, intercept=LD Score regression intercept, rg=genetic correlation in the same study sample.

Sample	Sex	N	h ² (SE)	Mean chi ²	Lambda	Intercept (SE)	rg male	rg female
UK Biobank	male	177.817	0.083 (0.007)	1,157	1,143	1.001 (0.008)	1	0.857 (0.051)
	female	208.716	0.092 (0.005)	1,233	1,210	1.011 (0.008)	0.857 (0.051)	1
23andMe	male	443.207	0.080 (0.004)	1,385	1,317	1.016 (0.008)	1	0.925 (0.022)
	female	501.270	0.090 (0.003)	1,580	1,460	1.046 (0.009)	0.925 (0.022)	1
Meta	male	621.024	0.067 (0.003)	1,460	1,382	1.024 (0.009)	1	0.919 (0.018)
	female	709.986	0.078 (0.003)	1,700	1,547	1.042 (0.009)	0.919 (0.018)	1

Extended Data Fig 1a-b. Manhattan and Q-Q plots of the genome-wide analysis of insomnia. Results are shown for the genome-wide analysis in (a) UK Biobank and (b) 23andMe.

Extended Data Fig. 3a-c. Sex-specific Manhattan plot and Q-Q plot of the insomnia meta-analysis in males and females (UK Biobank + 23andMe). (a) Miami plot showing sex-specific SNP association P-values for females on the upper side and males on the lower side. (b) Q-Q plot in females, and (c) in males.

Extended Data Fig. 4a-b. Q-Q plot and lead SNPs of the GWAS meta-analysis for insomnia. (a) QQ-plot of the insomnia meta-analysis showing the expected negative log10-transformed P -value distribution on the x-axis, and observed negative log10-transformed P -value on the y-axis, (b) effect size plot of the 248 lead SNP of the insomnia meta-analysis (log-transformed odds ratio and 95% confidence interval) in UK Biobank and 23andMe.

Extended Data Fig. 5. Risk of insomnia per polygenic risk score decile in three independent holdout samples (N=3x3000). Odds ratios and 95% confidence interval for deciles in polygenic risk score were calculated based on a logistic regression model, using the lowest polygenic risk score decile as the reference.

807

808 **Extended Data Fig. 6. Venn diagram showing the number of genes that were mapped by**
809 **four gene-mapping strategies.** Each square shows the number of overlapping genes between
810 three gene-mapping methods in FUMA (positional mapping, eQTL mapping and chromatin
811 interaction mapping) and significant genes in gene-based tests in MAGMA. The number of
812 genes in bold highlights the number of genes that were implicated by all four methods.
813

Extended Data Fig. 7a-b. Manhattan plot and Q-Q plot of the genome-wide analysis of morningness in UK Biobank and 23andMe. Results are shown for (a) UK Biobank and (b) 23andMe.

Extended Data Fig. 8a-f. Q-Q plots of the genome-wide analysis of six sleep related traits. (a) morningness (including UKB and 23andMe), (b) sleep duration, (c) ease of getting up, (d) daytime napping, (e) daytime dozing, (f) snoring. Manhattan plots of the genome-wide analyses are shown in Fig. 3.

Extended Data Fig. 9a-f. Genome-wide gene-based association analysis of six sleep-

related phenotypes. Manhattan plots genome-wide gene-based analysis (GWGAS) results

for (a) morningness (b) sleep duration (c) ease of getting up (d) daytime napping (e) daytime

dozing (f) snoring. GWGAS was performed in MAGMA. The analysis of morningness was

based on GWAS meta-analysis of UKB and 23andMe, while other sleep-related phenotypes

were analysed in UKB. The red line indicates Bonferroni corrected significance threshold

depending on the number of genes tested.

Supplementary Information includes:

- 1. Supplementary Methods**
 - 1.1 Sample description UK Biobank
 - 1.2 Sample description 23andMe
 - 1.3 Insomnia phenotype validation external sample
- 2. Supplementary Discussion**
 - 2.1. Sex-specific association results for insomnia
 - 2.2. GWAS meta-analysis results for insomnia
 - 2.3. Implicated genes for insomnia
 - 2.4. Gene-set association results for insomnia
 - 2.5. Results sleep-related phenotypes
 - 2.6 Mendelian Randomization
- 3. Supplementary Figures (1 to 2)**
- 4. Supplementary Tables (1 to 28)**

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