

# Shared Genetic Background for Regulation of Mood and Sleep: Association of *GRIA3* with Sleep Duration in Healthy Finnish Women

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**Study Objectives:** Sleeping 7 to 8 hours per night appears to be optimal, since both shorter and longer sleep times are related to increased morbidity and mortality. Depressive disorder is almost invariably accompanied by disturbed sleep, leading to decreased sleep duration, and disturbed sleep may be a precipitating factor in the initiation of depressive illness. Here, we examined whether, in healthy individuals, sleep duration is associated with genes that we earlier found to be associated with depressive disorder.

**Design:** Population-based molecular genetic study.

**Setting:** Regression analysis of 23 risk variants for depressive disorder from 12 genes to sleep duration in healthy individuals.

**Participants:** Three thousand, one hundred, forty-seven individuals (25-75 y) from population-based Health 2000 and FINRISK 2007 samples.

**Measurements and Results:** We found a significant association of rs687577 from *GRIA3* on the X-chromosome with sleep duration in women (permutation-based corrected empirical  $P = 0.00001$ ,  $\beta = 0.27$ ; Bonferroni corrected  $P = 0.0052$ ;  $f = 0.11$ ). The frequency of C/C genotype previously found to increase risk for depression in women was highest among those who slept for 8 hours or less in all age groups younger than 70 years. Its frequency decreased with the lengthening of sleep duration, and those who slept for 9 to 10 hours showed a higher frequency of C/A or A/A genotypes, when compared with the midrange sleepers (7-8 hours) (permutation-based corrected empirical  $P = 0.0003$ , OR = 1.81).

**Conclusions:** The *GRIA3* polymorphism that was previously found to be associated with depressive disorder in women showed an association with sleep duration in healthy women. Mood disorders and short sleep may share a common genetic background and biologic mechanisms that involve glutamatergic neurotransmission.

**Keywords:** Sleep duration, short sleep, long sleep, depressive disorder, *GRIA3*, glutamatergic

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## INTRODUCTION

Sleep is an important contributor to our general health and well-being. Sleeping from 7 to 8 hours per night is considered optimal for health, since, in cross-sectional studies, both shorter and longer sleep have been related to poor health outcomes, including obesity, heart disease, neuroticism, anxiety, and death,<sup>1-7</sup> as well as, in prospective studies, to increased morbidity and mortality.<sup>8-10</sup>

Both short and long sleep are common; according to a cross-sectional population-based study conducted in the United States, 28.3% of the respondents reported having a sleep duration of 6 hours or less, whereas 8.5% reported sleep durations of 9 hours or more.<sup>11</sup> The prevalence of short sleepers ( $\leq 6$  hours) in the Finnish adult population has been estimated to be 14.5% (16.7% of men and 12.5% of women) and that of long sleepers ( $\geq 9$  hours) to be 13.5% (10.5% of men and 16.1% of women).<sup>12</sup> The proportion of both long and short sleepers appears to be relatively stable, even when the average sleep

duration at the population level decreases.<sup>13</sup> However, these epidemiologic studies have not revealed what proportion of the short sleepers are natural short sleepers and which suffer have sleep restriction.

Animal studies have shown that sleep duration is at least partially under genetic control.<sup>14</sup> The *Shaker* mutation in *Drosophila melanogaster* reduces the daily 9- to 15-hour sleep duration to 4 to 5 hours,<sup>15</sup> and a mutation in a transcriptional factor, basic helix-loop-helix family, a member of  $\epsilon 41$ , *BHLHE41* (*DEC2*) gene, has been associated with short sleep in both humans and mice.<sup>16</sup> Sleep can be characterized by a range of attributes, such as sleep length, sleep intensity (slow wave sleep activity or electroencephalographic [EEG] spectral power), latency to sleep onset, early morning awakenings, and sleep quality. Many of these features have a strong genetic component; for example, the heritability of EEG power spectrum in non-rapid eye movement (NREM) sleep has been found to be as high as 96%.<sup>17</sup> According to twin studies in Finland and Australia, even self-reported sleep duration has a relatively high heritability estimate: 44% and 33%, respectively.<sup>18,19</sup>

Depressive disorder is almost invariably accompanied by disturbed sleep, typically with early morning awakenings, leading to decrease in sleep duration,<sup>20</sup> but, in some forms of depression (e.g., seasonal affective disorder), sleep duration can be increased.<sup>21</sup> In bipolar depression, a decrease in sleep duration can lead to a switch into mania.<sup>22</sup> Depressive disorder has also been linked with several genes and their variants, e.g., serotonin

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transporter.<sup>23</sup> However, these findings have been inconsistent and have not been replicated in other populations.<sup>24</sup> It is possible that, even with a genetic vulnerability for depressive disorder, one needs a triggering factor for the onset of the disease itself.

Our recent findings suggest that insufficient or disturbed sleep may be one of such triggering factors. To clarify whether disturbed sleep precedes depressed mood, we studied a nationwide cohort of same-sex Finnish twins at 5-year intervals. Those with sleep complaints at an earlier study point had an increased risk for developing life dissatisfaction, which is a surrogate for depressed mood, at a later time point, whereas life dissatisfaction at an earlier study point did not increase the risk for developing sleep complaints at the later study point. Furthermore, the risk of life dissatisfaction was significantly increased among those who slept less than 7 hours per night.<sup>25</sup> We observed that variants of a number of genes regulating serotonergic, glutamatergic, neural plasticity, the hypothalamic-pituitary-adrenal (HPA) axis, and circadian systems were associated with mood disorders with sleep disturbances, whereas those who slept normally did not show associations to the same extent.<sup>26,27</sup> The associated genes included *tryptophan hydroxylase 2 (TPH2)*; *glutamate decarboxylase 1 (GAD1)*; *glutamate receptor, ionotropic, AMPA 3 (GRIA3)*; *brain-derived neurotrophic factor (BDNF)*; *corticotropin releasing hormone receptor 1 (CRHR1)*; and *timeless homolog (Drosophila) (TIMELESS)*. This finding could be explained by the hypothesis that disturbed sleep, by leading to sleep loss, is a precipitating factor in the initiation of depression in those individuals who also have a genetic vulnerability to depression. Another possibility is that the same genetic factors that regulate mood also regulate sleep duration and quality; in other words, the genetic variants that associate with depression also associate with features of sleep.

In the present study, we asked whether sleep duration in healthy individuals associates with genes that we have previously found to be associated with depressive disorder. We tested this hypothesis in a population-based sample from Finland from which we selected all healthy individuals with information on their sleep duration. Our data demonstrated that one of the candidate genes, *GRIA3*, is strongly associated with sleep duration in healthy women, raising the possibility that some of the genetic mechanisms underlying the regulation of sleep duration contribute to depressive disorder.

## MATERIALS AND METHODS

### Study Samples

The participants were recruited from the population-based national health interview and examination survey Health 2000 (<http://www.terveys2000.fi/indexe.html>) and FINRISK study 2007 survey (<http://www.ktl.fi/portal/4168>) carried out in Finland. The health status of individuals was assessed with a health examination monitored by physicians and trained nurses at a local health care center.

### Health 2000

The health status of individuals was evaluated by the research version of the Composite International Diagnostic Interview using the DSM-IV criteria for psychiatric disorders.<sup>28</sup> All the included individuals had answered the sleep-duration questionnaire. Individuals with no depression and no complaint of dis-

turbed sleep, comprising the sample of 1135 healthy sleepers (610 women and 525 men) were selected for the study (Healthy-sleeper sample). In addition, 1357 individuals (690 women and 667 men) from the complete Health 2000 samples, originally selected for a case-control study on the metabolic syndrome, were included after exclusion of 141 cases with depressive disorder (Genmets (D-) sample). Within that sample, 285 women and 271 men had metabolic syndrome. The criteria for metabolic syndrome were defined as follows: the waist circumference had to be at least 94 cm in men and 80 cm in women. In addition, the subjects had to fulfill two of the following four criteria: (1) blood triglyceride levels of at least 1.7 mmol/l, (2) blood high-density lipoprotein cholesterol level in men less than 1.03 mmol/L or in women less than 1.29 mmol/L, (3) systolic blood pressure at least 130 mm Hg or diastolic blood pressure at least 85 mm Hg or medication for treating blood pressure, and (4) glucose concentration at least 5.6 mmol/L. Therefore, metabolic syndrome (n = 556) was controlled as a covariate in the analyses.

### FINRISK 2007

The sample was collected from the Helsinki-Vantaa region as part of the Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) study, an extension of the FINRISK 2007 study. A total of 655 healthy individuals (326 women and 329 men) with no cardiovascular disease were included in the study (FINRISK 2007 sample). All of them had answered the sleep-duration questionnaire.

These participants from the two population-based cohorts were also characterized for symptoms of depressed mood as defined by a quantitative sum score of Beck's Depression Inventory. The Beck Depression Inventory comprises 21 questions, of which we had 13 available in our data set, allowing us to assess the main depression component of the Beck Depression Inventory. The questions available were: feeling sad, hopelessness about the future, feelings about failing in life, dissatisfaction with life, disappointment in oneself, self criticism and uselessness, suicidal thoughts, lack of interest in other people, problems in decision making, disappointment in one's own appearance, professional capability, tiredness, and appetite. The study individuals were also characterized for symptoms of insomnia, which was evaluated in both cohorts with the question "Do you suffer from insomnia?"; answers ranging from *often*, *sometimes*, and *never* were used as variables in both cohorts. In the secondary analyses, symptoms of a depressed mood (n = 2691) as well as symptoms of insomnia (n = 1689) were used as covariates.

Finally, the complete study sample comprised 3147 healthy subjects: 1626 women (mean age 50 y) and 1521 men (mean age 49 y) (Table 1).

### Sleep Duration

Sleep duration was assessed with the question "How many hours do you sleep on average during 24 hours?" It was applied in statistical analyses as quantitative sleep duration. We also classified the subjects into those who sleep 6 hours or less (short sleepers), those who sleep 7 to 8 hours (midrange sleepers), and those who sleep 9 hours or more (long sleepers). Altogether, 195 women and 234 men were short sleepers, 1220 women and 1156 men were midrange sleepers, and 211 women and 131 men were long sleepers (Table 1). The particulars of the self-

**Table 1**—Distribution of sleep duration groups across study samples by sex (n = 3147).

Study Samples	Women				Men			
	All	Short sleepers	Midrange sleepers	Long sleepers	All	Short sleepers	Midrange sleepers	Long sleepers
Healthy Sleepers	610 (100%)	35 (6%)	502 (83%)	73 (11%)	525 (100%)	58 (11%)	431 (82%)	36 (7%)
Genmets (D-)	690 (100%)	114 (17%)	484 (70%)	92 (13%)	667 (100%)	130 (20%)	486 (73%)	51 (8%)
FINRISK 2007	326 (100%)	46 (14%)	234 (72%)	46 (14%)	329 (100%)	46 (14%)	239 (73%)	44 (13%)
All	1626	195	1220	211	1521	234	1156	131
Age, Mean ± SD	51 ± 13	54 ± 15	50 ± 13	47 ± 16	49 ± 13	49 ± 11	48 ± 13	55 ± 14

Short sleepers, ≤ 6 hours sleep; midrange sleepers, 7-8 hours sleep; long sleepers, ≥ 9 hours sleep.

reported sleep-duration measurement in the Health 2000 and FINRISK study surveys have been previously described.<sup>12,13</sup>

The study was approved by the Institutional Review Board of Helsinki and Uusimaa Hospital District. All the participants provided written informed consent for the collection of samples and subsequent analyses.

### Selection of SNPs and Genotyping

The genes that in our earlier studies had associated significantly ( $P < 0.05$ ) with depressive disorder and disturbed sleep were selected.<sup>26,27</sup> We examined 23 single nucleotide polymorphisms (SNPs) (Supplementary Table S1) from 12 genes; *TPH2* (rs12229394), *solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4)* (rs4251417), *GRIA3* (rs687577, rs526716), *disrupted in schizophrenia 1 (DISC1)* (rs3738401), *BDNF* (rs6265, rs1491850), *CRHR1* (rs173365), *neuronal PAS domain protein 2 (NPAS2)* (rs12712083), *nuclear factor, interleukin 3 regulated (NFIL3)* (rs1619450), *aryl hydrocarbon receptor nuclear translocator-like (ARNTL)* (rs6486121, rs3816358, rs969485), *aryl hydrocarbon receptor nuclear translocator-like 2 (ARNTL2)* (rs4964060, rs7304939, rs1037921, rs2289709), *TIMELESS* (rs2291738, rs1082214), and *RAR-related orphan receptor A (RORA)* (rs4774370, rs8027829, rs1568717, rs4774388).

After the single SNP analyses had shown a strong association between sleep duration and *GRIA3*, we selected 54 variants of this gene (Supplementary Table S2), genotyped within Genmets (D-) and FINRISK 2007 samples, for a post-hoc linkage disequilibrium block and haplotype analysis.

Genotyping of the sample of the healthy sleepers was performed using MassARRAY technology (Sequenom, Inc., San Diego, CA), as has been previously described.<sup>26,27</sup> The Genmets (D-) and FINRISK 2007 samples were genotyped with the Illumina 610 K platform (Illumina, Inc., San Diego, CA), with a greater than 95% call rate cutoff for both individuals and markers. The markers with Hardy-Weinberg equilibrium  $P > 1 \times 10^{-6}$  had been included in the analyses.

### Statistical Analysis

We tested the association of the selected SNPs with the self-reported habitual sleep duration in men and women separately

by using three different models. First, a linear-regression model was used to analyze sleep duration. Second, the groups with short sleep duration (≤ 6 hours) versus midrange sleep duration (7-8 hours) and, third, long sleep duration (≥ 9 hours) versus midrange sleep duration (7-8 hours) were analyzed using a logistic-regression model. We included age and metabolic syndrome status as covariates in all of these primary analyses. Association P values were corrected by simulating the data set 10,000 times, and P values were adjusted to the number of models (three models in both sexes, altogether six models) by the Bonferroni correction. Association was considered significant when both the permutation-based corrected empirical P values and Bonferroni corrected P values were  $P < 0.05$ . We implemented all these analyses in the PLINK software package, web-based version 1.06.<sup>29</sup>

Subsequently, we performed descriptive analyses of variant rs687577 from *GRIA3* that gave evidence for a significant association in the primary analyses. Using a linear regression model we analyzed separately those females who slept for less than 10 hours, and using a logistic regression model we compared those who slept for 9-10 hours to midrange sleepers (7-8 hours). We also performed secondary analyses in the complete study sample including symptoms for depressed mood and insomnia as covariates and analyzed sleep duration using a linear regression model.

We performed post-hoc haplotype based association tests using the sliding window approach as implemented in the PLINK (V.1.06),<sup>29</sup> and determined the LD structure of *GRIA3* by using the Haploview program (V.4.1).<sup>30</sup> For analysis of the transcription factor binding sites we used the tool ConSite, a platform-independent web resource.<sup>31</sup> We first retrieved the corresponding transcript region of humans and mice by using a genome browser EnsEMBL (www.ensembl.org) and then examined the transcription factor binding sites shared by this gene as described in Utge et al.<sup>27</sup>

## RESULTS

### Single SNP Analysis

Out of the 23 SNPs, seven showed suggestive associations (pointwise  $P < 0.05$ ) either with sleep duration or with short

**Table 2**—Single marker association analyses between sleep duration, short and long sleepers and allelic replication with depressive disorder

Chr	Gene	SNP	A1/A2	Gender	Sleep duration (linear model)			Short sleep (logistic model midrange sleepers as reference)			Long sleep (logistic model midrange sleepers as reference)			+Association with depression & disturbed sleep	
					MAF	$\beta$ (95% CI)	P	MAF	OR (95% CI)	P	MAF	OR (95% CI)	P	Group (OR)	Gender
15	RORA	rs4774388	A/G	Male	0.21	-0.08 (-0.18-0.02)	0.123	0.19	1.42 (1.08-1.86)	<b>0.010</b>	0.24	0.94 (0.63-1.38)	0.89	D+FAT+ (0.61)	Female
12	TPH2	rs12229394	G/A	Male	0.07	0.01 (-0.06-0.09)	0.697	0.08	1.28 (1.03-1.59)	<b>0.024</b>	0.10	1.33 (1.00-1.76)	<b>0.041</b>	D+ (1.28) D+EMA+ (1.40)	Female
12	TIMELESS	rs2291738	G/A	Male	0.11	-0.03 (-0.10-0.03)	0.339	0.11	1.22 (1.00-1.50)	<b>0.048</b>	0.08	1.05 (0.80-1.36)	0.706	D+FAT+ (1.52)	Female
12	TIMELESS	rs1082214	C/T	Male	0.48	0.08 (-0.05-0.21)	0.238	0.47	1.23 (0.85-1.78)	0.266	0.48	1.61 (1.04-2.50)	<b>0.026</b>	D+EMA+ (2.70) D+FAT+ (1.72)	Male
X	GRIA3	rs687577	C/A	Female	0.11	0.27 (0.16-0.38)	<b>0.00001*</b>	0.08	0.84 (0.57-1.23)	0.382	0.18	1.89 (1.42-2.52)	<b>0.00001**</b>	D+ (0.70)	Female
17	SLC6A4	rs4251417	G/A	Female	0.07	0.15 (0.01-0.28)	<b>0.027</b>	0.05	0.74 (0.46-1.19)	0.214	0.09	1.33 (0.92-1.92)	0.114	D+ (1.49) D+FAT+ (1.46)	Female
15	RORA	rs4774370	T/C	Female	0.30	0.05 (-0.03-0.14)	0.211	0.31	0.72 (0.53-0.97)	<b>0.037</b>	0.29	1.06 (0.82-1.38)	0.616	D+ (0.75)	Female

Chr, Chromosome; A1/A2, Major allele/Minor allele; MAF, Minor allele frequency;  $\beta$ , Regression coefficient; OR, Odds ratio. 95% CI, Lower and upper bound confidence interval for  $\beta$  or odds ratio. SNPs (single nucleotide polymorphisms) which showed suggestive association from quantitative and dichotomous analysis ( $P < 0.05$ ) are marked with **bold** type. \*Permutation-based corrected empirical  $P = 0.00001$ , Bonferroni  $P = 0.000038$ ,  $P$ -values adjusted to the number of models (three models in both genders, altogether six models) (Bonferroni corrected  $P = 0.0052$ ) for association with sleep duration in females. \*\*Permutation-based corrected empirical  $P = 0.0003$ , Bonferroni  $P = 0.00028$ ,  $P$ -values adjusted to the number of models (three models in both genders, altogether six models) (Bonferroni corrected  $P = 0.039$ ) for association with long sleep in females. †The two right-most columns show data from our previous publications.<sup>26,27</sup> D+, patients with depression; D+EMA+, depressed patients with early morning awakenings; D+FAT+, depressed patients with fatigue.

or long sleep (Table 2, whole data presented in Supplementary Table S1). Association with only one variant, rs687577 from *GRIA3*, survived correction for multiple testing in females. We found the strongest evidence for association with sleep duration (complete study sample: pointwise  $P = 0.00001$ , permutation-based corrected empirical  $P = 0.00001$ ,  $\beta = 0.27$ ; Bonferroni corrected  $P = 0.0052$ ,  $f = 0.11$ ), and with long sleep in females (complete study sample: pointwise  $P = 0.00001$ , permutation-based corrected empirical  $P = 0.0003$ , OR = 1.89; Bonferroni corrected  $P = 0.039$ ,  $f = 0.18$ ). The distribution of the rs687577 minor allele and genotype frequencies in the whole sample, in short sleepers, midrange sleepers, and long sleepers is provided in Supplementary Table S3.

The frequency of rs687577 genotype major/major (C/C) was highest among those women who slept  $\leq 8$  hours (Figure 1A) in all age groups except those over 70 years of age (Figure 1B). The complete data with the number of subjects in each group of Figure 1A and Figure 1B are given in Supplementary Table S4. Figure 1A also shows that the proportion of C/C genotypes decreases by lengthening sleep duration. Although the relative decrease was found to be most striking among those sleeping  $\geq 11$  hours, the association with sleep duration remained significant also after excluding the extreme long sleepers (permutation-based corrected empirical  $P = 0.0002$ ,  $\beta = 0.21$ ). Females who slept for 9-10 hours, as compared to midrange sleepers (7-8 hours), showed a higher proportion of

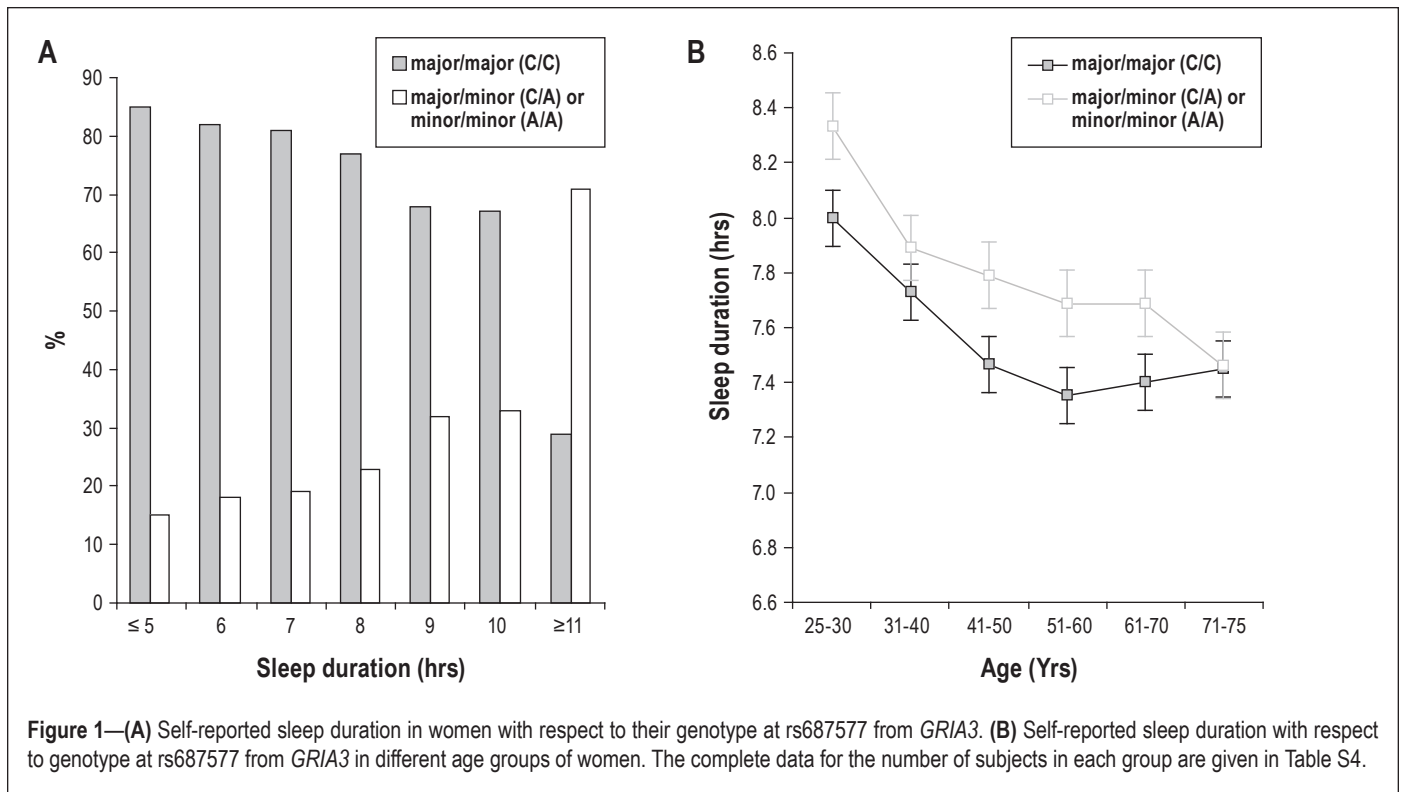
C/A or A/A genotypes (permutation-based corrected empirical  $P = 0.0003$ , OR = 1.81).

The association of rs687577 with sleep duration was robust, and it emerged in females in all three subsamples (“Healthy sleepers” sample: permutation-based corrected empirical  $P = 0.002$  and  $\beta = 0.24$ , “Genmets (D-)” sample: permutation-based corrected empirical  $P = 0.004$  and  $\beta = 0.30$ , and “FINRISK 2007” sample: permutation-based corrected empirical  $P = 0.04$  and  $\beta = 0.31$ ). The results also remained significant when models were adjusted for symptoms of depressed mood (complete study sample: permutation-based corrected empirical  $P = 0.0001$ ,  $\beta = 0.28$ ), and symptoms of insomnia (complete study sample: permutation-based corrected empirical  $P = 0.0001$ ,  $\beta = 0.28$ ).

### Haplotype Analysis of *GRIA3*

We then proceeded to demarcation of the region in *GRIA3* behind the association signal in single SNP analysis by defining the LD structure of the gene and by haplotype analysis. With the aid of all available ( $N = 54$ ) genotyped variants of *GRIA3* in the “Genmets (D-)” and “FINRISK 2007” samples, we identified 11 haploblocks by using the algorithm solid spine.<sup>30</sup> RS687577 was located on haploblock 9 that ranged from intron 11 to 12 (Supplementary Figure S1, whole haploblocks presented in Supplementary Figure S2) Haplotype analysis utilizing rs3848874 and rs687577 (pairwise LD:  $D' = 0.84$ ,  $r^2 = 0.018$ ) in block 9 revealed an equivalent association signal





to sleep duration as compared with that obtained with rs687577 alone in females from the “Healthy sleepers” subsample of the Health 2000 cohort (G-A of rs3848874 and rs687577,  $P = 0.0011$ ,  $\beta = 0.24$ ). Addition of rs526716 from block 8 (Supplementary Figure S1) dismantled the signal between two allelic haplotypes that differed at rs526716 (A-G-A or G-G-A of rs526716-rs3848874-rs687577,  $P = 0.030$ ,  $\beta = 0.31$  and  $P = 0.007$ ,  $\beta = 0.25$ , respectively). Similarly, haplotype analysis of the “Genmets (D-)” and “FINRISK 2007” samples demarcated the association signal to haplblock 9 (for example, A-A of rs10521721-rs687577,  $P = 0.00004$ ,  $\beta = 0.31$ ) while adding variants from adjacent blocks (A-A-A or G-A-A of rs526716-rs10521721-rs687577,  $P = 0.042$  and  $P = 0.0001$ ;  $\beta = 0.28$  and  $\beta = 0.39$ , respectively) diluted or dismantled the signal between different allelic haplotypes. Thus, the association signal initially observed by rs687577 in single SNP analysis to sleep duration in females apparently reflects allelic diversity on haplblock 9 ranging from intron 11 to intron 12 of *GRIA3* (Supplementary Figure S1).

## DISCUSSION

In the present study we assessed the shared genetic background for regulation of mood and sleep. Out of variants from the 12 candidate genes that had been identified in our previous studies,<sup>26,27</sup> *GRIA3*, encoding for ionotropic glutamatergic receptor, associated strongly with sleep duration in healthy females. This finding was statistically robust and consistent in all three subpopulations included in the study. The major allele C of *GRIA3* variant rs687577, previously associated with depression in females<sup>26</sup>, associated here for short sleep and minor allele A robustly associated in the present study with long sleep in healthy females (Table 2). The distribution of rs687577 genotypes also differed significantly in relation to sleep dura-

tion in women. We observed a systematic decrease in the proportion of C/C genotype carriers according to lengthening in sleep duration per each hour. Thus, females with C/A or A/A genotypes were more likely to have longer sleep duration. The difference in average sleep duration between women with C/C genotype as compared to those with C/A or A/A was present in all age groups below 70 years. The absence of correlation in the oldest age group is likely to reflect the age-related changes in sleep<sup>32</sup> and the effect of medical comorbidities on sleep duration and quality.<sup>33</sup>

According to epidemiological studies, poor quality of sleep and insomnia are predictive for depression.<sup>34</sup> To further elucidate the mechanism of *GRIA3* in the interplay of regulation of mood, insomnia, and sleep duration, we adjusted the analyses on sleep duration with reported symptoms of depressive mood and insomnia. The effect of rs687577 on sleep duration was maintained even when symptoms of depressive mood and insomnia were taken into account. These findings suggest that the association of an allelic variant of *GRIA3* is specific to sleep duration.

Glutamate is the main excitatory neurotransmitter in the central nervous system. Approximately 70% of the synapses in the mammalian brain contain N-methyl-D-aspartate (NMDA) or  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors,<sup>35</sup> which are also potential therapeutic targets for medication of major depressive disorder.<sup>36</sup> *GRIA3* (GluR3) is one of the four AMPA receptor subunits, and it is expressed e.g., in the reticular nucleus of the thalamus and the cerebral cortex,<sup>37,38</sup> which are important areas in the regulation of sleep and wakefulness.<sup>39</sup> In a genome-wide scan aiming to identify the natural gene variants that contribute to quantitative traits, *Gria3* was found to affect aggressive behavior in inbred mouse strains.<sup>40</sup> Recently, its functional role has been

addressed by using GluR3-knockout mice, which demonstrate reduced motor coordination and reduced exploratory behavior but increased mobility in the forced swimming test, as well as increased seizure activity.<sup>41</sup> The EEG recordings evidenced decreased power in the lower frequency ranges (0.5-4 Hz) particularly during NREM sleep, suggesting that the GluR3 subunit may play a role in the generation of cortical slow oscillations.<sup>41</sup> On the other hand, the mRNA level of GluR3 in the cortex was elevated during sleep deprivation and declined during sleep.<sup>42</sup> Activation of NMDA and AMPA receptors using their respective agonists induce robust waking,<sup>43,44</sup> whereas the infusion of the antagonists has the opposite effect.<sup>44</sup> Thus the role of the glutamatergic system in the brain is to induce or maintain wakefulness. This is the first study showing that human genetic variation of *GRIA3* gene is associated with the regulation of sleep. *GRIA3* has been previously associated with schizophrenia,<sup>45</sup> bipolar disorder,<sup>46</sup> mental retardation,<sup>46,47</sup> migraine,<sup>48</sup> sexual dysfunction during major depressive disorder,<sup>49</sup> and to suicidal ideation emerging during citalopram treatment of major depression.<sup>50,51</sup> However, all these associations locate towards the 5' end of the gene, whereas haplotype analysis in the present study demarcated the associating region of sleep duration to a haploblock ranging from intron 11 to intron 12, towards the 3' end of the gene (Supplementary Figure S1). In a previous study we identified suggestive association of variants from the same haploblock of *GRIA3*, the minor allele A from rs3848874 and the major allele C of rs687577 as putative susceptibility factors for depression with sleep disturbance while, haplotype G-A of rs3848874 and rs687577 appeared as protective for depression.<sup>26</sup> Here, in coherence with findings from the single SNP analysis, we found an association between the haplotype G-A to longer sleep duration. Interestingly, we did not observe any association between variants from *GRIA3* gene, located on X-chromosome, with sleep duration in males. Similarly, in our previous work on depression<sup>27</sup> and in accordance with findings from twin studies,<sup>52</sup> a consistent difference between the genetic liability factors for depression in the two genders has been observed.

These sex differences can be explained by several molecular and neurobiological mechanisms. Gender-related differences in norepinephrine neurons at locus coeruleus may render women more vulnerable to developing stress-related pathologies.<sup>53</sup> Various neurotransmitter systems interact with estrogen and can induce sex-specific changes in mood and sleep.<sup>54</sup> Estrogen also regulates expression of corticotrophin-releasing factor, a primary mediator of the stress response.<sup>55</sup>

Interestingly, at the promoter region of *GRIA3*, there is a binding site for *GATA-3* (*GATA binding protein 3*) (Supplementary Figure S1), a transcription factor that plays an important role in normal cellular development<sup>56</sup> and contains one estrogen receptor (ER $\alpha$ ) binding site near the 3' end.<sup>57</sup> In addition, gender influences methylation of genes on X-chromosome and autosomes, and sex-specific differences in *GRIA3* methylation have been detected.<sup>58</sup> Previous studies have also identified a methylation sensitive site (*HpaII-site*) at the promoter region of *GRIA3* that has been found to be involved in X-chromosome inactivation in a female patient with bipolar disorder and mental retardation,<sup>46</sup> and in a male patient with mental retardation.<sup>47</sup> It remains to be clarified

whether these sites are involved in the mechanisms of gender-specific regulation of sleep via the *GRIA3* haplotype revealed in the present study.

According to both epidemiologic and experimental studies, short sleep is associated with several unfavorable health outcomes, including obesity, compromised cardiovascular health, type 2 diabetes, anxiety, substance use, and a depressed mood.<sup>59-62</sup> Short sleepers reported higher rates of difficulty for falling to sleep, awakenings across the nights, awakening in the very early morning, awakening unrefreshed, and feeling sleepy during the day.<sup>2</sup> We have previously identified a time-bound link between short sleep and depressed mood so that among individuals with normal mood at baseline, the risk for depressed mood was significantly, albeit modestly (1.31-fold), increased for those who slept less than 7 hours per night at the baseline.<sup>25</sup> A disadvantage of epidemiological studies is that the only available data is the self-reported sleep duration. One limitation of the current study is that we do not have information on possible naps during the day. Whether the self-reported sleep duration represents genuine sleep need (true short sleepers) or results from voluntary restriction of sleep remains open. The present results raise the possibility that mood disorders and short sleep may share at least partially common mechanisms and a genetic background which involve glutamatergic neurotransmission. Our findings also make *GRIA3* an appealing candidate gene to study a number of nonpsychiatric (somatic) features and traits related to short sleep, such as obesity, cardiovascular disease, and type 2 diabetes.

## ABBREVIATIONS

*GRIA3* (GluR3), glutamate receptor, ionotropic, AMPA 3  
BHLHE41 (DEC2), basic helix-loop-helix family, member e41  
EEG, electroencephalographic  
NREM, non-rapid eye movement  
HPA, hypothalamic pituitary adrenal  
TPH2, tryptophan hydroxylase 2  
GAD1, glutamate decarboxylase 1  
BDNF, brain-derived neurotrophic factor  
CRHR1, corticotropin releasing hormone receptor 1  
TIMELESS, timeless homolog (*Drosophila*)  
CIDI, Composite International Diagnostic Interview  
DSM-IV, Diagnostic and Statistical Manual of Mental Disorders IV  
DILGOM, Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome  
SLC6A4, solute carrier family 6 (neurotransmitter transporter, serotonin), member 4  
DISC1, disrupted in schizophrenia 1  
NPAS2, neuronal PAS domain protein 2  
NFIL3, nuclear factor, interleukin 3 regulated  
ARNTL, aryl hydrocarbon receptor nuclear translocator-like  
ARNTL2, aryl hydrocarbon receptor nuclear translocator-like 2  
RORA, RAR-related orphan receptor A  
LD, linkage disequilibrium  
SNP, single nucleotide polymorphism  
NMDA, N-methyl-d-aspartate  
AMPA,  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid  
GATA-3, GATA binding protein 3

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## DISCLOSURE STATEMENT

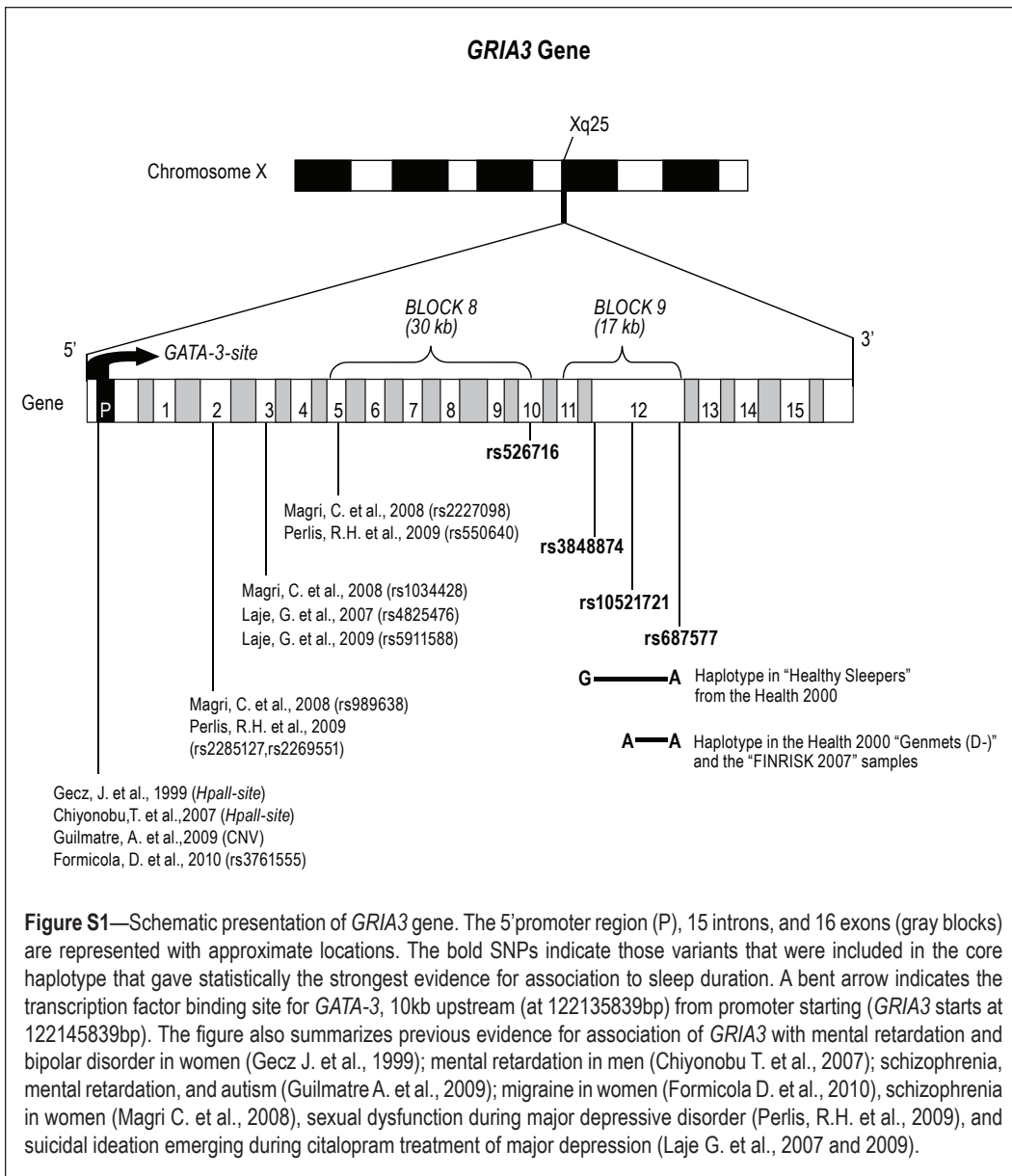
This was not an industry supported study. **Dr. Partonen** has participated in speaking engagements for Janssen-Cilag, Leiras Finland, Nordea, and Servier Finland. The other authors have indicated no financial conflicts of interest.

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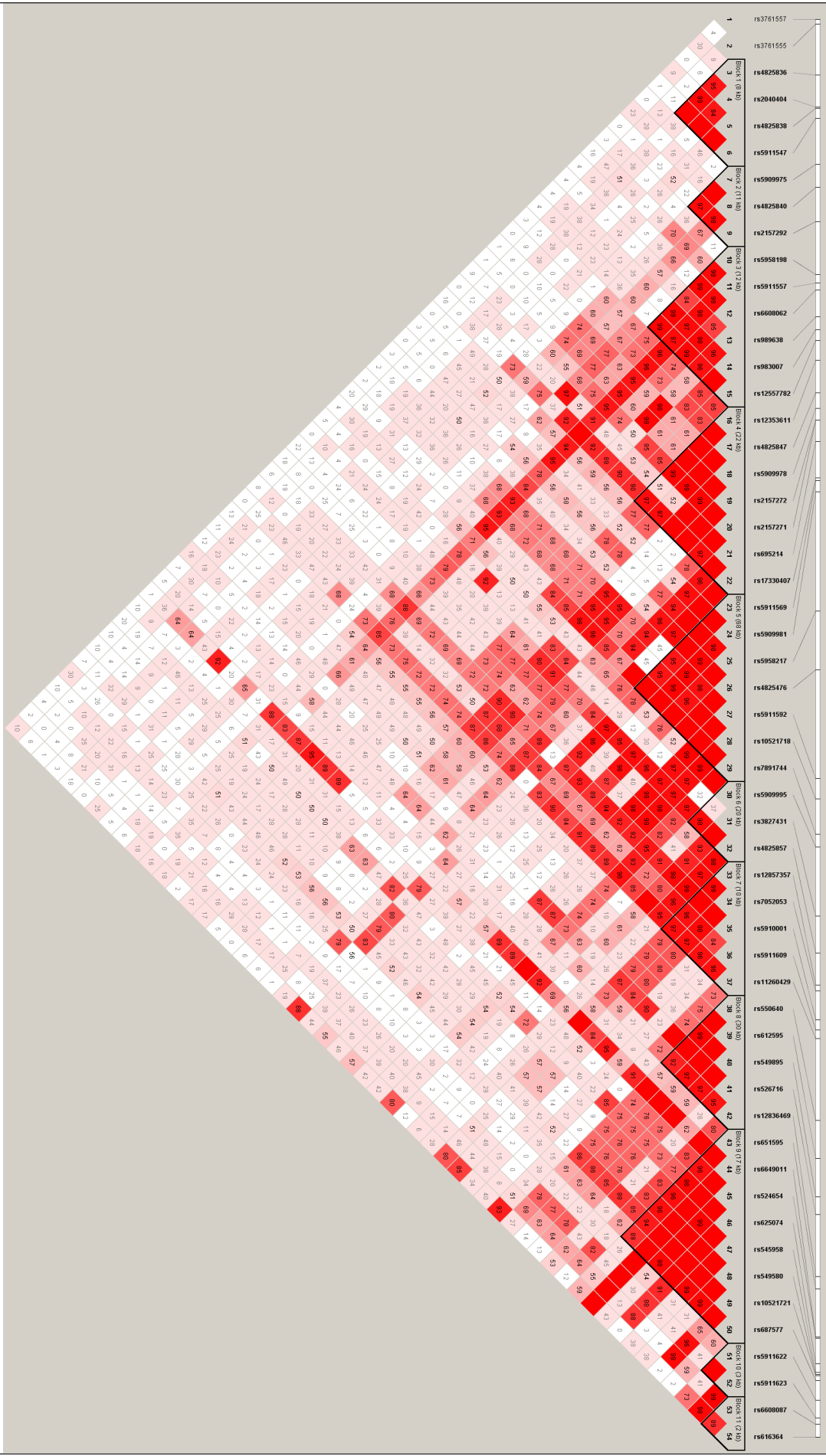
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**Figure S1**—Schematic presentation of *GRIA3* gene. The 5'promoter region (P), 15 introns, and 16 exons (gray blocks) are represented with approximate locations. The bold SNPs indicate those variants that were included in the core haplotype that gave statistically the strongest evidence for association to sleep duration. A bent arrow indicates the transcription factor binding site for *GATA-3*, 10kb upstream (at 122135839bp) from promoter starting (*GRIA3* starts at 122145839bp). The figure also summarizes previous evidence for association of *GRIA3* with mental retardation and bipolar disorder in women (Gecz J. et al., 1999); mental retardation in men (Chiyonobu T. et al., 2007); schizophrenia, mental retardation, and autism (Guilmatre A. et al., 2009); migraine in women (Formicola D. et al., 2010), schizophrenia in women (Magri C. et al., 2008), sexual dysfunction during major depressive disorder (Perlis, R.H. et al., 2009), and suicidal ideation emerging during citalopram treatment of major depression (Laje G. et al., 2007 and 2009).



**Figure S2**—Linkage disequilibrium and haplotype structure of *GRIA3* gene. The linkage disequilibrium and haplotype structure of 54 SNPs of *GRIA3* in the Genets (D) and FINRISK 2007 samples created using the algorithm solid spine.<sup>30</sup> Blocks 1-3 are positioned within intron 2. Block 4 includes variants from intron 2 and 3. Block 5 is the largest block spanning a 68kb region located within introns 3 and 4. Block 6 ranges from intron 4 to 5. Block 7 is located within intron 5. Block 8 includes variants from introns 5,7,10, and 11. Block 9 extends over a 17kb region and includes variants from introns 11 and 12, and the most significantly associated variant, rs687577, is located within this block. Blocks 10 and 11 are positioned in the region of intron 12.

**Table S1**—Complete results for single marker association analyses of sleep duration as well as short and long sleep in females (N = 1626) and males (N = 1521) from the population-based Health 2000 and FINRISK study 2007 samples.

Chr	Gene	SNPs	BP	A1/A2	Females						Males											
					Sleep duration <sup>a</sup>			Short sleep vs. midrange sleep <sup>b</sup>			Long sleep vs. midrange sleep <sup>b</sup>			Sleep duration <sup>a</sup>			Short sleep vs. midrange sleep <sup>b</sup>			Long sleep vs. midrange sleep <sup>b</sup>		
					MAF	$\beta$ (95% CI)	P	MAF	OR (95% CI)	P	MAF	OR (95% CI)	P	MAF	$\beta$ (95% CI)	P	MAF	OR (95% CI)	P	MAF	OR (95% CI)	P
1	D/SC1	rs3739401	228137030	G/A	0.28	-0.051 (-0.12,-0.025)	0.190	0.31	1.11 (0.88-1.40)	0.353	0.26	0.90 (0.71-1.13)	0.374	0.29	0.005 (-0.07,-0.08)	0.891	0.29	1.02 (0.82-1.27)	0.819	0.30	1.05 (0.79-1.39)	0.705
2	NPAS2	rs12721083	100875331	A/G	0.43	0.005 (-0.06,-0.076)	0.873	0.43	0.96 (0.77-1.2)	0.740	0.42	0.92 (0.75-1.14)	0.479	0.43	0.052 (-0.02,-0.12)	0.154	0.42	0.94 (0.77-1.15)	0.595	0.43	1.05 (0.81-1.37)	0.689
9	NFL/3	rs1619450	93208976	T/C	0.14	-0.008 (-0.122,-0.105)	0.887	0.15	1.18 (0.83-1.67)	0.353	0.16	1.3 (0.94-1.78)	0.108	0.13	-0.021 (-0.13,-0.09)	0.721	0.17	1.01 (0.73-1.40)	0.909	0.12	1.11 (0.74-1.65)	0.627
11	ARNTL	rs6486121	13312346	C/T	0.10	-0.016 (-0.087,-0.054)	0.648	0.11	1.05 (0.84-1.31)	0.630	0.12	1.12 (0.91-1.38)	0.288	0.10	-0.035 (-0.10,-0.03)	0.330	0.10	1.01 (0.83-1.24)	0.855	0.11	0.95 (0.73-1.23)	0.708
11	ARNTL	rs3816358	13348048	C/A	0.49	0.013 (-0.088,-0.116)	0.789	0.48	0.84 (0.60-1.17)	0.325	0.49	0.99 (0.73-1.35)	0.996	0.49	0.042 (-0.05,-0.14)	0.418	0.49	0.95 (0.71-1.27)	0.748	0.49	1.05 (0.73-1.52)	0.768
11	ARNTL	rs969485	13359619	A/G	0.14	-0.053 (-0.137,-0.030)	0.204	0.13	1.13 (0.88-1.46)	0.325	0.13	0.96 (0.75-1.24)	0.804	0.14	0.0005 (-0.08,-0.08)	0.991	0.13	0.89 (0.70-1.14)	0.374	0.14	1.02 (0.75-1.39)	0.895
11	BDNF	rs6265	27636492	C/T	0.22	0.038 (-0.06,-0.134)	0.461	0.24	1.05 (0.77-1.42)	0.742	0.21	1.05 (0.79-1.40)	0.696	0.22	-0.043 (-0.14,-0.05)	0.382	0.20	1.2 (0.92-1.56)	0.176	0.22	1.04 (0.72-1.49)	0.822
11	BDNF	rs1491850	27706301	T/C	0.15	0.067 (-0.003,-0.139)	0.060	0.15	0.87 (0.69-1.09)	0.232	0.15	1.03 (0.84-1.28)	0.715	0.15	-0.038 (-0.10,-0.03)	0.286	0.17	1.18 (0.97-1.45)	0.090	0.14	1.08 (0.83-1.40)	0.549
12	ARNTL2	rs4964060	27424634	G/A	0.41	-0.006 (-0.078,-0.065)	0.862	0.38	1.03 (0.82-1.29)	0.758	0.42	0.90 (0.73-1.12)	0.381	0.43	0.019 (-0.05,-0.09)	0.599	0.47	1.03 (0.84-1.26)	0.732	0.44	0.88 (0.67-1.14)	0.348
12	ARNTL2	rs1304939	27435612	C/T	0.41	0.033 (-0.088,-0.155)	0.586	0.41	0.83 (0.55-1.24)	0.395	0.39	0.87 (0.60-1.25)	0.461	0.43	-0.066 (-0.18,-0.05)	0.276	0.44	1.16 (0.84-1.60)	0.337	0.39	0.73 (0.44-1.20)	0.228
12	ARNTL2	rs1037921	27444833	A/G	0.09	0.006 (-0.127,-0.140)	0.921	0.07	0.82 (0.52-1.28)	0.394	0.08	0.88 (0.59-1.33)	0.563	0.09	-0.097 (-0.23,-0.03)	0.155	0.11	1.21 (0.85-1.71)	0.289	0.07	0.67 (0.38-1.18)	0.163
12	ARNTL2	rs2289709	27464900	C/T	0.07	0.006 (-0.101,-0.115)	0.898	0.06	1.03 (0.73-1.44)	0.851	0.06	0.94 (0.68-1.31)	0.739	0.07	-0.029 (-0.14,-0.08)	0.604	0.09	1.01 (0.74-1.37)	0.941	0.05	0.73 (0.46-1.15)	0.179
12	TIMELESS	rs2291738	55101548	G/A	0.11	0.025 (-0.046,-0.097)	0.491	0.11	0.81 (0.65-1.02)	0.081	0.10	0.97 (0.78-1.21)	0.844	0.11	-0.035 (-0.10,-0.03)	0.339	0.11	1.22 (1.00-1.50)	0.048	0.08	1.05 (0.80-1.36)	0.706
12	TIMELESS	rs1082214	55132757	C/T	0.44	-0.037 (-0.164,-0.089)	0.560	0.40	1.24 (0.86-1.78)	0.242	0.44	1.05 (0.72-1.53)	0.775	0.48	0.082 (-0.05,-0.21)	0.238	0.47	1.23 (0.85-1.78)	0.266	0.48	1.61 (1.04-2.50)	0.026
12	TPH2	rs12229394	70679181	G/A	0.07	-0.002 (-0.077,-0.073)	0.956	0.09	1.04 (0.83-1.32)	0.681	0.07	0.92 (0.73-1.16)	0.521	0.07	0.015 (-0.06,-0.09)	0.697	0.08	1.28 (1.03-1.59)	0.024	0.10	1.33 (1.00-1.76)	0.041
15	RORA	rs4774370	58680723	T/C	0.30	0.056 (-0.032,-0.145)	0.211	0.31	0.72 (0.53-0.97)	0.037	0.29	1.06 (0.82-1.38)	0.616	0.30	0.060 (-0.02,-0.15)	0.184	0.34	0.86 (0.66-1.11)	0.257	0.35	1.07 (0.78-1.47)	0.653
15	RORA	rs8027829	58961163	C/T	0.20	-0.015 (-0.090,-0.059)	0.687	0.15	0.96 (0.76-1.21)	0.774	0.21	0.99 (0.79-1.24)	0.975	0.19	0.005 (-0.06,-0.07)	0.877	0.17	0.90 (0.73-1.11)	0.332	0.21	0.99 (0.75-1.30)	0.952
15	RORA	rs1568717	59149739	G/T	0.35	-0.051 (-0.135,-0.031)	0.233	0.33	1.02 (0.78-1.32)	0.883	0.35	0.82 (0.63-1.07)	0.147	0.35	0.069 (-0.01,-0.15)	0.115	0.32	0.89 (0.69-1.15)	0.397	0.34	1.18 (0.87-1.60)	0.281
15	RORA	rs425417	59254290	A/G	0.23	0.005 (-0.09,-0.104)	0.905	0.24	1.12 (0.83-1.50)	0.438	0.20	1.13 (0.84-1.50)	0.403	0.20	-0.081 (-0.18,-0.02)	0.125	0.19	1.42 (1.08-1.86)	0.010	0.24	0.94 (0.63-1.38)	0.760
17	SLOC4	rs425417	25575984	G/A	0.07	0.153 (0.017-0.288)	0.027	0.05	0.74 (0.46-1.19)	0.214	0.09	1.33 (0.92-1.92)	0.114	0.08	0.053 (-0.07,-0.18)	0.422	0.07	0.96 (0.66-1.39)	0.824	0.09	1.27 (0.83-1.97)	0.281
17	CRHR1	rs173365	41256865	G/A	0.32	0.008 (-0.068,-0.085)	0.831	0.32	0.98 (0.77-1.25)	0.903	0.32	0.97 (0.77-1.23)	0.861	0.29	-0.014 (-0.09,-0.06)	0.722	0.31	1.10 (0.88-1.37)	0.388	0.30	1.10 (0.83-1.47)	0.492
X	GRIA3	rs681577	122304639	C/A	0.11	0.270 (0.160-0.381)	0.00001*	0.08	0.84 (0.57-1.23)	0.382	0.18	1.89 (1.42-2.52)	0.00001**	0.11	0.0003 (-0.16,-0.16)	0.995	0.08	0.68 (0.41-1.12)	0.129	0.07	0.62 (0.31-1.22)	0.186
X	GRIA3	rs282676	122374650	G/A	0.16	-0.008 (-0.103,-0.086)	0.860	0.18	1.20 (0.91-1.60)	0.181	0.18	1.19 (0.91-1.57)	0.187	0.17	-0.017 (-0.15,-0.11)	0.794	0.17	1.06 (0.73-1.54)	0.750	0.17	0.97 (0.59-1.59)	0.965

Chr, chromosome; BP, base pair, reported by NCBI dbSNP database build 125 to 130; A1/A2, Major allele / Minor allele; MAF, Minor allele frequency;  $\beta$ , Regression coefficient; OR, Odds ratio; 95% CI, Lower and upper bound confidence interval for  $\beta$  or odds ratio; \*Linear regression analysis with age and presence of metabolic disorder as covariates; \*\*Logistic regression analysis with age and presence of metabolic disorder as covariates; Gene and SNPs with P-values < 0.05 as well as the corresponding MAF; gOR, gOR values are bolded; \*Permutation-based corrected empirical P = 0.0001; Bonferroni P = 0.00038; P-values adjusted to the number of models (3 models in both genders, altogether 6 models) [Bonferroni corrected P = 0.0052] for association with sleep duration in females; \*\*Permutation-based corrected empirical P = 0.0003; Bonferroni P = 0.00028; P-values adjusted to the number of models (3 models in both genders, altogether 6 models) [Bonferroni corrected P = 0.039] for association with long sleep in females.

**Table S2**—List of 54 *GRIA3* SNPs genotyped in the Health 2000 “Genmets (D-)” and “FINRISK 2007” samples.

Chr	SNPs	BP	Region	A1/A2	MAF	Chr	SNPs	BP	Region	A1/A2	MAF
X	rs3761557	122143150	Promoter	G/A	0.311	X	rs10521718	122294219	INTRON4	G/A	0.229
X	rs3761555	122144118	Promoter	A/G	0.269	X	rs7891744	122301013	INTRON4	A/G	0.12
X	rs4825836	122153999	INTRON2	G/A	0.307	X	rs5909995	122303507	INTRON4	A/C	0.452
X	rs2040404	122160177	INTRON2	A/C	0.257	X	rs3827431	122316845	INTRON5	A/G	0.181
X	rs4825838	122160374	INTRON2	A/G	0.382	X	rs4825857	122324045	INTRON5	C/A	0.431
X	rs5911547	122162359	INTRON2	G/A	0.247	X	rs12857357	122330260	INTRON5	A/G	0.132
X	rs5909975	122171306	INTRON2	A/C	0.467	X	rs7052053	122331410	INTRON5	G/A	0.151
X	rs4825840	122175690	INTRON2	G/A	0.227	X	rs5910001	122336974	INTRON5	G/A	0.215
X	rs2157292	122182364	INTRON2	A/G	0.149	X	rs5911609	122338964	INTRON5	A/G	0.482
X	rs5958198	122192643	INTRON2	C/A	0.395	X	rs11260429	122340630	INTRON5	A/G	0.156
X	rs5911557	122194317	INTRON2	A/G	0.099	X	rs550640	122356484	INTRON5	G/A	0.292
X	rs6608062	122195582	INTRON2	A/G	0.395	X	rs612595	122363807	INTRON7	A/C	0.304
X	rs989638	122200788	INTRON2	A/G	0.329	X	rs549895	122371307	INTRON10	G/A	0.301
X	rs983007	122203281	INTRON2	G/A	0.417	X	rs526716	122374650	INTRON10	G/A	0.166
X	rs12557782	122205419	INTRON2	A/G	0.418	X	rs12836469	122386662	INTRON11	A/G	0.271
X	rs12353611	122209224	INTRON2	A/C	0.373	X	rs651595	122389038	INTRON11	G/A	0.356
X	rs4825847	122215515	INTRON3	A/C	0.379	X	rs6649011	122398392	INTRON12	C/A	0.064
X	rs5909978	122218494	INTRON3	A/C	0.128	X	rs524654	122398611	INTRON12	C/A	0.231
X	rs2157272	122220788	INTRON3	A/G	0.128	X	rs625074	122403594	INTRON12	G/A	0.125
X	rs2157271	122220831	INTRON3	A/G	0.347	X	rs545958	122405251	INTRON12	G/A	0.281
X	rs695214	122224942	INTRON3	G/A	0.138	X	rs549580	122405634	INTRON12	G/A	0.282
X	rs17330407	122232036	INTRON3	G/A	0.053	X	rs10521721	122405854	INTRON12	A/G	0.135
X	rs5911569	122232568	INTRON3	G/A	0.252	X	rs687577	122406785	INTRON12	C/A	0.116
X	rs5909981	122234703	INTRON3	A/G	0.307	X	rs5911622	122410604	INTRON12	G/A	0.433
X	rs5958217	122257803	INTRON3	A/G	0.257	X	rs5911623	122414093	INTRON12	G/A	0.369
X	rs4825476	122269160	INTRON3	A/G	0.309	X	rs6608087	122415252	INTRON12	G/A	0.492
X	rs5911592	122290243	INTRON4	G/A	0.231	X	rs616364	122417727	INTRON12	A/G	0.18

Chr, chromosome; BP, base pair, reported by NCBI dbSNP database build 125 to 130; A1/A2, Major allele / Minor allele; MAF, Minor allele frequency.

**Table S3**—Distribution of rs687577 minor allele frequency and genotype counts across the samples of sleep duration, short sleepers, long sleepers and midrange sleepers

Females					Males						
Samples	MAF	Genotype count		β/OR	P-value	Samples	MAF	Genotype count		β/OR	P-value
		CC/CA/AA						CC/CA/AA			
All females	0.11	1258 / 342 / 20 (N = 1620)	77.7% / 21.1% / 1.2%	0.27	0.00001	All males	0.11	1346 / 0 / 167 (N = 1513)	89.0% / 0% / 11.0%	0.0003	0.995
Female short sleepers	0.08	161 / 33 / 1 (N = 195)	82.6% / 16.9% / 0.5%	0.84	0.382	Male short sleepers	0.08	213 / 0 / 20 (N = 233)	91.4% / 0% / 8.6%	0.68	0.129
Female long sleepers	0.18	140 / 62 / 9 (N = 211)	66.4% / 29.4% / 4.3%	1.89	0.00001	Male long sleepers	0.07	120 / 0 / 10 (N = 130)	92.3% / 0% / 7.7%	0.62	0.186
Female midrange sleepers	0.11	957 / 247 / 10 (N = 1214)	78.8% / 20.3% / 0.8%	na	na	Male midrange sleepers	0.11	1013 / 0 / 137 (N = 1150)	88.1% / 0% / 11.9%	na	na

MAF, minor allele frequency (major allele ‘C’, minor allele ‘A’); β, Regression coefficient; OR, Odds ratio; na, not applicable. The β/OR and P-values were generated by using linear regression model (for sleep duration) and logistic regression model (for short sleepers vs. midrange sleepers as well as long sleepers vs. midrange sleepers).



**Table S4**—The number of subjects in each group for Figure 1A and Figure 1B

**Self-reported sleep duration in females in respect to their genotype at rs687577 from *GRIA3* (Figure 1A)**

Hours of sleep	MAJ/MAJ (C/C)			MAJ/MIN (C/A) or MIN/MIN (A/A)		
	Number of subjects	Age (Average ± SD)	Frequency of C/C	Number of subjects	Age (Average ± SD)	Frequency of C/A or A/A
≤ 5	29	58.65 ± 11.38	85%	5	60.2 ± 14.72	15%
6	132	53 ± 14.66	82%	29	52.34 ± 16.02	18%
7	450	50.37 ± 12.70	81%	107	47.48 ± 14	19%
8	507	49.66 ± 12.81	77%	150	47.38 ± 13.48	23%
9	114	46.51 ± 16.14	68%	54	44.68 ± 16.40	32%
10	24	52.41 ± 18.68	67%	12	48.75 ± 13.53	33%
≥ 11	2	45 ± 12.72	29%	5	46.6 ± 15.59	71%

**Self-reported sleep duration in respect to genotype at rs687577 from *GRIA3* in different age group of females (Figure 1B)**

Age groups	MAJ/MAJ (C/C)		MAJ/MIN(C/A) or MIN/MIN (A/A)	
	Number of subjects	Sleep duration (Average ± SD)	Number of subjects	Sleep duration (Average ± SD)
25-30	23	8 ± 1.04	15	8.33 ± 1.17
31-40	268	7.74 ± 0.87	94	7.89 ± 1.22
41-50	338	7.46 ± 0.88	92	7.79 ± 0.84
51-60	302	7.35 ± 0.96	80	7.68 ± 1.38
61-70	235	7.4 ± 1.04	61	7.68 ± 1.28
71-75	80	7.45 ± 1.13	13	7.46 ± 1.33