

## Supplementary Methods

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ERF: The Erasmus Rucphen Family study is a family based study that includes over 3000 participants descending from 22 couples living in the Rucphen region in the 19<sup>th</sup> century. All living descendants of these couples and their spouses were invited to take part in the study. 1700 individuals from this population were assessed for sleep latency. After strict quality control and removing individuals on medications that could affect sleep patterns 940 were available for the analysis, of which 740 individuals (58% females) were used in the genome-wide analysis.(Aulchenko and others 2004)

EGCUT: The Estonian Genome Center, University of Tartu (EGCUT) is a bio-bank consisting of data of 40000 individuals from a population based Estonian cohort aged 18 years and older (67% females).(Metspalu 2004) For the current study, GWAS was performed on 933 subjects with both Illumina HumanCNV370 genotype (array according to Illumina protocol in Estonian Biocenter Genotyping Core Facility) and MCTQ questionnaire data available. The age range was 18-86 years (mean 39.8 (SD 16.1) years). The current sample consists of 412 males (mean age 38.4 (SD=16.2) years) and 521 females (mean age 41.0 (SD=15.9) years).

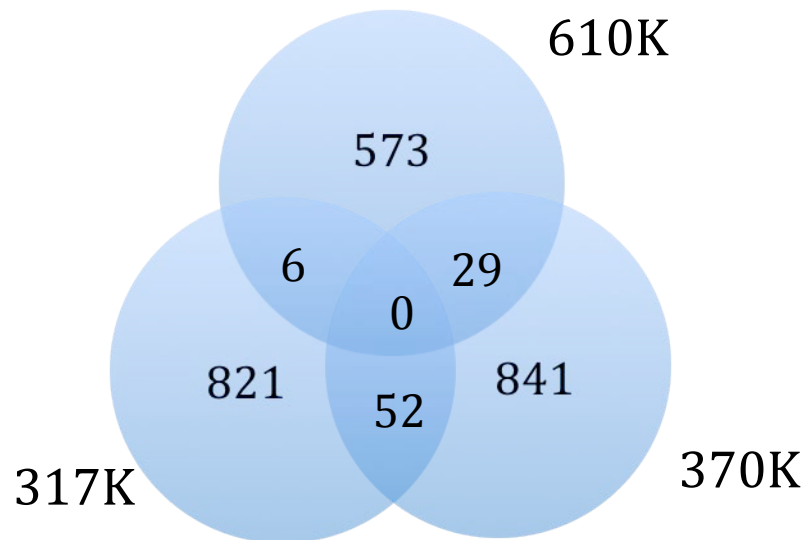
*Cooperative Health Research in the Augsburg Region (KORA):* The KORA F4 study is a follow-up study to the KORA-Survey 2000 (S4, 10/1999 – 7/2001). It was conducted between October 2006 and May 2008. It was conducted between October 2006 and May 2008. From KORA F4 survey (full cohort  $n = 3080$ ), 1814 individuals between 32 to 81 years were selected for Genotyping on Affymetrix 1000K.(Wichmann and others 2005) The current study includes 548 individuals that provided both sleep information and genotypes and who passed quality control.

**MICROS:** The Micro-isolates in South Tyrol Study (MICROS) study is part of the genomic health care program 'GenNova' and was carried out in three villages of the Val Venosta on the populations of Stelvio, Vallelunga and Martello. This study was an extensive survey carried out in South Tyrol (Italy) in the period 2001-2003. An extensive description of the study is available elsewhere.(Pattaro and others 2007) Briefly, study participants were volunteers from three isolated villages located in the Italian Alps, in a German-speaking region bordering with Austria and Switzerland. Information on the health status of participants was collected through a standardized questionnaire. Laboratory data were obtained from standard blood analyses. Genotyping was performed on just under 1,400 participants with 1,334 available for analysis after data cleaning.

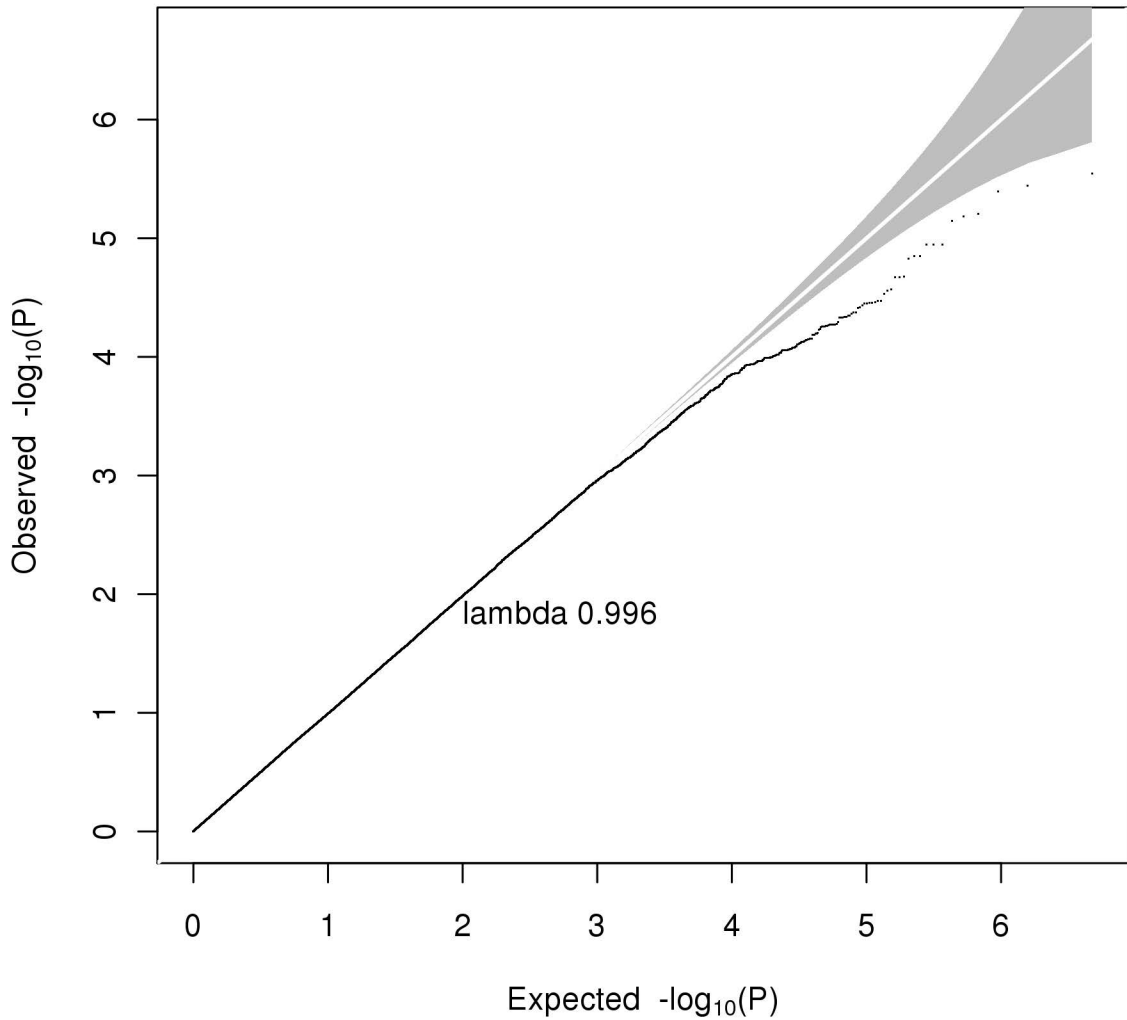
**NESDA:** The Netherlands Study of Depression and Anxiety includes 1763 unrelated cases with a current or remitted major depressive disorder and healthy controls. For the present study 540 subjects without a current major depressive disorder who passed phenotype and quality control were used for the analysis. The average age of the included participants was 41.3 years (66% females).(Boomsma and others 2008)

**ORCADES:** The Orkney Complex Disease Study is a family-based cross sectional genetic epidemiological study in the isolated Scottish islands of Orkney. Genetic diversity is decreased compared to the mainland Scottish samples, consistent with high extent of endogamy. The current study included 424 individuals (37% females) that had both directly observed and imputed genotypes and were also assessed for sleep patterns with MCTQ questionnaire.

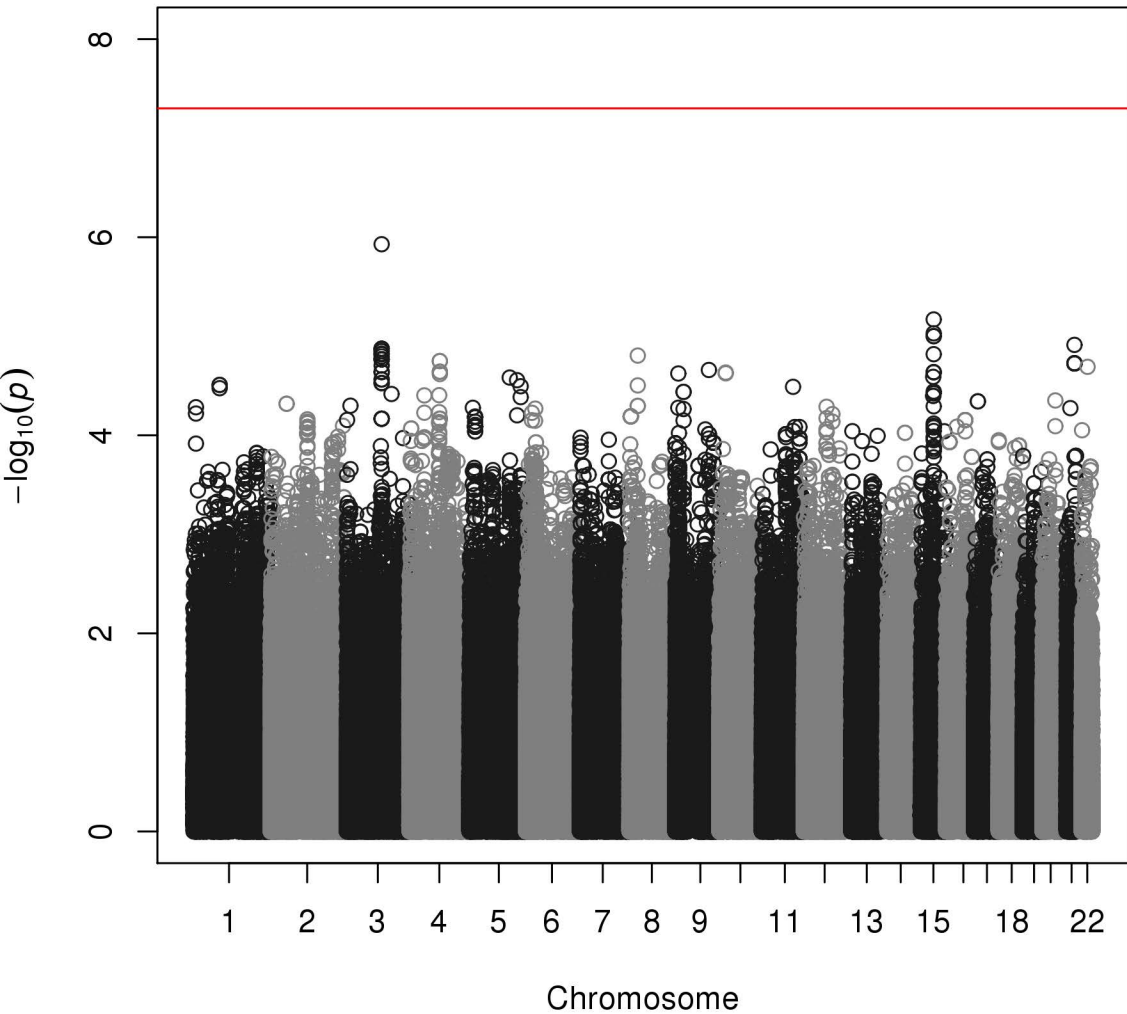
Supplementary Figure 1. Venn diagram showing the breakdown of the sample by Illumina genotyping platform.



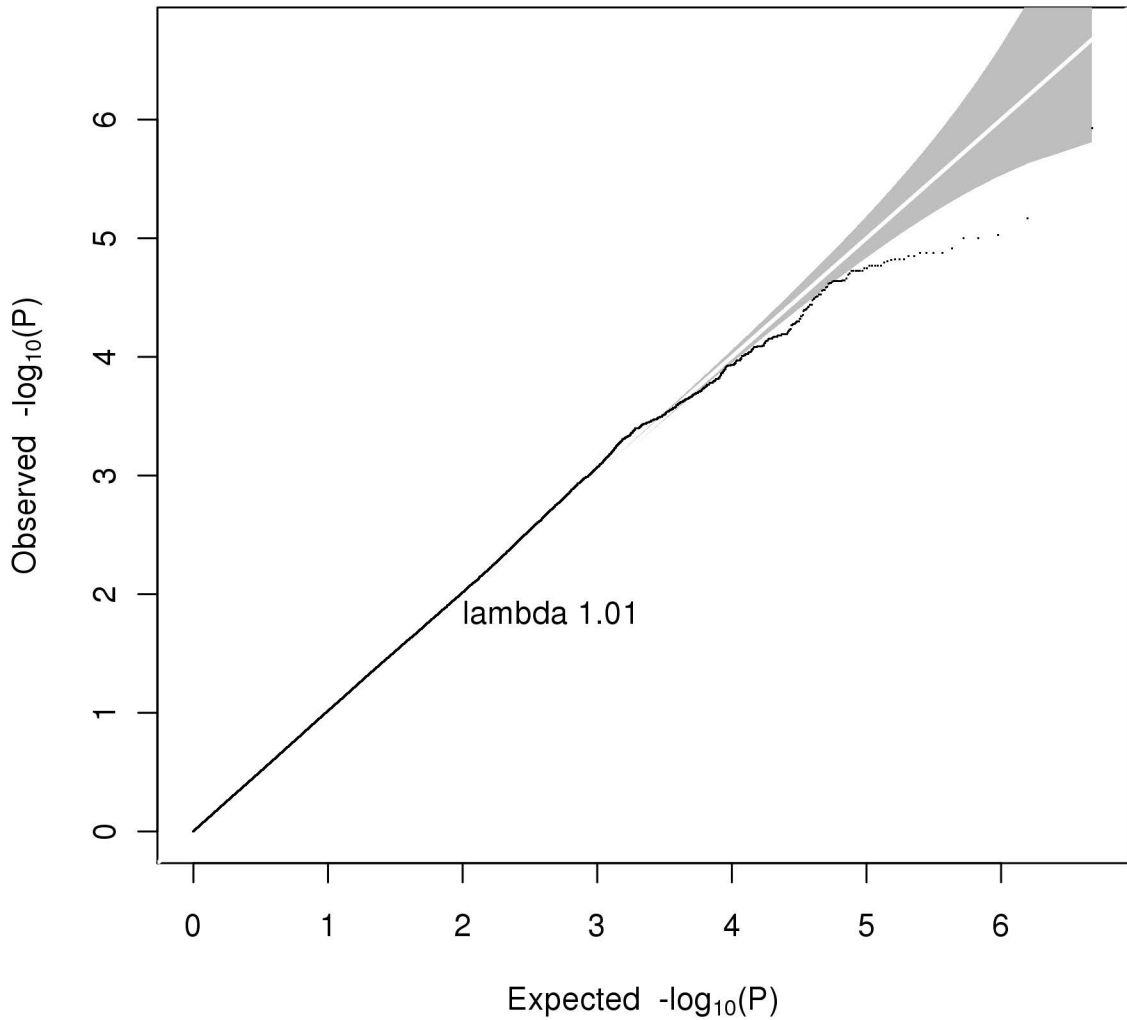
# Bedtime



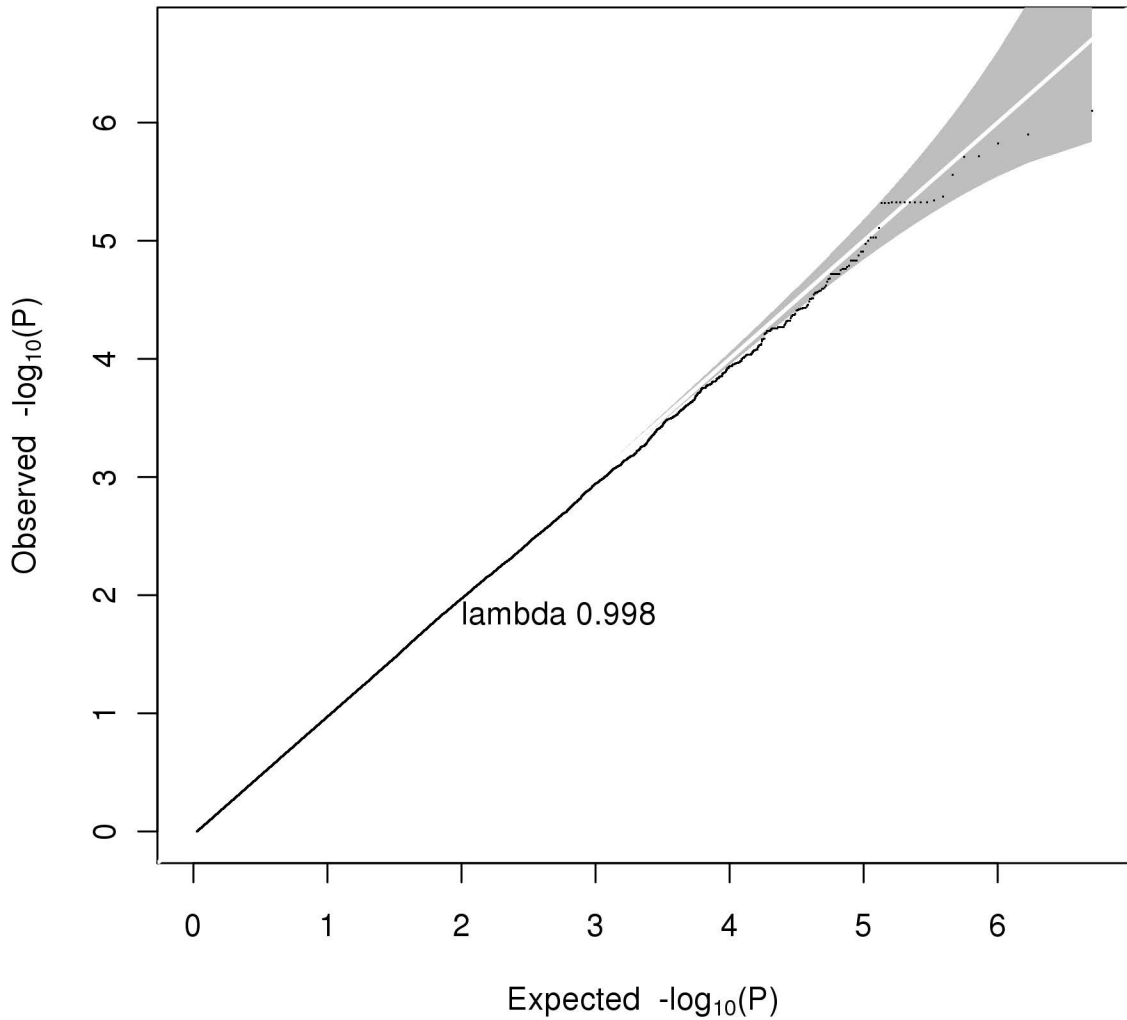
# Sleep Depth



# Depth

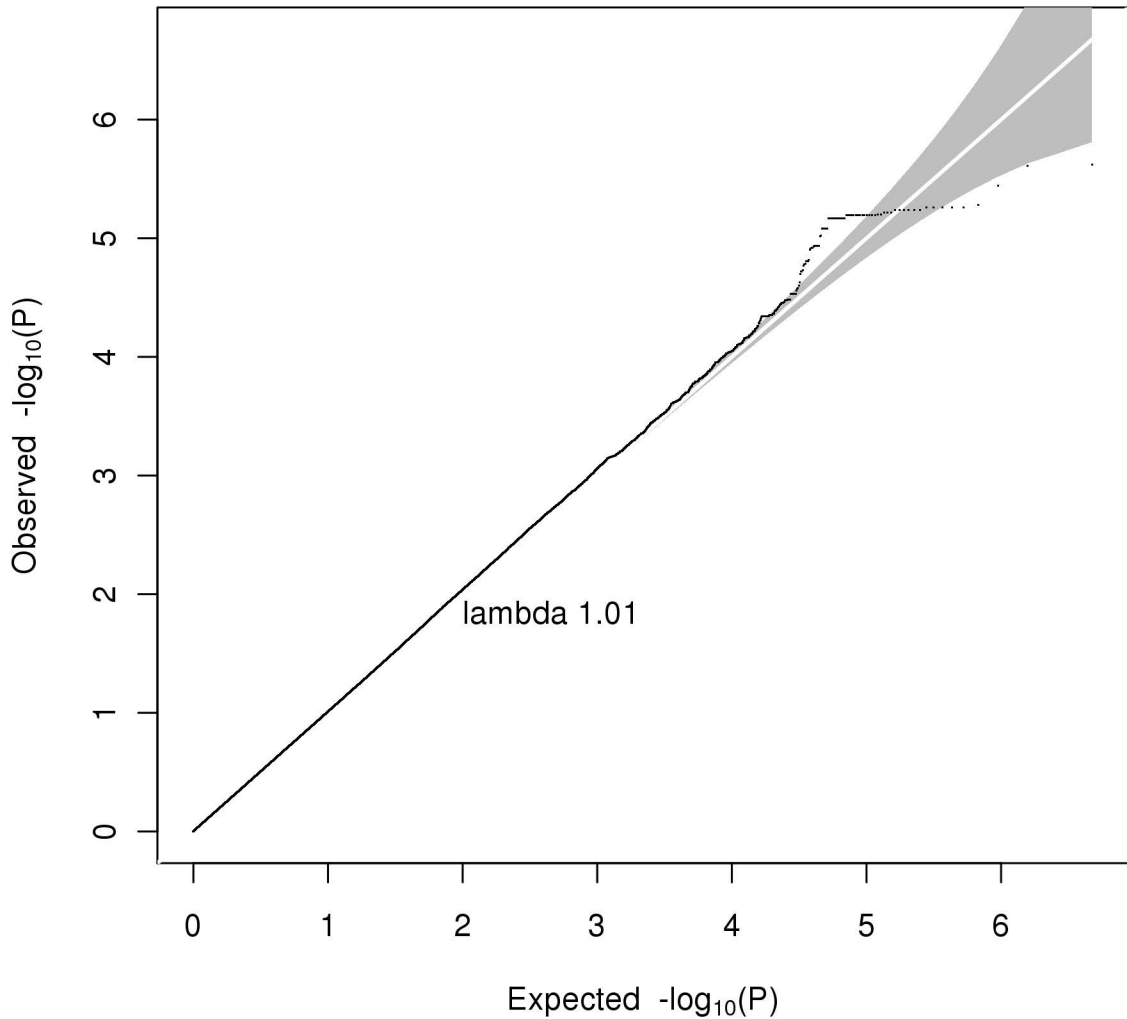


# Sleep Duration

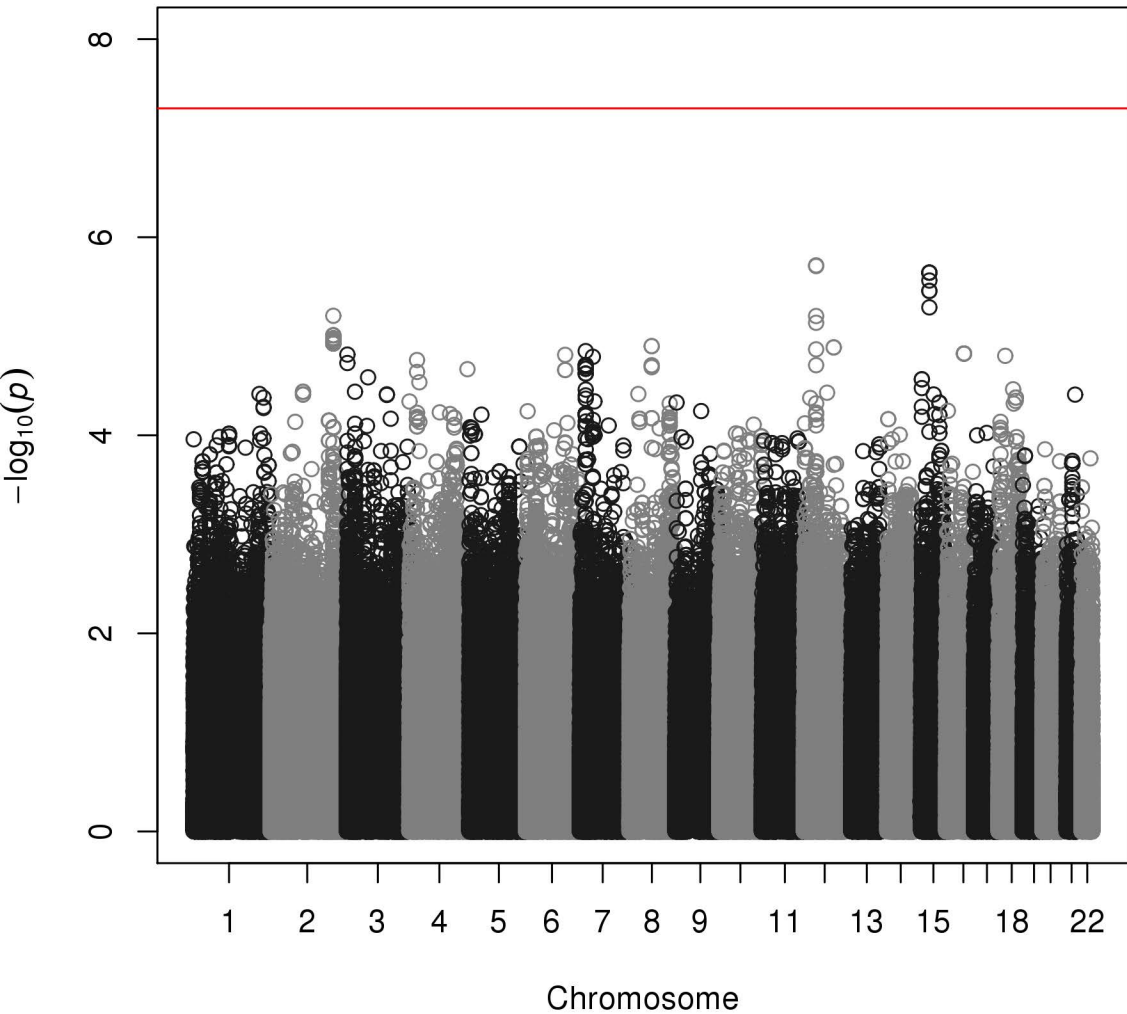




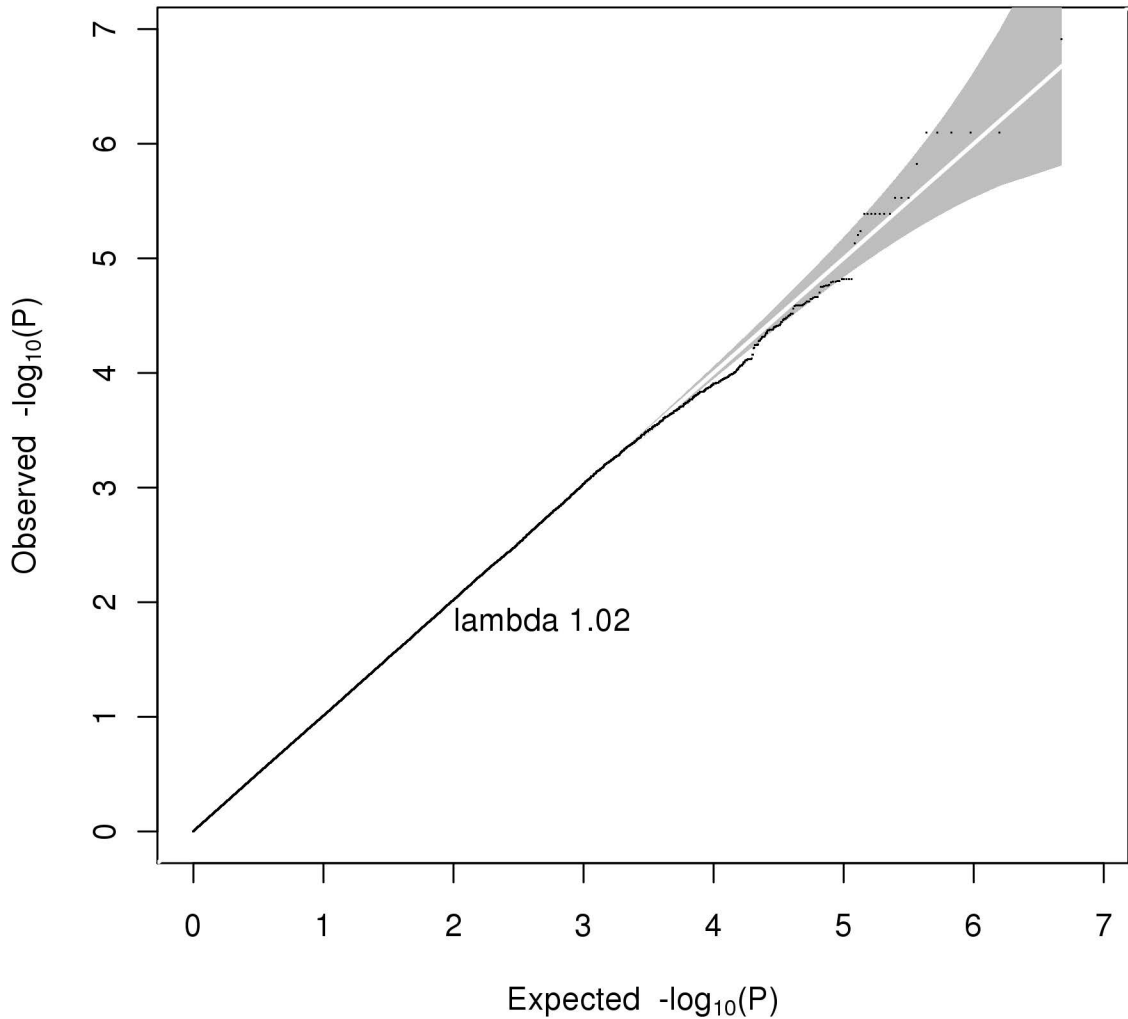
# Insomnia Factor Score



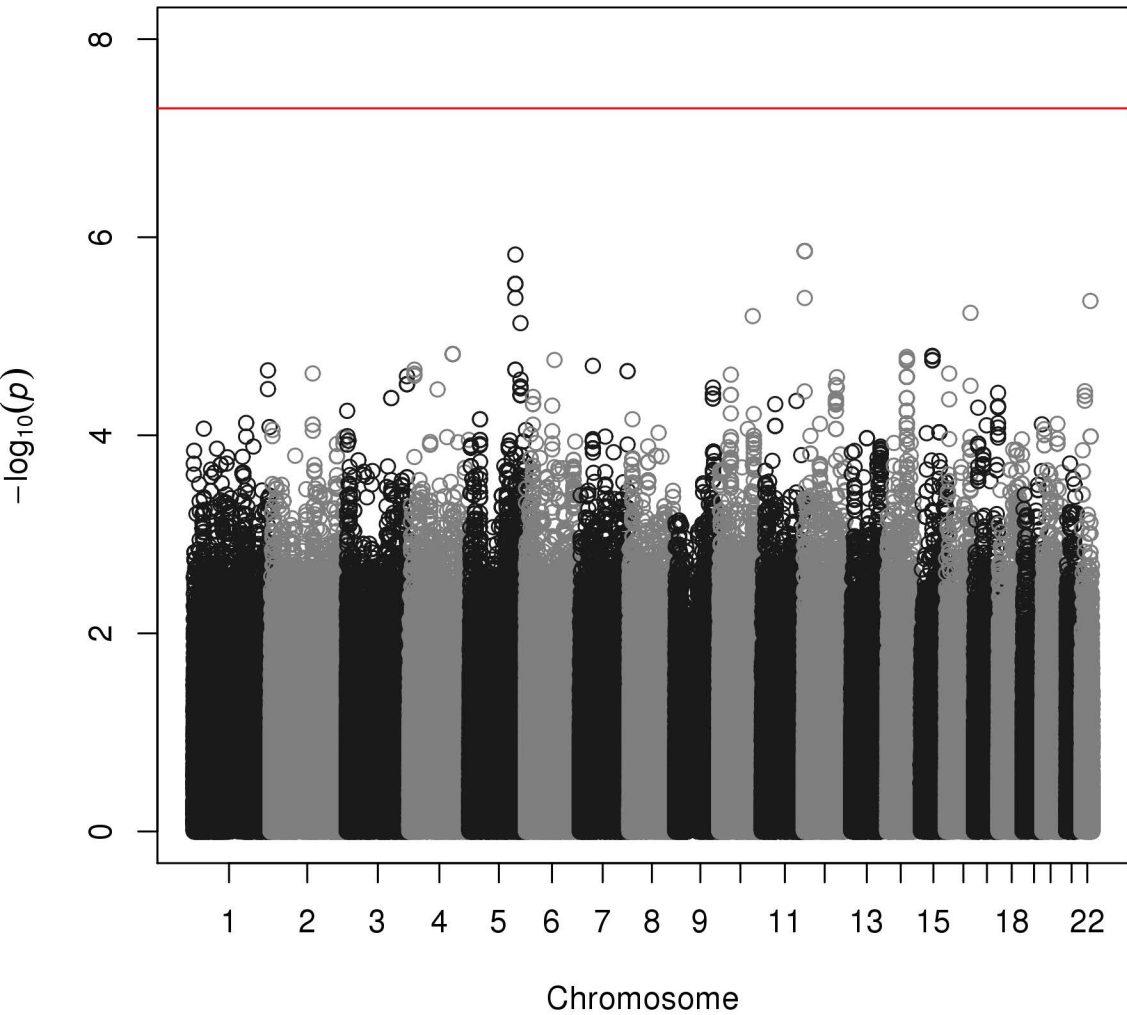
# Insomnia Factor Score



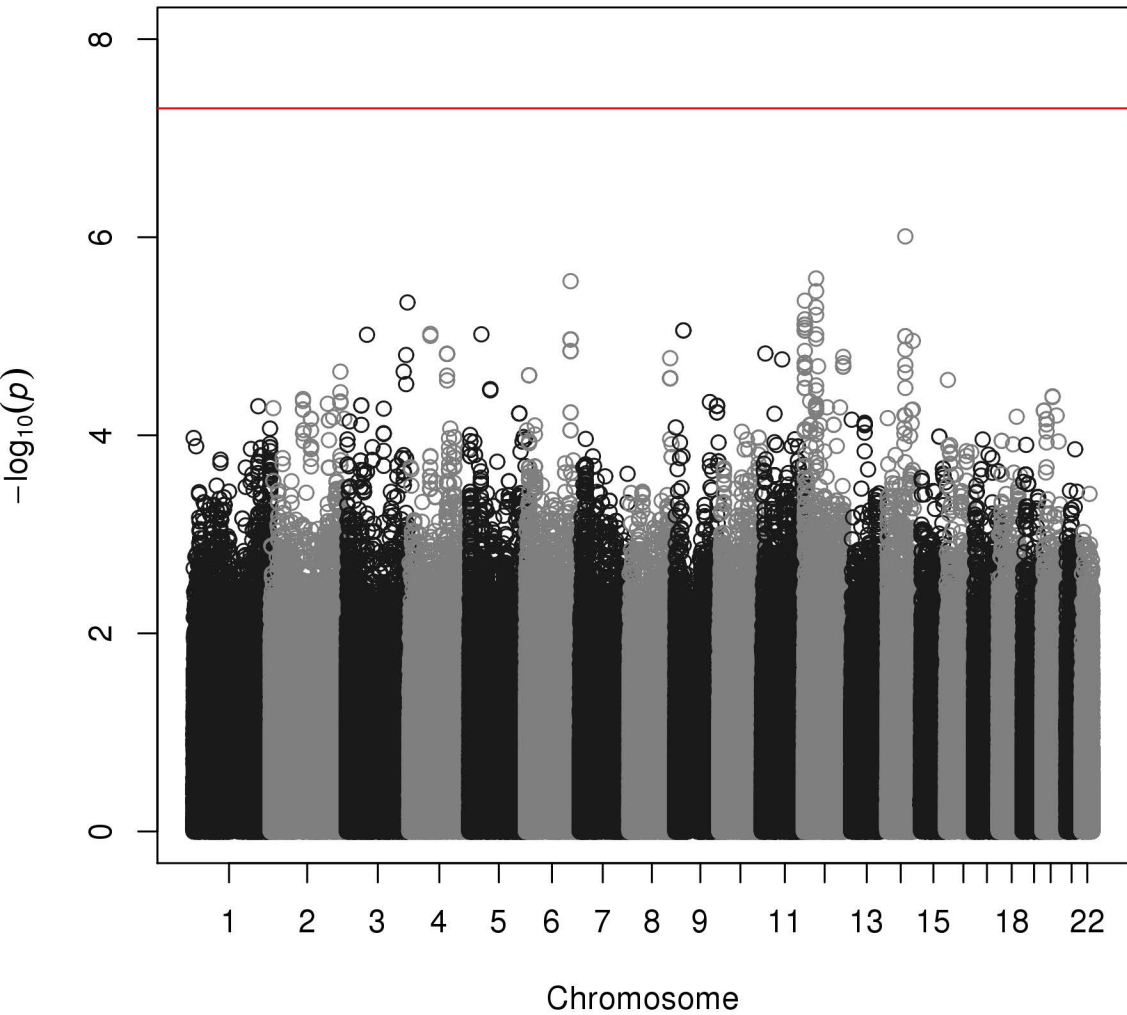
# Latency



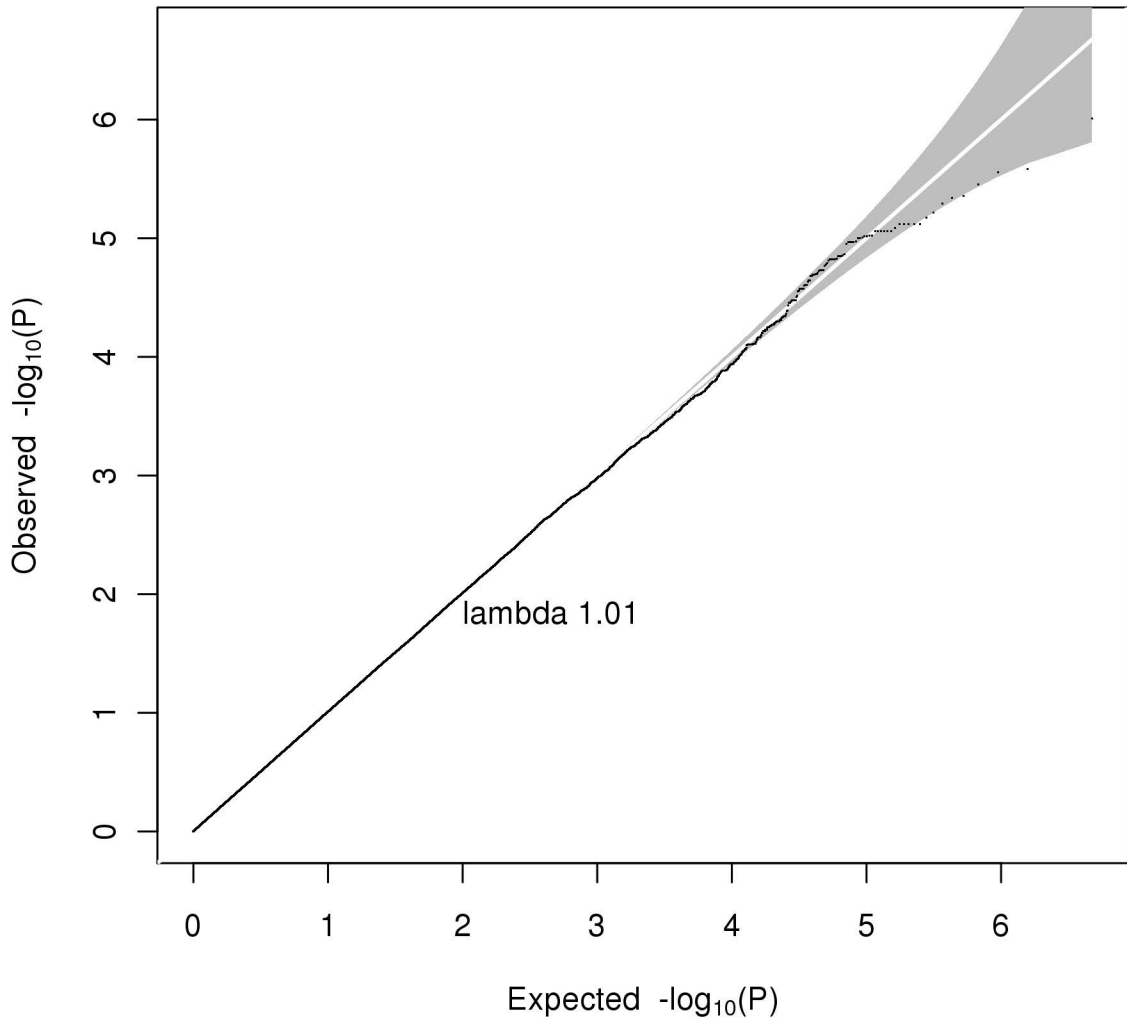
# Sleep Latency



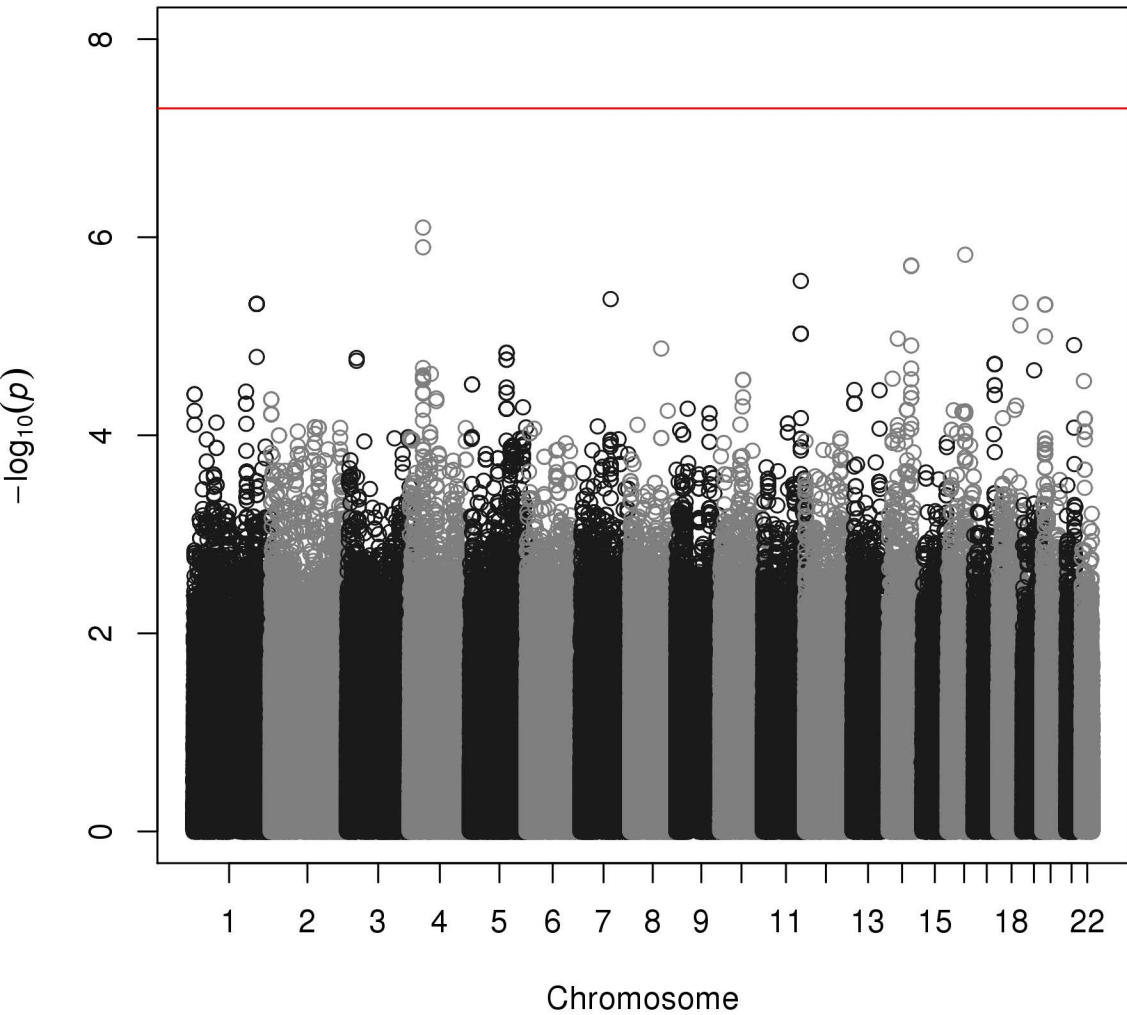
# Sleep Quality



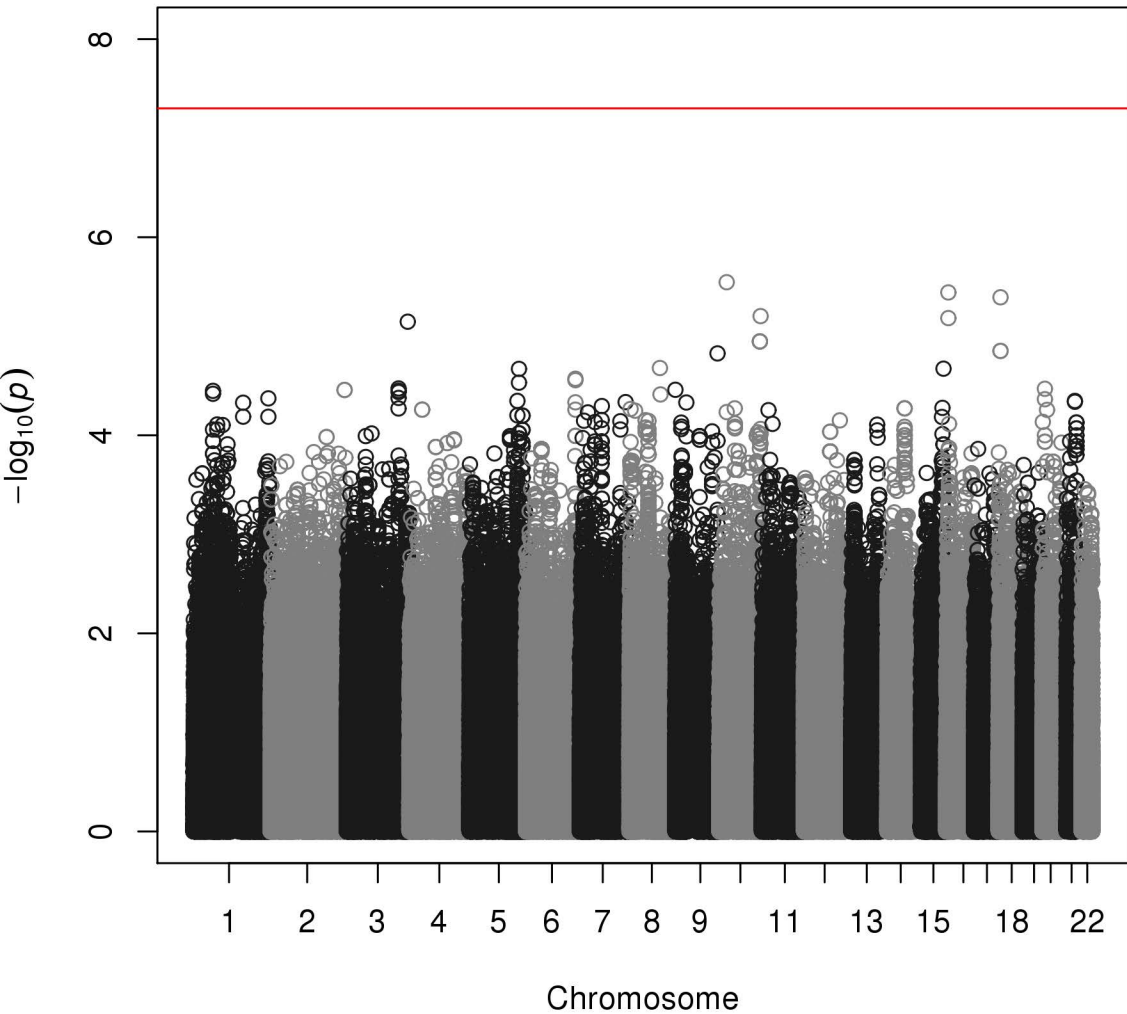
# Quality



# Sleep Duration



# Self-report Sleep Onset Time





Supplementary Table 1. Correlation between the 6 variables analysed. All correlations were significant with  $p < 0.01$

|           | Duration | Latency | Sleeptime | Quality | Depth | I.F.S. |
|-----------|----------|---------|-----------|---------|-------|--------|
| Duration  | 1        |         |           |         |       |        |
| Latency   | -0.23    | 1       |           |         |       |        |
| Sleeptime | -0.36    | 0.14    | 1         |         |       |        |
| Quality*  | -0.23    | 0.43    | 0.07      | 1       |       |        |
| Depth*    | -0.13    | 0.19    | -0.07     | 0.263   | 1     |        |
| I.F.S.    | -0.26    | 0.63    | 0.076     | 0.759   | 0.432 | 1      |

\* Higher scores for the Quality and Depth variables indicate poorer quality and lighter depth of sleep

Supplementary Table 2.

Power to detect variants with sample size used in present study explaining different proportions of the phenotypic variance in a phenotype with total heritability of 30%.

Calculations assume that causal variant is directly genotyped or is tagged by a SNP in complete LD and with the same minor allele frequency

| QTL variance (%) | Power (%) | Sample size for 80% power (singletons) |
|------------------|-----------|----------------------------------------|
| 5                | 100%      | 772                                    |
| 3                | 98.91%    | 1300                                   |
| 2                | 80.44%    | 1960                                   |
| 1                | 19.40%    | 4260                                   |

Supplementary Table 3. Results from Ingenuity Pathway Analysis of sleep traits showing the number of genes with  $p < 0.05$  from gene-based test and top functions and pathways

| Trait    | No. of genes | Top Molecular and Cellular Functions                                                                     | Top Canonical Pathways                                                                                                 |
|----------|--------------|----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|
| Latency  | 525          | RNA Post-transcriptional Modification<br>Post-translational Modification<br>Organ Morphology             | Antiproliferative Role of Somatostatin Receptor 2<br>Endothelin-1 signalling<br>Eicosanoid Signalling                  |
| Timing   | 513          | Thermoregulation<br>Inflammatory Response<br>Lipid Metabolism                                            | NRF2-mediated Oxidative Stress Response<br>Type 1 Diabetes Mellitus Signalling<br>Aminophosphonate Metabolism          |
| Quality  | 547          | Nucleic Acid Metabolism<br>Cellular Assembly and Organization<br>Nervous System Development and Function | Germ Cell-Sertoli Cell Junction Signalling<br>Regulation of eIF4 and p7OS6K Signalling<br>IL-22 Signalling             |
| Depth    | 575          | Cellular Assembly and Organisation<br>Hematological System Development and Function<br>Hematopoiesis     | Graft-versus-Host Disease Signalling<br>Crosstalk between Dendritic Cells and NK cells<br>Antigen Presentation Pathway |
| Duration | 475          | Cell Signalling<br>Cellular Function and Maintenance<br>Cardiovascular System Development and Function   | Phenylalanine Metabolism<br>Tyrosine Metabolism<br>PI3K/AKT signalling                                                 |
| I.F.S.   | 557          | Cellular Movement<br>Embryonic Development<br>Nervous System Development and Function                    | IL-22 signalling<br>Inhibition of Angiogenesis by TSP1<br>iCOS-iCOSL Signalling in T Helper Cells                      |

Supplementary Table 4. SNPs in or near candidate genes for sleep/wake regulation

with  $p < 0.001$

| Trait     | Chr | Gene     | Best SNP   | Best SNP p-value | MAF  | Best SNP Gen/Imp | Effect Size | S.E  |
|-----------|-----|----------|------------|------------------|------|------------------|-------------|------|
| Latency   | 11  | CRY2     | rs939105   | 5.72E-04         | 0.09 | Imp              | -0.13       | 0.05 |
| Latency   | 4   | CLOCK    | rs471546   | 6.84E-04         | 0.21 | Imp              | -0.08       | 0.03 |
| Latency   | 20  | TSHZ2    | rs8121971  | 4.12E-04         | 0.04 | Imp              | 0.20        | 0.07 |
| Latency   | 11  | OPCML    | rs12577796 | 6.16E-05         | 0.12 | Imp              | -0.14       | 0.04 |
| Latency   | 3   | FHIT     | rs2205042  | 1.19E-04         | 0.07 | Imp              | -0.13       | 0.06 |
| Latency   | 5   | PDE4D    | rs17782374 | 8.60E-04         | 0.19 | Imp              | -0.09       | 0.04 |
| Sleeptime | 10  | NPS      | rs10734107 | 1.13E-05         | 0.48 | Imp              | 7.40        | 1.69 |
| Sleeptime | 1   | EPB41    | rs12045777 | 2.43E-04         | 0.50 | Gen              | 6.30        | 1.72 |
| Sleeptime | 10  | PRF1     | rs10999432 | 7.40E-04         | 0.31 | Imp              | 6.19        | 1.83 |
| Sleeptime | 3   | FHIT     | rs1447971  | 1.02E-04         | 0.35 | Imp              | 6.84        | 1.76 |
|           |     |          |            |                  | 0.03 | Imp              | -           | 5.12 |
| Sleeptime | 10  | HABP2    | rs11592949 | 8.49E-04         |      |                  | 17.07       |      |
| Quality   | 7   | AHR      | rs10247158 | 7.50E-04         | 0.14 | Imp              | 0.14        | 0.04 |
| Quality   | 15  | GABRB3   | rs7168574  | 8.90E-04         | 0.37 | Imp              | -0.09       | 0.03 |
| Quality   | 2   | NPAS2    | rs2117714  | 7.99E-04         | 0.27 | Gen              | -0.10       | 0.03 |
| Quality   | 20  | TSHZ2    | rs6097170  | 1.16E-04         | 0.12 | Gen              | -0.16       | 0.04 |
| Quality   | 7   | ING3     | rs17617539 | 8.60E-04         | 0.04 | Imp              | 0.25        | 0.07 |
| Depth     | 11  | PARVA    | rs10500757 | 5.12E-04         | 0.44 | Imp              | 0.07        | 0.02 |
| Depth     | 4   | HTT      | rs1419043  | 5.91E-04         | 0.08 | Imp              | 0.13        | 0.04 |
| Depth     | 11  | OPCML    | rs10894671 | 8.35E-04         | 0.05 | Imp              | 0.14        | 0.04 |
| Duration  | 10  | KCNMA1   | rs573712   | 7.15E-05         | 0.25 | Imp              | -0.14       | 0.03 |
| Duration  | 16  | CSNK2A2  | rs9937047  | 2.06E-04         | 0.36 | Gen              | 0.11        | 0.03 |
| Duration  | 14  | FOS      | rs12433287 | 2.64E-04         | 0.02 | Imp              | -0.36       | 0.10 |
| Duration  | 7   | IL6      | rs7458109  | 4.26E-04         | 0.04 | Imp              | 0.26        | 0.07 |
| Duration  | 5   | PDE4D    | rs12654485 | 4.66E-04         | 0.06 | Imp              | -0.21       | 0.06 |
| Duration  | 14  | PTGDR    | rs893298   | 6.18E-04         | 0.36 | Imp              | 0.10        | 0.03 |
| Duration  | 8   | EYA1     | rs10092844 | 7.22E-04         | 0.41 | Gen              | 0.10        | 0.03 |
| Duration  | 10  | ADARB2   | rs2820623  | 7.82E-04         | 0.06 | Imp              | 0.21        | 0.06 |
| Duration  | 12  | TNFRSF1A | rs1860545  | 8.42E-04         | 0.40 | Gen              | 0.10        | 0.03 |
| I.F.S.    | 7   | AHR      | rs3757824  | 7.42E-04         | 0.23 | Imp              | 0.08        | 0.03 |
| I.F.S.    | 10  | ADARB2   | rs11598577 | 6.83E-04         | 0.03 | Imp              | -0.25       | 0.07 |
| I.F.S.    | 10  | KCNMA1   | rs10762759 | 9.43E-04         | 0.14 | Imp              | 0.11        | 0.03 |
| I.F.S.    | 3   | FHIT     | rs4679599  | 5.20E-04         | 0.12 | Imp              | -0.12       | 0.03 |

Supplementary Table 5. SNPs listed as most strongly associated from Gottlieb et al that show nominal association with sleep traits analysed out of 34 SNPs genotyped or imputed in present sample.

| Trait     | CHR | SNP        | Allele | Freq | Beta  | S.E. | P-value | Gen/Imp |
|-----------|-----|------------|--------|------|-------|------|---------|---------|
| Latency   | 14  | rs434052   | A      | 0.70 | 0.08  | 0.03 | 0.01    | Imp     |
| Latency   | 9   | rs10512058 | C      | 0.89 | -0.09 | 0.04 | 0.04    | Imp     |
| Latency   | 1   | rs2985334  | G      | 0.71 | 0.06  | 0.03 | 0.04    | Imp     |
| Sleeptime | 1   | rs2985334  | G      | 0.71 | 5.08  | 1.86 | 0.01    | Imp     |
| Sleeptime | 21  | rs2247614  | G      | 0.89 | 5.31  | 2.69 | 0.05    | Imp     |
| Sleeptime | 3   | rs10510835 | G      | 0.76 | 4.71  | 2.02 | 0.02    | Gen     |
| Quality   | 3   | rs10510835 | G      | 0.76 | 0.08  | 0.03 | 0.02    | Gen     |
| Quality   | 1   | rs10489832 | G      | 0.85 | 0.08  | 0.04 | 0.05    | Gen     |
| Depth     | 10  | rs932650   | T      | 0.66 | -0.05 | 0.02 | 0.03    | Gen     |
| Depth     | 5   | rs1823068  | A      | 0.86 | 0.06  | 0.03 | 0.04    | Gen     |
| Depth     | 7   | rs1725021  | T      | 0.63 | 0.04  | 0.02 | 0.04    | Gen     |
| Duration  | 7   | rs2189829  | G      | 0.77 | -0.09 | 0.03 | 0.01    | Imp     |
| Duration  | 9   | rs10512058 | C      | 0.89 | 0.10  | 0.05 | 0.03    | Imp     |