Genome-wide association analyses of chronotype in 697,828 individuals provides new insights into circadian rhythms in humans and links to disease

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Abstract

Using data from 697,828 research participants from 23andMe and UK Biobank, we identified 351 loci associated with being a morning person, a behavioural indicator of a person's underlying circadian rhythm. These loci were validated in 85,760 individuals with activity-monitor derived measures of sleep timing: the mean sleep timing of the 5% of individuals carrying the most "morningness" alleles was 25.1 minutes (95% CI: 22.5, 27.6) earlier than the 5% carrying the fewest. The loci were enriched for genes involved in circadian rhythm and insulin pathways, and those expressed in the retina, hindbrain, hypothalamus, and pituitary (all FDR<1%). We provide some evidence that being a morning person was causally associated with reduced risk of schizophrenia (OR: 0.89; 95% CI: 0.82, 0.96), depression (OR: 0.94; 95% CI: 0.91, 0.98) and a lower age at last childbirth in women (β: -0.046 years; 95% CI: -0.067, -0.025), but was not associated with BMI (β: -4.6×10^{-4}; 95% CI: -0.044, 0.043) or type 2 diabetes (OR: 1.00; 95% CI: 0.91, 1.1). This study offers new insights into the biology of circadian rhythms and disease links in humans.

Introduction

Circadian rhythms are fundamental cyclical processes that occur in most living organisms, including humans. These daily cycles affect a wide range of molecular and behavioural processes, including hormone levels, core body temperature and sleep-wake patterns[1]. Chronotype, often referred to as circadian preference, describes an individual's proclivity for earlier or later sleep timing and is a physical and behavioural manifestation of the coupling between internal circadian cycles and the need for sleep, driven by sleep homeostasis. Significant natural variation exists amongst the human population with chronotype often measured on a continuous scale[2], though individuals are traditionally separated into "morning people" (or "larks") who prefer going to bed and waking earlier, "evening people" (or "owls") who perform best with a later bedtime and later rising time, and "intermediates" who lie between the two extremes[3,4]. Age and gender, as well as environmental light levels explain a substantial proportion of variation in chronotype, but genetic variation is also an important contributor [5,6,7,8].

There is evidence that alterations to circadian timing are linked to disease development, particularly metabolic and psychiatric disorders[9,10]. Animal model studies have shown that mutations in, and altered expression of, key circadian rhythm genes can cause obesity, hyperglycaemia and defective beta-cell function leading to diabetes[11–13]. In humans, there are many reported associations between disrupted circadian rhythms and disease[14,15], but the evidence for a causal role of chronotype on disease is limited[16]. For example, evening people have an increased frequency of obesity[17], type 2 diabetes[18] and depression[19] independent of sleep disturbance, and studies of shift workers show an increased risk of diabetes, depression and other diseases[20]. However, these associations could be explained by reverse causality (diseases affecting sleep patterns or dictating job options) or confounding (common risk factors influencing both chronotype and disease). Genetic analyses identifying variants robustly associated with putative risk factors, such as chronotype, can improve causal understanding by providing genetic instruments for use in Mendelian Randomization (MR) analyses, which minimise the effect of both reverse causality and bias caused by confounding. Identifying genetic variants associated with chronotype and sleep timing will also provide new insights into the biological processes underlying circadian rhythms and sleep homeostasis.

Three previous genome-wide association studies (GWAS)[21–23], using a maximum of 128,286 individuals, identified a total of 22 variants associated with self-report chronotype. In this study, we performed a GWAS meta-analysis of a substantially expanded set of 697,828 individuals, including 248,098 participants from 23andMe Inc., a personal genetics company

and 449,734 participants from UK Biobank. We were able to validate self-report chronotype genetic associations using estimates of sleep midpoint derived from activity monitors worn continuously for up to 7 days by 85,760 UK Biobank participants. We offer new insights into the genetics behind natural variation in chronotype and identify causal relationships between variation in human circadian rhythms and multiple traits and diseases.

Results

351 loci associated with morning chronotype from a GWAS meta-analysis including 697,828 individuals. We performed a GWAS of self-report chronotype using 11,977,111 imputed variants in 449,734 individuals of European ancestry from the UK Biobank and 11,947,421 variants in 248,098 European-ancestry 23andMe research participants. We identified 351 independent loci at $P<5x10^{-8}$, of which 258 reached $P<6x10^{-9}$, a correction for the significance threshold based on permutation testing (**Supplementary Methods**). The meta-analysis and individual study results are shown in **Figure 1** and **Supplementary Table 1.** Conditional analysis identified 49 loci with multiple independent signals (**Supplementary Table 2**). A sensitivity analysis was performed in the UK Biobank data alone, excluding shift workers and those either on medication or with disorders affecting sleep (see the **Methods** section and **Supplementary Methods** for details). Effect sizes were similar to those in the full UK Biobank GWAS (**Supplementary Table 1** and **Supplementary Figure 1**).

Validation of chronotype associated variants in 85,760 individuals with activity monitor data. Self-report assessments of sleep and chronotype can be subject to reporting bias[24–27]. To validate the self-report chronotype associations using activity monitor data available in UK Biobank, we tested the association of the chronotype-associated variants with derived estimates of sleep timing, duration and quality and estimates of circadian activity rhythms. Derived phenotypes included sleep efficiency, sleep duration and timings of sleep midpoint, midpoint of the least active 5 hours of the day (L5 timing) and midpoint of the most active 10 hours of the day (M10 timing). Summary statistics of these derived phenotypes and their associations with self-report morningness are presented in **Supplementary Table 3**, and their associations with the newly identified chronotype SNPs are provided in **Supplementary Table 4**. To avoid inflation of associations with our GRS, we performed an additional meta-analysis excluding all UK Biobank individuals with activity monitor data. Of the 292 lead chronotype variants reaching $P<5x10^{-8}$ from this meta-analysis that were available in the UK Biobank imputed genotype data, 258 had a consistent direction of effect for sleep midpoint (two-sided binomial test $P=8.8x10^{-44}$), 262 with L5 timing ($P=2.1x10^{-47}$) and 260 with M10 timing ($P=1.5x10^{-45}$). A genetic risk score (GRS) of these 292 variants was associated with earlier sleep midpoint, L5 timing and M10 timing ($P=4x10^{-}$

[128], $P=1x10^{-182}$ and $P=7x10^{-130}$ respectively). There was little evidence of association between the chronotype GRS and the activity monitor sleep phenotypes that estimate sleep duration and fragmentation (**Supplementary Table 5**), indicating a specific effect of the chronotype SNPs on sleep timing and circadian metrics. As further validation, the 109 lead variants identified from the independent 23andMe morningness GWAS, which are also represented in the UK Biobank, showed the same directional consistency pattern for sleep midpoint (100/109; binomial $P=1.4x10^{-20}$), L5 timing (100/109; $P=1.4x10^{-20}$) and M10 timing (96/109; $P=8.3x10^{-17}$), with a GRS derived using these variants also being strongly associated with earlier sleep midpoint ($P=3.3x10^{-72}$), L5 timing ($P=2x10^{-100}$) and M10 timing ($P=4.9x10^{-69}$) and not with the other measures (**Supplementary Table 5**). Using the activity-monitor derived estimates of sleeping timing, the 5% of individuals carrying the most "morningness" alleles at the 292 associated loci (and weighted by self-reported effect size) had L5 timing shifted, on average, by 25.1 minutes (95% CI: 22.5, 27.6) compared to the 5% carrying the fewest morningness alleles: a mean L5 time of 03:06 rather than 03:32. A similar effect of 26.1 minutes (95% CI: 23.1, 29.2) was seen for M10 timing, corresponding to a peak of activity at 13:29 for the 5% of people carrying the most morningness alleles as opposed to 13:55 for those carrying the least.

Variants associated with circadian rhythms, obesity and insulin secretion are amongst the associated loci. Well-documented circadian rhythm genes were among the most strongly associated loci (**Supplementary Table 1**). These genes included the previously reported loci containing *RGS16*, *PER2*, *PER3*, *PIGK/AK5*, *INADL*, *HCRTR2* and *HTR6*[21–23], and newly associated loci containing known circadian rhythm genes *PER1, CRY1 and ARNTL* (**Supplementary Figure 2**). Additional associations were identified within *MEIS1*, *BTBD9* and *PTPRD*, three of four genes currently implicated in restless legs syndrome (RLS)[28] though the reported lead RLS variants did not reach genome-wide significance. At the *PER3* locus, two highly correlated low frequency missense variants (rs150812083 and rs139315125, MAF=0.5%), previously reported to be a monogenic cause of familial advanced sleep phase syndrome[29], were associated with self-reported morningness (OR=1.44 for minor allele; $P=2x10^{-38}$) but with a lower magnitude of effect on sleep timing than expected in the activity-monitor derived measures of chronotype, advancing sleep timing by only 8 minutes (95% CI: 4, 13, $P=4.3x10^{-4}$) as opposed to the 4.2 hours reported in the previous study[29]. The allele in *FTO* (rs1558902) previously associated with higher BMI[30,31] was also associated with being a morning person (OR=1.04, $P= 4.9x10^{-32}$). A missense variant in the *MADD* gene (rs35233100) previously associated with lower proinsulin levels[32] (but not type 2 diabetes) was strongly associated with eveningness (OR= 1.05, $P=4.4x10^{-12}$).

Circadian rhythm, insulin pathway and brain and pituitary expressed genes are strongly enriched at the associated loci. We next used MAGMA[33,34], PASCAL[35], MAGENTA[36] and DEPICT[37] to identify biological pathways and tissues enriched for genes at the associated loci (**Supplementary Tables 6-11**)**.** We identified strong enrichment of the circadian rhythm and insulin-regulation pathways (FDR<5%), as well as G protein signalling and activation, (**Figures 2 and 3**) and enrichment of genes expressed in tissues of the brain, pituitary and retina, amongst the associated loci (**Figure 4, Supplementary Tables 7 and 11**).

Integration of GWAS data with RNAi data highlights potentially causal genes at associated loci. We next assessed the overlap of genes at the chronotype associated loci with 343 putative clock genes identified from RNAi knockdown experiments in a human cellular clock model[38]. Of 353 genes mapped to the chronotype-associated loci at $P<0.05$ by DEPICT, there was no enrichment of the genes identified in the RNAi screen, with only 6 of the 353 genes overlapping ($P=0.16$). However, the overlapping genes included *FBXL3*, known to have a role in circadian oscillations through ubiquitination and degradation of *CRY1* and *CRY2*, as well as *GFAP*, encoding a filament protein important in the development of astrocytes in the central nervous system (**Supplementary Table 12**).

Chronotype is heritable and demonstrates strong genetic correlation with several metabolic and psychiatric traits. Using LD score regression, we estimated the heritability of chronotype to be 0.106 (0.003) in the meta-analysis. This compares to the heritability of 0.135 (0.001) estimated by BOLT-LMM in the UK Biobank data alone. We also performed LD-score regression analyses against a range of other diseases and traits where GWAS summary statistics are publicly available (**Supplementary Table 13**). The most genetically correlated trait was subjective well-being, which was positively correlated with being a morning person ($r_G=0.17$, $P=6\times10^{-9}$). Psychiatric traits schizophrenia ($r_G=-0.11$, $P=1\times10^{-7}$), depressive symptoms ($r_G=-0.16$; $P=2\times10^{-6}$), major depressive disorder ($r_G=-0.19$; $P=3\times10^{-5}$) and intelligence ($r_G=-0.11$; $P=8\times10^{-6}$) were all negatively correlated with the morning chronotype. Metabolic traits fasting insulin ($r_G=-0.09$, $P=0.03$) and HOMA-IR ($r_G=-0.12$, $P=0.009$) were nominally negatively correlated with being a morning person. BMI ($r_G=0.007$, $P=0.74$) and T2D ($r_G=0.02$, $P=0.60$) were not genetically correlated.

Mendelian randomisation (MR) analyses provide evidence for a causal link between chronotype and mental health and fecundity. Altered sleep patterns are associated with many disease and health outcomes in observational studies but there is limited evidence for

causality. To test for a causal effect of chronotype on disease and related outcomes we performed Mendelian randomization analyses against 367 metabolic, psychiatric and other phenotypes in the UK Biobank and with external GWAS datasets using MR Base[39] and the corresponding R package TwoSampleMR[40]. We used 109 (of the total 115) variants identified at $P<5x10^{-8}$ in the 23andMe study alone (**Supplementary Table 14**) for the UK Biobank MR analyses. For most traits, even those with strong observational associations, we did not find evidence of a causal effect of genetically influenced chronotype. For example, there was no evidence that morningness or eveningness leads to higher BMI (P=0.94 in UK Biobank) or risk of Type 2 diabetes (P=0.83) (**Supplementary Figures 3 and 4** and **Supplementary Table 15**). A small number of Mendelian randomisation tests survived correction for multiple testing, including morningness leading to a younger age at which women had their last child: a genetically-determined unit log-odds increase in morningness (equivalent to a morningess odds ratio of ~2.7) was associated with a 16.8-day (95% CI: 12.8, 24.5) younger age of last child birth (IVW $P=3x10^{-5}$) (**Supplementary Figure 5**). There was nominal evidence that morningness causally decreases risk of schizophrenia, depression and multiple pregnancy loss: a genetically-determined unit log-odds increase in morningness was associated with odds ratios of 0.89 (0.82, 0.96; IVW P=0.004), 0.94 (0.91, 0.98; IVW P=0.002) and 0.91 (0.86, 0.96; IVW P=0.002) respectively (**Supplementary Figures 6-8, Supplementary Tables 15 and 16**).

Nominal evidence that type 2 diabetes, insulin secretion and BMI causally influence chronotype. We next used Mendelian randomisation to test whether or not disease related traits could alter chronotype (**Supplementary Table 17**). We selected 40 traits where large-scale GWAS had identified multiple robustly associated SNPs and where the traits have been associated with chronotype in the literature or were observationally associated in the UK Biobank study. We found only nominal evidence that diseases and related traits alter chronotype: a genetically determined 1 unit log odds increase in type 2 diabetes, 1 SD increase in favourable adiposity[41] (increased subcutaneous-to-visceral adiposity but lower insulin levels), 1 SD increase in insulin secretion and 1 SD increase in BMI were associated with morningness odds ratios of 1.02 (95%CI: 1.00, 1.04; IVW P=0.01), 0.86 (0.76, 0.97; IVW P=0.02), 1.10 (1.01, 1.20; IVW P=0.04) and 1.06 (1.00, 1.13; IVW P=0.05) respectively. For BMI, the association did not remain after excluding the chronotype *FTO* variant which has a primary effect on BMI (OR = 1.02; 95%CI: 0.96, 1.08; IVW P=0.58) (**Supplementary Figures 9-11**).

Discussion

We performed a GWAS of chronotype in 697,828 individuals from 23andMe and the UK Biobank study and increased the number of associated loci from 22 to 351. The variants associated with self-report chronotype were also associated with activity-monitor derived estimates of sleep timing but not measures of sleep quantity or quality. The validation using activity-based estimates of sleep timing was important because self-report traits may result in reporting biases. Our data suggests that self-report sleep measures strongly relate to an individual's sleep timing and therefore provide valid instruments for MR and compelling insights into circadian biology.

Chronotype-associated loci were strongly enriched for known circadian genes, as well as genes more distally involved in circadian processes. Variants at these loci included the low frequency (MAF=0.5%) coding variants (P415A/H417R) in *PER3* previously identified as a cause of familial advanced sleep phase syndrome[29]. Although it had a large effect on chronotype (OR=1.44), we found that the minor allele of this variant was associated with an average earlier sleep timing of only 8 minutes, suggesting this is a low penetrance disease variant and not a monogenic cause of delayed sleep. By integrating information from tissue expression and previous RNAi experiments we identified additional genes likely to be involved in circadian timing, for example *LSM7* which encodes core components of the spliceosomal U6 small nuclear ribonucleoprotein complex for which some previous studies have suggested a role in circadian timing[38,42].

Despite a substantial body of observational epidemiology literature on the association between circadian rhythm variation and disease, we found no convincing evidence for a causal effect of morningness on many of these disorders in our MR analyses. Of the traits we tested, we found some evidence that being a morning person may result in a younger age at last childbirth and a reduced risk of multiple pregnancy losses, schizophrenia and depression. Although these results need replication, there is existing evidence of the relationship between circadian rhythms and fertility in women[43–45], and between circadian rhythms and mental health suggesting that this an area for further investigation.

Previously reported observational associations of chronotype with metabolic diseases are particularly strong[46,47], but we found no evidence for a causal effect of morningness on type 2 diabetes or BMI and could exclude the observational association effect sizes. We found nominal evidence of effects in the opposite direction. For example, MR analyses showed that there was nominal evidence of a causal role of higher T2D risk, and both higher insulin secretion and BMI, on being a morning person. This is exemplified by a missense variant in

the *MADD* gene (rs35233100) previously associated with lower proinsulin levels[32] (but not type 2 diabetes) being associated with eveningness. The BMI association, however, appears to be driven by the obesity associated allele in *FTO* (rs1558902) previously associated with higher BMI[30,31] being strongly associated with being a morning person. There is substantial evidence of a link between appetite, feeding and insulin pathways and circadian rhythms[48,49] supported by our pathway analyses which demonstrates an enrichment of insulin secretion regulation pathways; however, it is unclear why the causal association is not seen in the opposite direction.

The response to UK Biobank participation was < 5% and this can result in bias, including in GWAS and MR[50]. Here GWAS results replicated those of 23andMe, a study that may also suffer from selection bias but of a different nature to UK Biobank, and our MR results in UK Biobank were consistent with those from using two-sample MR in publicly-available aggregated data, based on consortia of studies that had considerably greater response rates.

In conclusion, we have identified hundreds of novel loci that regulate circadian rhythms and sleep timing in humans and provide new insights into the causal nature of the observational associations with disease.

Materials and Methods

Data availability

Summary statistics for the top 10,000 chronotype meta-analysis variants are provided in **Supplementary Table 18** and the full set of UK Biobank-only chronotype and morning person GWAS summary statistics can be found at: http://www.t2diabetesgenes.org/data/.

Ethics Statement

Details of patient and public involvement in the UK Biobank are available online at www.ukbiobank.ac.uk/about-biobank-uk/ and https://www.ukbiobank.ac.uk/wp-content/uploads/2011/07/Summary-EGF-consultation.pdf. Participants were not involved in setting research questions or any outcome measures, nor were they involved in planning, design, recruitment for or implementation of this study. Participants also had no involvement in analysing data, interpreting results or preparing this manuscript. There are no specific plans to disseminate the results of the research to study participants, though the UK Biobank disseminates key findings from projects on its website.

Cohorts

The UK Biobank is described in detail elsewhere[51]. We used data on 451,454 individuals from the full UK Biobank data release that we identified as White European and that had genetic data available. To define a set of White Europeans, we performed Principal Components Analysis (PCA) in the 1000 Genomes (1KG) reference panel using a subset of variants that were of a high quality in the UK Biobank. We projected these principal components into the set of related UK Biobank participants to avoid the relatedness confounding the principal components. We then adopted a k-means clustering approach to define a European cluster, initializing the ethnic centres defined by the population-specific means of the first four 1KG principal components. This analysis was performed only within individuals self-reporting as “British”, “Irish”, “White” or “Any other white background. Because association analyses are performed using linear mixed-model (LMM) method, we included related individuals.

We used summary statistics from a morning chronotype GWAS performed by 23andMe of 248,100 (N_{case}=120,478; $N_{control}$=127,622) participants with a minimum of 97% European ancestry. GWAS analysis was performed in a maximal set of unrelated participants, where pairs of individuals were considered related if they shared 700cM IBD of genomic segments, roughly corresponding to first cousins in an outbred population. The 23andMe cohort is described in more detail elsewhere[21].

Activity Monitor Data

A subset of the UK Biobank cohort was invited to wear a wrist-worn activity monitor for a period of one week. Individuals were mailed the device and asked to wear it continuously for seven days, including while bathing, showering and sleeping. In total, 103,720 participants returned their activity monitor devices with data covering at least three complete 24-hour periods. We downloaded the raw activity monitor data (data-field 90001) for these individuals, in the form of binary Continuous Wave Accelerometer (cwa) files. Further information, along with details of centrally-derived variables, is available elsewhere[52]. Detailed protocol information can be found online at http://biobank.ctsu.ox.ac.uk/crystal/docs/PhysicalActivityMonitor.pdf and a sample instruction letter at http://biobank.ctsu.ox.ac.uk/crystal/images/activity_invite.png (UKB Resources 131600 and 141141, respectively; both accessed January 30th 2018). We converted the .cwa files to .wav format using the open-source software "omconvert", recommended by the activity monitor manufacturers Axivity, which is available online (see https://github.com/digitalinteraction/openmovement/tree/master/Software/AX3/omconvert). To process the raw accelerometer data, we used the freely available R package "GGIR" (v1.5-12)[53,54]. The list of our GGIR settings is provided in the **Supplementary File** and the full list of variables produced by GGIR can be found in the CRAN GGIR reference manual (see https://cran.r-project.org/web/packages/GGIR/GGIR.pdf).

Genotyping and quality control

The 23andMe cohort was genotyped on one of four custom arrays: the first two were variants of the Illumina HumanHap550+ BeadChip (N_{case}=4,966; $N_{control}$=5,564), the third a variant of the Illumina OmniExpress+ BeadChip (N_{case}=53,747; $N_{control}$=61,637) and the fourth a fully custom array (N_{case}=61,765; $N_{control}$=60,421). Successive arrays contained substantial overlap with previous chips. These genotypes were imputed to ~15.6M variants using the September 2013 release of the 1000 Genomes phase 1 reference panel. For analyses, we used ~11.9M imputed variants with imputation $r^2 \geq 0.3$, MAF ≥ 0.001 (0.1%) and that showed no sign of batch effects.

The UK Biobank cohort was genotyped on two almost identical arrays. The first ~50,000 samples were genotyped on the UK BiLEVE array and the remaining ~450,000 samples were genotyped on the UK Biobank Axiom array in two groups (interim and full release). A total of 805,426 directly-genotyped variants were made available in the full release. These variants were centrally imputed to ~93M autosomal variants using two reference panels: a combined UK10K and 1000 Genomes panel and the Haplotype Reference Consortium

(HRC) panel. For all analyses, we used ~12.0M Haplotype Reference Consortium (HRC) imputed variants with an imputation $r^2 \geq 0.3$, MAF ≥ 0.001 (0.1%) and with a Hardy–Weinberg equilibrium $P>1x10^{-12}$. We excluded non-HRC imputed variants on advice from the UK Biobank imputation team. Further details on the UK Biobank genotyping, quality control and imputation procedures can be found elsewhere[55].

Self-Reported Phenotypes

Chronotype (UK Biobank)

The UK Biobank collected a single self-reported measure of Chronotype ("Morning/evening person (chronotype)"; data-field 1180). Participants were prompted to answer the question "Do you consider yourself to be?" with one of six possible answers: "Definitely a 'morning' person", "More a 'morning' than 'evening' person", "More an 'evening' than a 'morning' person", "Definitely an 'evening' person", "Do not know" or "Prefer not to answer", which we coded as 2, 1, -1, -2, 0 and missing respectively. Prior to association testing, we adjusted the phenotype for age, gender and study centre (categorical). Of the 451,454 white European participants with genetic data, 449,734 were included in the GWAS (had non-missing phenotype and covariates).

Morning Person (UK Biobank)

In order to provide interpretable odds ratios for our genome-wide significant variants, we also defined a binary phenotype using the same data-field as for Chronotype. Participants answering "Definitely an 'evening' person" and "More an 'evening' than a 'morning' person" were coded as 0 (controls) and those answering "Definitely a 'morning' person" and "More a 'morning' than 'evening' person" were coded as 1 (cases). Participants answering "Do not know" or "Prefer not to answer" were coded as missing. A total of 403,195 participants were included in the GWAS (252,287 cases and 150,908 controls).

Morning Person (23andMe)

Responses to two identical questions were used to define the dichotomous morning person phenotype in the 23andMe cohort, with one question having a wider selection of neutral options. More details are given in Supplementary Table 2 of the 23andMe morning person GWAS[21]. Morning people were coded as 1 (cases; N=120,478) and evening people were coded as 0 (controls; N=127,622).

Activity monitor Phenotypes

Identifying the sleep period window

The software package GGIR[54,56] produces quantitative and timing measures relating to both activity levels and sleep patterns, with a day-by-day breakdown, as well averages across the period of wear. A new algorithm, implemented in version 1.5-12 of the GGIR R package and validated using PSG in an external cohort[57], allows for detection of sleep periods without the use of a sleep diary and with minimal bias. Briefly, for each individual, median values of the absolute change in z-angle (representing the dorsal-ventral direction when the wrist is in the anatomical position) across 5-minute rolling windows were calculated across a 24-hour period, chosen to make the algorithm insensitive to activity monitor orientation. The 10th percentile was incorporated into the threshold to distinguish movement from non-movement. Bouts of inactivity lasting ≥30 minutes are recorded as inactivity bouts. Inactivity bouts that are <60 minutes apart are combined to form inactivity blocks. The start and end of longest block defines the start and end of the sleep period time-window (SPT-window).

Activity monitor exclusions and adjustments

The UK Biobank made multiple activity monitor data-quality variables available. From our activity monitor phenotypes, we excluded 4,925 samples with a non-zero or missing value in data field 90002 (“Data problem indicator”). We then excluded any individuals with the “good wear time” flag (field 90015) set to 0 (No), “good calibration” flag (field 90016) set to 0 (No), “calibrated on own data” flag (field 90017) set to 0 (No), “data recording errors” (field 90182) > 788 (Q_3 + 1.5xIQR) or a non-zero count of “interrupted recording periods” (field 90180). Phenotypes determined using the SPT-window (all phenotypes except L5 and M10 timing) had additional exclusions based on short (<3 hours) and long (>12 hours) mean sleep duration and too low (<5) or too high (>30) mean number of sleep episodes per night (see below). These additional exclusions were to ensure that individuals with extreme (outlying), and likely incorrect, sleep characteristics were not included in any subsequent analyses. Prior to association testing, we adjusted all phenotypes for age activity monitor worn (derived from month and year of birth and date activity monitor worn), gender, season activity monitor worn (categorical; winter, spring, summer or autumn; derived from date activity monitor worn) and number of valid measurements (SPT-windows for sleep phenotypes, number of valid days for diurnal inactivity or number of L5 or M10 detections).

L5 and M10 timing (UK Biobank)

L5 and M10 refer to the least-active five and the most-active ten hours of each day, and are commonly studied measures relating to circadian activity and sleep. L5 (M10) defines a five-hour (ten-hour) daily period of minimum (maximum) activity, as calculated by means of a moving average with a five-hour (ten-hour) window. We defined our L5 (M10) timing phenotype as the number of hours elapsed from the previous midnight to the L5 (M10)

midpoint, averaged over all valid wear days. Of the 103,711 participants with activity monitor data, there were 85,205 and 85,670 with valid L5 and M10 timing measures respectively, covariates and genetic data. Basic summaries of these and other raw activity monitor phenotypes are given in **Supplementary Table 3**.

Sleep midpoint (UK Biobank)

Sleep midpoint was calculated as the time directly between the start and end of the SPT-window, and is defined as the number of hours elapsed since the previous midnight (e.g. 02:30 = 26.5). Our sleep midpoint phenotype represented the average over all valid sleep periods. After exclusions and adjustments, 84,810 participants had valid sleep midpoint, covariates and genetic data.

Sleep duration (UK Biobank)

Sleep episodes within the SPT-window were defined as periods of at least 5 minutes with no change larger than 5° associated with the z-axis of the activity monitor, as described previously[54]. The summed duration of all sleep episodes provided the sleep duration for a given SPT-window. We took both the mean and standard deviation of sleep duration across all valid SPT-windows to provide a measure of average sleep quantity and a measure of variability. After exclusions and adjustments, we had 85,449 (84,441) participants with valid sleep duration mean (S.D.), covariates and genetic data.

Sleep efficiency (UK Biobank)

This was calculated as a ratio of sleep duration (defined above) to SPT-window duration. The phenotype represented the mean across all valid SPT-windows and after exclusions and adjustments, left us with 84,810 participants with valid sleep efficiency, covariates and genetic data.

Number of sleep episodes (UK Biobank)

This is defined as the number of sleep episodes separated by last least 5 minutes of wakefulness within the SPT-window. The phenotype represents the mean across all SPT-windows and can be interpreted as a measure of sleep disturbance or fragmentation. After exclusions and adjustments, we had 84,810 participants with valid sleep efficiency, covariates and genetic data.

Diurnal inactivity duration (UK Biobank)

The total daily duration of estimated bouts of inactivity that fall outside of the SPT-window. This includes both periods of inactivity and sleep (naps), as it is impossible to separate these

without detailed activity diaries. The phenotype is calculated as the mean across all valid days and, after exclusions and adjustments, we were left with 84,757 participants with a valid measure, covariates and genetic data.

Genome-wide Association Analysis

We performed all association test using BOLT-LMM[58] v2.3, which applies a linear mixed model (LMM) to adjust for the effects of population structure and individual relatedness, and allowed us to include all related individuals in our white European subset, boosting our power to detect associations. This meant a sample size of up to 449,734 individuals, as opposed to the set of 379,768 unrelated individuals. BOLT-LMM approximates relatedness within a cohort by using LD blocks and avoids the requirement of building a genetic-relationship matrix (GRM), with which calculations are intractable in cohorts of this size. From the ~805,000 directly-genotyped (non-imputed) variants available, we identified 524,307 "good-quality" variants (bi-allelic SNPs; MAF≥1%; HWE $P>1x10^{-6}$; non-missing in all genotype batches, total missingness<1.5% and not in a region of long-range LD[59]) for inclusion in BOLT-LMM's mixed model, and we used the 1000 Genomes European LD-Score table provided with the software for information on LD structure. We forced BOLT-LMM to apply a non-infinitesimal model, which provides better effect size estimates for variants with moderate to large effect sizes, in exchange for increased computing time. Prior to association testing, continuous phenotypes were first adjusted for relevant covariates, as indicated above, and at runtime we included "release" (categorical; UKBiLEVE array, UKB Axiom array interim release and UKB Axiom array full release) as a further covariate. The binary morning person phenotype was adjusted at runtime for age, gender, study centre and release.

In the 23andMe morning person GWAS, the summary statistics were generated through logistic regression (using an additive model) of the phenotype against the genotype, adjusting for age, gender, the first four principal components and a categorical variable representing genotyping platform. Genotyping batches in which particular variants failed to meet minimum quality control were not included in association testing for those variants, resulting in a range of sample sizes over the whole set of results. A λ_{GC} of 1.325 was reported for this GWAS.

Sensitivity Analysis

To avoid issues with stratification, we performed a sensitivity GWAS, in UK Biobank alone, to assess whether any of the associations were driven by a subset of the cohort with specific conditions. We excluded those reporting shift or night shift work at baseline, those taking

medication for sleep or psychiatric disorders and those with either with a HES ICD10 or self-reported diagnosis of depression, schizophrenia, bipolar disorder, anxiety disorders or mood disorder (see **Supplementary Methods** for further details). Results for the 341 lead chronotype variants available in the UK Biobank are provided in **Supplementary Table 1** alongside the main meta-analysis results.

Meta-analysis of GWAS Results

Meta-analysis was performed using the software package METAL[60]. To obtain the largest possible sample size, and thus maximising statistical power, we performed a sample-size meta-analysis, using the results from the UK Biobank chronotype GWAS and the 23andMe morning person GWAS. Genomic control was not performed on each set of summary statistics prior to meta-analysis but instead the meta-analysis chi-squared statistics were corrected using the LD score intercept (I_{LDSC} = 1.0829), calculated by the software LDSC, as using λ_{GC} is considered overly conservative and the LD score intercept better captures inflation due to population stratification[61]. For interpretable results, we reported the odds ratio from a secondary effect size meta-analysis between our dichotomous UK Biobank morning person GWAS and the 23andMe morning person GWAS. The primary meta-analysis produced results for 15,880,618 variants in up to 697,828 individuals.

Post-GWAS Analyses

Pathway analysis and tissue-enrichment

We used MAGENTA[36], DEPICT[37], PASCAL[35] and MAGMA[33]. For MAGENTA and DEPICT, we included all variants from the meta-analysis, whereas for PASCAL, we included only those with an RSID as the software assigns variants to genes using their RSID. For the MAGENTA analysis, we used upstream and downstream limits of 110Kb and 40Kb to assign variants to genes by position, we excluded the HLA region from the analysis and set the number of permutations for gene-set enrichment analysis (GSEA) to 10,000. For DEPICT, we used the default settings and the annotation and mapping files provided with the software.

Genetic correlation and heritability analyses

We used the LD Score Regression (LDSC) software, available at https://github.com/bulik/ldsc/, to quantify the genetic overlap between the trait of interest and 222 traits with publicly available GWA data. Details of methodology are available elsewhere[62]. We considered any correlation as statistically significant if it had a Bonferroni corrected $P<0.05$.

Mendelian Randomisation Analyses

Given the large number of studies reporting multi-variable associations of chronotype and sleep traits with multiple health outcomes, we undertook MR analyses to explore both the effect of chronotype on different outcomes and the effect of different exposures on chronotype as an outcome. These two-sample MR analyses can be summarised by:

1. Chronotype exposure using 109 variants and effect sizes discovered in 23andMe against 250 outcomes from unpublished parallel UK Biobank GWAS (**Supplementary Table 15**)
2. Chronotype exposure using 351 variants and effect sizes discovered in the meta-analysis against 671 outcomes from published studies (**Supplementary Table 16**)
3. 39 exposures using variants from published studies against UK Biobank chronotype as an outcome (**Supplementary Table 17**).

Analyses 1 and 3 were performed using a custom pipeline (designed to use only summary data of both exposure and outcome) in which we tested four MR methods:

a. Inverse-variance weighting (IVW)[63]
b. MR-Egger[63]
c. Weighted median (WM)[64] estimation
d. Penalised weighted median (PWM)[64] estimation

Analysis 2 was performed using the R package TwoSampleMR using aggregated summary statistics available through the MR-Base platform[39]. We implemented the same four methods as with analyses 1 and 3, and also included the MR-Egger bootstrap to provide better estimates of the effect sizes and standard errors as compared to the MR-Egger method. Analyses 1 and 2 both investigated the effect of chronotype on different traits or diseases, yet they had different strengths. Analysis 1 took advantage of the availability of summary level data in ~39 million HRC-imputed positive-strand SNPs for multiple outcomes. This means that the statistical power of the MR tests was less variable from one phenotype to another, as the number of genetic chronotype instruments used was consistent across all outcomes. The caveat was that we had to use 109 variants (and effects) discovered in 23andMe (and not UK Biobank) to avoid biasing our results away from the null, reducing the number of chronotype instruments and thus reducing our power to detect causal associations. As analysis 2 relied upon published summary statistics, we were able to use all 351 variants discovered in the chronotype meta-analysis as genetic instruments (thus providing greater statistical power), though the number of these available in the summary statistics of published GWAS varied greatly, leading to a large heterogeneity in the statistical

power available to detect causal associations with the different outcomes. Consistent evidence of association for the same outcomes between analyses 1 and 2 would provide strong evidence for causality.

We used the inverse variance weighted approach as our main analysis method and MR-Egger, weighted median estimation and penalised weighted median estimation as sensitivity analyses in the event of unidentified pleiotropy of our genetic instruments. MR results may be biased by horizontal pleiotropy, i.e. where the genetic variants that are robustly related to the exposure of interest (here chronotype) independently influence the outcome, through association with another risk factor for the outcome. IVW assumes that there is either no horizontal pleiotropy (under a fixed effect model) or, if implemented under a random effects model after detecting heterogeneity amongst the causal estimates, that:

i. The strength of association of the genetic instruments with the risk factor is not correlated with the magnitude of the pleiotropic effects
ii. The pleiotropic effects have an average value of zero

MR-Egger provides unbiased causal estimates if just the first condition above holds, by estimating and adjusting for non-zero mean pleiotropy. However, MR-Egger requires that the InSIDE (Instrument Strength Independent of Direct Effect) assumption[65] holds, in that it needs the pleiotropy of the genetic instruments to be uncorrelated with the instruments' effect on the exposure. The weighted median approach is valid if less than 50% of the weight in the analysis stems from variants that are pleiotropic (i.e. no single SNP that contributes 50% of the weight or a number of SNPs that together contribute 50% should be invalid because of horizontal pleiotropy). Given these different assumptions, if all methods are broadly consistent this strengthens our causal inference.

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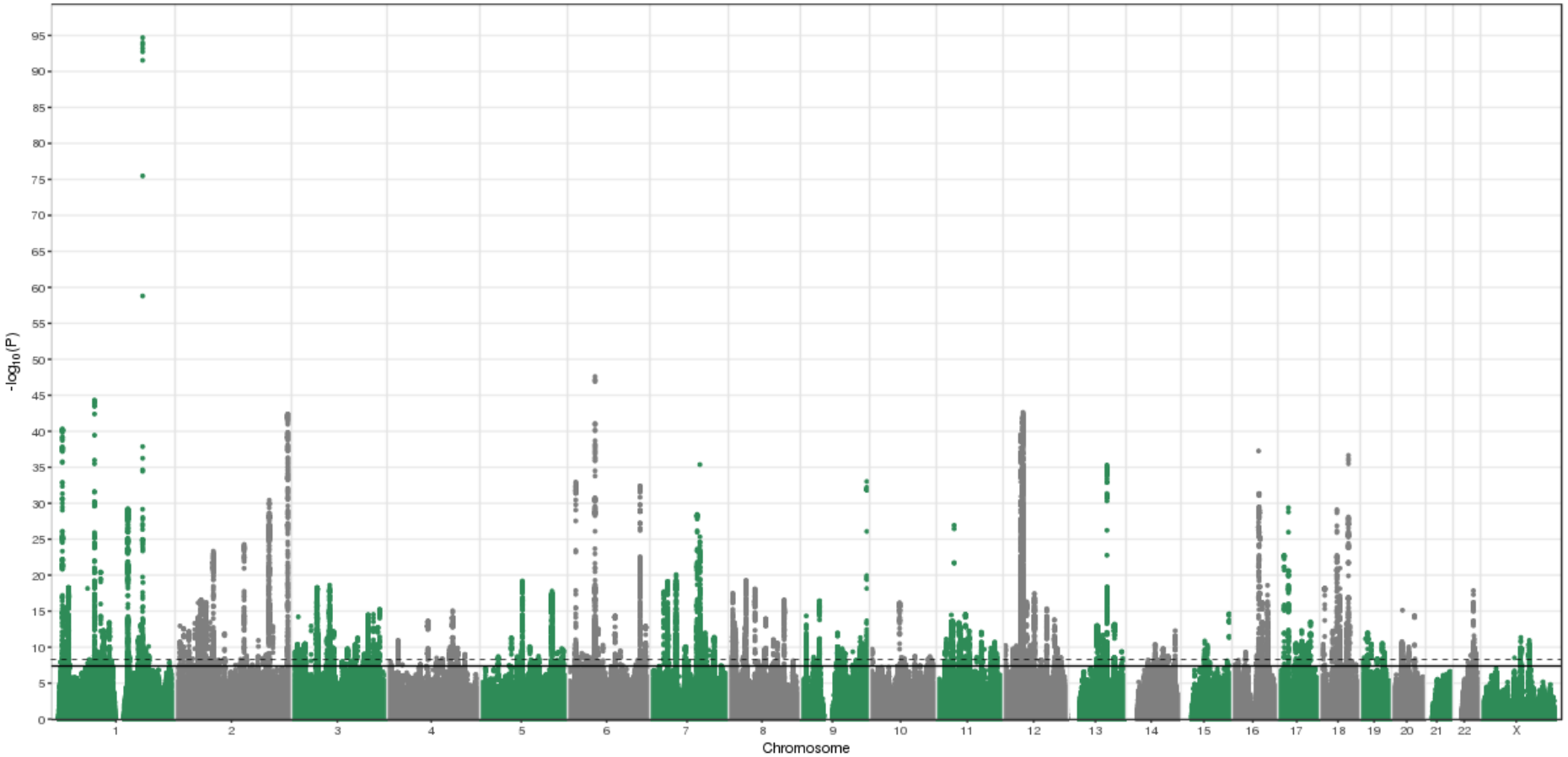

Figure 1. Manhattan plot of the chronotype meta-analysis GWAS. The solid and dashed black lines respectively indicate typical and our permutation-testing genome-wide significance thresholds of $P=5x10^{-8}$ and $P=6x10^{-9}$.

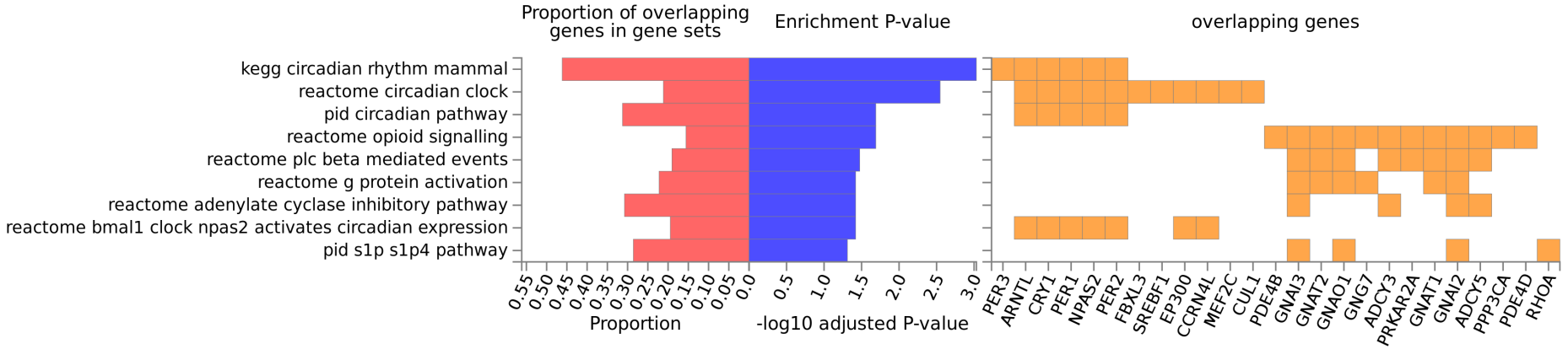

Figure 2. Reactome gene sets overlapping Chronotype genes identified using positional and eQTL mapping (and not MAGMA) in FUMA's GENE2FUNC process. Note that these results may differ to those produced by MAGMA.

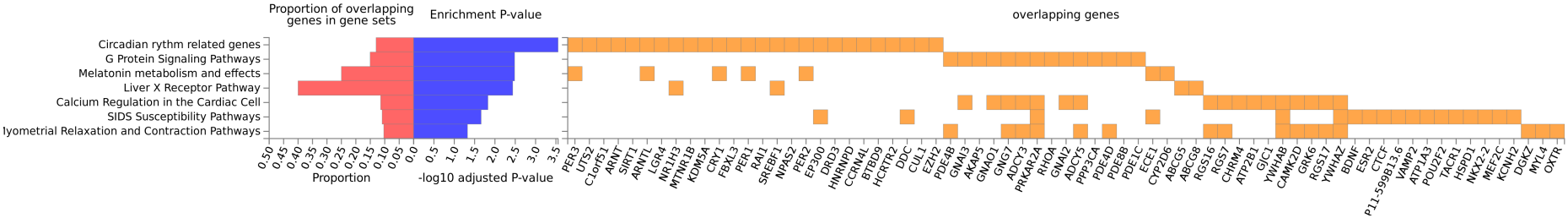

Figure 3. WikiPathways gene sets overlapping Chronotype genes identified using positional and eQTL mapping (and not MAGMA) in FUMA's GENE2FUNC process. Note that these results may differ to those produced by MAGMA.

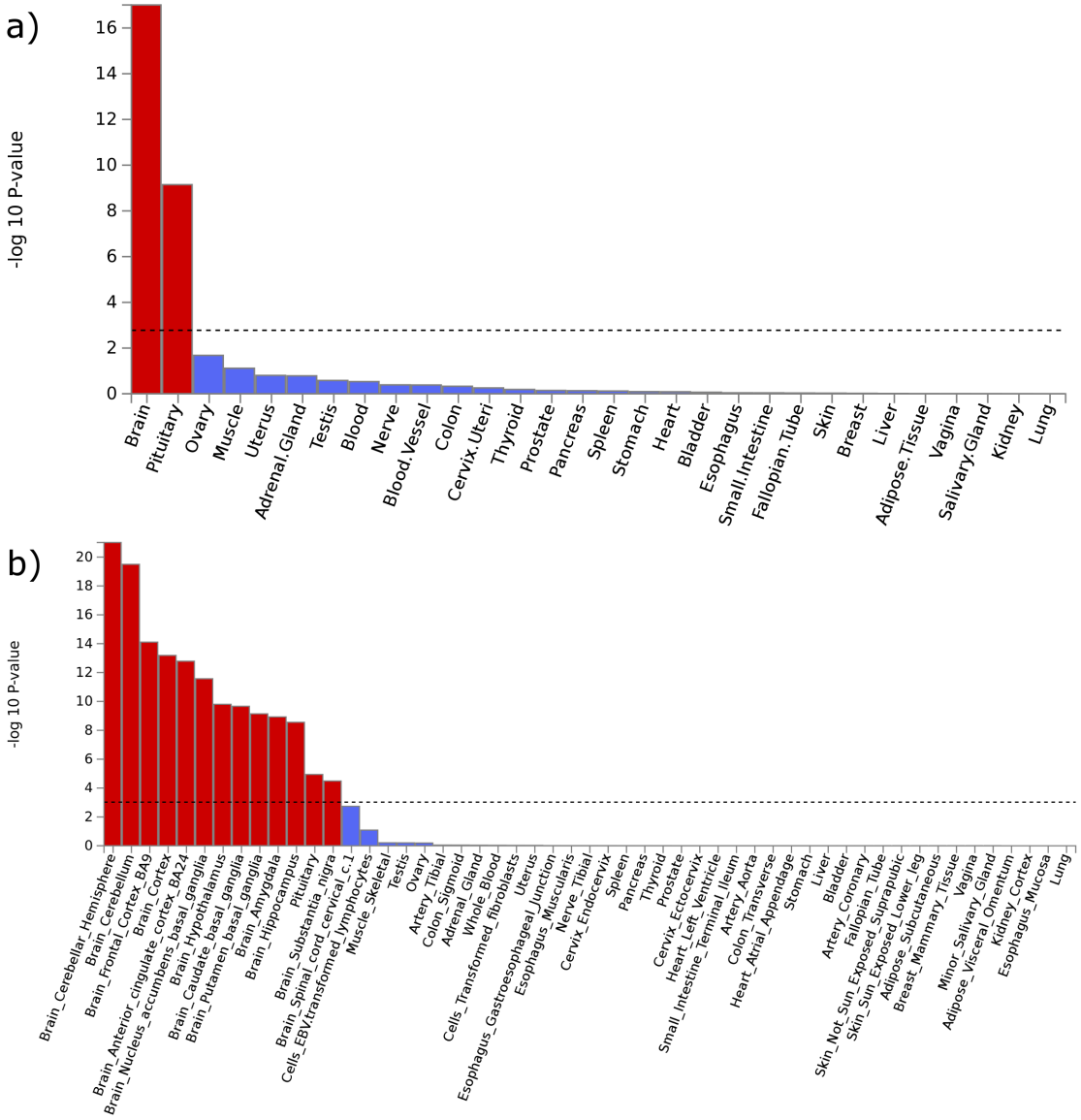

Figure 4. MAGMA tissue expression analysis using gene expression per tissue based on GTEx RNA-seq data for a) 30 general and b) 53 specific tissue types.

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