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ORIGINAL ARTICLE

A K_{ATP} channel gene effect on sleep duration: from genome-wide association studies to function in *Drosophila*

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Humans sleep approximately a third of their lifetime. The observation that individuals with either long or short sleep duration show associations with metabolic syndrome and psychiatric disorders suggests that the length of sleep is adaptive. Although sleep duration can be influenced by photoperiod (season) and phase of entrainment (chronotype), human familial sleep disorders indicate that there is a strong genetic modulation of sleep. Therefore, we conducted high-density genome-wide association studies for sleep duration in seven European populations ($N=4251$). We identified an intronic variant (rs11046205; $P=3.99 \times 10^{-8}$) in the *ABCC9* gene that explains $\approx 5\%$ of the variation in sleep duration. An influence of season and chronotype on sleep duration was solely observed in the replication sample ($N=5949$). Meta-analysis of the associations found in a subgroup of the replication sample, chosen for season of entry and chronotype, together with the discovery results showed genome-wide significance. RNA interference knockdown experiments of the conserved *ABCC9* homologue in *Drosophila* neurons renders flies sleepless during the first 3 h of the night. *ABCC9* encodes an ATP-sensitive potassium channel subunit (SUR2), serving as a sensor of intracellular energy metabolism.

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Keywords: *ABCC9*; chronotype; Munich ChronoType Questionnaire; seasonality; sleep duration; SUR

Introduction

The fact that all higher animals sleep, preventing them from collecting resources and reproducing, indicates that this process is essential for survival.¹ Sleep has three major characteristics—quality, timing and duration; the latter two are influenced by sleep

pressure, that is, previous length of wakefulness (homeostasis), as well as by the circadian clock and its phase of entrainment (chronotype).¹ Because light is the most important *zeitgeber* (synchroniser) for the circadian system, it is not surprising that seasonal changes in photoperiod can also influence sleep/wake behaviour.^{2–5} Abrupt changes in social times, such as jetlag or daylight savings time transitions, interfere with the seasonal adaptation of sleep and its entrained phase, resulting in circadian misalignment and sleep deprivation.⁴ A number of psychiatric disorders are associated with abnormal sleep and circadian behaviour in humans, and/or

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with genes involved in the regulation of these phenotypes in model organisms,⁶ suggesting common pathways. The influence of season on neurobehavioral disorders⁶ may also be linked to gene–environment interactions underlying sleep and circadian behaviour.⁵ Too little or too much sleep is also associated with health deficits, including high body mass index (BMI), hypertension, cardiovascular diseases, type-2 diabetes and general mortality.⁷ Insufficient sleep, as a consequence of sleep deprivation, has also been linked to sub-optimal learning and memory.⁸

Although sleep duration strongly depends on age and gender, large inter-individual differences remain, even when sleep duration is normalised for these covariates,⁹ and indicating the involvement of multiple genes (twin studies indicate a 40% heritability).¹⁰ A polygenic basis for sleep duration is also suggested by quantitative trait locus studies in mice and *Drosophila*.^{11,12} In mice, sleep characteristics (both timing and duration) are altered in clock gene knock-outs (for example *NPAS2*, *BMAL1* and *CLOCK*), supporting the involvement of the circadian clock in sleep regulation.¹³ The association of a mutation in *hDEC2* (a regulator of *CLOCK* and *BMAL1*) with sleep duration in a family-based study was confirmed in transgenic mice and *Drosophila*.¹⁴ A small-scale genome-wide scan¹⁵ identified a linkage peak in the *hPROK2* gene (controlled by the *CLOCK*-*BMAL1* complex), and a common gene variant of *hCLOCK* showed a significant association ($P < 0.05$) with sleep duration in a candidate clock gene study.⁹

To identify novel genes associated with sleep duration, we performed genome-wide association studies (GWAS) for the average weekly sleep duration, that is, across work and free days, in seven populations ($N=4251$). Genotypes were imputed for ≈ 2.5 Mio autosomal single-nucleotide polymorphisms (SNPs) from the HapMap2 CEU panel. The meta-analysis of these discovery cohorts revealed a genome-wide significant signal in the *ABCC9* locus that encodes a pore-forming subunit of an ATP-sensitive potassium channel (K_{ATP}), known to be involved in energy metabolism and in the aetiology of cardiomyopathies.¹⁶ This signal was replicated when meta-analysing the results of the discovery cohorts with two additional cohorts: an *in silico* (GWAS data) sample and a subgroup population of a large *de-novo* (single genotyping) sample. The phenotype of the large additional sample showed dependencies on season and chronotype, which were used as criteria to select a sub-population for replication analysis. We confirmed the conserved role of *ABCC9* by knocking down its homologue in *Drosophila*, which shortened night time but not daytime sleep duration.

Materials and methods

Study cohorts

Data from seven GWAS consisting of 4251 phenotyped and genotyped individuals (all of European ancestry and informative for sleep duration) were

meta-analysed in the discovery phase (Table 1). A description of the participating cohorts is provided in the Supplementary Material and Methods (Supplementary Table 1). Informed consents were obtained from all participants, and the respective local ethics committees approved the study designs. The data of the individual GWAS regarding sleep duration are reported here for the first time. 5949 *de novo* (TaqMan, Applied Biosystems, Life Technologies GmbH, Darmstadt, Germany) and 536 *in silico* (OmniExpress 700K, Illumina 370CNV-quad, San Diego, CA, USA) genotyped subjects from EGCUT (Supplementary Table 1, and Supplementary Figure 1 for study design), who were not genotyped in the initial GWAS, were used for the replication study.

Phenotyping

Average weekly sleep duration (SD_{av}) was assessed with the short version of the Munich ChronoType Questionnaire,^{4,9} showing a normal distribution in all investigated cohorts. The Munich ChronoType Questionnaire asks a series of simple questions regarding sleep times on workdays (mostly depending on work times or timing of social activities), and on free days (associated with the individual's endogenous circadian rhythm). To assess chronotype, the mid-sleep phase on free days was corrected for the sleep-duration deficit of workdays.¹⁷ Inclusion criteria were: 1) no use of an alarm clock on free days; 2) no shift-work during the last three months; and 3) no use of sleep medication (benzodiazepines and other pharmacological agents that influence sleep; see Supplementary Table 2).

Genotyping and imputation

Genotyping was done on a variety of platforms. Imputation of non-genotyped SNPs in the HapMap2 CEU v21a or v22 reference panels was carried out for each study using MACH¹⁸ or IMPUTE¹⁹ (Supplementary Table 3). The overall quality-control criteria excluded individuals with low call rates, excess heterozygosity and gender mismatch. Three *ABCC9* SNPs were selected for replication (see Table 1). Linkage disequilibrium (LD) extent was based on pair-wise r^2 values from HapMap2 CEU. *De novo* genotyping ($N=5949$; EGCUT) was performed using a TaqMan assay (Applied Biosystems; Supplementary Table 4).

Statistical analysis

Genotypes consisting of both directly typed and imputed SNPs (as specified in Supplementary Table 3) were used for the individual GWAS. To avoid over-inflation of test statistics due to population structure or relatedness, we applied genomic controls for the independent studies and meta-analysis (Supplementary Table 3; Supplementary Figure 2). Analyses were performed using linear regression (under an additive model), with sleep duration as the dependant variable, SNP allele dosage as predictor and with age, gender and BMI as covariates. A fixed-effects meta-analysis was conducted using the inverse-

Table 1 Meta-analysis top cluster-index SNPs ($P < 10^{-5}$) for associations across all investigated autosomal chromosomes

| Chr | SNP ID (position) | Effect allele | CEU allele freq | Freq range | Genome-wide association studies (N) - Beta coefficients - | | | | | Meta-analysis P | Cluster SNPs (KB) | Locus (role), nearby <i>loci</i> ^a | SNP predicts expression of <i>loci</i> ^b ($P < 0.0001$) | | | |
|-----|--------------------------------------|---------------|-----------------|-------------|---|---------------------|--|--|---------------------|---------------------|---------------------|--|--|-------------|----------------------------------|------------------------|
| | | | | | EGCUT (924) | ERF (740) | KORA F4 (548) | KORCULA (600) | MICROS (693) | | | | | NESDA (540) | ORCADES (206) | |
| 1 | rs11118271 (217,555,382) | A | 0.108 | 0.07–0.104 | 0.2331 | 0.2906 | 0.1148 | 0.1527 | 0.1378 | 0.0889 | 0.0951 | 0.1701 (0.037) | 6.12×10^{-6} | 25 (118) | — | TUSC2 |
| 2 | rs2697804 (233,088,264) | A | 0.208 | 0.217–0.268 | 0.0694 | 0.0498 | 0.1295 | 0.1844 | 0.1812 | 0.1502 | 0.1105 | 0.1193 (0.026) | 4.46×10^{-6} | 4 (34) | TIGD1, EIF4E2, CHRN3, CHRNA1 | FARP1 |
| 2 | rs2245601 (233,099,181) | A | 0.526 | 0.422–0.526 | -0.0871 | -0.1187 | -0.1752 | -0.1309 | -0.1299 | NA | 0.0358 | -0.1162 (0.025) | 4.13×10^{-6} | 1 (0) | CHRNA1 (coding), CHRNA1 (intron) | NA |
| 3 | rs13092077 (157,720,046) | A | 0.364 | 0.267–0.337 | 0.1279 | 0.0779 | 0.0970 | 0.1633 | 0.1189 | 0.1238 | 0.0806 | 0.1121 (0.024) | 2.60×10^{-6} | 30 (63) | KCNAB1 (intron) | NA |
| 3 | rs963354 (158,876,464) | A | 0.667 | 0.649–0.815 | 0.1037 | 0.0977 | 0.1066 | 0.2117 | 0.1019 | 0.1363 | 0.1371 | 0.1195 (0.024) | 8.41×10^{-7} | 6 (20) | — | NA |
| 12 | rs11046205 (21,883,593) | A | 0.173 | 0.148–0.206 | 0.1269 ^c 0.1076 ^d | 0.1747 ^c | 0.1452 ^c 0.2034 ^d | 0.1779 ^e 0.0387 ^d | 0.0848 ^c | 0.3303 ^e | 0.1387 ^c | 0.1669 (0.030) ^{c,e} / 0.1565 (0.028) ^{c,d,e} | 3.68×10^{-8} ^{c,e} 3.99×10^{-8} ^{c,d,e} | 3 (20) | ABCC9 (intron) | NA |
| 12 | rs11046211 (21,894,989) | T | 0.059 | 0.042–0.119 | 0.1273 | 0.3727 | 0.2404 | 0.1341 | 0.0579 | 0.4249 | 0.1060 | 0.2001 (0.045) | 9.90×10^{-6} | 13 (68) | ABCC9 (intron) | NA |
| 12 | rs11046209 ^f (21,888,233) | A | 0.948 | 0.881–0.958 | -0.1288 | -0.3730 | -0.2334 | -0.1515 | -0.0617 | -0.4266 | -0.0609 | -0.1951 (0.045) | 1.42×10^{-5} | 13 (68) | ABCC9 (intron) | NA |
| 12 | rs10506103 (33,158,210) | T | 0.208 | 0.191–0.280 | -0.1155 | -0.3388 | -0.1436 | -0.0269 | -0.0437 | -0.0654 | -0.1002 | -0.1273 (0.026) | 1.15×10^{-6} | 33 (123) | — | SLC39A4 |
| 13 | rs9515329 (110,423,204) | A | 0.664 | 0.624–0.739 | -0.0401 | -0.1026 | -0.0315 | -0.1365 | -0.1057 | -0.2557 | -0.0986 | -0.1013 (0.023) | 9.11×10^{-6} | 10 (8) | — | LZIC, LOC286126, NRBP2 |
| 17 | rs9911832 (71,701,120) | A | 0.225 | 0.213–0.311 | -0.1587 | -0.0525 | -0.0471 | -0.0842 | -0.1813 | -0.0850 | -0.2064 | -0.114 (0.026) | 8.72×10^{-6} | 8 (33) | RNF157 (intron), FOXJ1 | STT3A |

Abbreviations: Chr, chromosome; EGCUT, Estonian Genome Center, University of Tartu; ERF, Erasmus Rucphen Family Study; GWAS, genome-wide association studies; KORA F4, Cooperative Health Research in the Region of Augsburg; KORCULA, The Korcula study in Croatia; MAF, minor allele frequency; MICROS, Microisolates in South Tyrol; NA, not available; NESDA, The Netherlands Study of Depression and Anxiety; ORCADES, The Orkney Complex Disease Study; SNP, single-nucleotide polymorphism.

^aThe nearest reference locus is in bold typeface if the SNP cited is within it, and the role of the SNP is given between brackets. Reference *loci* are shown if any SNP of the cluster is located in a specific *locus* or within 20 KB up or downstream of it. Chromosomes positions are based on the NCBI build 36. Total N for the meta-analysis was 4251, except for rs2245601 (N = 371), for which results were not available for the NESDA GWA.

^bGene expression annotation from SCAN database.

^cImputed based on linkage-disequilibrium of genotyped SNPs with HAPMAP SNPs.

^dGenotyped by TaqMan.

^eGenotyped by the GWAS platform. The effect (beta) was calculated based on either the minor or the common allele, depending on the statistics packages used for the GWA analyses.

^frs11046209 is a perfect proxy of rs11046211, used for technical replication.

Index SNPs from ten different associated genomic regions are shown. All SNPs listed are in Hardy–Weinberg equilibrium (HWE, $P > 0.05$). Criteria for clustering SNPs were physical distance threshold of 250 KB in relation to the index SNP, the extent of linkage disequilibrium with it ($r^2 > 0.5$), and association $P < 0.01$.

variance-weighted method as implemented in METAL. All SNPs with low MAF (<0.01) and low imputation quality (Rsq/proper_info <0.3) were dropped from the meta-analysis. Genomic control correction was applied to all cohorts before meta-analysis. Corresponding to Bonferroni adjustment for one million independent tests,²⁰ we specified a threshold of $P < 5 \times 10^{-8}$ for genome-wide significance. To assess the number of independent loci associated with SD_{av} at the $P < 10^{-5}$ (Table 1), correlated SNPs were grouped using a LD-based result clumping procedure (PLINK). Linear regression (PLINK) for associations with SD_{av} was used to analyse the *de novo* and *in silico* replication datasets, with age, sex and BMI used as covariates. The *de novo* replication dataset was analysed separately for summer (day-light savings time — DST period) and winter (actual zone time), and the first or second half (early and late half, respectively) of the chronotype distribution (normalised for age and sex — MSF_{sasc} ⁴). Season-comparisons in similar subgroups were conducted at the phenotypic level in seven of the nine investigated cohorts (for ERF and NESDA individual entry dates of assessments were not available). We used non-parametric statistics so as to not violate any assumptions of normality in the subgroup analyses. Analyses were performed with SPSS version 18 (SPSS, Inc. 2010, Chicago, IL, USA). The final meta-analysis was conducted including all discovery phase results, the *in silico* replication, and the early chronotype sub-population of the large replication dataset sampled during the winter (Supplementary Figure 1). To plot phenotype means per genotypes, we normalised SD_{av} for age and sex, yielding the final quantitative phenotype: SD_{asc} . Algorithms for the normalisation were based on the SD_{av} distribution of a large EGCUT-phenotyped sample ($N \approx 12\,000$).⁹ The variance of the phenotype explained by the leading meta-analysis SNP genotypes was calculated based on SD_{asc} .

Fly strains and behavioural assays

An *elav-gal4* strain was crossed to two independent RNA interference (RNAi) *UAS-Sur* lines (*V104241* and *V6750*), targeted against distinct portions of the *dSur* gene, from the Vienna *Drosophila* RNAi Centre. The knockdown was quantified and found to be $\sim 90\%$, although the sleep analysis was performed on 5-min bins (Supplementary Methods). Control experiments were performed with *V10424* and *V6750*, *elav-gal4* and two additional strains, *Canton-S* (*CS*) and a natural isolate *HU*.²¹ *Drosophila* Activity Monitoring System devices from Trikinetics (Waltham, MA, USA) were used to collect locomotor activity and sleep data in 1-min bins. Two-day-old males (15–32 flies per genotype) were acclimatised in 12:12 h light/dark cycles at 25 °C for 2 days, and activity was recorded under these conditions for further 2 days before release into constant darkness and activity recording for an additional 5–7 days. The data were analysed using BeFly!, an in-house (Department of Genetics, University of Leicester) sleep/circadian statistical package.

Results

After genomic control analyses, no population stratification was observed in any of the cohorts (inflation factors λ , in the independent GWAS: 0.99–1.03; for the overall genome-wide meta-analysis λ : 0.99), as shown on the quantile–quantile plots (Supplementary Figure 2). The meta-analysing analysis of the discovery-phase GWAS generated a single genome-wide significant association with SD_{av} ($P = 3.68 \times 10^{-8}$; Table 1, Figure 1a, and Supplementary Figures 3 and 5), corresponding to a SNP (rs11046205) within the *ABCC9* gene (ATP-binding cassette, sub-family C, member 9). The largest effect was observed in a cohort with typed (not imputed) genotypes (NESDA). To correct for potential imputation bias (poorly imputed genotypes due to different genotyping platforms;²² see Supplementary Table 3 and Supplementary Figure 4 for details), we genotyped rs11046205 on an individual basis in three of the discovery cohorts (which had initially only imputed data for this SNP; Table 1; Supplementary Table 4). This produced comparable meta-analysis results ($P = 3.99 \times 10^{-8}$; Supplementary Table 4). As the leading SNP (rs11046205) only showed weak LD ($r^2 < 0.4$; Supplementary Figure 5) with the second-best hit (rs11046211) in this locus, we tested the independence of their effects on the GWAS results, by conditional-association analysis, in the three cohorts that produced the largest effects (NESDA, EGCUT and ERF) and found that the rs11046211 effect disappeared when adjusted for rs11046205. Seven additional intronic variants in perfect LD ($r^2 = 1$; Supplementary Figures 5 and 6) with rs11046211 ranked among the top 100 association signals ($P = 9.90 \times 10^{-6}$ – 1.5×10^{-5} ; Supplementary Table 5). A summary of the best ranking loci based on P values and number of correlated SNPs (Materials and methods) is listed in Table 1 (see Figure 2 for functional descriptions and Supplementary Figure 7 for forest plots); there were only 10 other known genes (with no established relationship to sleep) among the top 100 association signals (Supplementary Table 5).

CSNK1A1, recently characterized as a clock regulator kinase (CK1 α),²³ showed the best associations ($P < 0.001$) among 19 clock genes (Supplementary Figure 8, and Supplementary Table 6). Our results also indicate that: (i) common variants having a small contribution for sleep duration—such as *CLOCK* gene variants⁹ (earlier associations could not be followed up because of low-quality imputation in the region; Supplementary Figure 8), may have a significant effect only in combination with other variants, and (ii) rare variants with large effects, for example: *DEC2*,¹⁴ will not explain the variation of sleep duration in general populations, which should be rather modulated by frequent variants from several loci. We selected thereafter three of the *ABCC9* SNPs for replication, the leading SNP on the meta-analysis, the second best SNP within the same locus, and a

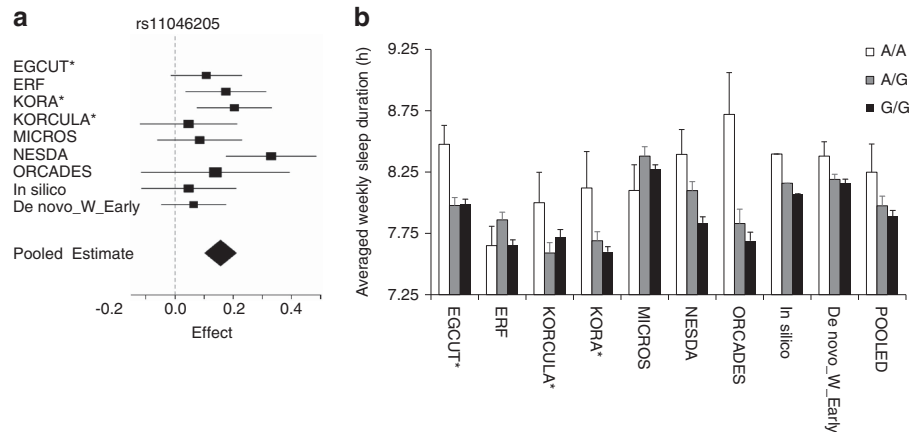


Figure 1 Contribution and direction of the effects from each discovery-phase study. **(a)** Forest plots for rs11046205. Contributing effects (beta at 95% CI) are represented by squares, with confidence intervals indicated by horizontal lines. **(b)** Averaged weekly sleep duration (normalised for age and sex; SD_{asc}) per genotype category, for each independent discovery and replication cohorts and for the pooled average sleep duration across all cohorts. SD_{asc} means per genotypes only reflect partially the effect direction, as these are not adjusted for body mass index (BMI), which was a covariate in the association analyses. Error bars indicate standard errors of the means. *Discovery cohorts that were *de novo* genotyped.

perfect proxy of it for technical replication (Table 1), but found no significant association with SD_{av} in the replication sample.

Because non-replication does not conclusively discredit discovery-phase results, we hypothesised that other influences on sleep duration and sleep timing could be responsible for the non-replication. Given that many studies in various different organisms, including humans, show the influence of season on circadian entrainment^{4,24–26} and sleep behaviour,^{5,27,28} we examined possible interactions between season and chronotype with the genetic associations detected at the *ABCC9* locus. Limitations due to small sample sizes influenced the power to directly examine these interactions in the discovery cohorts. We, nevertheless, were able to analyse the effect of these on SD_{asc} for both the discovery and replication cohorts (see Materials and methods) and found significant seasonal and phase of entrainment influences solely in the large replication cohort (EGCUT); with significantly shorter sleep in summer than in winter (Mann–Whitney U -test $P=0.01$; Supplementary Table 7), as well as in the early vs the late half of the chronotype distribution, both in summer and in winter (Mann–Whitney U -test $P<0.001$). A season-specific difference in SD_{asc} between the early and late chronotypes distribution was observed for two other cohorts (KORCULA and MICROS; Supplementary Table 7) contributing with the smallest effects (beta coefficients; see Table 1) in the discovery phase meta-analysis, but no seasonal differences were found for those contributing with larger effects (KORA, EGCUT and ORCADES).

There were significant differences in SD_{asc} between the smaller EGCUT discovery sample and the large EGCUT replication sample (t -test $P<0.0001$), which could be due to environmental influences or different strength of correlation of phenotypes across samples. Indeed, season and chronotype influences on sleep

duration (SD_{av}) were reflected in the genetic associations, for all three *de novo*-genotyped SNPs of the large EGCUT sample. In the dataset collected during winter (defined as the period without DST), their effect directions were consistent with the meta-analysis results, whereas in the dataset collected during summer (during DST) these were opposite in relation to the meta-analysis (Supplementary Table 8). Similarly, when analysing early and late chronotypes of the winter replication sample separately (Materials and methods and Supplementary Figure 1), the early half of the distribution replicated the effect directions of the three *ABCC9* SNPs found in the discovery phase, whereas these were opposite for late chronotypes (Supplementary Table 8). As we observed that sleep duration is influenced by season and chronotype, and potentially by the confounding factor of sleep deprivation due to DST, we considered only the early (chronotype) half of the winter collection as a valid replication sample (Supplementary Figure 1). Although the replication signal based on this population subgroup was still not significant ($P>0.05$), meta-analysing its results with the discovery and the *in silico* replication results (Supplementary Figure 1), validated the association of the discovery phase leading SNP with average sleep duration (Beta = 0.12, $P=7.9 \times 10^{-8}$; Supplementary Table 9).

The pooled SD_{asc} data from the homozygous carriers of the effect allele A (less frequent allele; Table 1; discovery $SD_{asc}=8.13$ h) in the discovery sample differed by 24 min (16 min in the replication phase) in relation to carriers of the reference (common) allele G (discovery $SD_{asc}=7.49$ h), a reduction of $\approx 5\%$ ($\approx 3\%$ in the replication phase; Figure 1b).

To investigate the functional relevance of SUR2 (the protein encoded by *ABCC9* in humans) for sleep duration, we knocked down the expression of its *Drosophila* homologue (*dSur*) in the flies' nervous system (both central and peripheral). Unlike humans,

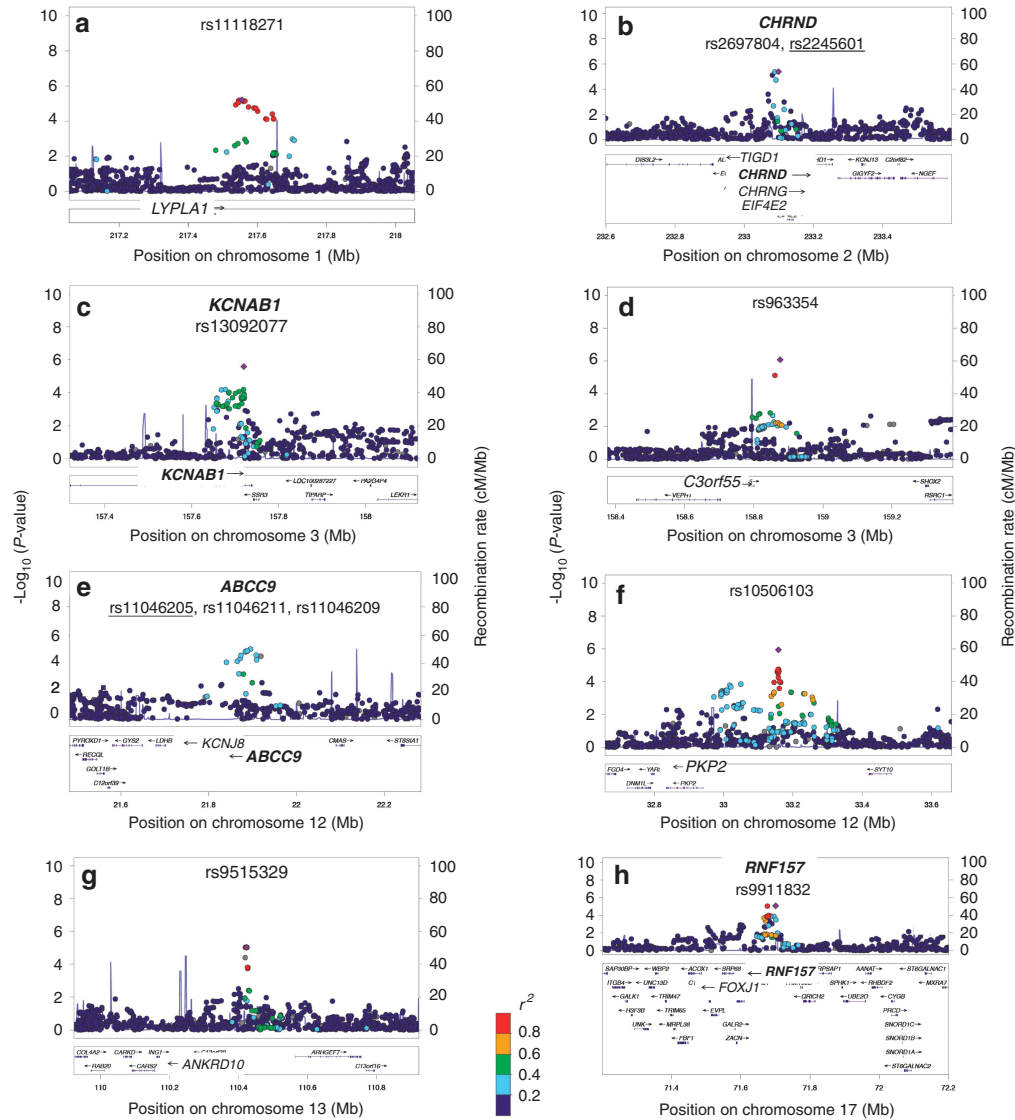


Figure 2 Regional plots of the SNPs listed in Table 1 (500 kb up and downstream of the associated SNP; $-\log_{10} P$ values on the y axis and the SNP genomic position on the x axis). Estimated recombination rates are plotted at the base of the graphic (blue line). In each panel, the index SNP (underlined when more SNPs in the same region) is denoted with a purple diamond. SNPs are coloured to reflect LD with the index SNP (pairwise r^2 values from HapMap CEU). Description of SNP locus per panel: (a) The nearest gene (*LYPLA1*) is associated with waist-hip ratio,⁵¹ adiposity and fat distribution.⁵² (b) *CHRND* encodes the delta subunit of the nicotinic acetylcholine receptors of muscle, which mediates the opening of an ion-conducting channel across the plasma membrane. (c) *KCNAB1* encodes a β -subunit of a K^+ voltage-gated channel that interacts with the pore-forming α -subunits encoded by Shaker. The channel is activated by changes in electrical potential differences, allowing a rapid and co-ordinated neuronal depolarisation. (d) No gene in the associated region. (e) *ABCC9*, encoding a K_{ATP} -sensitive channel subunit, had the best association signal in our meta-analysis. Protein structure suggests a role as the drug-binding channel-modulating subunit of the extrapancreatic K^+ ATP channels. It is involved with the development of human cardiopathies and diabetes¹⁶ and is connected to several disease and metabolic processes pathways (Figure 4). (f) The nearest gene (*PKP2*) encodes a protein (plakophilin 2) necessary for desmosomes formation, structures which provide strength to the myocardium and signalling between neighbouring cells. (g) The nearest gene (*ANKRD10*) appears to produce several proteins, mostly in brain, lung and eye, with no sequence overlap. (h) *RNF157* is a RING finger protein of the ubiquitin conjugating system. Plots were generated with LocusZoom and gene annotations with the NCBI genome browser. SNP, single nucleotide polymorphism; LD, Linkage disequilibrium.

flies are active predominantly around dawn and dusk and show two large sleep episodes during the day and during the night. Knockdown of *dSur* dramatically reduced night-sleep, particularly during the first half of the dark period, but had little effect on the flies'

day-sleep (Figure 3). This was true for both *elavgal4 > UAS-Sur* RNAi genotypes compared with their parental control strains as well as in comparison to two, unrelated wild-type lines, the laboratory *Canton-S* and a recent isolate from *Holland, HU*²¹ (Figure 3,

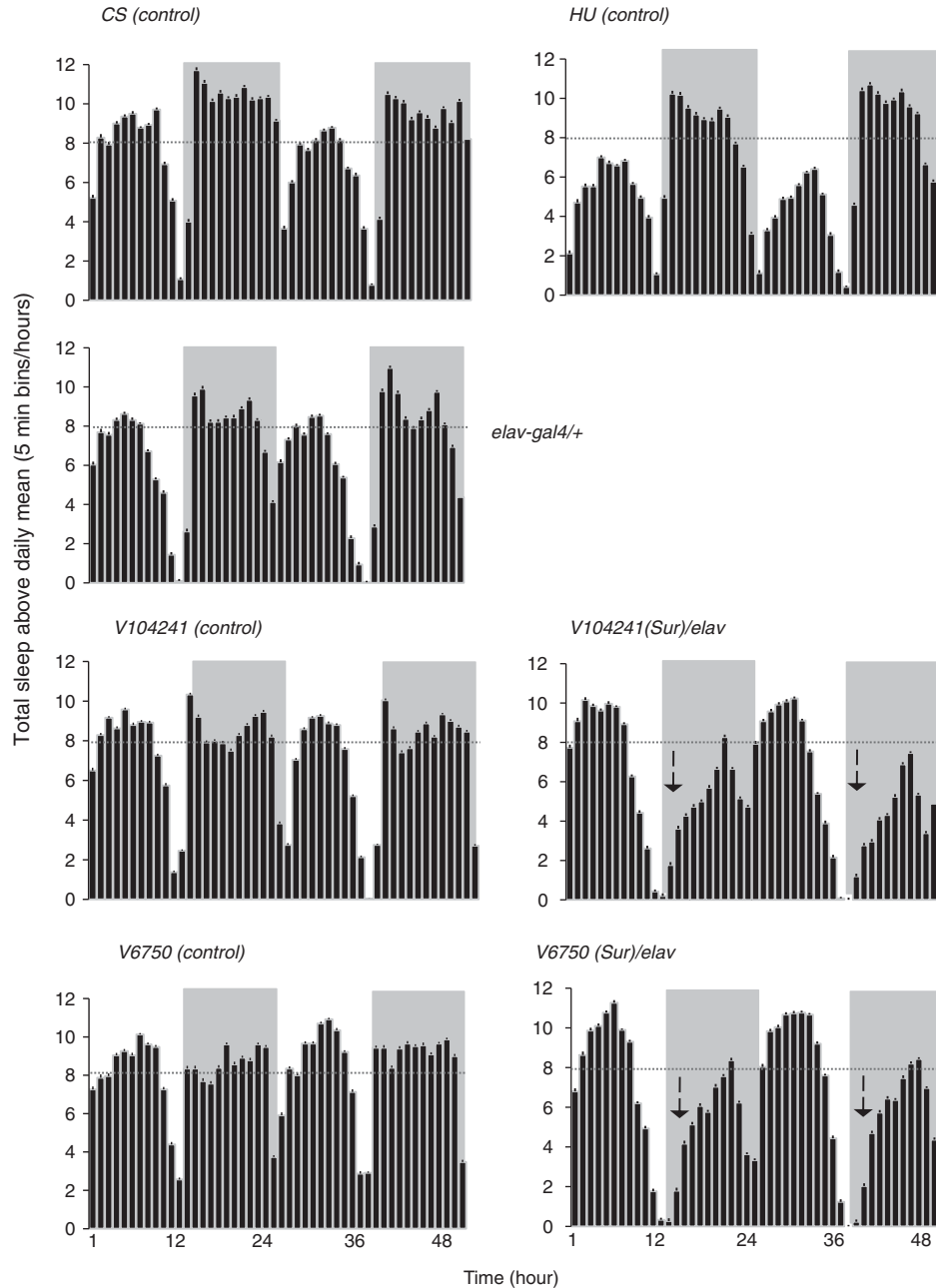


Figure 3 Pan-neuronal knockdown of *dSur* in *Drosophila* decreases night sleep duration. *elav-gal4* was crossed to each of two independent *UAS-Sur* RNAi lines (*V104241* and *V6750*). Plots of circadian immobility (> daily mean, in 5-min bins) of experimental and control males in 12:12 h light/dark cycles over 2 days. Experimental flies show reduced sleep (defined as 5 min of immobility) in the first half of the night (see arrows *V104241(Sur)/elav* and *V6750(SUR)/elav*, $F_{2,79} = 29.7$, $P = 2.43 \times 10^{-10}$, and $F_{2,82} = 32.1$, $P = 5.01 \times 10^{-11}$, respectively), compared with corresponding controls for the dark phase; grey background and dashed threshold line), whereas rest duration in the light period was similar among cases and controls. Sleep bouts in experimental flies were not significantly shorter in length nor more numerous than for control flies.

and Supplementary Figure 9a and b). These differences in sleep duration were not due to changes in the circadian clock, as the period of the free-running locomotor activity rhythm in constant darkness (≈ 24 h) was indistinguishable between knockdown flies and all controls (Supplementary Figure 10 and Supplementary Methods). Furthermore, no systematic differences in activity levels were found between experimental genotypes and controls (Supplementary

Figure 11a). Thus, the major effect of knocking down the *ABCC9* homologue in flies is shortening nighttime sleep due to a delay of its onset by 3 h (Figure 3 and Supplementary Figure 11b).

Discussion

The genome-wide significant signal of the discovery meta-analysis (rs11046205) maps to intron 27 of

ABCC9, proximal to exon 27. *ABCC9* encodes one of the 17 trans-membrane domains of the pore-forming subunit of an adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channel (SUR2, sulfonylurea receptor). In the replication phase, the effect direction of the associations of *ABCC9* SNPs with sleep duration was shown to be dependant on season and phase of entrainment, which interfered with the SD_{asc} distribution in this cohort. Photoperiod influences the circadian metabolism^{24,29} (that is, clock genes expression) and the modulation of sleep timing,^{5,25,30} indirectly influencing sleep duration.⁴ Beyond photoperiod and phase of entrainment interactions, the average sleep duration is altered during the DST period.⁴ DST advances the social clock (that is, work simply starts an hour earlier), increasing the discrepancy between social and the circadian timing, a phenomenon called social jetlag.¹⁷ Social jetlag and the resulting sleep deprivation are obviously more pronounced in late than in early types, and late types have greater difficulties in adjusting to the DST changes than early types.⁴ These socio-biological influences on sleep duration are reflected in our results. Early chronotypes, collected for the replication during the winter months, showed the same effect directions for all three *ABCC9* SNPs as found in the discovery populations. Collections during DST in

summer and late types, irrespective of season, were however inconsistent in their effect direction.

The influences of photoperiod and chronotype on sleep duration depend on latitude; the further people live from the equator, the later their chronotype (Allebrandt and Roenneberg unpublished). The large differences between winter and summer photoperiods in Estonia (7:50 vs 16:40 h), the origin of the EGCUT samples (from latitude 57° to 59°N), is a likely explanation for the seasonal influence on sleep duration, an effect that will be detectable only in large samples.⁴ These geo-sociobiological interactions have implications for association studies investigating the circadian/sleep phenotypes: (i) different strength of correlation of phenotypes across populations or population subgroups; and/or (ii) population-specific effects generated under differential gene-environment interactions.³¹ Therefore, GWAS scanning only for main effects on sleep duration, or on neurobehavioral disorders influenced by season and chronotype, could miss important genetic variants specific to subgroups of the population.³²

In an attempt to corroborate our findings, we combined our genetic association strategy in humans with a functional analysis in *Drosophila*. *Drosophila* has a single invertebrate ATP-binding SUR protein (*dSur*), which is homologous to SUR2.^{33–35} Other

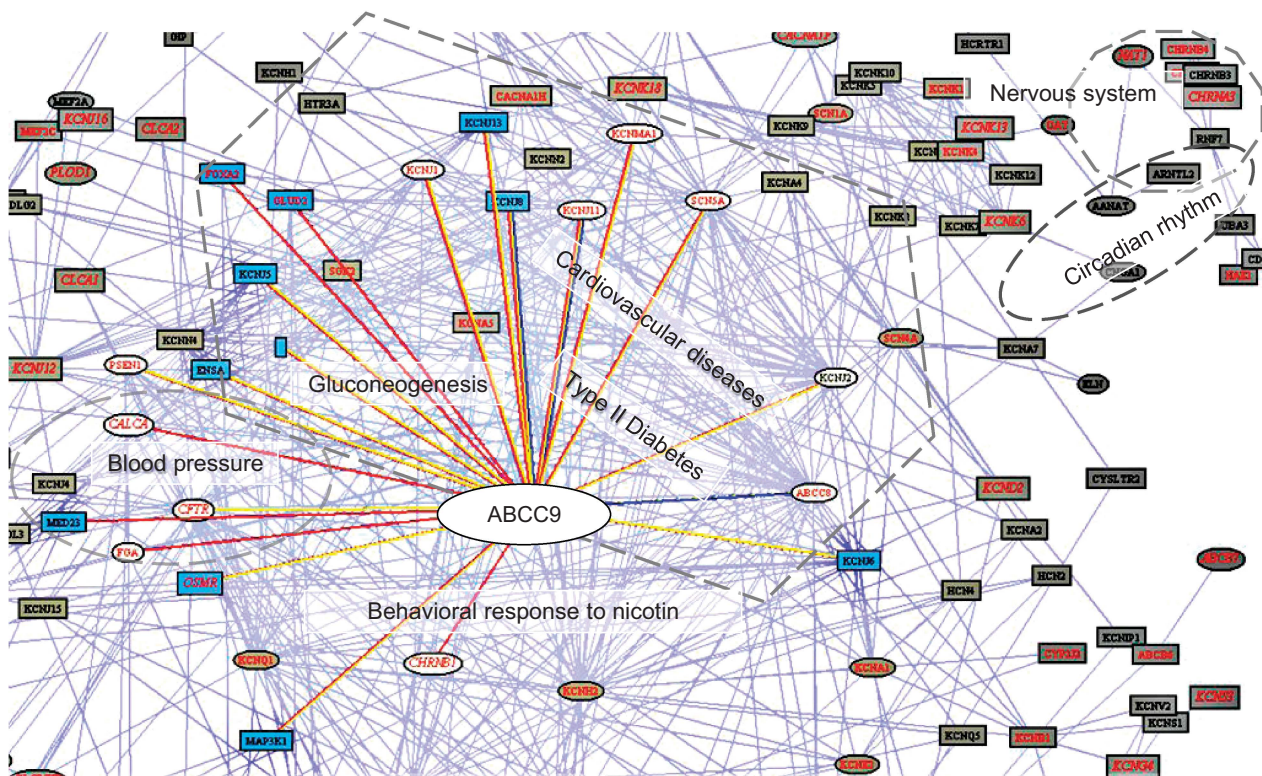


Figure 4 *ABCC9* genetic interaction network (generated with SNPs 3D). Oval nodes represent genes associated with diseases, and rectangular nodes represent other genes. Genes in red have deleterious SNPs, or deleterious common SNPs (MAF > 1%; in italic). The length and colour of the edges represent the strength of the link between pairs of genes. Red edges link genes sharing the same abstracts. Short edges link genes sharing a large number of biological keywords. The diseases or metabolic process that these genes (dashed areas) are involved with are highlighted in the white rectangles. MAF = minor allele frequency.

potassium-channel regulatory proteins, encoded by *Shaker*, *Hyperkinetic* and *Sleepless*, have been shown to influence sleep duration in *Drosophila*.^{36,37} Our study supports the role of *Hyperkinetic* in modulating sleep duration, as its human homologue, *KCNAB1*, is the second best signal in the discovery meta-analysis of our association study. Additionally, K⁺ channel activity is enhanced when nAChR is blocked in the rat hypothalamus³⁸ (relationship indicated in the *ABCC9* interaction network, Figure 4), and one of the nAChR genes (*CHRND* expressed in muscle tissue) was the third best ranking gene in our meta-analysis (Figure 2). This is the first large-scale genome-wide association study showing a potential role of several K⁺ channel regulatory proteins in modulating sleep duration in humans, strengthened by supporting evidence from *Drosophila*.

In vertebrates, *ABCC9* is expressed in various tissues (in mammals mostly in heart, skeletal muscle, ovary, brain, tongue and pancreatic islets) and has various splice isoforms, indicating its functional diversity and genetic complexity.^{35,39,40} It is genomically located within a cluster of genes (*ARNTL2*, *ABCC9*, *KCNJ8* and *LDHB*), on chromosome 12, that has corresponding paralogues on chromosome 11 (*ABCC8*, *ARNTL*, *KCNJ11* and *LDHA*) indicating an ancient duplication. All eight genes maintain their relative chromosomal order (synteny) from zebrafish to humans.⁴¹ *ABCC8* encodes SUR1, expressed in pancreatic β cells, and shares 70% identity with SUR2,⁴² whereas *KCNJ8* and *KCNJ11*, encode the two subtypes of K_{ATP} channel pore-forming subunits (K_{IR}6.1 and K_{IR}6.2, respectively) that pair with SUR2 and SUR1 subunits to form the channel.¹⁶ SUR2A/K_{IR}6.2 pores regulate action potential duration in the heart, whereas SUR2B / K_{IR}6.1 pores regulate action potential duration and vasodilatation in vascular smooth muscle, depending on the state of intracellular ATP and glucose metabolism in voluntary striated muscle.³⁵ The functional relationships of these channel subunits suggest a higher order regulation that may have driven the preservation of their synteny in the vertebrate lineage.

In the brain, K_{ATP} channel action potentials mediate the state of cortical arousal modulated by neurons involved in slow-wave oscillations (during deep sleep), via a K_{IR}6.2 subunit.⁴³ High glucose levels induce a significant decrease in the K_{IR}6.2 mRNA level, reversible by lower glucose concentration.⁴⁴ Activation of hypothalamic K_{IR}6.2/SUR1 channels restrains hepatic gluconeogenesis (generation of glucose), whereas inhibition of K_{IR}6.2/SUR-2B channels in the ventromedial hypothalamus are indicated to amplify the counter regulatory responses to acute hypoglycemia,⁴⁵ providing a link between the CNS, liver metabolism and the development of diabetic hyperglycaemia.

In contrast, orexin neurons, which innervate the arousal system in the brain helping to promote and sustain wakefulness,⁴⁶ express K_{IR}6.1/SUR1 channels.⁴⁷ Unlike the hunger-induced arousal mechanism (that is, under glucose deprivation), K_{ATP} channels

mediate hyperpolarization of orexin neurons, thereby promoting sleep and exerting a neuro-protective mechanism during severe energy depletion.⁴⁸ In this sense, non-functional K_{ATP} channels in these neurons could prolong wakefulness during energy depletion, as indicated by patch clamping of rat brain slices treated with K_{ATP} blockers in the absence of glucose.⁴⁸ This supports an important role of K_{ATP} channels in balancing adaptive response to stress and the metabolic resources to ensure survival.⁴⁹ Additionally, K_{ATP} channels are indicated to mediate the action of leptin on the regulation of food intake and body weight.⁵⁰

In conclusion, our study shows that variants in the SUR2 gene (*ABCC9*) associate with epidemiological variation in human sleep duration, which is also influenced by inter-individual differences in seasonal adaptation and chronotype. The knockdown of its (single) homologue in flies shortens sleep duration, demonstrating this locus is relevant for the modulation of sleep across species. SUR2 is involved in energy metabolism and in the aetiology of cardiomyopathies (Figure 4),¹⁶ which is closely related to endophenotypes (BMI and hypertension) that by themselves correlate with sleep duration.⁷ The relevance of this locus for sleep duration regulation has therefore implications for dissecting the relationships between sleep, BMI, hypertension and disease.⁷

Conflict of interest

The authors declare no conflict of interest.

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URLs: MACH, <http://www.sph.umich.edu/csg/abecasis/MaCH>; LocusZoom, <http://csg.sph.umich.edu/locuszoom/>; SNPs 3D, <http://www.snps3d.org/>; SCAN, <http://www.scandb.org/>; METAL, <http://www.sph.umich.edu/csg/abecasis/metal/index.html>; PLINK, <http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml>.

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