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Common variants in *DRD2* are associated with sleep duration: the CARe consortium

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Abstract

Sleep duration is implicated in the etiologies of chronic diseases and premature mortality. However, the genetic basis for sleep duration is poorly defined. We sought to identify novel genetic components influencing sleep duration in a multi-ethnic sample. Meta-analyses were conducted of genetic associations with self-reported, habitual sleep duration from seven Candidate Gene Association Resource (CARe) cohorts of over 25 000 individuals of African, Asian, European and Hispanic American ancestry. All individuals were genotyped for ~50 000 SNPs from 2000 candidate heart, lung, blood and sleep genes. African-Americans had additional genome-wide genotypes. Four cohorts provided replication. A SNP (rs17601612) in the dopamine D2 receptor gene (*DRD2*) was significantly associated with sleep duration ($P = 9.8 \times 10^{-7}$). Conditional analysis identified a second *DRD2* signal with opposite effects on sleep duration. In exploratory analysis, suggestive association was observed for rs17601612 with polysomnographically determined sleep latency ($P = 0.002$). The lead *DRD2* signal was recently identified in a schizophrenia GWAS, and a genetic risk score of 11 additional schizophrenia GWAS loci genotyped on the IBC array was also associated with longer sleep duration ($P = 0.03$). These findings support a role for *DRD2* in influencing sleep duration. Our work motivates future pharmacogenetics research on alerting agents such as caffeine and modafinil that interact with the dopaminergic pathway and further investigation of genetic overlap between sleep and neuro-psychiatric traits.

Introduction

Sleep occupies a substantial portion of our lives and is increasingly being recognized for its role in a number of diseases. Large meta-analyses have confirmed a role of sleep duration in cardiovascular diseases and all-cause mortality (1,2). The indirect and direct impact of sleep disorders and deprivation within the USA has an estimated financial magnitude of hundreds of billions of dollars (3). However, despite intensive study, there are basic gaps in our knowledge of mechanisms controlling sleep, and although habitual sleep duration varies widely across individuals, the factors that influence this are not understood. Sleep is likely to be among the more complicated phenotypes to study genetically due to connections with a broad range of physiologic mechanisms and variation with age (4), gender (5) and ethnicity (6).

Although self-reported sleep duration is heritable with estimates ranging from 9 to 44% (7–9), and there have been prior studies of genetic associations for sleep duration in humans, these studies have been limited to single cohorts (7,8,10,11) or single ethnic groups (12,13). Recently, the CHARGE Consortium identified two genome-significant ($P < 5.0 \times 10^{-8}$) signals near *IER3* and *PAX8* in European ancestry individuals. Genetic association studies of other complex traits including height have uncovered hundreds of significant loci when analysing hundreds of thousands of individuals (14), suggesting that novel loci significantly associated with sleep duration may emerge with increased sample size.

We identified an opportunity to examine sleep duration genetics across multi-ethnic US populations to further understand the etiology of sleep duration across populations, which may impact the morbidity of cardiovascular and other diseases and provide insights into pharmacogenetic approaches for improving sleep and alertness. Therefore, we conducted a large-scale, multi-ethnic meta-analysis using a customized gene chip (15) to identify novel polymorphisms associated with sleep duration.

Results

The study population comprised 25 465 individuals from seven ethnically diverse cohorts [5842 African Americans (AA); 546 Asian Americans (AsA); 18 026 European Americans (EA) and 1051 Hispanic Americans (HA)], with mean self-reported habitual weekday sleep duration ranging from 6.27 to 7.37 h (Table 1). Heritability estimates for sleep duration based on analysis of family data were $h^2 = 0.131$ (SE 0.022) for EA adults in FHS and 0.043 (0.093) for AA adults in CFS. Heritability estimated using GCTA-based analysis (16) for unrelated individuals and applied to 2058 AAs in CARDIA, JHS, and MESA in whom genome-wide data were available was 0.134 (SE = 0.149).

European and multi-ethnic meta-analysis identifies *DRD2* SNP association with sleep duration

Single SNP genetic analysis was performed in a cohort-specific regression framework adjusting for age, age^2, sex, age × sex and 10 population principal components. Ethnicity-specific meta-analyses showed little evidence of genomic inflation (lambda = 0.965–1.022; Supplementary Material, Fig. S1).

Gene-centric multi-ethnic meta-analysis of 40 520 IBC array SNPs in 25 465 individuals identified the SNP rs17601612 within intron 1 of *DRD2* (dopamine D2 receptor) that reached a study-wide significance threshold of $P = 1.9 \times 10^{-6}$ (17) with the derived C allele associated with shorter sleep duration [meta $P = 1.83 \times 10^{-6}$, beta (SE) = −3.66 (0.72) min per C allele; Table 2; Fig. 1]. The association was primarily driven by EAs, our largest ethnic group, and the direction of association was consistent in AsA and HA, but not in AA (Table 2).

Regional association testing conditional on rs17601612 revealed a second independent signal of association at this locus [lead SNP rs11214607 G $P_{unadjusted} = 8.26 \times 10^{-6}$, beta (SE) = 4.08 (0.90) min, $P_{conditional} = 8.8 \times 10^{-4}$, beta (SE) = 2.91 (0.88) min; Fig. 1]. This SNP was largely uncorrelated with rs17601612 ($r^2 < 0.05$) in 1000 Genome African (AFR), East Asian (ASN) and European (EUR) samples, although weak correlation was observed in CARe EA cohorts ($r^2 < 0.075$).

Effect estimates did not appreciably change after adjustment for covariates that may influence sleep duration through independent or pleiotropic pathways (e.g. depression, obesity, smoking and alcohol; Supplementary Material, Table S1). No SNPs previously reported to be associated with sleep duration present on the IBC array were nominally significantly associated in our study (Supplementary Material, Table S2).

Replication of lead SNPs from both *DRD2* signals was attempted in independent cohorts comprised primarily of elderly individuals of European American descent: MAP + ROS, MrOS, SOF (mean ages 83.2, 76.7, 83.9; $n = 1705$, 2348, 1502, respectively) (18–23). Stronger statistical significance was seen in meta-analysis of the discovery and replication studies (rs17601612 $P = 9.75 \times 10^{-7}$;

Table 1. Characteristics of the multi-ethnic discovery sample

Ethnic group	Cohort	Median age (IQR)	Percent female	Mean BMI (SD)	Mean self-reported sleep duration (SD)	SNPs ≥ 1% MAF	*N*
African American (AA)	CARDIA	40 (7)	59.2	30.56 (7.4)	6.27 (1.4)	865 755	1171
	CFS[a]	44 (24)	57.6	34.23 (9.7)	7.12 (1.8)	858 698	524
	CHS	76 (8)	66.7	28.40 (5.4)	7.09 (1.7)	42 283	588
	JHS	49 (14)	60.9	32.28 (7.7)	6.41 (1.4)	894 684	2168
	MESA	66 (15)	54.3	30.10 (5.9)	6.54 (1.4)	907 215	1391
Asian American (AsA)	MESA	66 (16)	49.8	24.06 (3.4)	6.75 (1.2)	31 213	546
European American (EA)	ARIC	62 (9)	54.1	28.97 (5.2)	7.20 (1.1)	33 429	4414
	CARDIA	41 (5)	53.3	27.14 (5.9)	6.77 (1.0)	34 527	1333
	CFS[a]	44 (26)	54.5	31.19 (8.2)	7.19 (1.4)	34 397	552
	CHS	78 (7)	59.9	26.72 (4.6)	7.37 (1.4)	34 312	2942
	FHS[a]	48 (18)	53.6	27.32 (5.4)	7.29 (1.1)	34 228	6764
	MESA	67 (17)	51.7	27.85 (5.2)	7.01 (1.2)	34 957	2021
Hispanic American (HA)	MESA	65 (16)	52.6	29.71 (5.4)	6.68 (1.4)	39 936	1051

Seven studies included 25 465 individuals (5842 AA; 546 AsA; 1051 HA; 18 026 EA). African American *N* depended on Affymetrix 6.0 and CARe IBC chip coverage of individual SNPs.
[a]Family cohort.

Table 2. Ethnicity-specific and multi-ethnic meta-analysis of significant ($P < 1.9 \times 10^{-6}$) and suggestive ($P < 1 \times 10^{-5}$) signals of association with habitual sleep duration

Population	SNP	Gene	Derived allele	DAF	Beta (SE), min	*P*	Direction	P_{het}	*N*
European Americans	rs17601612	*DRD2*	C	0.37–0.39	−3.66 (0.72)	7.52×10^{-7}	−−−−−−	0.085	18 022
	rs11214607	*DRD2*	G	0.15–0.16	4.08 (1.02)	4.37×10^{-5}	++++++	0.9	18 024
Asian Americans	rs17601612	*DRD2*	C	0.028	−16.14 (13.56)	0.233	−	N/A	546
	rs11214607	*DRD2*	G	0.38	2.04 (4.32)	0.64	+	N/A	546
Hispanic Americans	rs17601612	*DRD2*	C	0.28	−8.46 (3.90)	0.031	−	N/A	1051
	rs11214607	*DRD2*	G	0.30	5.46 (3.96)	0.169	+	N/A	1050
African Americans	rs17601612	*DRD2*	C	0.12–0.15	2.46 (2.34)	0.30	−++++	0.7	5546
	rs11214607	*DRD2*	G	0.07–0.09	3.60 (3.00)	0.23	+−+++	0.9	5546
Discovery meta-analysis	rs17601612	*DRD2*	C	0.028–0.39	−3.32 (0.70)	1.83×10^{-6}	−−−−−−−−−++++	0.054	25 165
	rs11214607	*DRD2*	G	0.072–0.38	4.08 (0.90)	8.26×10^{-6}	+++++++++−+++	1.00	25 166
Replication[a]	rs17601612	*DRD2*	C	0.37–0.39	−2.05 (1.41)	0.15	+−−−	0.55	5 529
	rs11214607	*DRD2*	G	0.16	3.64 (1.94)	0.061	+++−	0.44	5 529
Combined meta-analysis	rs17601612	*DRD2*	C	0.028–0.39	−3.07 (0.63)	9.75×10^{-7}	−−	0.54	30 694
	rs11214607	*DRD2*	G	0.072–0.38	4.04 (0.90)	7.76×10^{-6}	++	0.60	20 695

DAF, derived allele frequency range in component studies.
[a]Proxy rs2471854 with pairwise $r^2 = 0.90$ in EUR used for replication studies.

rs11214607 $P = 7.76 \times 10^{-6}$; Table 2). Haplotype analyses confirmed two independent signals (Supplementary Material, Table S3). In contrast, no generalizability was observed in a Korean cohort (age 40–69, $n = 8842$) with a different pattern of linkage disequilibrium at the locus [rs17601612 beta (SE) 0.44 (0.19), $P = 0.02$; rs11214607 beta (SE) 0.013 (0.02), $P = 0.51$].

Correlated functional SNPs in *DRD2* region: bioinformatics data

Gene-based association analysis using VEGAS (24) confirmed *DRD2* as the only significantly associated gene in Europeans after correction for multiple testing ($P = 8.0 \times 10^{-6}$; $P_{adj} = 0.042$; Supplementary Material, Table S4). The two next best non-significant genes (*ANKK1* and *TTC12*) are located near *DRD2*.

To localize genic regions with putative functional significance, we performed multi-ethnic regional fine mapping by 1000 Genomes Project (1KG) imputation. The most significant association was observed for the imputed SNP rs4274224 (imputation quality score $r^2 \geq 0.967$, $P = 3.22 \times 10^{-7}$, 1000 Genomes EUR r^2 with rs17601612 = 0.648; Supplementary Material, Table S5). The most significant SNP from the secondary association signal was rs11214607 ($P = 7.56 \times 10^{-6}$). Twenty-three candidate regulatory SNPs were in high LD (EUR $r^2 \geq 0.95$) with one of the top SNPs contributing to each of the two association signals in *DRD2* [Supplementary Material, Table S6 (25–27)]. If these SNPs contribute additive effects on multiple regulatory elements, then transferability of these effects may differ across individual haplotypes and populations.

DRD2 sleep duration SNP association with polysomnography-determined traits

As exploratory analyses to provide insight into mechanisms influencing association with sleep duration, we tested for association of the two independent *DRD2* signals with 15 additional polysomnographic and 4 self-reported sleep traits across 6 domains (Fig. 2, Supplementary Material, Table S7). Consistent

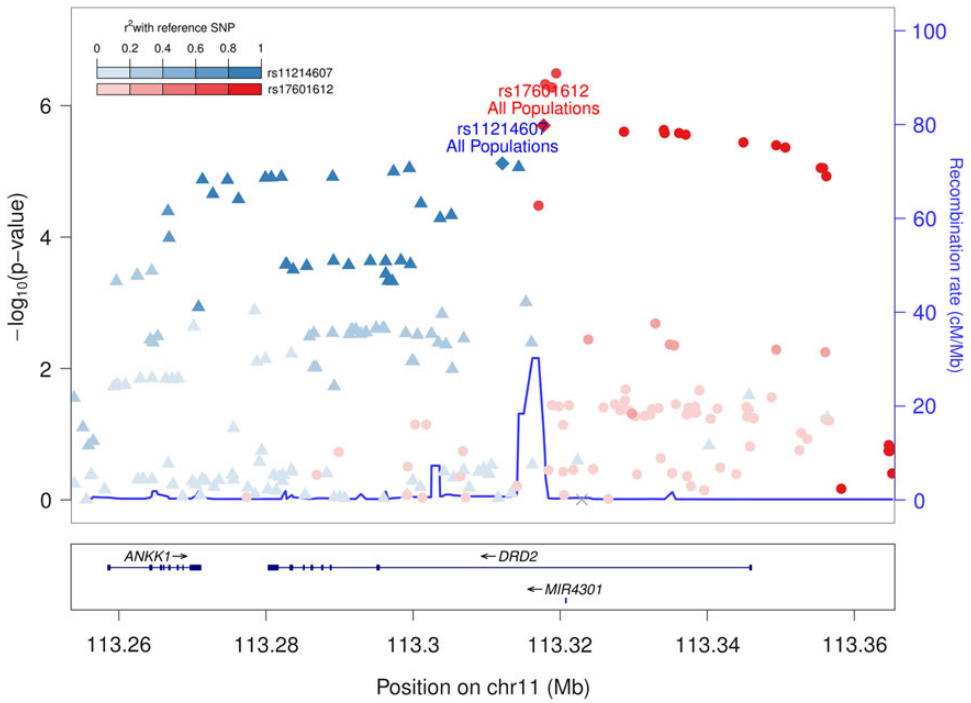

Figure 1. Regional association plot of *DRD2* Sleep duration multi-ethnic association results for the discovery sample. $-\log_{10}$ *P*-value of the multi-ethnic (AA + AsA + EA + EA) association results is plotted on the *y*-axis, and chromosomal position is plotted on the *x*-axis encompassing the *DRD2* locus. Individual directly genotyped SNPs are binned based on the stronger linkage disequilibrium relationship with either rs17601612 (blue) or rs11214607 or (red). Note the recombination hotspot separating the two independent association signals. LD was calculated based on 1000 Genomes European population data.

with the primary results, and despite a limited available sample size, the strongest results varied between both signals. rs17601612 C was most significantly associated with objectively measured shorter sleep latency [i.e. time to fall asleep after 'lights off'; beta (SE) −2.08 (0.69) min, $P = 2.4 \times 10^{-3}$, $n = 2096$, Fig. 2A], while rs11214607 G displayed trends with indices of sleep depth [i.e. lower Stage 1 percentage; −0.19 (0.12) percent, $P = 0.099$, $n = 3740$, Fig. 2B].

Association of a schizophrenia genetic risk score with sleep duration

Several candidate associations have been reported for European proxy SNPs of the second *DRD2* signal (represented by rs11214607) with various neurobehavioral traits (Supplementary Material, Table S8), including differences in long/short DRD2 isoform ratios with distinct pre- and post-synaptic isoform localization and roles (28,29). Notably, a recent study reported genome-wide significant association of 108 loci with schizophrenia in GWAS from individuals of largely European ancestry, including multiple variants within *DRD2* (rs17601612, $P = 1.71 \times 10^{-8}$) (30). The lead SNP (rs2514218, $P = 4.09 \times 10^{-10}$) is in strong LD with rs17601612 (EUR $r^2 = 0.74$), suggesting a pleiotropic effect, with the rs17601612 C protective for schizophrenia and associated with short sleep duration in our study. While several SNPs were outside the region that was successfully imputed in our study, rs17601612 and 10 additional genome-significant SNPs for schizophrenia were imputed in our sleep duration study (rs7948028, rs4337071, rs4630328, rs11601054, rs61902787, rs7110440, rs35277073, rs61902807, rs7121986, rs6589377). These SNPs showed suggestive association to sleep duration in AA–AsA–EA–HA cohorts with $P < 1.0 \times 10^{-5}$ but were not significant and showed opposite direction for most African American cohorts, as observed for rs17601612. Given this potential pleiotropic association, and given that disturbances in sleep, including total sleep time and sleep latency, have been associated with schizophrenia in meta-analysis (31), we constructed a weighted genetic risk score of the 12/108 schizophrenia variants (or $r^2 > 0.8$ proxies in CEU) that were genotyped on the IBC array and tested this genetic risk score for association with sleep duration in EA. The schizophrenia genetic risk score was associated with longer sleep duration with and without including the lead *DRD2* variant rs17601612 [beta (SE) 0.86(0.24) min per allele, $P = 2.4 \times 10^{-4}$, beta (SE) 0.54(0.25) min/allele, $P = 0.03$; $n = 18\,026$].

Discussion

In this study, we have demonstrated a novel association between sleep duration and common polymorphisms within the dopamine receptor *DRD2* in over 25 000 individuals from a multi-ethnic US population. While genome-wide significance for each association signal was not reached, several lines of evidence provide strong support that these associations are valid: (i) conditional analysis reveals two independent *DRD2* association signals with opposite effect directions, each in linkage disequilibrium with putatively functional SNPs, (ii) a consistent direction of allelic effects is observed in at least three of four ethnic groups, (iii) gene-based analysis confirms significant association of *DRD2*, (iv) suggestive association is observed with objective sleep traits including effects on sleep latency and sleep architecture and (v) the lead SNP is in high LD with a recently identified genetic variant for schizophrenia, another neurobehavioral phenotype. Our findings, including secondary analysis of polysomnographic sleep measures, were robust to alternative statistical models that considered potential sources of phenotype

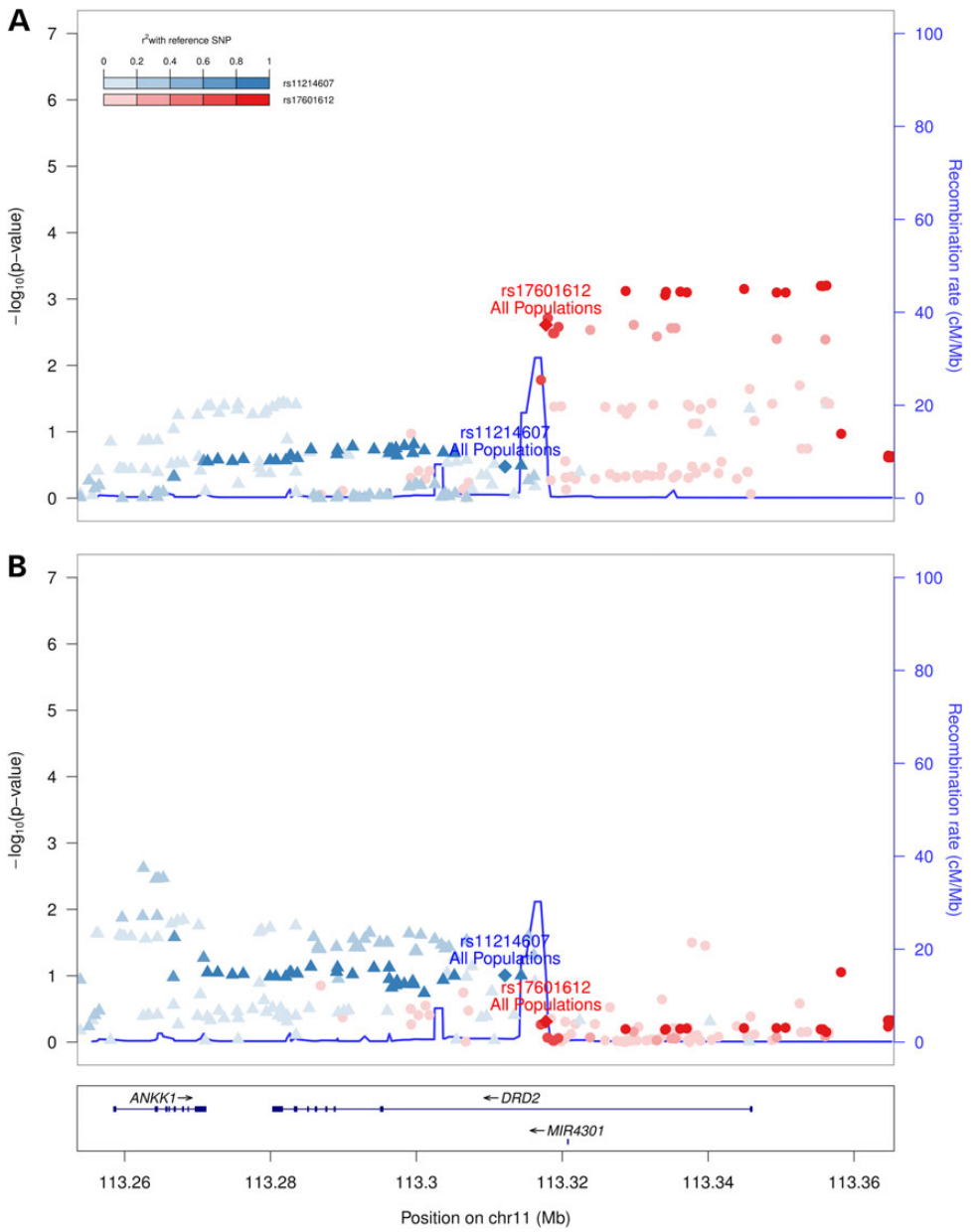

Figure 2. Regional association plot of *DRD2* multi-ethnic association results for sleep latency and Stage 1 sleep. Combined AA–EA results are shown for each trait: (**A**) sleep latency and (**B**) Stage 1 sleep. Phenotypes were collected on a subset of Sleep Heart Health Study I (ARIC, CHS, FHS) and CFS participants. rs17601612 and rs11214607 results are listed in Supplementary Material, Table S7. The axes and linkage disequilibrium pattern are as presented in Figure 1.

heterogeneity. However, available external replication was limited, requiring some caution in generalization of findings from our large European American sample to multiple ethnic groups.

DRD2 has a wide range of influence, including over 80 human biological processes observed in the Gene Ontology (32). The literature provides strong biological support for gene-level effects of *DRD2* on sleep and circadian rhythms phenotypes, dating to 1917 (33,34). The dopamine receptor agonist apomorphine has been shown to induce yawning in rats, which was blocked by treatment with a preferential D2 antagonist (35). A common medicine for promoting alertness for disorders of hypersomnolence, including narcolepsy, is modafinil, which enhances wakefulness, at least in part, through effects on dopamine transporter (*DAT*) function (36) and DRD2/DRD3 receptor availability as measured by [11-C]raclopride-binding potential (37). *Dat* knockout mice display increased sleep fragmentation and decreased NREM sleep time relative to wild-type littermates (36). Both *Drd2* pre-synaptic autoreceptors and post-synaptic heteroreceptors influence dopamine expression levels in mice (38). Drd2-binding efficiency effects may be due to DAT/DRD2 interactions and/or DRD2 modulation of DAT cell surface expression through the MAPK pathway (29,39,40). *Drd2* knockout mice display significant differences in wakefulness, NREM sleep and sleep latency (41).

Secondary analyses of polysomnographic indices also showed variation in objective measures of sleep. Our primary *DRD2* signal associated with shorter sleep latency that may reflect a greater homeostatic drive concurrent with reduced sleep duration, while the second independent *DRD2* signal tended to be associated with improved sleep quality as measured by

decreased time in stage N1 and may indicate a positive effect on both sleep duration and sleep depth.

There are a number of pathways by which *DRD2* may influence sleep (as well as alertness), including through a complex involving the adenosine receptor *ADORA2A* (a target of caffeine) (42–44). Both proteins are prominently expressed in the human striatum (45). Caffeine ingestion increases [11-C]raclopride binding to DRD2/DRD3 in the human ventral striatum (46). Two weeks of increasing melatonin exposure have been shown to increase the affinity of Drd2 receptors in rat striatum by 48%, with minimal impact on receptor density (47). A single night of sleep deprivation reduced raclopride binding in the human striatum (48). Drd2 is linked to the rhythmicity of the key circadian gene *Per2* in the rat striatum (49). It is possible that neuroinflammatory processes and sleep inter-relate given a central neuroinflammation role for Drd2 (50,51). Interestingly, rs17601612 and the 5 SNPs with the lowest *P*-values in our primary fine-mapped *DRD2* association signal overlap an enhancer region in astrocytes (Supplementary Material, Table S6; rs4245146, rs4245147, rs4936271, rs4936272, rs4274224; $P = 3.22 \times 10^{-7}$–5.31×10^{-7}). DRD2 also forms complexes with a number of other receptors including NTS1, which impacts mammalian sleep and circadian phase-shifting (52–54). DRD2 affects clock output through altered ARNTL/CLOCK complex activity (55). The secondary signal splice-site SNP rs1076560 affects phosphorylation of the circadian gene GSK3B and has been modestly associated with eyes-closed waking electroencephalographic activity (56,57). Despite this rich literature implicating *DRD2* in sleep and circadian rhythms, this is the first large-scale genetic analysis to show an association between *DRD2* variants and human sleep.

The SNPs we identified are likely to have functional effects. DRD2 receptor availability, density and/or location vary with different secondary signal *DRD2* splice SNP (rs1076560 and rs2283265) variant isoforms (28,58). The pre-synaptic short isoform variant has differential effects on DRD2 autoreceptor function and striatal release of GABA and glutamate (reviewed in Ref. 29). We show novel associations between sleep duration and these SNPs (Supplementary Material, Table S8). Other prominent SNPs include rs1800497 (Taq1A, AsA–EA–HA $P = 2.29 \times 10^{-5}$) and rs1079597 (Taq1B, AsA–EA–HA $P = 9.81 \times 10^{-6}$) from the secondary signal, which have been associated with human striatum receptor density differences (59,60). rs1800497 is a missense *ANKK1* polymorphism located outside of *DRD2*. Its effects on *DRD2* are suggested to be mechanistically related to the two splice SNPs (28). SNPs corresponding to our primary signal are in low LD with rs1800497 (1000 Genomes AFR, ASN and EUR $r^2 < 0.01$), are physically proximal (<1500 bp) and individually overlap epigenetic enhancer evidence from 9–51 cell lines (Supplementary Material, Table S6). A study of anxiety disorders and clock genes identified rs4245146 as the most significant SNP across all assayed genes (61). rs4274224, the most significant imputed SNP from our primary signal, was implicated in depression and associated with dorsolateral prefrontal cortex activation during anticipation of reward and stress-induced dopamine release (62,63). SNPs corresponding to our primary signal were recently associated with schizophrenia (30), including rs17601612 and rs61902807 (located within *DRD2* and overlapping promoter histone marks in 12 brain cell lines and 22 additional cell lines in a region of mammalian conservation). The schizophrenia-associated SNPs extend beyond the range of our imputed genotypes. These convergent cellular and imaging finding studies suggest multiple functional effects for *DRD2* SNPs, many of which likely relate to sleep–wake traits, that should be investigated further in future studies.

The effect size of each allele, while relatively modest, is within the range of other reports of genetic associations for sleep duration (10,13). The size of the effect approximately doubles when considering the combined contributions of both *DRD2* loci. Small effect sizes are generally found for individual loci in other complex traits such as height (14). It is also likely that random misclassification introduced by use of questionnaire-based information on sleep duration (rather than objective data) attenuated the strength of actual associations. Future corroboration with objective sleep data is likely to improve estimates of effect sizes.

Study strengths include the relatively large sample size from multiple ethnic groups. Subtle population stratification effects were controlled using genomic control and 10 population principal components. Detailed polysomnography data in a subset of the sample amplified the primary questionnaire-based results, suggesting that the top SNPs for sleep duration also were associated with sleep latency and sleep architecture. Fine mapping by imputation provided insights into possible physiological mechanisms, including numerous SNPs in regulatory regions, which are suggested to have 'pervasive involvement' in human disease (64).

Weaknesses of this study include the use of a self-reported measure of sleep duration, which is the trait most available for analysis of large numbers of individuals. Although likely contributing to random misclassification, self-reported data have been shown to predict health outcomes in numerous epidemiological studies (1,2). Sleep duration is likely influenced by multiple environmental, social and biological factors. Although we controlled for many covariates while examining our *DRD2* associations, we did not have detailed psychiatric or mood data to further explore pleiotropic relationships. Of particular interest was the observed association between a schizophrenia genetic risk score and longer sleep duration, a phenotype that is poorly understood, but often occurs in individuals with mood disorders or psychiatric diseases and can predict a number of adverse cardiometabolic health outcomes. Research aimed at dissecting the potential shared neurological bases for sleep and psychiatric traits may help to clarify the extent to which variation in sleep traits with chronic diseases reflect causal associations, or rather, are manifestations of disturbances in overlapping pathways. Genotyping with the IBC chip allowed dense coverage of ~2000 genes suspected to play a role in primarily cardiovascular disease, but did not provide adequate coverage of regulatory regions or gene deserts. Although consistency across cohorts and populations were observed for some associations, in other cases there was heterogeneity, which may have resulted from any number of causes. External replication data were limited to elderly cohorts. Sleep duration, human *DRD2* H3K4me3 epigenetic marks, mRNA levels and DRD2/3 receptor availability are all impacted by age (4,65–67). Epistasis with other dopamine-related polymorphisms is possible (40,68,69). While further studies are required to determine the generalizability limits of our findings, given the plausible mechanisms, past findings and the importance of *DRD2* across related phenotypes, future mechanistic investigation into the role of this gene in sleep regulation is warranted.

In summary, the findings of this study highlight the importance of a gene involved in a number of neurological processes in also influencing sleep duration, a trait that impacts many health outcomes such as obesity and cardiometabolic diseases. Although further replication of our results is required, future studies that address the influence of *DRD2* on physiology may be advanced by also considering the influence of *DRD2* on sleep-related outcomes. *DRD2* and dopamine functional interactions

with caffeine and modafinil suggest that these variants are likely to play an important role in inter-individual differences in drug efficacy, thus providing a new avenue for pharmocogenetics research in sleep and alerting medications.

Materials and Methods

Participating studies

The discovery sample included data from over 25 000 individuals of European, African, Hispanic and Asian ancestries from seven cohorts participating in the Candidate gene Association Resource (CARe) Consortium (70): Atherosclerosis Risk In Communities (ARIC), Coronary Artery Risk Development in Young Adults (CARDIA), Cleveland Family Study (CFS), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), Jackson Heart Study (JHS) and Multi-Ethnic Study of Atherosclerosis (MESA). The Sleep Heart Health Study (SHHS) further contributed ARIC, CHS and FHS subsets from Visit 1 (1995–98) (71). Population characteristics are displayed in Table 1. Children under the age of 18 were omitted from analyses. Replication cohorts included the Rush Memory and Aging Project + Religious Orders Study (MAP/ROS), Osteoporotic Fractures in Men (MrOS), the Study of Osteoporotic Fractures (SOF) and the Korean Genome and Epidemiology Study (KoGES). Local IRB approval was obtained for the CARe study.

The Atherosclerosis Risk in Communities Study (ARIC) was primarily designed to investigate cardiovascular risk factors and atherosclerosis across four communities: Forsyth County, NC; Jackson, MS; suburban Minneapolis, MN and Washington County, MD. Cohort component visits began in 1987. All sleep duration data were obtained through the SHHS exam, which included a subgroup of EA individuals recruited from the Minneapolis ($n = 1000$) and Washington County ($n = 750$) sites. Sleep duration information was obtained through a SHHS Sleep Habits Questionnaire (SHQ) administered to EAs.

The Coronary Artery Risk Development in Young Adults (CARDIA) study longitudinally examines cardiovascular health in four communities: Birmingham, AL; Chicago, IL; Minneapolis, MN and Oakland, CA. Examinations have occurred from 1985 to 2010. All AA and EA results were obtained from a sleep questionnaire administered in 2005–06 (Year 20).

The Cleveland Family Study (CFS) is a family-based longitudinal study designed to examine the genetic basis of Obstructive Sleep Apnea (OSA) in AAs and EAs studied between 1990 and 2006. Index probands with confirmed OSA were recruited, along with additional family members and neighborhood control families from Northeast Ohio. Data were used from the last examination available for each individual. The final wave of data also included overnight in-laboratory polysomnography. Sleep duration was assessed from a standardized questionnaire (72).

The Cardiovascular Health Study (CHS) assessed adults aged 65 or greater through a series of examinations from 1989 to 1999, with field centers in Washington County, MD; Allegheny County, PA; Sacramento County, CA and Winston-Salem, NC. Of these subjects, 3280 individuals from Allegheny, Sacramento and Washington Counties participated in the SHHS and had data from the Sleep Habits Questionnaire available for this analysis. Additional phenotype and covariate data obtained from CHS visits in years 9–11 were used for 1424 CHS individuals who did not participate in the SHHS examination.

The Framingham Heart Study (FHS) began data collection in 1948, with a primary focus on identifying factors contributing to cardiovascular disease. Based in Framingham MA, the study includes second- and third-generation cohorts from which current study data were obtained. Approximately 700 second-generation individuals (Offspring cohort) were recruited for SHHS polysomnography. The majority of Offspring information was obtained from the Sleep Habits Questionnaire ($n = 2148$), with remaining data ($n = 758$) obtained from questionnaires administered at FHS Exams 7 (1998–2001) and 8 (2005–08). Generation 3 data ($n = 3875$) were obtained from Exam 1 (2002–05).

The Jackson Heart Study (JHS) is investigating factors influencing cardiovascular disease in individuals and families in Jackson, MS. All data used in the present analyses were obtained from Exam 1 from 2004, with JHS data analyzed independently of other original ARIC site data.

The Multi-Ethnic Study of Atherosclerosis (MESA) is investigating the characteristics related to the progression of subclinical to clinical cardiovascular disease, with an emphasis on understanding any possible ethnic, age and gender differences in the risk factors for and the progression of subclinical cardiovascular disease. MESA is following individuals from six communities: Baltimore, MD; Chicago, IL; Los Angeles, CA; New York, NY; Minneapolis/St Paul, MN and Winston-Salem, NC. Examinations began in 2000 with five exams completed and follow-up ongoing. The current data derive from questionnaires administered in Exam 4 (2004–05).

The Osteoporotic Fractures in Men Study (MrOS) (21,22) is a prospective cohort of 5994 males who were aged 65 or older at the baseline visit. Recruitment occurred between 2000 and 2002 in six communities: Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Monongahela Valley, PA; Portland, OR and San Diego, CA. An ancillary study to MrOS, Outcomes of Sleep Disorders in Older Men, enrolled 3135 participants in an examination that included administration of sleep questionnaires, actigraphy and polysomnography between 2003 and 2005. The Study of Osteoporotic Fractures (SOF) is an analogous study involving 9704 women aged 65 and older (23). The study commenced in 1986 with recruitment in Baltimore, MD; Monongahela Valley, PA; Minneapolis, MN and Portland, OR. Standardized sleep measures were collected from 4727 SOF participants at the eighth clinic visit, which took place between 2002 and 2004.

The Rush Memory and Aging Project (MAP) (18) is a longitudinal investigation of genetic and environmental risk factors for chronic conditions of old age in community-dwelling older individuals (19) recruited from the Chicago, IL region beginning in 1997. The current replication data are derived from participants who underwent actigraphy testing, which was added in 2005.

The Religious Orders Study (ROS) is a prospective study investigating the association of risk factors for Alzheimer's disease, mild cognitive impairment and other neurocognitive outcomes (20). Examinations of members of over 40 religious communities from multiple ethnicities (88% non-Hispanic EAs) began in 1994.

The Korean Genome and Epidemiology Study (KoGES) (73,74) was designed to investigate chronic diseases within an industrial city (Ansan) and a rural community (Ansung) in South Korea. This population-based study began studying adults aged 40–69 in 2001. The initial baseline examination comprised 2497 females and 2523 males.

Phenotype and covariate definition

The primary phenotype was a measure of self-reported habitual nightly weekday sleep duration interrogated using single questions from standardized questionnaires [$n = 21\,373$ individuals; largely using or adapting the Pittsburgh Sleep Quality Index question 'During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spend in bed.)' (75)] or calculated from self-reported sleep

onset and offset times via the SHHS Sleep Habits Questionnaire [$n = 5641$ individuals (71)] and harmonized to provide comparable data across cohorts. Exact questionnaire text is provided in Supplementary Material, Table S9. Implausible values (sleep duration ≤3 h or ≥14 h) or values of sleep onset between 4 AM and 6 PM (indicating possible shift work) were excluded from analysis. Data were extracted from multiple visits to maximize information. When sleep duration was reported from multiple visits, exams that included more detailed questionnaires (e.g. bed and wake times) were prioritized over exams with less complete information.

Missing and discordant gender information was inferred based on X chromosome SNP heterozygosity checks performed within PLINK (76). For secondary analyses to examine the effects of environmental exposures and comorbidities on the *DRD2* SNPs (Supplementary Material, Table S1), alcohol, Type 2 diabetes, hypertension, retirement status and smoking covariates were expressed as binary variables. Depression status was determined by harmonizing within-cohort single-question ordinal scales typically considering the last 1–4 weeks (Supplementary Material, Table S10). Snoring (a symptom of obstructive sleep apnea) was dichotomized according to frequency (≥3 times, 'several nights', or 'often' per week).

Polysomnographic data [available from SHHS (71) and CFS (77)] and self-reported sleep traits used the same visit as the sleep duration questionnaire collection whenever possible. Traits across six domains were tested with the fine-mapped SNPs as exploratory analyses to provide insight into the primary sleep duration results: sleepiness (Epworth Sleepiness Scale); sleep apnea (minimum oxygen saturation across the night, apnea hyponea index within NREM); sleep latency; sleep timing [weekend average sleep (when reported), latency to first REM episode, self-reported sleep midpoint (weekday and weekend), time in bed, total sleep time]; arousability [sleep maintenance efficiency, arousal index (across the night, within NREM, within REM), wake after sleep onset]; and sleep stage percentages (Stages 1, 2, 3 + 4 and REM) (AA + EA $n = 2096$–9332).

Genotyping and quality control

Genotyping was performed by the Broad Institute and included an Affymetrix 6.0 chip for AAs and a customized IBC candidate gene chip on all individuals. The IBC chip targeted 2016 loci of cardiovascular, metabolic, inflammatory and sleep interest based on literature searches, pathway analyses, mouse eQTL data and multiple large GWAS. Coverage of these regions was designed to be dense and cosmopolitan. A large number of low MAF SNPs were included, and r^2 performance at lower MAF thresholds was generally equal to the Illumina 1 M array in the three HapMap populations (15).

Quality control for CARe has previously been reported (70). Briefly, for the Illumina IBC array, SNPs were called by Beadstudio and for the Affymetrix 6.0 array by Birdseed. SNPs in linkage disequilibrium ($r^2 > 0.3$) were pruned, and Eigenstrat (78) was used to compute 10 principal components on the subset of individuals passing quality control for use as covariates and to remove population outliers. Samples were also excluded for cryptic relatedness and sample call rate <90% (PLINK). SNPs were excluded for SNP call rate <95%, MAF <1%, heterozygous haploid genotypes and discordant alleles on both arrays. Genotypes with a minor allele frequency (MAF) ≥1% were analyzed.

Statistical analysis

The primary model for large-scale genetic analysis and GWAS of habitual sleep duration as a quantitative trait included adjustment for age, age^2, sex, age × sex and 10 population principal components (70). Secondary models were constructed to examine the effects of environmental exposures and comorbidities on the *DRD2* SNPs (Supplementary Material, Table S1): Model 2 (alcohol, smoking, years of education and retirement status); and Model 3 (BMI, BMI^2, depression, diabetes, hypertension and snoring), with each higher order model including prior variables. Covariate-adjusted residual values for sleep duration were calculated in each cohort using R (79). Individual cohort analyses (sub-divided by ancestry) were performed using an additive model within PLINK (76) and GWAF (80) for non-family and family cohorts. A fixed-effect, inverse variance-weighted meta-analysis was performed in METAL using a standard error model with genomic control applied at the meta-analysis level (81). A *P*-value of 1.9×10^{-6} was considered IBC array-wide significant (17), while the genome-wide significance threshold was used for AA analyses ($P = 5.0 \times 10^{-8}$). Ethnicity-specific specific QQ and Manhattan plots are provided in Supplementary Material, Figure S1. LocusZoom (82) was used for figure visualizations. Gene-based association analysis was performed using VEGAS (24), and Bonferroni correction for 5275 annotated genes with $n = 2$ or $n > 2$ SNPs was applied to determine the statistical significance threshold (9.48×10^{-6}).

DRD2 locus analysis

Conditional linear regression was performed using GCTA (83). Twenty-five IBC SNPs were located within 50 kb of rs17601612. The minimal Hardy–Weinberg equilibrium *P*-value for any SNP in this region was 0.0001 (CARDIA AAs, rs7131440). *DRD2* fine mapping imputation was performed in MACH and Minimac (84) using 1000 Genomes Phase 1 (Release 3) (85) reference haplotypes. Individual cohort genotypes (minimal MAF 1%) were phased across all of Chromosome 11 with a 10 MB segment used for imputation. MAF-dependent Rsq quality score cutoffs (<10% MAF: 0.75, <20%: 0.70, <30%: 0.66, <40%: 0.60, <50%: 0.55) were based on guidelines (86) that found a specificity of 98.1% and a sensitivity of 96.4%. Linkage disequilibrium was calculated using Haploview (87) for directly assayed SNPs and vcftools (88) for European 1000 Genomes SNPs. Genomic control was not used on fine-mapping results due to the strengthened, non-normal distribution of *P*-values within the region. As such, *P*-values are not directly comparable between assayed and imputed results. Imputed SNP haplogroup membership was assessed via EUR r^2 with the top EA assayed SNPs rs2471854 and rs17601612. Secondary analysis tested association of 20 imputed SNPs at the *DRD2* locus with 15 phenotypes assessed by polysomnography and 4 questionnaire-based measures described above. Epigenetic and transcription factor binding site regions were based on HaploReg v3 (26) annotations of ENCODE (25,89–91) and NIH Roadmap Epigenomics (27) data using an imputed model generated on 14 February 2015. 1000 Genomes Phase 1 EUR r^2 cutoffs of 0.95 were used for candidate functional SNPs, as calculated internally using vcftools.

Schizophrenia genetic risk score analysis

We identified proxies with $r^2 > 0.8$ in CEU for 12/108 schizophrenia sentinel SNPs on the IBC array. We constructed a genetic risk score weighted by the scaled schizophrenia effect estimate for each SNP and evaluated combined association of the genetic risk score with sleep duration in the EA meta-analysis, as implemented in GTX (92).

Replication analysis

Four primarily European American and 1 Korean cohort were used for replication and generalizability of top SNPs for both signals. Meta-analysis was performed using a fixed-effects inverse variance model in METAL.

Data availability

Meta-analysis results are freely available and have been posted to https://sleepgenetics.org/downloads

Supplementary Material

Supplementary material is available at *HMG* online.

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