

## ORIGINAL ARTICLE

## Novel loci associated with usual sleep duration: the CHARGE Consortium Genome-Wide Association Study

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Usual sleep duration is a heritable trait correlated with psychiatric morbidity, cardiometabolic disease and mortality, although little is known about the genetic variants influencing this trait. A genome-wide association study (GWAS) of usual sleep duration was conducted using 18 population-based cohorts totaling 47 180 individuals of European ancestry. Genome-wide significant association was identified at two loci. The strongest is located on chromosome 2, in an intergenic region 35- to 80-kb upstream from the thyroid-specific transcription factor *PAX8* (lowest  $P = 1.1 \times 10^{-9}$ ). This finding was replicated in an African-American sample of 4771 individuals (lowest  $P = 9.3 \times 10^{-4}$ ). The strongest combined association was at rs1823125 ( $P = 1.5 \times 10^{-10}$ , minor allele

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Received 11 November 2013; revised 1 September 2014; accepted 4 September 2014; published online 2 December 2014

frequency 0.26 in the discovery sample, 0.12 in the replication sample), with each copy of the minor allele associated with a sleep duration 3.1 min longer per night. The alleles associated with longer sleep duration were associated in previous GWAS with a more favorable metabolic profile and a lower risk of attention deficit hyperactivity disorder. Understanding the mechanisms underlying these associations may help elucidate biological mechanisms influencing sleep duration and its association with psychiatric, metabolic and cardiovascular disease.

*Molecular Psychiatry* (2015) **20**, 1232–1239; doi:10.1038/mp.2014.133; published online 2 December 2014

## INTRODUCTION

Usual sleep duration is an important determinant of daytime sleepiness; moreover, both short and long sleep duration have been consistently associated with psychiatric illness, hypertension, diabetes mellitus, coronary heart disease and mortality, although the mechanisms underlying these associations are poorly understood. Significant heritability of usual sleep duration has been reported from twin studies, with heritability estimates generally in the range of 0.40–0.55.<sup>1–3</sup> A number of neurotransmitters and neuropeptides are known to regulate sleep–wake behavior, and genetic screens in non-mammalian vertebrates have demonstrated an important role of ion channels, which regulate neural activity (reviewed in Sehgal and Mignot<sup>4</sup>). Polymorphisms in the human period 2 (*PER2*) and casein kinase 1d (*CSNK1D*) genes, known elements of the circadian molecular clock, are associated with autosomal dominant advanced sleep phase syndrome in isolated pedigrees.<sup>5,6</sup> The genetic basis for heritability of usual sleep duration, however, remains largely unknown. Candidate-gene studies have inconsistently implicated genes associated with the mammalian circadian clock, including *BHLHE41* (*DEC2*) and *CLOCK*,<sup>7–9</sup> and the glutamate receptor-encoding *GRIA3*.<sup>10</sup> In a small genome-wide association study (GWAS) of usual sleep duration in 749 Framingham Heart Study participants, no genome-wide significant associations were identified.<sup>11</sup> Recently, a GWAS in over 4000 individuals in seven European cohorts identified a polymorphism in *ABCC9*, encoding an ATP-sensitive potassium channel, which explained approximately 5% of the variance in usual sleep duration.<sup>12</sup> This finding was not replicated in other cohorts; however, knockdown of this gene in *Drosophila* results in lack of sleep during the first 3 h of the night. To date, no replicated associations between common genetic variants and sleep duration (or other sleep parameters) have been reported

from GWAS. In this study, we utilize self-report data on usual sleep duration, collected by 18 community-based cohort studies that have genotyped their cohorts, in order to identify common genetic variants associated with sleep duration. This study comprises a community-based sample of 47 180 individuals, approximately 10-fold larger than all previously reported GWAS of this phenotype,<sup>11,12</sup> and is the first to show replication in an independent sample.

## MATERIALS AND METHODS

### Cohorts

Participating cohorts were prospective studies that had collected self-report data on usual sleep duration. The analysis was initiated by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE),<sup>13</sup> but was extended beyond that initial group in order to obtain sufficient power for the analysis. Eighteen cohorts were ultimately included in the discovery sample: the Atherosclerosis Risk in Communities (ARIC) Study,<sup>14</sup> Cardiovascular Health Study (CHS),<sup>15</sup> Framingham Heart Study (FHS),<sup>16–18</sup> Health Aging and Body Composition (HABC) Study,<sup>19</sup> Helsinki Birth Cohort Study (HBCS),<sup>20</sup> Invecchiare in Chianti (InCHIANTI),<sup>21</sup> Osteoporotic Fractures in Men (MrOS) Study,<sup>22,23</sup> Quebec Family Study (QFS),<sup>24</sup> Queensland Institute of Medical Research Twins Study (QIMR),<sup>2</sup> Rotterdam Study I and II (RS I and RS II),<sup>25</sup> Study of Health in Pomerania (SHIP),<sup>26</sup> Study of Osteoporotic Fractures (SOF),<sup>27</sup> TwinsUK,<sup>28</sup> Wisconsin Sleep Cohort (WiSC),<sup>29</sup> Young Finns Study (YFS),<sup>30</sup> and genotyped subsets of the Health Professionals Follow-up Study (HPFS)<sup>31</sup> and the Nurses Health Study (NHS).<sup>32</sup> As shown in Table 1, the discovery cohorts were located in Europe, Australia and North America. This analysis included only participants of European ancestry, as determined by self-report, with additional exclusion in some cohorts for failure to cluster with European samples in principal components analysis or multidimensional scaling (ARIC, HPFS, MrOS, NHS, RS I and II, SHIP, SOF, TwinsUK and WiSC). Replication of the findings of this meta-analysis was sought in the

**Table 1.** Characteristics of the discovery cohorts

Cohort name	N	Location	Age	Sex, %F	Usual sleep duration, h	Reference
Atherosclerosis Risk in Communities (ARIC)	3578	US	62.6 (5.6)	53.2%	7.4 (1.1)	14
Cardiovascular Health Study (CHS)	1515	US	77.9 (4.6)	62.1%	7.3 (1.3)	15
Framingham Heart Study (FHS)	7531	US	51.3 (13.2)	54.1%	7.9 (1.3)	16–18
Health Aging and Body Composition (HABC)	1661	US	73.8 (2.8)	47.0%	7.0 (1.2)	19
Helsinki Birth Cohort Study (HBCS)	1175	Finland	69.0 (2.7)	60.7%	8.2 (1.1)	20
Health Professionals Follow-Up Study (HPFS)	3542	US	56.0 (8.7)	0.0%	7.2 (0.9)	31
Invecchiare in Chianti (InCHIANTI)	1205	Italy	68.3 (15.5)	55.4%	6.8 (1.5)	21
Osteoporotic Fractures in Men Study (MrOS)	2354	US	76.7 (5.7)	0.0%	7.0 (1.2)	22, 23
Nurses Health Study (NHS)	6638	US	54.4 (6.7)	100.0%	7.0 (0.9)	32
Quebec Family Study (QFS)	865	Canada	41.1 (15.4)	56.3%	7.7 (1.1)	24
Queensland Institute of Medical Research Twins Study (QIMR)	2286	Australia	34.5 (14.3)	74.2%	7.7 (1.0)	2
Rotterdam Study I (RS I)	2834	The Netherlands	76.1 (6.3)	59.5%	6.8 (1.3)	25
Rotterdam Study II (RS II)	1425	The Netherlands	68.9 (7.6)	57.6%	6.9 (1.3)	25
Study of Health in Pomerania (SHIP)	2859	Germany	49.4 (16.5)	57.9%	7.5 (1.3)	26
Study of Osteoporotic Fractures (SOF)	3303	US	77.0 (5.1)	100.0%	7.0 (1.2)	27
TwinsUK	1531	UK	53.1 (12.6)	86.1%	6.8 (0.8)	28
Wisconsin Sleep Cohort Study (WiSC)	850	US	55.7 (7.5)	45.6%	7.1 (0.9)	29
Young Finns Study (YFS)	2028	Finland	37.7 (5.0)	54.9%	7.4 (0.8)	30

African-American participants of the Candidate Gene Association Resource (CARE), which included the Cleveland Family Study (CFS), the Coronary Artery Risk Development in Young Adults (CARDIA) study, the Jackson Heart Study (JHS) and the Multi-Ethnic Study of Atherosclerosis (MESA).<sup>33</sup>

### Phenotype definition

Phenotype data were obtained from standardized personal interviews or self-completion questionnaires (Supplementary Table 1). The most widely available measure of sleep duration in the participating cohorts was self-report of usual hours of sleep at night; response options were typically whole number values. A smaller number of cohorts also collected self-reported usual bed and rise times, from which time in bed could be calculated. The relation between self-reported usual sleep duration and calculated time in bed was assessed in 9400 participants in the ARIC, CHS and FHS cohorts with data for both measures. Although the correlation between measures was fairly high at  $r=0.70$ , values differed by at least 1 h in 24.3% of subjects and by at least 2 h in 7.1% of subjects. Based on these differences, and for consistency on phenotype definition, calculated time in bed was not used as a proxy for self-reported usual sleep duration in meta-analyses.

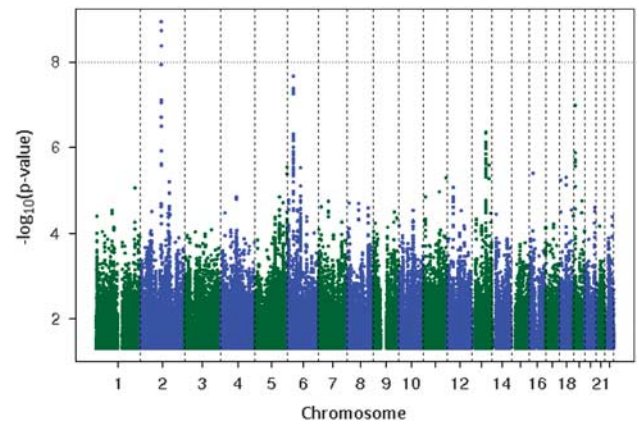
Measures of sleep duration were also ascertained separately for weekday nights and weekend nights in some cohorts. In advance of performing genetic association testing, the heritability of weekday versus weekend sleep duration, assessed by the questions, 'How many hours of sleep do you usually get at night (or your main sleep period) on weekdays or workdays?' and 'How many hours of sleep do you usually get at night (or your main sleep period) on weekends or your non-workdays?' was explored in the Framingham Heart Study Offspring cohort, which includes many sibling pairs, using the program SOLAR.<sup>34</sup> Based on 2388 individuals in 726 sibships, the estimated heritability of age- and sex-adjusted usual sleep duration on weekday nights was 23.6% (s.d. 7.7%), somewhat lower than the previously reported heritability of sleep duration from twin studies,<sup>1-3</sup> while for weekend nights heritability was estimated at only 12.3% (s.d. 7.2%). Therefore, where weekday and weekend night sleep duration were both available, weekday night sleep duration was analyzed. In order to exclude subjects in whom night shift work might lead to spurious estimates of sleep duration, subjects reporting a usual bedtime between 0500 and 1800 hours were excluded from analysis, where these data were known. Those whose usual sleep duration differed by >2h between weekdays and weekends were also excluded from analysis, where this difference was known, as behavioral factors were presumed to have a major influence on this measure.

### Genotyping and association analysis

Genotyping arrays and cohort-specific quality control filters are provided in Supplementary Tables 2a and 2b. Allele dosage was imputed using the software indicated. Association testing was performed independently in each of the contributing cohorts, using an additive model and untransformed sleep duration, adjusted for age and sex, which are both strong predictors of sleep duration, plus any covariates used by the individual cohorts to account for likely sources of population stratification or for relatedness among subjects (Supplementary Table 3). A fixed-effects meta-analysis of the cohort-specific results was performed using the inverse variance-weighted method in METAL,<sup>35</sup> with a total of 2 033 301 single-nucleotide polymorphisms (SNPs) tested. Genomic control correction was applied at the time of meta-analysis; individual cohort inflation factors ranged from 0.98 to 1.05. Only SNPs with minor allele frequency >0.05 and without significant heterogeneity across cohorts at  $P < 0.01$  were considered. A threshold  $P < 5 \times 10^{-8}$  was specified for statistical significance, corresponding to a Bonferroni correction for an estimated 1 million independent tests. All SNPs for the replication in African Americans were present on the Affymetrix 6.0 SNP array (Affymetrix, Inc., Santa Clara, CA, USA) used to genotype the CARE African-American sample, and were thus directly genotyped rather than imputed. Association analyses were adjusted for age, sex and the first 10 principal components to control for population stratification, and results of the four cohorts combined using fixed-effects meta-analysis in METAL. Conditional multi-SNP association testing was performed using GWAS summary statistics, as previously described.<sup>36</sup> Power analyses were performed using Quanto v1.2.<sup>37</sup>

### Evaluation of possible SNP function

Evidence that SNPs significantly associated with sleep duration, and those in linkage disequilibrium (LD) with these SNPs at  $r^2 > 0.5$  in HapMap Utah



**Figure 1.** Manhattan plot for genome-wide association with usual sleep duration in cohorts of European descent.

residents with ancestry from northern and western Europe (CEU), as defined by SNP Annotation and Proxy Search (SNAP) queries,<sup>38</sup> had an influence on gene expression was sought in the expression quantitative trait locus (eQTL) database of the Pritchard Lab (eqtl.uchicago.edu, accessed 18 November 2012) and in a separate query of significant results from >50 gene expression data sets covering multiple tissues (Supplementary Table 4). Evidence for an effect of SNPs of interest on thyroid function was sought through a look-up of results of the Meta-Thyroid consortium.<sup>39</sup> Cohorts in the consortium include several participating in the current analysis of sleep duration (CHS, FHS, HBCS, InCHIANTI, Rotterdam Study and UK Twins), as well as multiple additional cohorts. Data on glycemic traits were contributed by MAGIC investigators and downloaded from [www.magicinvestigators.org](http://www.magicinvestigators.org). Data on type 2 diabetes mellitus were contributed by DIAGRAM investigators and downloaded from [diagram-consortium.org](http://diagram-consortium.org). Data on psychiatric illnesses were obtained from published GWAS analyses of the Psychiatric Genomics Consortium (PGC), with data visualized using the Ricopili tool (<http://www.broadinstitute.org/mpg/ricopili/>) and downloaded from the PGC website (<http://www.med.unc.edu/pgc/data-sharing#SharingOpp>).

## RESULTS

Cohort-specific genome-wide association analyses of self-reported usual sleep duration from 18 population-based cohorts were meta-analyzed (Figure 1). All included subjects from these discovery cohorts were European or of European descent (Table 1). No evidence of population stratification was noted in the meta-analysis of self-reported usual sleep duration (Supplementary Figure 1; overall  $\lambda=1.06$ ; the range of inflation factors for individual cohorts was 0.98–1.05). Two independent loci showed genome-wide significant association with usual sleep duration (Table 2, Figures 2 and 3).

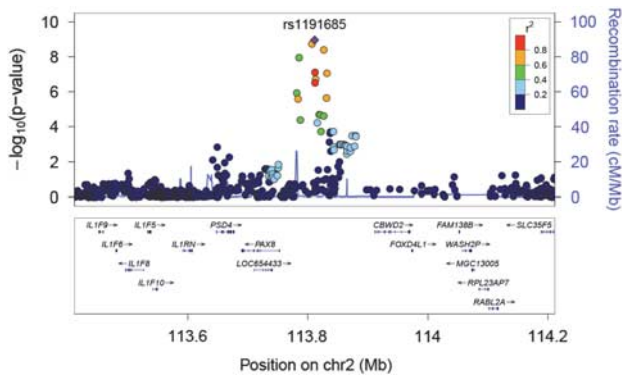
### Identification and replication of a novel sleep duration locus on chromosome 2

The most strongly associated locus is located between two genes on chromosome 2: 30- to 80-kb upstream from paired box gene 8 (*PAX8*) and, on the opposite strand, 80- to 130-kb upstream from cobalamin synthase W domain-containing protein 2 gene (*CBWD*) (Figure 2). *PAX8* is a well-characterized transcription factor essential to the formation of thyroxine-producing follicular cells during thyroid development. *PAX8* mutations produce thyroid dysgenesis, but the transcription factor is more widely expressed and may have other functions. In contrast, *CBWD2* is a poorly characterized gene highly expressed in the brain. The intergenic region also overlies a poorly characterized, predicted non-coding RNA (LOC101927400). This locus contains four SNPs meeting pre-specified criteria for genome-wide significance: rs1191685 ( $P=1.1 \times 10^{-9}$ ), rs1823125 ( $P=1.7 \times 10^{-9}$ ), rs1807282 ( $P=3.9 \times 10^{-9}$ ) and rs1964463 ( $P=1.1 \times 10^{-8}$ ), with minor allele

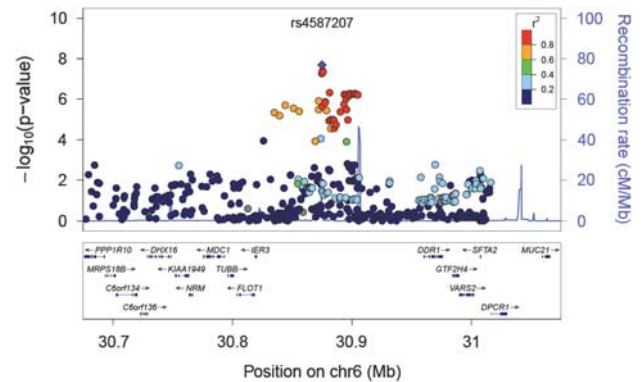
**Table 2.** SNPs with genome-wide significant association with usual sleep duration in discovery cohorts in two independent loci

SNP ID	Chr	Position, bp	Effect allele	Allele frequency	N	Coefficient ( $\beta$ ), min	SE $_{\beta}$ , min	P-value
rs1191685	2	113 811 454	C	0.37	44 563	2.87	0.47	$1.06 \times 10^{-9}$
rs1823125	2	113 806 882	G	0.26	45 281	3.01	0.50	$1.71 \times 10^{-9}$
rs1807282	2	113 826 506	T	0.26	46 805	2.89	0.49	$3.91 \times 10^{-9}$
rs1964463	2	113 785 491	G	0.25	45 281	2.84	0.50	$1.07 \times 10^{-8}$
rs4587207	6	30 874 924	G	0.20	46 807	-3.14	0.56	$2.02 \times 10^{-8}$
rs4248149	6	30 875 606	C	0.20	46 810	-3.08	0.56	$3.95 \times 10^{-8}$
rs2394403	6	30 875 848	T	0.20	46 811	-3.07	0.56	$4.39 \times 10^{-8}$

Abbreviations: bp, base pair; Chr, chromosome; N, number of individuals contributing to analysis for each SNP; SE $_{\beta}$ , standard error of the estimated coefficient; SNP, single-nucleotide polymorphism.



**Figure 2.** Chromosome 2 regional association plot for usual sleep duration in cohorts of European descent. Figure was constructed using the Broad Institute SNAP tool (<http://www.broadinstitute.org/mpg/snap/>).



**Figure 3.** Chromosome 6 regional association plot for usual sleep duration in cohorts of European descent. Figure was constructed using the Broad Institute SNAP tool (<http://www.broadinstitute.org/mpg/snap/>).

frequencies of 0.25–0.37, which were associated with an increase in self-reported usual sleep duration of 2.8 (s.e. 0.5) to 3.0 (s.e. 0.5) min per night per copy of the minor allele, explaining an estimated 0.07% of phenotypic heterogeneity. LD between the most strongly associated SNP and each of the other three significantly associated SNPs at this locus was modest, with  $r^2$  values between 0.51 and 0.64 in the HapMap 2 CEU sample. Conditional association testing was performed using summary-level statistics from the meta-analysis as previously described<sup>36</sup> with LD estimates derived from a representative sample of 4000 unrelated Australians of European descent. Conditioning on rs1191685, the effect sizes for the other SNPs reported above were reduced by approximately 60% and were no longer genome-wide significant (range of  $P$ -values 0.003–0.01).

The direction of effect was positive in all but one cohort (Supplementary Figure 2). Although there was no significant heterogeneity across cohorts, in nine of the European-descent cohorts, the estimated effect was  $>5.0$  min per night per copy of the minor allele, whereas in eight of the cohorts the estimated effect was  $<2.6$  min per night. The former cohorts were on average substantially older, with a mean age of 70 (s.d. 8) years, versus a mean age of 50 (s.d. 12) years in the latter group, and there was a strong correlation between mean age of cohort participants and estimated effect size ( $r=0.72$ ,  $P=0.001$ ). Although most of the participating cohorts excluded related individuals, two were twin studies (QIMR and TwinsUK) and two were family studies (FHS and QFS). A sensitivity analysis excluding these cohorts from the meta-analysis found a somewhat stronger effect size for all four SNPs, with effect estimates of 3.3–3.7 min per night. The strongest association in this sample was at rs1807282 ( $P=2.4 \times 10^{-10}$ ).

Three of the significantly associated SNPs in this region were directly genotyped in the Candidate-Gene Association Resource

(CARE)<sup>33</sup> African-American sample (rs1823125, rs1807282 and rs1964463); a fourth directly genotyped SNP (rs1191684) was in perfect LD with rs1191685 in the HapMap 2 Yoruba in Ibadan, Nigeria (YRI) sample. Interestingly, these four SNPs have very little LD in the HapMap 2 YRI sample, with  $r^2$  values of 0.001–0.04 (Supplementary Figure 3). Association testing in this sample of 4771 individuals replicated the finding from the discovery cohorts (Table 3), with effect sizes in the African-American sample that were in the same direction and somewhat larger than those seen in the discovery sample in three of the four SNPs, with two out of four SNPs reaching significance in the replication sample after Bonferroni correction. The strongest association in African Americans was at rs1807282, with an effect size 11.2 (s.e. 3.4) min per night per copy of the minor allele ( $P=9.34 \times 10^{-4}$ ), explaining 0.15% of phenotypic variance in this sample. When meta-analyzed together, the strongest association was with SNP rs1823125, the minor allele of which was associated with a sleep duration 3.1 min per night longer ( $P=1.47 \times 10^{-10}$ ; Table 3).

The locus of significant association is in the vicinity of an enhancer that is associated with an *in vitro* increase in *PAX8* gene expression of up to 250-fold.<sup>40</sup> None of the SNPs that were significantly associated with sleep duration are associated with significant differential expression of *PAX8* in published gene expression databases; no thyroid tissue gene expression databases are available, however. A look-up of these SNPs in the Meta-Thyroid consortium analysis of over 20 000 individuals of European ancestry<sup>39</sup> found no evidence for association of any of these SNPs with blood levels of either thyroid-stimulating hormone or free thyroxine. The SNP rs1191685 is significantly associated with differential expression in skin of a transcript of *IL1RN* ( $P=3.30 \times 10^{-5}$ ), which encodes the interleukin-1 receptor antagonist.<sup>41</sup> This eQTL signal peaks at rs1191683, which is in high LD with rs1191685 ( $r^2=0.80$ ); a signal was not seen in a smaller skin eQTL data set.<sup>42</sup>

**Table 3.** Replication of chromosome 2 locus association with sleep duration in African-American cohorts

SNP ID	Chr	Position, bp	Effect allele	Allele frequency	N	Coefficient ( $\beta$ ), min	SE $_{\beta}$ , min	P-value	Coefficient ( $\beta$ ), min	P-value
<b>African-American sample</b>										
rs1823125	2	113 806 882	G	0.12	4747	7.28	2.67	$6.35 \times 10^{-3}$	3.14	$1.47 \times 10^{-10}$
rs1191684 <sup>a</sup>	2	113 811 749	C	0.25	4770	3.70	2.02	$6.78 \times 10^{-2}$	2.90	$2.32 \times 10^{-10}$
rs1807282	2	113 826 506	T	0.08	4771	11.15	3.37	$9.34 \times 10^{-4}$	3.05	$3.35 \times 10^{-10}$
rs1964463	2	113 785 491	G	0.07	4766	0.78	3.42	$8.14 \times 10^{-1}$	2.80	$1.25 \times 10^{-8}$

Abbreviations: CARE, Candidate Gene Association Resource; LD, linkage disequilibrium; YRI, Yoruba in Ibadan, Nigeria; other abbreviations see Table 2. <sup>a</sup>SNP rs1191685 was not genotyped in the CARE sample. SNP rs1191684 is located 295 bp away and is in perfect LD with rs1191685 in the HapMap release 22 YRI sample, and is used as a proxy in this analysis; the effect allele is C at both rs1191685 and rs1191684.

**Table 4.** Additional loci associated with usual sleep duration not reaching genome-wide significance

Chr	Position, bp	# Of SNPs at $P < 10^{-5}$	SNP ID of strongest association at locus	MAF	N	Coefficient ( $\beta$ ), min	SE $_{\beta}$ , min	P-value	Function	Closest gene
1	215 423 024	1	rs2221285	0.29	46 062	2.15	0.49	$8.60 \times 10^{-6}$	Intergenic	ESRRG
2	158 551 264	1	rs6437122	0.10	45 283	-3.25	0.72	$6.30 \times 10^{-6}$	Intergenic	UPP2
5	178 328 456	2	rs11741688	0.45	46 084	1.96	0.42	$2.91 \times 10^{-6}$	Intergenic	ZNF454
6	70 547 232	2	rs9346353	0.41	42 595	-2.03	0.43	$3.00 \times 10^{-6}$	Intron	LMBRD1
11	124 764 510	1	rs731716	0.48	43 616	1.96	0.43	$5.18 \times 10^{-6}$	Intron	PKNOX2
12	31 890 261	1	rs2128614	0.38	46 802	1.87	0.42	$8.71 \times 10^{-6}$	Intergenic	LOC440093
13	79 449 564	26	rs9531006	0.31	44 567	2.43	0.48	$4.22 \times 10^{-7}$	Intergenic	SPRY2
13	97 373 844	2	rs9517132	0.31	44 567	-2.62	0.56	$2.61 \times 10^{-6}$	Intergenic	RANBP5
18	5 568 982	1	rs11664536	0.18	46 075	-3.19	0.70	$5.53 \times 10^{-6}$	Intergenic	EPB41L3
18	34 934 497	2	rs12165098	0.16	44 568	-3.18	0.70	$5.26 \times 10^{-6}$	Intergenic	BRUNOL4
19	9 820 014	7	rs2287838	0.47	46 079	-2.21	0.41	$1.05 \times 10^{-7}$	Intron	PIN1

Abbreviation: MAF, minor allele frequency; other abbreviations see Table 2.

As a result of a strong association of short sleep duration with diabetes mellitus and other glycemic traits,<sup>43</sup> we performed a look-up of these SNPs in published GWAS analyses of these traits. Three of the SNPs (rs1807282, rs1823125 and rs1964463) showed nominally significant association with the homeostatic model assessment of beta-cell function (HOMA- $\beta$ ,  $P=0.04$ ),<sup>44</sup> and with glycated hemoglobin (HgbA<sub>1c</sub>,  $P=0.008-0.011$ ).<sup>45</sup> In each case, the minor allele, which is associated with longer sleep duration, is associated with a more favorable metabolic profile, that is, higher HOMA- $\beta$  and lower HgbA<sub>1c</sub>. No association of these SNPs with type 2 diabetes mellitus was present in data from the DIAGRAM consortium (DIAGRAMv3.2012DEC17). As sleep disturbance is a common symptom in a number of psychiatric illnesses, we also performed a look-up of these SNPs in published PGC GWAS analyses. Attention deficit hyperactivity disorder (ADHD) was associated with SNPs rs1823125, rs1807282 and rs1964463 ( $P=0.03$  for each).<sup>46</sup> In each case, the allele that was associated with longer usual sleep duration in this study was associated with a lower ADHD risk in the PGC analysis. No significant association of these SNPs, or any in LD with these SNPs at  $r^2 > 0.4$ , was present for schizophrenia, depression or bipolar disorder. The association of ADHD has two local peaks, at rs1191694 and rs13032628 (each  $P=0.004$ ), which are in very low LD with one another ( $r^2=0.07$ ). The sleep duration-associated SNPs rs1823125 and rs1807282 are approximately 5600 and 2800 bp, respectively, from these ADHD-associated SNPs ( $r^2=0.12-0.13$  for LD between sleep duration-associated SNPs and rs1191694;  $r^2=0.06$  for LD between sleep duration-associated SNPs and rs13032628).

Identification of a second novel sleep duration locus on chromosome 6

The second region of genome-wide significant association in the cohorts of European descent is located on chromosome 6 in an intergenic region approximately 50-kb upstream of *IER3* and *FLOT1* (Figure 3), which also contains a long intergenic non-coding

RNA (LINC00243) of uncertain function. The three SNPs with genome-wide significant association span only 924 bp and are in perfect LD in both the CEU and YRI samples. In the discovery cohorts, the minor allele frequency was 0.20 and the strongest estimated association with sleep duration was 3.1 (s.e. 0.6) minutes less sleep per night per copy of the minor allele, explaining 0.07% of phenotypic heterogeneity. These SNPs are also in perfect LD with two SNPs (rs4713380 and rs4713385) that are significantly associated with the expression in peripheral whole blood of transcripts of the genes *IER3* ( $P=8.40 \times 10^{-23}$ ), *FLOT1* ( $P=4.00 \times 10^{-17}$ ), *VARS2* ( $P=4.60 \times 10^{-12}$ ) and *TUBB* ( $P=4.20 \times 10^{-5}$ ),<sup>47</sup> and in both skin ( $P=1.60 \times 10^{-9}$ ) and B-lymphoblastoid cell lines ( $P=6.49 \times 10^{-5}$ ) with expression of *IER3*.<sup>41</sup> A look-up in the PGC cross-disorder GWAS, which analyzed the association of genotype jointly with five psychiatric disorders (ADHD, autism spectrum disorders, bipolar disorder, major depressive disorder and schizophrenia), indicated that the three SNPs on chromosome 6 that are associated with sleep duration are significantly associated with psychiatric disorders ( $P=0.0003-0.001$ ).<sup>48</sup> These SNPs are part of a LD block spanning approximately 30 000 bp, and are in complete LD ( $r^2=1.0$ ) with 10 additional SNPs in this block, each of which is associated with the psychiatric disorders at  $P=4.7-9.9 \times 10^{-5}$ . This association was driven by associations with major depressive disorder ( $P=0.02-0.05$ )<sup>49</sup> and schizophrenia ( $P=0.04-0.08$ ).<sup>50</sup> In each case, the allele associated with shorter sleep duration in the present analysis was associated with increased depression and schizophrenia risk in the PGC analyses. Notwithstanding these suggestive correlates, this region did not replicate in the African-American sample. The effect was in the same direction but somewhat smaller (2.2-2.6 min less sleep per night per copy of the minor allele), and not statistically significant (lowest  $P=0.39$ ), although given a MAF of 0.09-0.10, the power to detect a significant replication of an effect of the magnitude seen in the discovery sample was low (range 17-18%).

#### Additional novel sleep duration loci and evaluation of candidate genes

An additional 11 loci were associated with usual sleep duration in the GWAS of the discovery cohorts at a nominal  $P < 10^{-5}$  (Table 4); none was significantly associated with sleep duration in the replication cohort, albeit with low power to detect significant effects (power  $< 30\%$  for each locus). A number of genes have been associated with sleep duration or chronotype through smaller candidate gene or GWAS that had no overlap with the cohorts included in this study. The present GWAS included multiple SNPs in these genes and other core mammalian clock genes, including *ABCC9* (131 SNPs), *PER2* (22 SNPs), *PER3* (56 SNPs), *CLOCK* (110 SNPs), *ARNTL* (114 SNPs), *ARNTL2* (106 SNPs) and *CSNK1D* (6 SNPs). No SNPs in *BHLHE41* were present in the GWAS. There was no evidence for association at any of these SNPs with usual sleep duration, using a liberal threshold of  $P < 0.01$ . The SNP rs12649507 in *CLOCK*, previously reported to be associated with usual sleep duration with an effect size of approximately 5-min shorter sleep per night in homozygotes for the minor allele than in homozygotes for the major allele,<sup>8</sup> had a smaller estimated effect in this study of 0.9-min shorter sleep per night per copy of the minor allele ( $P = 0.03$ ). The SNP rs11046205 in *ABCC9*, previously reported to be associated with usual sleep duration with an effect size of approximately 10-min longer sleep per night per copy of the minor allele,<sup>11</sup> had an estimated effect in the present analysis of only 0.9-min longer sleep per night per copy of the minor allele ( $P = 0.11$ ).

#### DISCUSSION

This GWAS identified two loci with genome-wide significant age- and sex-adjusted association to self-reported usual sleep duration in a large, multi-national sample of adults from Europe or of European descent. One of these loci was replicated in a sample of African Americans, strengthening the finding. This first locus is located approximately 30- to 80-kb upstream from the thyroid-specific transcription factor *PAX8* and, at a somewhat greater distance, upstream from *CBWD2*. It also overlies a predicted non-coding RNA LOC101927400 and is approximately 200 kb from an interleukin-1 gene cluster. Although little is known about the function of *CBWD2*, *PAX8* is a transcription factor that is most highly expressed in thyroid tissue, where it is important both in thyroid development and in maintaining adult thyroid function.<sup>51</sup> Thyroid-stimulating hormone levels are reduced by sleep deprivation,<sup>52,53</sup> with both higher<sup>52</sup> and lower<sup>53</sup> free thyroxine levels reported. Hypothyroidism is associated with excessive sleepiness<sup>54</sup> and with reductions in slow-wave sleep that can be corrected with hormone replacement,<sup>55</sup> whereas hyperthyroidism is associated with insomnia.<sup>56</sup> These findings suggest a role for thyroid hormone in sleep-wake regulation and thus a plausible role for *PAX8* effects on sleep duration. The locus of interest is in the vicinity of an enhancer that is associated with an *in vitro* increase in *PAX8* gene expression of up to 250-fold.<sup>40</sup> As no thyroid-specific eQTL databases are available, and *PAX8* is expressed in adults primarily in the thyroid gland, it was not possible to assess whether these variants are associated with changes in *PAX8* expression; however, these SNPs were not associated with measures of thyroid function in a GWAS, albeit in a smaller sample.<sup>39</sup> An alternative possibility is suggested by the association of rs1191685 with expression of *IL1RN*, as its product, the interleukin-1 receptor antagonist, has been shown to block the somnogenic effect of interleukin-1,<sup>57</sup> which is hypothesized to be involved in the physiologic regulation of sleep. The *IL1RN* is located 100-kb downstream of *PAX8* and a regulatory element for this receptor could be distantly located between *PAX8* and *CBWD2* (Figure 1). Although the mechanisms discussed above remain speculative, the cross-racial replication of the association suggests a true effect of this locus on usual sleep duration.

Although the magnitude of the association of the SNPs upstream of *PAX8* with sleep duration appears modest, with each copy of the minor allele associated with an estimated increase in usual sleep duration of approximately 3 min per night, the 0.07% of variance in sleep duration explained by this variant is typical of GWAS. For example, of 32 loci showing genome-wide significant association with body mass index in the GIANT Consortium analysis of almost 250 000 individuals, only four explained  $> 0.07\%$  of the variance in body mass index, with the two most strongly associated loci (*FTO* and *TMEM18*) explaining 0.34% and 0.15% of variance, respectively, and the remaining 30 variants explaining an average of 0.03% of variance each.<sup>58</sup> Moreover, self-reported usual sleep duration is likely to be an imprecise correlate of the underlying biological construct of interest, which is the innate sleep period of the individual free of environmental constraints. This reflects both technical factors, including imprecision in self-report estimates of sleep duration compared with objective measures such as actigraphy<sup>59</sup> and the coarse-grained response options typical of sleep duration questionnaires, as well as extensive socioenvironmental influences on sleep behaviors, including the widespread consumption of caffeine and alcohol, the impact of medical and psychiatric illness on sleep, and most importantly the impact of work and social schedules that are often unrelated to individual differences in optimal sleep duration. In older individuals, retirement from work and lack of childrearing responsibility often reduce the impact of social demands on sleep schedule, perhaps explaining the stronger effect observed in the older cohorts included in this analysis.

Although this study is  $> 10$ -fold larger than the two previously published GWAS of sleep duration, it remains small compared with recent studies of traits such as body mass index and hypertension, which are more widely available in large population-based cohorts. The replicated locus upstream from *PAX8* is therefore likely to represent the first of a larger number of associations that will appear as future population-based GWAS of sleep duration benefit from more rigorous phenotyping and larger sample size. Self-reported short sleep duration and experimental sleep restriction are strongly associated with impaired glucose metabolism.<sup>43</sup> Sleep disturbance and short sleep duration are also common in psychiatric disorders, including ADHD. It is therefore of interest that the alleles upstream from *PAX8* that are associated with longer sleep duration in this study were in prior GWAS associated with higher HOMA- $\beta$  and lower HgbA<sub>1c</sub><sup>44,45</sup> and with lower ADHD risk.<sup>46</sup> As sleep duration is associated with other important illnesses, including incident post-traumatic stress disorder,<sup>60</sup> obesity, hypertension and coronary heart disease, as well as with mortality, elucidating the molecular pathways that regulate sleep duration may both identify novel mechanisms affecting sleep regulation and help to explain its association with psychiatric and cardiometabolic disease.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### ACKNOWLEDGMENTS

The *Atherosclerosis Risk in Communities Study* is carried out as a collaborative study supported by National Heart, Lung and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, N01-HC-55022, R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. The *Cardiovascular Health Study* was supported by NHLBI contracts HHSN268201200036C, N01-HC-85239, N01-HC-55222, N01-HC-85079, N01-HC-85080, N01-HC-85081, N01-HC-85082, N01-HC-85083, N01-HC-85086; and NHLBI grants HL080295, HL087652, HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was

provided through AG023629 from the National Institute on Aging (NIA). DNA handling and genotyping was supported in part by the National Center for Research Resources grant UL1RR033176, now at the National Center for Advancing Translational Sciences CTSI grant UL1TR000124; the National Institute of Diabetes and Digestive and Kidney Disease grant DK063491 to the Southern California Diabetes Endocrinology Research Center. This research was conducted in part using data and resources from the *Framingham Heart Study* of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARe) project. This work was partially supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (contract no. N01-HC-25195) and its contract with Affymetrix, Inc for genotyping services (contract no. N02-HL-6-4278). A portion of this research utilized the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. The *Health, Aging and Body Composition Study* supported by NIA contracts N01-AG-62101, N01-AG-62103 and N01-AG-62106 and NIA grants 1R01AG032098-01A1 and 1R01AG030474-01A1. Genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268200782096C. This research was supported in part by the Intramural Research Program of the NIH, NIA. The *Helsinki Birth Cohort Study* has been supported by grants from the Academy of Finland, the Finnish Diabetes Research Society, Folkhälsan Research Foundation, Novo Nordisk Foundation, Finska Läkarsällskapet, Signe and Ane Gyllenberg Foundation, University of Helsinki, Ministry of Education, Ahokas Foundation, Emil Aaltonen Foundation, Juho Vainio Foundation, and Wellcome Trust (grant number WT089062). The *Nurses Health Study* and *Health Professional Follow-Up Study* GWAS were supported by grants from the National Institutes of Health [NCI (CA40356, CA087969, CA055075, CA98233), NIDDK (DK058845, DK070756), NHGRI (HG004399), NHLBI (HL35464)] with additional support from Merck/Rosetta Research Laboratories, North Wales, PA. The *Invecchiare in CHIANTI* study baseline (1998–2000) was supported as a 'targeted project' (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (Contracts: 263 MD 9164 and 263 MD 821336). The *Osteoporotic Fractures in Men (MrOS)* Study is supported by National Institutes of Health funding. The following institutes provide support: the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute on Aging (NIA), the National Center for Research Resources (NCRR), and NIH Roadmap for Medical Research under the following grant numbers: U01 AR45580, U01 AR45614, U01 AR45632, U01 AR45647, U01 AR45654, U01 AR45583, U01 AG18197, U01-AG027810, and UL1 RR024140. The National Heart, Lung, and Blood Institute (NHLBI) provides funding for the MrOS Sleep ancillary study 'Outcomes of Sleep Disorders in Older Men' under the following grant numbers: R01 HL071194, R01 HL070848, R01 HL070847, R01 HL070842, R01 HL070841, R01 HL070837, R01 HL070838, and R01 HL070839'. The NIAMS provides funding for the MrOS ancillary study 'GWAS in MrOS and SOF' under the grant number RC2AR058973. The *Quebec Family Study* was funded by multiple grants from the Medical Research Council of Canada and the Canadian Institutes for Health Research. This work was supported by a team grant from the Canadian Institutes for Health Research (FRN-CCT-83028). Funding for the *Queensland Institute of Medical Research Twin Study* was provided by the Australian National Health and Medical Research Council (241944, 339462, 389927, 389875, 389891, 389892, 389938, 442915, 442981, 496739, 552485, 552498), the Australian Research Council (A7960034, A79906588, A79801419, DP0770096, DP0212016, DP0343921), the FP-5 GenomEUtwin Project (QLG2-CT-2002-01254) and the US National Institutes of Health (NIH grants AA07535, AA10249, AA11998, AA13320, AA13321, AA13326, AA14041, MH66206). A portion of the genotyping on which this study was based (Illumina 370K scans) was carried out at the Center for Inherited Disease Research, Baltimore (CIDR), through an access award to our late colleague Dr Richard Todd (Psychiatry, Washington University School of Medicine, St Louis). Statistical analyses were carried out on the Genetic Cluster Computer, which is financially supported by the Netherlands Scientific Organization (NWO 480-05-003). EMB is supported by NHMRC grant 613608. The generation and management of GWAS genotype data for the *Rotterdam Study* are supported by the Netherlands Organisation of Scientific Research NWO Investments (no. 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) project nr. 050-060-810. The Rotterdam Study is funded by Erasmus MC and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. Henning Tiemeier was supported by the VIDJ grant of ZonMw (2009-017.106.370). Karin Hek was supported by a grant from BavoEuroport. *Netherlands Twin Registry* funding was obtained from the Netherlands Organisation for Scientific Research (NWO: MagW/ZonMw grants 904-61-090, 985-10-002, 904-61-193,480-04-004, 400-05-717, Addiction-31160008, Middelgroot-911-09-032, Spinozapremie 56-464-14192), Center for Medical

Systems Biology (CSMB, NWO Genomics), NBIC/BioAssist/RK(2008.024), Biobanking and Biomolecular Resources Research Infrastructure (BBMRI -NL, 184.021.007), VU University's Institute for Health and Care Research (EMGO+ ) and Neuroscience Campus Amsterdam (NCA), European Science Foundation (ESF, EU/QLRT-2001-01254), the European Community's Seventh Framework Program (FP7/2007-2013), ENGAGE (HEALTH-F4-2007-201413); European Science Council (ERC 230374), Rutgers University Cell and DNA Repository (NIMH U24 MH068457-06), the Avera Institute, Sioux Falls, South Dakota (USA), and the National Institutes of Health (NIH, R01D0042157-01A, Grand Opportunity grants 1RC2MH089951-01 and 1RC2 MH089995-01). Part of the genotyping and analyses were funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health. The *Study of Health in Pomerania* is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103 and 01ZZ0403), the Ministry of Cultural Affairs and the Social Ministry of the Federal State of Mecklenburg-West Pomerania. Genome-wide data have been supported by the Federal Ministry of Education and Research (grant no. 03ZIK012) and a joint grant from Siemens Healthcare, Erlangen, Germany and the Federal State of Mecklenburg-West Pomerania. The University of Greifswald is a member of the 'Center of Knowledge Interchange' program of the Siemens AG and the Caché Campus program of the InterSystems GmbH. The *Study of Osteoporotic Fractures* is supported by National Institutes of Health funding. The National Institute on Aging (NIA) provides support under the following grant numbers: R01 AG005407, R01 AR35582, R01 AR35583, R01 AR35584, R01 AG005394, R01 AG027574, R01 AG027576, and R01 AG026720. The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) provides funding for the ancillary study 'GWAS in MrOS and SOF' under the grant number RC2AR058973. *TwinsUK* was funded by the Wellcome Trust; European Community's Seventh Framework Programme (FP7/2007-2013), ENGAGE project grant agreement (HEALTH-F4-2007-201413). The study also receives support from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London. Genotyping was performed by the Wellcome Trust Sanger Institute, support of the National Eye Institute via an NIH/CIDR genotyping project. This research was supported for the *Wisconsin Sleep Cohort Study* by the National Heart, Lung, and Blood Institute (R01HL62252) and National Center for Research Resources (1UL1RR025011) and by NS23724. The *Young Finns Study* has been financially supported by the Academy of Finland: grants 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendli), and 41071 (Skidi), the Social Insurance Institution of Finland, Kuopio, Tampere and Turku University Hospital Medical Funds (grant 9M048 for 9N035 for TeLeht), Juho Vainio Foundation, Paavo Nurmi Foundation, Finnish Foundation of Cardiovascular Research and Finnish Cultural Foundation, Tampere Tuberculosis Foundation and Emil Aaltonen Foundation.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)