Impact of common diabetes risk variant in *MTNR1B* on sleep, circadian and melatonin physiology

Short Title: Impact of *MTNR1B* rs10830963 on melatonin physiology

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Abstract

The risk of type 2 diabetes (T2D) is increased by abnormalities in sleep quantity, quality, circadian alignment, and melatonin regulation. A common genetic variant in a receptor for the circadian-regulated hormone melatonin (*MTNR1B)* is associated with increased fasting blood glucose and risk of T2D, but whether sleep or circadian disruption mediates this risk is unknown. We aimed to test if *MTNR1B* diabetes risk variant rs10830963 associates with measures of sleep or circadian physiology in intensive in-laboratory protocols (n=58 to 96) or cross-sectional studies with sleep quantity, quality and timing measures from self-report (n=4,307 to 10,332), actigraphy (n=1,513) or polysomnography (n=3,021). In laboratory studies, we found significant association with a substantially longer duration of elevated melatonin levels (41 min) and delayed circadian phase of dim-light melatonin offset (1.37h), partially mediated through delayed offset of melatonin synthesis. Furthermore, increased T2D risk in *MTNR1B* risk allele carriers was more pronounced in early risers vs. late risers as determined by 7 days of actigraphy. Our results provide the surprising insight that the *MTNR1B* risk allele influences dynamics of melatonin secretion, generating a novel hypothesis that the *MTNR1B* risk allele may extend the duration of endogenous melatonin production later into the morning and early waking may magnify the diabetes risk conferred by the risk allele.

Keywords *MTNR1B* · Melatonin · Sleep · Type 2 diabetes · Circadian · genetic association · SNP

Introduction

Increased risk of type 2 diabetes (T2D) is associated with abnormalities in sleep quantity (1) and quality (1,2), circadian alignment (3,4) and melatonin regulation (5,6). Common variation at *MTNR1B* (7,8) was identified by genome-wide association studies to associate with diabetic traits. The mechanism whereby these variants lead to elevated T2D risk is unknown.

MTNR1B is one of two high-affinity receptors for the pineal hormone melatonin, which is released exclusively at night, and plays a role in glucose homeostasis (6,9). In humans, the release of melatonin occurs concurrent with overnight fasting during sleep. Elevated melatonin levels during an oral glucose load during the day causes impaired glucose tolerance (6,10). Common variants in *MTNR1B* associate with increased risk of T2D, fasting glucose (FBG) levels (7,8) and lower glucose stimulated insulin secretion in non-diabetic individuals (11). Functional studies have established that *MTNR1B* rs10830963 is the likely causal variant (12). Strong independent association of rare, loss-of-function variants in *MTNR1B* with increased risk of T2D further implicates *MTNR1B* as the most likely causal gene in the region (13).

Although *MTNR1B* plays a role in glucose homeostasis [reviewed in (14) and (15)], it is currently unknown how *MTNR1B* rs10830963 may alters its normal role in glucose metabolism. In this study, we explored the hypothesis that the association of rs10830963 with T2D may be mediated via effects on melatonin endocrinology, sleep timing/physiology, and/or the circadian system. Understanding these intermediate trait associations may lead to further insights into mechanisms by which risk variants influence

glycemic traits and point towards new avenues of therapeutic intervention. We tested association of rs10830963 with sleep, circadian and melatonin traits in two study populations with complementary strengths: 1) intensive in-laboratory protocols (n=58 to 96) with participants assessed for precise measures of circadian physiology, and 2) cross-sectional studies (Candidate-gene Association Resource, CARe) with sleep quantity, quality and timing measures from questionnaires (n=4,307-10,332), actigraphy (n=1,513) and overnight polysomnography (n=3,021).

RESEARCH DESIGN AND METHODS

Laboratory Studies (n=193)

Study Participants

Participants included 193 healthy individuals (a subset of 58-96 for whom measures of circadian physiology were available) from completed research studies in the Intensive Physiologic Monitoring Unit, Center for Clinical Investigations, Brigham and Women's Hospital from 2001-2011 as previously described and donated a blood sample for genetic analysis (16). To promote a stable circadian rhythm, all participants maintained an 8-hour sleep schedule of their choice at home for 1-3 weeks prior to admission. Compliance was verified with a sleep diary, calling in, and wrist actigraphy. Participants also completed a morningness-eveningness questionnaire (MEQ) (17). The genetic sample collection and analyses were approved by the Partners Health Care Human Research Committee. Separate informed consent was obtained for enrollment in genetic studies.

Circadian Phenotypes

Of the 193 participants studied in the laboratory, a subset of 58-96 were assessed in intensive protocols with precise measures of endogenous circadian physiology. Measures of circadian rhythm timing (phase), magnitude (amplitude), length (period), and melatonin physiology were measured in the in-laboratory samples using hourly plasma melatonin concentrations and core body temperature (1-minute epochs) collected over a minimum of 24 hours (Supplementary Table 1). We used baseline data from individual studies where subjects had undergone either a constant routine (CR) or posture (CP) protocol (n=96) (18-20) or a forced desynchrony protocol (FD) (n=63) ((21-23),Supplementary Figure 1A-1C). Melatonin phase measures collected included: dim light melatonin onset (DLMO) and dim-light melatonin offset (DLMOff) calculated as the time of the melatonin profile fitted curve at which levels crossed 25% of peak upward and downward, respectively, melatonin synthesis offset calculated from a linear model fitted to each melatonin profile (24), and the midpoint of melatonin calculated as the midpoint between DLMO and DLMOff (DLMO+(DLMOff-DLMO)/2). Phase was also measured by core body temperature (CBT) nadir, the time when the fitted circadian curve of CBT was at its minimum (18). To assess the difference between internal and external timing, phase angles were calculated as the time between sleep midpoint and either dim-light melatonin onset, dim-light melatonin offset, or CBT nadir. Circadian amplitude of melatonin and CBT were calculated as 50% of the difference between the minimum and peak of the fitted circadian curve. Measures of melatonin stability were calculated from a linear model fitted to each melatonin profile generating plasma melatonin clearance rate and half-life. The duration of melatonin secretion was measured as the difference between DLMO and DLMOff. Sleep timing

phenotypes included bedtime, wake time, midpoint of sleep and sleep duration derived from seven days of time-stamped call-ins during which subjects were required to maintain a self-selected, but fixed 8-h sleep schedule prior to laboratory admission. Procedures for determination of circadian phase, phase angle, amplitude, and period have been previously described (17,19,21,24).

Sample Genotyping

DNA was extracted from whole blood using standard methods (Qiagen). All samples were genotyped for rs10830963 and 58 African-American and Hispanic ancestry informative markers to test and correct for population stratification. Genotyping was performed using the Sequenom platform (Broad Institute, Cambridge, Massachusetts). Quality control steps excluded samples with <60% call rate and SNPs with <90% call rate, departure from HWE equilibrium ($p<10^{-7}$) or minor allele frequency <1%. In-lab samples acquired since the original genotyping effort (n=9) and samples that failed QC in the previous round (n=49) were whole genome amplified and re-genotyped for all SNPs.

Evaluation of population stratification

SmartPCA in Eigenstrat (25) was used to calculate principal components after merging with HapMap 3 CEU, YRI, ASW and CHB populations, and outliers 4 SD from the mean of the CEU population in the first three principal components were removed. Concordance between self-reported "non-Hispanic white" ancestry and included samples of European ancestry was 90.6%.

Association Testing

Genetic association analyses were performed in PLINK (26) using an additive genetic model and adjusting for age, sex, and 5 significant principal components that capture ancestry information. The significance threshold was set at p=0.05. No correction was performed for multiple phenotypes tested.

Candidate-gene Association Resource (CARe) Study (n=10,322)

Study Participants

Briefly, participants in the CARe study included >40,000 multi-ethnic individuals from 9 NHLBI cohorts with genotype and phenotype data, described by Musunuru et al. (27). We utilized data from up to 10,322 individuals of European ancestry from the Atherosclerosis Risk in Communities (ARIC) study (28), the Coronary Artery Risk Development in Young Adults study (CARDIA) (29), the Cardiovascular Health Study (CHS) (30), the Framingham Heart Study (FHS) (31), the Multi-Ethnic Study of Atherosclerosis (MESA) (32), and the ancillary Sleep Heart Health Study (SHHS) (33), selected based on the availability of genotyping data, glycemic traits, sleep questionnaire and polysomnography (PSG,n=3,021). We also utilized data from the ancillary MESA Sleep Study conducted at Examination 5 (n=1,513) based on the availability of genotyping data, wrist actigraphy (34) and glycemic traits.

Sleep Phenotypes

Self-reported sleep measures were assessed via questionnaire covering sleep behavior over the month leading up the questionnaire in each parent cohort (32,33,35). Individual cohort

questions (Supplementary Table 2) were harmonized across CARe cohorts into the following self-reported sleep phenotypes: weekday/weekend bedtime, wake time and midpoint of sleep, weekday/weekend, and weekly sleep duration, average sleep latency, and the binary questions: difficulty falling asleep, wake after sleep onset, early morning awakenings, frequent napping, and excessive daytime sleepiness.

PSG sleep measures were available in the SHHS cohorts (n=3,021). PSG was conducted during an unattended overnight home session as previously described (36). Participants were fitted with sensors by a certified technician, and data were captured overnight. Sleep stages were scored using guidelines described by Rechtschaffen and Kales (37). Total sleep time and total time in bed were available from the FHS component of SHHS (n=556) and percentage of sleep time in each stage was available for all three SHHS cohorts (FHS, CHS, and ARIC,n=3,021). Percentage of sleep time in each stage was computed by dividing time in sleep stage by recorded sleep time.

The MESA Sleep Study protocol included 7-day actigraphy (Actiwatch Spectrum, Philips Respironics, Murrysville, PA) together with sleep diary and questionnaire (n=1,513). Actigraphy data during 30 second intervals were scored as sleep or wake by Actiware-Sleep v.5.59 analysis software. Subject bedtime, sleep midpoint, and wake time from weekday, weekend, and weekly averaged data was calculated from actigraphy using the sleep log as an upper and lower bounds. Sleep duration was defined as the average duration of sleep between sleep onset (sleep start time) and morning wakening (sleep end time) while in bed after "lights off."

Type 2 Diabetes Phenotypes

Information on demographics, age, sex, and race/ethnic group was obtained by questionnaire. Height, weight, and fasting glucose levels were measured at visit 5. Use of diabetes medications was determined by questionnaire and from medication containers (32). T2D was defined as a fasting glucose ≥7.0 mmol/l (126 mg/dl), or insulin/oral hypoglycemic medications.

Sample Genotyping

The ITMAT-Broad-CARE (IBC) array v2 genotype data included rs108309638 (27,38). Using Illumina Beadstudio software, SNPs were clustered into genotypes. Quality control filters for SNPs and samples were applied separately within each cohort using PLINK (26). SNPs were excluded for Hardy-Weinberg equilibrium $P<10^{-7}$ and call rates <95% and samples for individual call rates <90%, sex mismatch and duplicate discordance. To control for relatedness, estimates of pairwise identity-by-descent (IBD) were calculated, and individuals with values >0.125 were pruned from the sample.

Evaluation of population stratification

Self-reported ethnicity was verified by multidimensional scaling (MDS) analysis of identity-by-state distances as implemented in PLINK, including HapMap panels as reference standards. SNPs in linkage disequilibrium ($r^2 > 0.3$) were pruned and Eigenstrat was used to compute 10 principal components on the subset of individuals passing quality control for use as covariates in the regression analyses (25).

Association Testing

Power calculations were performed using Quanto in independent subjects using the gene-only setting (39). Linear and logistic regression analysis was performed in PLINK adjusting for age, sex, BMI and PCs (26). A fixed effects, inverse-variance meta-analysis was performed in METAL (40). For primary analysis, significance threshold was set at p=0.05 (as only one hypothesis was tested). No correction was performed for multiple phenotypes tested. For interaction analyses, the significance was set at p<0.05, as only one hypothesis was tested. Interaction analysis adjusting for age, sex, and BMI was performed in PLINK (26). Interaction plots were generated in R using the effects package.

Results

Association with later dim-light melatonin offset and longer melatonin duration in laboratory studies

Descriptive characteristics of the laboratory study population are shown in Table 1. We tested rs10830963 for association with sleep and circadian traits (Tables 2). In the laboratory studies, we found significant associations between *MTNR1B* diabetes risk variant rs10830963 G and timing of the melatonin rhythm: an allelic dose-dependent delayed dim-light melatonin offset by 1h and 22min (Beta 1.36 h, 95% CI 0.28-2.44, N=95, p=0.015, R^2=19%) and a longer duration of elevated melatonin levels by 41 min during constant routine protocols [defined as the difference between dim-light melatonin onset and offset] (Beta 41 min, 95% CI 4.2-78, N=94, p=0.032, R^2=2.5%). This association is driven by the delay in dim-light melatonin offset, as we did not see an association with dim-light melatonin onset (p=0.236) (Table 2, Figure 1).

We then asked if melatonin synthesis offset accounts for the relationship between rs10830963 and delayed dim-light melatonin offset. Suggestive association of the risk allele with delayed melatonin synthesis offset (Beta 1.05h, 95%CI -0.17-2.28, N=82, p=0.097) was observed, and after conditioning on melatonin synthesis offset, the effect of rs10830963 on dim-light melatonin offset was halved ($Beta_{conditional}$ 0.65 h, 95%CI -0.066-1.366 h, *p-val 0.079,p-val* $_{ANOVA}$ <0.001), suggesting partial mediation. Additional adjustment for season of study had no effect (not shown).

We tested if chronotype or sleep timing contributes to the association between rs10830963 and dim-light melatonin offset. We find significant mediation by sleep timing (bedtime 82.3%, p=0.05, midpoint 84.4%, p=0.04, wake time 85.9%, p=0.05, chronotype (MEQ) 47.7%, p=0.13), although a small portion of the effect is independent of sleep timing. The relationship between rs10830963 and melatonin duration is not mediated by sleep duration (p=0.24).

Association with glycemic traits and modification of type 2 diabetes risk by sleep timing in CARe

Descriptive characteristics of the CARe cohort are shown in Table 1. *MTNR1B* variant rs10830963 was significantly associated with T2D and fasting blood glucose (FBG) in CARe (T2D: OR 1.08, 95% CI 1.01-1.16, N=2,516 cases/17,293 controls, p=0.01,FBG: Beta 1.52 mmol/L, 95% CI 1.30-1.74, N=17,252 non-diabetic individuals, p=1.41 x 10^{-41}).

No significant association was observed between *MTNR1B* rs10830963 and self-reported, 7-day actigraphy or PSG measures of sleep timing, quality or duration (Table 3). Notably,

no comparable measures of melatonin secretion were available in CARe cohorts, therefore our laboratory findings could not be evaluated in this study population.

However, given that sleep timing under a controlled sleep duration schedule largely mediated the association with dim-light melatonin offset, we tested if rs10830963 association with T2D is modulated by sleep timing in CARe. If true, this would be consistent with the hypothesis that risk allele carriers with earlier wake times would be more likely to have elevated melatonin levels than non-carriers at times of T2D diagnostic testing and morning meal consumption, and this difference between genotypes would be less apparent in participants with later wake times. Objectively measured sleep timing (7-day actigraphy) significantly modified the effect of rs10830963 on T2D risk, such that earlier sleep timing in combination with the G allele carries an increased risk compared to later sleep timing (N=1,513, bedtime p_{int}= 0.053, sleep midpoint p_{int}= 0.0176, wake time p_{int}=0.024, Table 4, Figure 2). In analyses of participants of European descent stratified by median bedtime, midpoint, and wake time, a significant association between rs10830963 genotype and T2D was seen in early sleep timing (bedtime <23:12, N=310, OR [95%CI] 1.48 [1.01-2.18], p= 0.044,midpoint <02:58, N=310, OR [95%CI] 1.80 [1.00-3.22], p= 0.0492,wake time < 06:41, N=303, OR [95%CI] 1.88 [1.04-3.40], p=0.035) but not in late sleep timing (bedtime ≥ 23:12, N=310, OR [95%CI] 1.29 [0.77-2.16], p=0.337,midpoint ≥ 02:58, N=310, OR [95%CI] 1.34 [0.79-2.29], p=0.277,wake time ≥ 06:41, N=320, OR 1.25 [0.74-2.13], p=0.406), independent of sleep duration. Thus, the effect of rs10830963 on risk of T2D may be modified by sleep timing, with risk allele carriers with earlier sleep timing at an increased risk.

Discussion

We hypothesized that a common T2D risk variant in *MTNR1B* would be associated with melatonin, sleep or circadian traits. We found the *MTNR1B* diabetes risk variant (rs10830963G) was associated with a later melatonin offset and a longer duration of elevated melatonin levels in highly-controlled laboratory studies. Furthermore, we demonstrate that the increased T2D risk in rs10830963G carriers is more pronounced in early sleep timing, and almost absent in late sleep timing, in whom an extended morning melatonin profile would be obscured by the later rise time. Thus, taken together, our data suggests that *MTNR1B* rs10830963G extends the duration of melatonin production later into the morning and waking up earlier in the morning magnifies the diabetes risk with *MTNR1B* genotype.

The impact of *MTNR1B* rs10830963G on dim light melatonin offset is significantly mediated by sleep timing, suggesting that *MTNR1B* variation may influence dim light melatonin offset through changes in sleep timing, or that *MTNR1B* variation may influence sleep timing through changes in the timing of the melatonin profile. No significant associations with other sleep and circadian traits were observed, consistent with previous studies of narrower scope showing no *MTNR1B* risk allele effect on self-reported sleep disturbances (41,42). While our observations must be regarded as preliminary due to the limited sample size with detailed circadian measures and melatonin profile, they collectively add new insights linking *MTNR1B* to T2D. Melatonin receptor 1B (known as Mel1B or MT2) is one of two trans-membrane receptors for melatonin, a hormone that acts as a signal for the biological night. *MTNR1B* rs10830963G allele carriers have been reported to show increased Mel1B receptor expression in the pancreatic beta-cell (11). Melatonin signaling during the night, when diurnal humans are fasting, inhibits basal and

glucose-stimulated insulin secretion (5,43,44). Delayed dim light melatonin offset and a longer duration of melatonin in risk allele carriers may result in an increased risk for food intake to coincide with elevated melatonin levels in the morning, leading to decreased glucose tolerance and possibly elevated diabetes risk. Consistently, risk allele carriers with earlier sleep timing have an increased T2D risk, possibly due to concomitant food intake and elevated melatonin levels in the morning. In addition to the adverse effects of an increase in melatonin levels into daytime, a reduction in nighttime melatonin signaling also appears to be deleterious. Reduced nighttime melatonin signaling, either by *MTNR1B* receptor rare loss of function variants (13) or reduced nighttime melatonin levels (5), is associated with an increased risk of T2D. Future studies are warranted to test causality and to assess how the impact of rs10830963G on melatonin offset and duration alters the proper timing and magnitude of basal and postprandial insulin secretion and glucose control.

The strength of our study comes from the depth and breadth of phenotypes available in our cohorts. The laboratory study was limited in size due to the nature of the intensive physiologic studies required to obtain precise phenotypes. This sample is one of the largest of its kind, and contains precisely measured endogenous circadian measures that require multi-day studies under highly-controlled laboratory conditions to assess endogenous circadian control of plasma melatonin and core body temperature. These results need to be interpreted in light of the biases of this study, however, as only young healthy subjects were studied and held to self-selected sleep schedules prior to the in-laboratory portion of the study (8 hour sleep duration) that may not reflect biological preference. Although the sample size is limited, the phenotypes were measured in great depth, minimizing misclassification and maximizing specificity to biological processes of interest. Sleep

timing in MESA is measured objectively across multiple days, minimizing phenotype measurement error. The sample size, however, is limited. In all future studies, it will also be important to measure melatonin levels at the time of glucose assessment in the morning by genotype.

The CARe study is a large well-powered study encompassing one of the largest epidemiologic studies of sleep habits (Sleep Heart Health Study) with self-reported and objective overnight PSG-measured sleep phenotypes. Notably, our study did not identify significant associations with the available measures of sleep quality or quantity in CARe, consistent with previous studies (41, 42). It is important to recognize, however, that the indices of sleep quality, duration and timing available in these cohort studies likely are measured with modest to moderate error, misclassification would attenuate any true associations. Previous studies demonstrate a 50% reduction in power associated with measurement error equivalent to one standard deviation of the trait (45). This may even be true for PSG measures phenotypes in CARe, where first night effects influence sleep measures taken during a single unsupervised over-night PSG episode. This study establishes that diabetes-risk variants in *MTNR1B* are unlikely to play a role in central sleep behaviors and thus future research should emphasize evaluation of their role in peripheral tissues of relevance to T2D.

Given the clear and adverse effects of sleep disruption and circadian disruption on glucose control and diabetes risk, our new evidence linking sleep and circadian-related *MTNR1B* gene variant with altered melatonin physiology and indicating how this might impact

glucose control and diabetes risk is an important advance. Moving forward, the association of *MTNR1B* rs10830963 with melatonin rhythm phenotypes should be followed up with further in-depth mechanistic studies on a tissue level and phenotyping in individuals pre-selected based on genotypes of interest. In general, circadian metabolic assessments as well as targeted interventions (e.g., with light intervention or pharmacological doses of melatonin) may be useful strategies for probing the functional consequences of the variant on circadian rhythms, sleep physiology and metabolism. Ultimately this research could lead us towards new therapeutic interventions which reduce the impact of extended elevated melatonin levels into the morning perhaps via alterations to melatonin dynamics, melatonin-mediated insulin secretion or timing of food intake.

Author Contributions

The study was designed by AMC, OMB, JFD, SAS, FAJLS and RS. The following co-authors contributed to phenotype data collection in study cohorts: In-laboratory studies (DA, SWC, CAC, EBK, CA, NS, JJG, SWL, MM, SMWR, MR, KS, EVR, OMB and JFD), CARe cohorts: ARIC (NP), CARDIA (DL), CHS (SAG), FHS (DJG), MESA (PZ) and SHHS (BCE, SP, SR). AMC and MAH performed analyses to generate in-laboratory phenotypes. JML, AB and RS performed genetic analyses. JML, FAJLS and RS wrote the manuscript and all co-authors helped interpret data, reviewed and edited the manuscript, before approving its submission. RS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1. Cohort characteristics

Laboratory	
Phenotype (n)	*Mean(SD)*
N	198
Females, n†	70 (35%)
Age, yr†	25.41 (9.58)
Owl/lark questionnaire, numeric score (193)†	52.18 (12.15)
Bed-time,clock time (151)*	23:49 (1.49)
Sleep midpoint, clock time (151)*	03:59 (1.41)
Wake-time, clock time (151)*	08:10 (1.46)
Calculated sleep duration, hours (151)*	8.04 (0.13)
Phase of dim-light melatonin onset, clock time (96)	10:31 (1.86)
Phase angle between melatonin onset and sleep midpoint, hours (93)	5.58 (1.09)
Midpoint of melatonin secretion, clock time (95)	03:36 (1.82)
Phase of dim-light melatonin offset, clock time (95)	08:38 (1.98)
Phase angle between melatonin offset and sleep midpoint, hours (93)	4.53 (1.20)
Phase of melatonin synthesis offset, clock time (82)	6:31 (1.95)
Duration of melatonin secretion, hours (94)	10.11 (1.08)
Plasma melatonin clearance rate, min-1 (80)	0.03 (0.02)
Plasma melatonin clearance half-life, min (80)	34.17 (20.57)
Circadian melatonin amplitude, pg/ml (95)	38.30 (23.02)
Circadian period of melatonin, hours (58)	24.17 (0.19)
Phase of circadian core body temperature nadir, clock time (90)	04:59 (2.09)
Phase angle between core body temperature nadir and sleep midpoint, hours (88)	0.77 (1.37)
Circadian core body temp amplitude, degrees F (89)†	0.52 (0.15)
Circadian period of core body temperature, hours (64)†	24.15 (0.20)

CARe	
Phenotype (n)	*Mean(SD)*
N	10,332
Females, n	5,683 (55%)
Age, yr	64.65 (12.49)
BMI, kg/m2	27.34 (5.04)
Self-report average weekly sleep duration, hours (8,380)	7.24 (2.77)
Self-report average weekday sleep duration, hours (6,508)	7.1 (1.15)
Self-report average weekend sleep duration, hours (4,517)	7.48 (1.22)
Calculated average weekly sleep duration, hours (4,476)	7.48 (1.05)
Calculated average weekday sleep duration, hours (4,505)	7.39 (1.10)
Calculated average weekend sleep duration, hours (4,488)	7.72 (1.19)
Bedtime, weekday, clock time (hours) (4,542)	22:56 (1.05)

Bedtime, weekend, clock time (hours) (4,528)	23:11 (1.07)
Sleep midpoint, weekday, clock time (hours) (4,511)	02:32 (0.84)
Sleep midpoint, weekend, clock time (hours) (4,502)	02:55 (0.84)
Wake time, weekday, clock time (hours)(4,527)	06:19 (1.16)
Wake time, weekend, clock time (hours) (4,530)	06:54 (1.24)
Sleep latency, minutes (4,495)	16.61 (17.14)
Total sleep time, PSG measured, hours (556)	6.39 (0.97)
Total time in bed, PSG measured, hours (556)	7.50 (0.84)
REM sleep percent, PSG measured (3,026)	19.51 (6.64)
Stage 1 sleep percent, PSG measured (3,026)	5.26 (3.87)
Stage 2 sleep percent, PSG measured (3,026)	57.02 (13.13)
Stage 3/4 sleep percent, PSG measured (3,026)	18.22 (12.24)

	Cases, n (%)
Frequent daytime Sleepiness	2,778 (26.97%)
Frequent difficulty Falling Asleep	3,455 (33.49%)
Frequent wake after sleep onset	5,760 (55.79%)
Frequent early Awakening	3,747 (36.56%)
Frequent Naps	2,415 (42.41%)

MESA	
Phenotype (n)	*Mean(SD)*
N	1,513
Females, n	853 (56%)
Age, yr	69.18 (9.21)
BMI, kg/m2	28.73 (5.63)
Objectively measured bedtime, clock time (hours)	23:31 (1.40)
Objectively measured sleep midpoint, clock time (hours)	03:07 (1.19)
Objectively measured wake time, clock time (hours)	06:42 (1.38)

Data are shown as mean (standard deviation) or N (%). *indicates measures were collected via call-ins during a one week schedule of 8 hour sleep prior to in-laboratory studies. All in-laboratory measures except for those indicated with † were from subjects on a study protocol with restricted 8-hour time in bed. ‡ indicates sleep duration was calculated from self-reported bedtime and wake time.

Table 2. *MTNR1B* rs10830963 association with sleep, circadian and melatonin traits in Laboratory Studies.

	N	Beta	SE	*P*
Owl/lark questionnaire, numeric score	193	-0.345	2.389	0.885
Bed-time,clock time	151	30.36	19.38	0.119
Sleep midpoint, clock time	151	31.8	19.38	0.103
Wake-time, clock time	151	33.12	19.5	0.091
Calculated sleep duration, hours	151	0.046	0.033	0.165
Phase of Dim-light melatonin onset, clock time	96	0.648	0.542	0.236
Phase angle between melatonin onset and sleep midpoint, hours	93	0.165	0.338	0.627
Midpoint of melatonin secretion, clock time	95	1.014	0.514	0.052
Phase of dim-light melatonin offset, clock time	**95**	**1.361**	**0.55**	**0.015**
Phase angle between melatonin offset and sleep midpoint, hours	93	0.45	0.382	0.242
Phase of melatonin synthesis offset, clock time	82	1.047	0.624	0.097
Duration of melatonin secretion, hours	**94**	**0.684**	**0.314**	**0.032**
Plasma melatonin clearance rate, min-1	80	-0.006	0.007	0.382
Plasma melatonin clearance half-life, min	80	4.753	7.048	0.502
Circadian melatonin amplitude, pg/ml	95	0.058	7.258	0.994
Circadian period of melatonin, hours	57	0.01	0.067	0.885
Phase of circadian core body temperature nadir, clock time	90	0.627	0.575	0.279
Phase angle between core body temperature nadir and sleep midpoint, hours	88	-0.242	0.413	0.559
Circadian core body temp amplitude, degrees F	89	-0.027	0.042	0.521
Circadian period of core body temperature, hours	63	0.003	0.069	0.964

Results are from linear regression analysis in whites adjusted for age, sex and 5 PCs of ancestry. Significant results are shown in bold, no correction was applied for multiple phenotypes. Allele frequency of rs10830963 in the laboratory studies was 0.32.

Table 3. *MTNR1B* rs10830963 association with sleep traits in CARe.

	rs10830963 G allele				
	N	Effect/OR [95%CI]	SE	*P*	Min. Effect detectable†
Bedtime, weekday, mins	4,359	-0.72	1.50	0.63	3.93
Bedtime, weekend, mins	4,346	-0.84	1.56	0.60	4.2
Sleep midpoint, weekday, mins	4,329	-0.78	1.20	0.51	3.3
Sleep midpoint, weekend, mins	4,321	-0.66	1.20	0.58	3.3
Wake time, weekday, mins	4,344	-1.08	1.62	0.51	4.5
Wake time, weekend, mins	4,347	-0.78	1.80	0.66	4.8
Self-report average weekly sleep duration, hrs	6,406	0.01	0.03	0.62	0.135
Self-report average weekday sleep duration, hrs	6,321	0.02	0.02	0.45	0.065
Self-report average weekend sleep duration, hrs	4,333	0.01	0.03	0.69	0.08
Calculated average weekly sleep duration, hrs	4,295	0.00	0.03	0.99	0.07
Calculated average weekday sleep duration, hrs	4,323	-0.01	0.03	0.85	0.07
Calculated average weekend sleep duration, hrs	4,307	0.01	0.03	0.83	0.08
REM sleep percent, PSG measured	3,021	0.22	0.19	0.23	0.53
Stage 1 sleep percent, PSG measured	**3,021**	**0.18**	**0.11**	**0.08**	0.31
Stage 2 sleep percent, PSG measured	3,021	0.15	0.35	0.67	1.045
Stage 3/4 sleep percent, PSG measured	**3,021**	**-0.55**	**0.32**	**0.08**	0.97
Sleep latency, minutes	6,316	0.99	0.01	0.38	1.15
Objectively measured bedtime, mins	1,513	-2.20	3.54	0.54	9.7
Objectively measured sleep midpoint, mins	1,513	-1.92	0.75	0.53	8.1
Objectively measured wake time, mins	1,513	-1.64	3.50	0.64	9.6
Frequent difficulty Falling Asleep	9,846	1.01 [0.94 - 1.07]		0.88	1.052
Frequent early Awakening	9,808	0.98 [0.92 - 1.05]		0.66	1.051

Frequent daytime Sleepiness	9,977	0.98 [0.91 - 1.05]	0.65	1.05
Frequent Naps	6,457	1.06 [0.98 - 1.15]	0.15	1.0875
Frequent wake after sleep onset	9,855	0.97 [0.91 - 1.04]	0.43	1.064

Results are from linear/logistic regression analysis adjusting for age, gender, BMI, and ancestry. †Minimum detectable effect at 80% power, alpha=0.05. Allele frequency was 0.27.

Table 4. Interaction results of rs10830963 with sleep timing on type 2 diabetes risk.

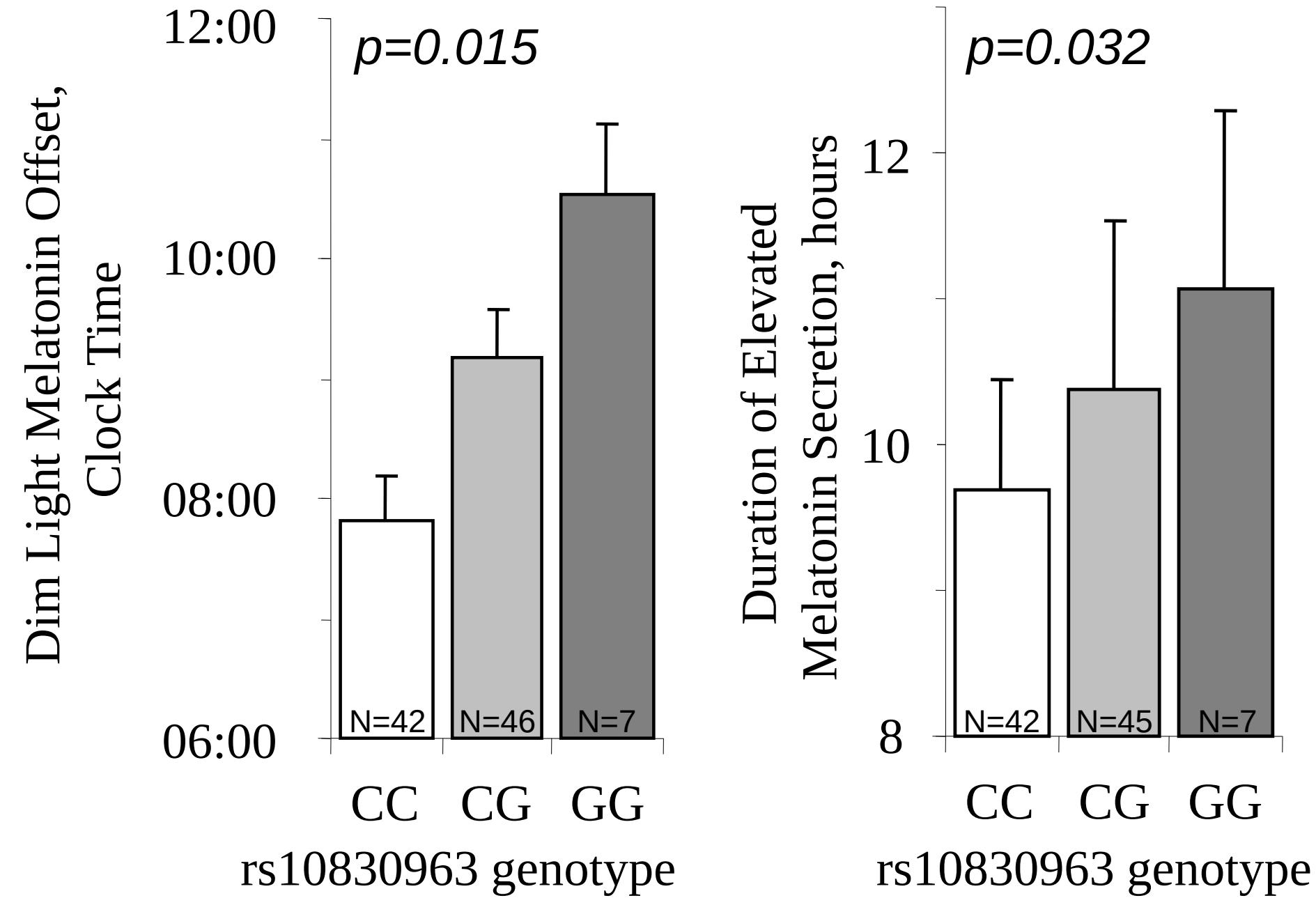
Interaction Phenotype	CHR	SNP	Effect Allele	Ethnicity	MAF	N	Early Timing OR [95%CI]	Late Timing OR [95% CI]	SE	*p*-int
Bedtime	11	rs10830963	G	Whites	0.26	619	1.48 [1.01-2.18]	1.29 [0.77-2.16]	0.1446	0.1907
				Asians	0.42	167	2.38 [0.88-6.46]	0.70 [0.24-2.07]	0.2356	0.1170
				Blacks	0.08	370	1.11 [0.56-2.19]	1.34 [0.60-3.00]	0.1867	0.9468
				Hispanics	0.19	357	1.07 [0.57-2.04]	1.02 [0.55-1.89]	0.1490	0.2639
				META	0.22	1,513	1.38 [1.03-1.83]	1.14 [0.81-1.60]	0.0846	0.0533
Sleep Midpoint	11	rs10830963	G	Whites	0.26	619	1.80 [1.00-3.22]	1.34 [0.79-2.29]	0.1783	0.0549
				Asians	0.42	167	2.45 [0.89-6.70]	0.97 [0.37-2.49]	0.3007	0.0626
				Blacks	0.08	370	1.07 [0.57-2.02]	1.02 [0.55-1.89]	0.2118	0.5954
				Hispanics	0.19	357	1.30 [0.59-2.90]	1.05 [0.53-2.07]	0.1668	0.4665
				META	**0.22**	**1,513**	**1.49 [1.05-2.13]**	**1.13 [0.81-1.57]**	**0.0996**	**0.0180**
Wake Time	11	rs10830963	G	**Whites**	**0.26**	**619**	**1.88 [1.04-3.40]**	**1.25 [0.74-2.13]**	**0.1720**	**0.0320**
				Asians	0.42	167	2.09 [0.85-5.06]	0.84 [0.37-2.63]	0.2672	0.1616
				Blacks	0.08	370	1.33 [0.61-2.94]	0.94 [0.47-1.87]	0.1808	0.3368
				Hispanics	0.19	357	0.84 [0.44-1.60]	1.26 [0.67-2.37]	0.1490	0.8488
				META	**0.22**	**1,513**	**1.32 [0.95- 1.84]**	**1.13 [0.82- 1.57]**	**0.0900**	**0.0290**

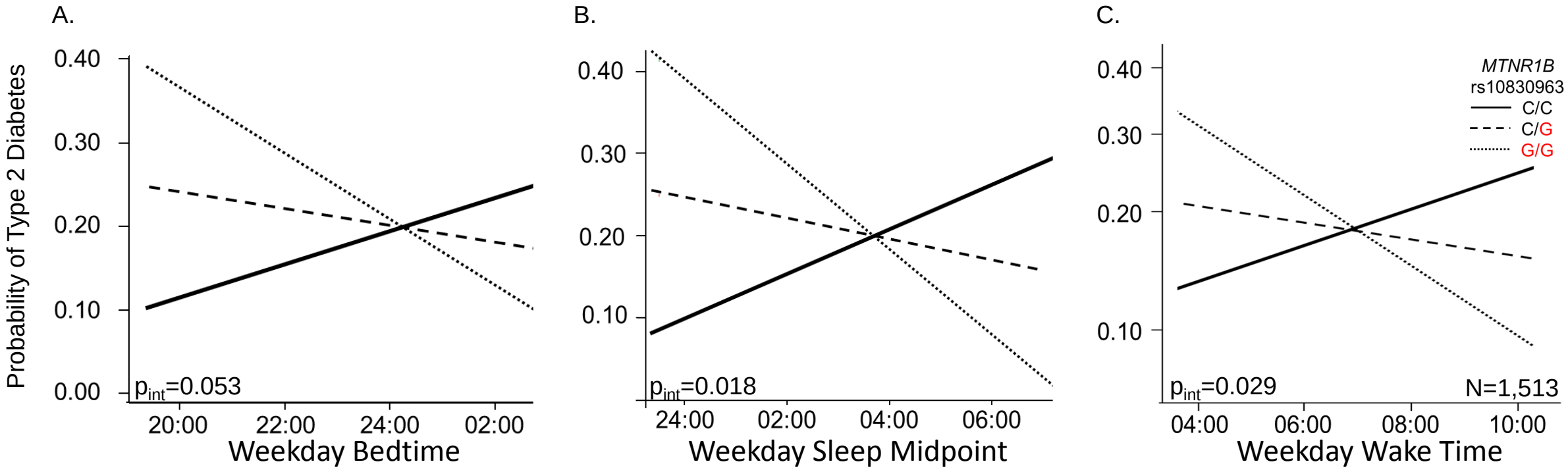
Results are from logistic regression with an interaction term. Model is adjusted for age, sex, and BMI. Wake time was measured by actigraphy across a minimum of three days. MAF = minor G allele frequency, OR=odds ratio, CI=95% confidence interval, SE=standard error. Odds of T2D are shown stratified by the median bedtime, midpoint, and wake time into "early" and "late". Significant results ($p<0.05$) are shown in bold.

Figure Legends

Figure 1. **Circadian phase of dim light melatonin offset (DLMOff) and duration of elevated melatonin levels vary by *MTNR1B* genotype in the InLaboratory cohort**. Adjusted mean and standard error shown by rs10830963 genotype (T2D risk allele, G). *P* value derived from multiple linear regression test between genotype and phenotype adjusted for age, sex, and principal components of ancestry. A. Circadian phase of DLMOff (n=95, adjusted means (se) in clock time, C/C 07:49 (23 min), C/G 09:10 (24 min), G/G 10:32 (35 min)) B. Duration of melatonin production (n=94, adjusted means (se) in clock time, C/C 9.70 h (0.74), C/G 10.38 h (1.15), G/G 11.07 h (1.21)).

Figure 2. **Sleep timing modifies the effect of *MTNR1B* variant rs10830963 on risk of type 2 diabetes in multi-ethnic MESA** (n=1,513). The effect of sleep timing on T2D risk is shown by rs10830963 genotype. The rs10830963 G T2D risk allele is shown in red. Lines represent the genotype specific linear regression of rs10830963 x 7 day actigraphy measured bedtime (A.), midpoint (B.), and wake time (C.) in 1,513 subjects of multi-ethnic ancestry, adjusted for age, sex, and BMI.


p=0.015
12:00
10:00
08:00
06:00
Dim Light Melatonin Offset, Clock Time
N=42
N=46
N=7
CC
CG
GG
rs10830963 genotype
p=0.032
12
10
8
Duration of Elevated Melatonin Secretion, hours
N=42
N=45
N=7
CC
CG
GG
rs10830963 genotype


A.
B.
C.
Probability of Type 2 Diabetes
0.40
0.30
0.20
0.10
0.00
p_{int}=0.053
20:00
22:00
24:00
02:00
Weekday Bedtime
p_{int}=0.018
24:00
02:00
04:00
06:00
Weekday Sleep Midpoint
p_{int}=0.029
N=1,513
04:00
06:00
08:00
10:00
Weekday Wake Time
MTNR1B
rs10830963
C/C
C/G
G/G