Shared Genetic Control of Brain Activity During Sleep and Insulin Secretion: A Laboratory-Based Family Study

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Diabetes 2018;67:155–164 | https://doi.org/10.2337/db16-1229

Over the past 20 years, a large body of experimental and epidemiologic evidence has linked sleep duration and quality to glucose homeostasis, although the mechanistic pathways remain unclear. The aim of the current study was to determine whether genetic variation influencing both sleep and glucose regulation could underlie their functional relationship. We hypothesized that the genetic regulation of electroencephalographic (EEG) activity during non–rapid eye movement sleep, a highly heritable trait with fingerprint reproducibility, is correlated with the genetic control of metabolic traits including insulin sensitivity and β-cell function. We tested our hypotheses through univariate and bivariate heritability analyses in a three-generation pedigree with in-depth phenotyping of both sleep EEG and metabolic traits in 48 family members. Our analyses accounted for age, sex, adiposity, and the use of psychoactive medications. In univariate analyses, we found significant heritability for measures of fasting insulin sensitivity and β-cell function, for time spent in slow-wave sleep, and for EEG spectral power in the delta, theta, and sigma ranges. Bivariate heritability analyses provided the first evidence for a shared genetic control of brain activity during deep sleep and fasting insulin secretion rate.

Over the past 20 years, epidemiologic as well as laboratory evidence has linked sleep duration and sleep quality to the regulation of glucose homeostasis, although the mechanisms mediating this relationship remain unclear. Longitudinal epidemiologic studies have found an increased risk of type 2 diabetes with insufficient sleep and poor sleep quality, with an effect size similar to that of traditional risk factors (1–3). Furthermore, laboratory studies in healthy volunteers have consistently reported a decrease in insulin sensitivity without adequate compensatory increase in β-cell response after short-term experimental sleep restriction (1,4,5). Slow-wave activity (SWA) in non–rapid eye movement (NREM) sleep, also known as delta activity, is a marker of the depth or intensity of NREM sleep that may be quantified by spectral power of the electroencephalogram (EEG) in the low-frequency range typical of slow waves (0.75 to 4.5 Hz) (6,7). SWA is highly reproducible from night to night in a given individual (8–10) and is highly heritable (11,12). Experimental suppression of SWA, either by selective deprivation of slow-wave sleep (SWS) or by sleep fragmentation across all stages of sleep, has been shown to reduce insulin sensitivity without significantly increasing insulin secretion (13,14). Additionally, epidemiologic and clinical studies have demonstrated that obstructive sleep apnea, a sleep disorder characterized by poor sleep quality generally associated with low levels of SWA (15–17), is a risk factor for insulin resistance and type 2 diabetes, independent of BMI. Given this body of evidence, we hypothesized that common genetic pathways may be involved in the regulation of EEG activity during sleep, especially during deep NREM sleep, and in the regulation of glucose metabolism.

Type 2 diabetes, a multifactorial polygenic disease estimated to affect nearly 400 million people worldwide (18), is characterized by both reduced insulin sensitivity and inadequate compensatory insulin secretion by the pancreatic β-cells (19). Several studies have documented the heritability of parameters controlling glucose homeostasis (20–24), suggesting that the current diabetes epidemic results from

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Received 9 October 2016 and accepted 24 October 2017.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db16-1229/-/DC1.

both environmental and genetic factors. Genome-wide association studies have identified about 90 single nucleotide polymorphisms that increase the risk of type 2 diabetes; however, in keeping with a highly polygenic architecture, these risk alleles explain only about 10% of the heritability of type 2 diabetes (25). Recently, several genes involved in the circadian control of sleep regulation, such as melatonin receptor 1b (MTNR1b) (26), period circadian clock 3 (PER3) (27), and cryptochrome circadian clock 2 (CRY2) (28), have also been associated with increased diabetes risk, suggesting pleiotropy between genetic pathways underlying sleep regulation and glucose metabolism. Further, within the past year, three large-scale genome-wide studies demonstrated a genetic correlation between self-reported sleep characteristics and risk for type 2 diabetes (29–31). In each study, self-administered questionnaires were used to assess subjective sleep duration, quality, and/or timing in large samples ($N > 10{,}000$). As demonstrated by the findings of these studies, phenotypic assessments of sleep traits by self-report are powerful because they facilitate the investigation of very large samples required for the discovery of individual genetic variants with small effects. By the same token, survey tools inherently lack phenotypic precision thereby limiting the biologic insight of the research. In particular, subjective assessments of sleep quality correlate minimally, if at all, with objective EEG-based measurements. Quantitative phenotypes, such as blood glucose or insulin levels in response to a challenge or brain EEG activity during sleep measured under controlled laboratory settings, are obviously impracticable to collect on large samples. To overcome this limitation, we used a large three-generation family to examine the joint heritability of quantitative metabolic and sleep traits rigorously measured under laboratory conditions. We hypothesized that the genetic regulation of sleep EEG activity, and especially SWA, may be correlated with the genetic regulation contributing to the variance of metabolic traits including insulin sensitivity and pancreatic β-cell function. The findings suggest that the large body of epidemiologic and experimental evidence linking sleep disturbances and increased risk of diabetes may partly reflect pleiotropy in the genetic control of sleep and glucose metabolism.

RESEARCH DESIGN AND METHODS

Subjects

One hundred twenty-three members of a large pedigree comprising over 180 members spanning three generations were invited to participate in the study. Family members under 18 years of age were excluded. All enrolled subjects provided written informed consent and the study was conducted in accordance with the Declaration of Helsinki. The family members were all of self-reported European descent. Forty-eight subjects completed the study. The pedigree is shown in Fig. 1.

Study Protocol

The protocol involved two consecutive nights of polysomnographic recording and an oral glucose tolerance test (OGTT). Recordings were performed in the research laboratory of the Sleep, Metabolism and Health Center of The University of Chicago, except for eight subjects who were studied in an out-of-campus location (at their home or at a hotel) using ambulatory equipment. For 1 week prior to the recording session, participants were asked to maintain a standardized schedule of bedtimes, designed in accordance with their self-reported usual sleep-wake habits. They were instructed not to deviate from this schedule by more than 30 min and to wear a wrist activity monitor (Actiwatch; Philips Respironics, Murrysville, PA) at all times. On the morning following the second night of sleep recording, after a 12-h fast, a blood sample was collected for blood chemistry and hemoglobin A_{1c} (HbA_{1c}) and a 3-h OGTT was performed. In two subjects, the OGTT took place after the first night of sleep recording for logistic reasons. On the evening prior to metabolic testing, a standardized dinner was served at 1930, with 50% of the calories derived from carbohydrates. For each subject, the total calorie content of the standardized dinner (600, 700, 800, or 1,000 kcal) approximated one-third of the subject's calculated total energy expenditure, which was estimated using the Schofield equation (32), based on sex, age, and weight. During the OGTT, blood was collected at −15, 0, 30, 60, 90, 120, 150, and 180 min after ingestion of 75 g of a glucose solution. Results of the −15 and 0 time points were averaged to obtain a baseline value.

Sleep Analysis

Sleep recordings were performed using a digital EEG acquisition system (Neurofax EEG-1100 A; Nihon Kohden, Tokyo, Japan). Surface electrodes were used to record EEG signals (two central referential EEG leads [C3-A2 and C4-A1] and two occipital referential leads [O1-A2 and O2-A1]), bilateral electrooculogram, and submental electromyogram. The presence or absence of obstructive sleep apnea (OSA) was evaluated by measuring oronasal airflow signal by thermal flow sensor and nasal pressure transducer, respiratory effort signal by thoracic and abdominal piezoelectric belts, and arterial oxygen saturation by pulse oximetry. Assessment of OSA was conducted during the first night of recording, except for the two subjects who had an OGTT in the morning after the first night, who underwent recording of the respiratory variables during the second night. Polysomnographic recordings were visually scored at 30-s intervals in stages wake, 1 (N1), N2, N3 (SWS), and REM using standardized criteria (33) by two experienced scorers. Respiratory events, periodic limb movements, and microarousals were scored according to established criteria (33). The presence of OSA was defined by an apnea-hypopnea index (AHI) ≥5 events per hour of sleep.

Spectral analysis of the EEG recordings was performed on the central EEG leads (C3, C4) using the PRANA software (PhiTools, Strasbourg, France) as previously described (13). During acquisition, EEG signals were filtered (0.3–35 Hz) and sampled at 200 Hz with a 16-bit resolution. After removal of muscular, ocular, and movement artifacts by automated detection followed by visual inspection, a fast-Fourier

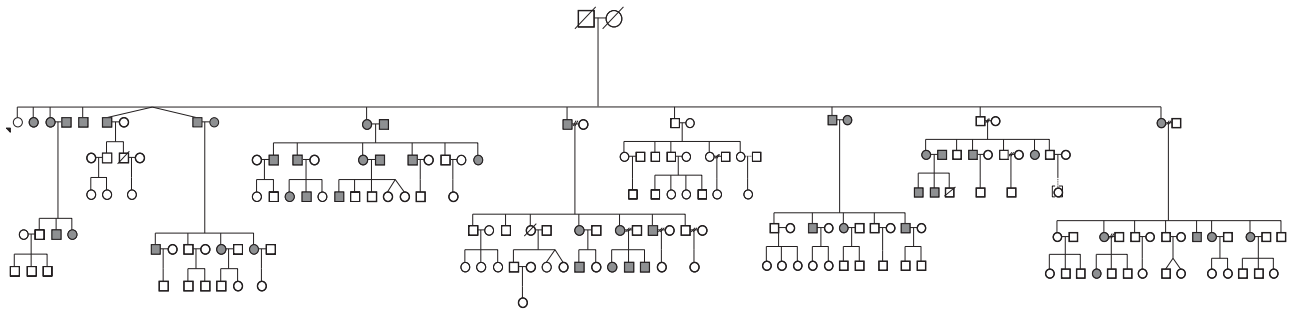

Figure 1—Pedigree. The shaded symbols represent the family members who completed the study. Squares represent men, circles represent women. The index subject is indicated with an arrow. The crossed-out symbols indicate deceased family members.

transform was computed on the EEG signal by using a Hanning window on consecutive 4-s intervals, resulting in a frequency resolution of 0.25 Hz. Power spectra of 15 consecutive 2-s intervals were averaged and matched with sleep scores. Delta (SWA), theta, alpha, and sigma activities were calculated as the absolute spectral power in the frequency bands 0.75–4.5 Hz, 4.5–8.5 Hz, 8.5–12 Hz, and 12.5–15 Hz, respectively. Average spectral power for each frequency was calculated in NREM sleep during the first 6 h of sleep using the second night of recordings. In four subjects, average spectral power was calculated from the first night of recording due to technical artifacts in the EEG signal from the second night of recording.

Metabolic Assays

Glucose, insulin, and C-peptide levels were assayed on all samples collected during the OGTT. HbA_{1c} was measured on a sample collected under fasting conditions. Plasma glucose levels were assayed by the glucose oxidase method; serum insulin and C-peptide levels were assayed by chemiluminescence assays using the Immulite 2000 System (Siemens Healthcare Diagnostics, Tarrytown, NY).

Metabolic Phenotypes

A number of metabolic phenotypes were derived from OGTT data. Fasting insulin resistance was estimated using the HOMA-IR ([fasting glucose in mg/dL × fasting insulin in μU/mL]/405) (34), and fasting insulin sensitivity was estimated by the QUICKI (1/[Ln(glucose in mg/dL) + Ln(insulin in μU/mL)]) (35). Insulin sensitivity during OGTT was estimated by the Matsuda index (10,000/√[fasting glucose in mg/dL × fasting insulin in μU/mL × mean glucose in mg/dL × mean insulin in μU/mL]) (36). Fasting β-cell function was estimated as the fasting HOMA-β

Table 1—Heritability of metabolic phenotypes

				Fixed-effect covariates		
				P value		
	n	h^2 (SE)	LRT *P* value	Age	Sex	OSA
HbA_{1c}	46	0.35 (0.33)	0.146	0.0114	ns	ns
Fasting values						
Glucose	46	0	0.5	ns	ns	ns
Insulin	48	0.60 (0.25)+	**0.015**	ns	ns	ns
C-peptide	48	0.90 (0.15)+	**0.0001**§	0.0004	ns	ns
ISR	42	0.79 (0.33)+	**0.02**	ns	ns	ns
HOMA-IR	48	0.67 (0.26)	**0.0125**	0.007	ns	ns
HOMA-β	48	0.30 (0.30)	0.165	ns	ns	ns
QUICKI	48	0.67 (0.26)	**0.013**	0.040	ns	ns
Postchallenge values						
Matsuda index	48	0	0.5	ns	ns	ns
Insulinogenic index	46	0	0.5	0.041	ns	ns
AUC glucose	46	0	0.5	0.0003		
AUC insulin	46	0	0.5	ns	ns	ns
IncrAUC glucose	46	0	0.5	0.0002	ns	ns
IncrAUC insulin	46	0	0.5	ns	ns	ns
IncrAUC insulin/IncrAUC glucose	46	0	0.5	0.038	ns	ns

SEs are shown in parentheses alongside all heritability estimates greater than zero. LRT *P* value: *P* values from LRT indicating whether the heritability estimated is significantly different from zero. Fixed-effect covariates *P* value: reported *P* values from LRT indicating whether the variable significantly impacted the heritability model. Age and sex were retained in the model regardless of their LRT *P* value, while BMI and OSA were dropped if $P > 0.1$ (reported in table as not significant [ns]) to result in a parsimonious model. Statistically significant data appear in boldface type. AUC, area under the curve; IncrAUC, incremental area under the curve. §*P* values exceeding the Bonferroni corrected threshold for significance of $P = 0.004$ (corrected for the number of traits analyzed). +BMI was a significant covariate and estimate was adjusted for BMI.

([360 – fasting insulin in μU/mL]/[fasting glucose in mg/dL – 63]) (34). The β-cell response to the oral glucose challenge was estimated by the insulinogenic index ($\Delta insulin_{0–30min}$ in pmol/L/$\Delta glucose_{0–30min}$ in mmol/L) (37) as well as the ratio of incremental area under the curve of insulin to that of glucose. Insulin secretion rates (ISRs) were derived from C-peptide concentrations by mathematical deconvolution using parameters individually adjusted for sex, age, and body surface area (38).

Variance-Component Model Assuming a Pedigree

The pedigree-based variance-component method assumes the following linear model:

$$y = \mu + Z\beta + \varepsilon$$

where y is the phenotype vector, μ is the baseline mean, Z is a matrix of observed additive factors, β is the vector of effect sizes, and ε is the residual vector. The model can include a sum of covariate effects. The residual vector is assumed to be Gaussian:

$$\varepsilon \sim N(0, 2\Phi\sigma_a^2 + \gamma\sigma_c^2 + \sigma_e^2)$$

centered at 0 and with covariance matrix that is a function of the kinship matrix Φ (wherein each entry shows the expected proportion of the genome shared between a pair of subjects), σ_a^2 is the additive genetic variance, γ is the matrix showing shared environment (between a pair of subjects), σ_c^2 is the variance attributable to the shared environment and σ_e^2 is the residual environmental variance. The univariate heritability is then defined as:

$$h^2 = \sigma_a^2 \Big/ \sigma_a^2 + \sigma_c^2 + \sigma_e^2$$

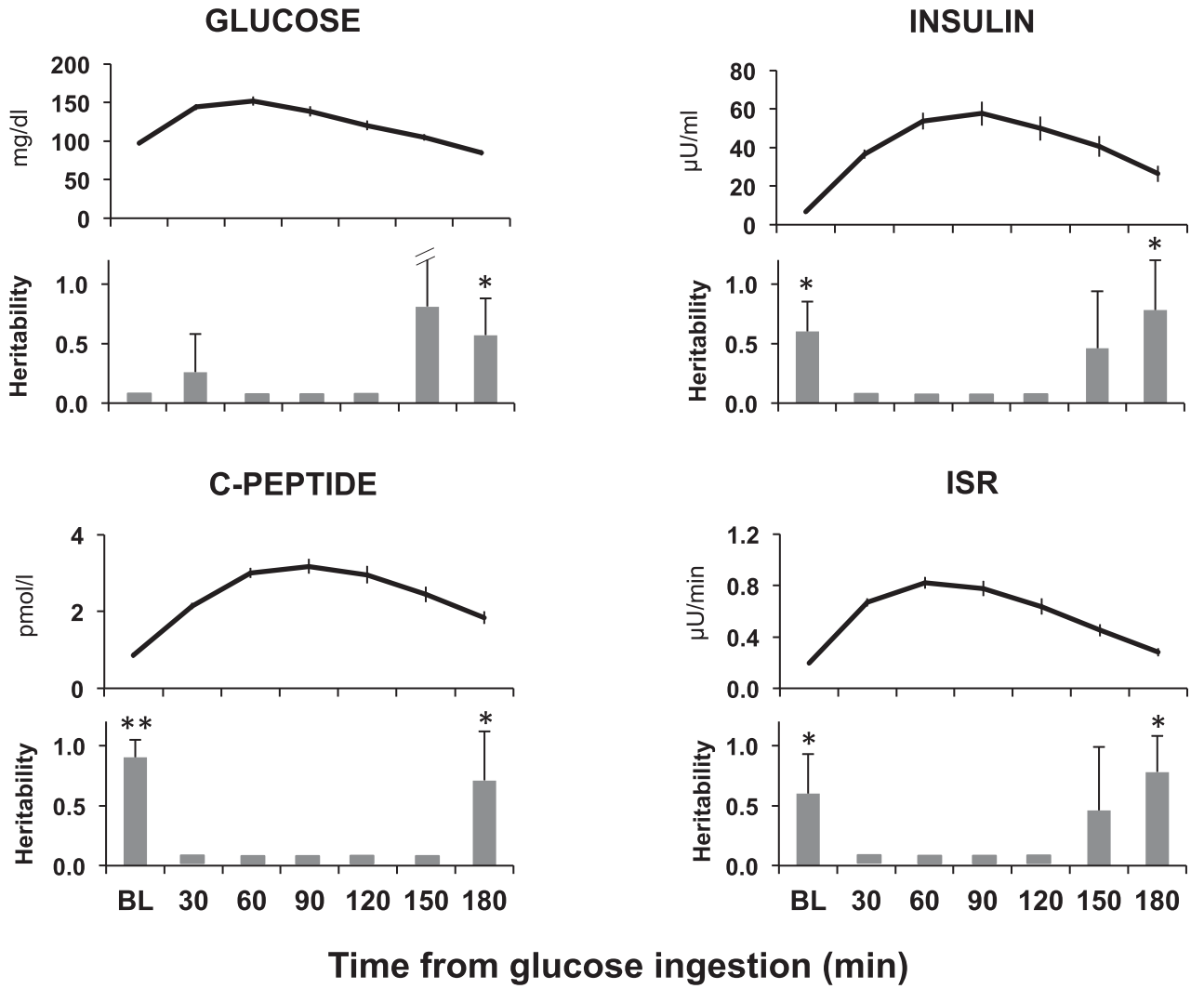

Figure 2—For glucose, insulin, C-peptide, and ISR, the upper panel illustrates the mean (± SEM) concentrations measured at each time point of the OGTT. Gray vertical bars in the lower panels show heritability for each time point. Fixed covariates included age and sex. BMI was included in the model if it significantly ($P < 0.1$) impacted the results based on a LRT comparing the models with and without BMI. Error bars are shown for all heritability estimates greater than zero. BL, baseline. *$P < 0.05$; **$P < 0.01$.

A likelihood ratio test (LRT), comparing the likelihood of a full model with that of a nested model (without the additive genetic component), is applied to test for a nonzero additive genetic variance.

Univariate Heritability Analysis

Heritability (h^2) estimates were obtained from a variance components model implemented in the Sequential Oligogenic Linkage Analysis Routine (SOLARv4.1.5) software package. Univariate heritability estimates indicate the proportion of trait variance explained by genetic variance estimated from familial relationships under an additive genetic model after accounting for important covariates. Both quantitative sleep and metabolic traits were inverse-normalized when the variable demonstrated high kurtosis. We used the SOLAR covariate screening procedure in which the effect of each covariate on the additive model was evaluated using a LRT. For all analyses, covariates age and sex were retained regardless of whether they significantly affected the model. Screened covariates included BMI and presence of OSA (yes/no) for heritability of metabolism analyses and BMI, presence of OSA (yes/no), and psychotropic medication for heritability of quantitative sleep trait analyses. These covariates were retained if they modestly impacted the model (LRT $P < 0.1$) and otherwise were dropped to create a more parsimonious model (Table 1).

Bivariate Analysis and Calculation of Standard Error

To test the hypothesis that quantitative sleep and metabolic traits share additive genetic risk, we performed pairwise bivariate heritability analyses between metabolic and sleep variables that showed significant univariate heritability. We tested fasting insulin, fasting C-peptide, ISR, and HOMA-IR against delta, theta, and sigma power, while retaining covariates that were included in either or both univariate analyses.

The bivariate analysis of two traits requires estimation of the corresponding variances σ_a^2, σ_c^2, and σ_e^2 for each trait (as defined above) but also the correlations from the corresponding components. In particular, we are interested in an estimate of the correlation r_a, which provides a measure of shared additive genetic influences on the two phenotypes, P_1 and P_2, under comparison and which is provided by SOLAR. Using a Taylor series expansion (39) for the sampling variance $\hat{r_a}$, the standard error (SE) of the estimated genetic correlation is approximately:

Table 2—Heritability of sleep phenotypes

				Fixed-effects covariates			
				P value			
	n	h^2 (SE)	LRT *P* value	Age	Sex	OSA (yes/no)	Medication
Total sleep time	46	0	0.5	0.0001	0.008	ns	ns
Sleep efficiency (%)	46	0.32 (0.40)	0.186	0.002	0.025	ns	ns
Sleep latency (min)	46	0.41 (0.25)	*0.054*	ns	ns	ns	ns
Total wake (min)	46	0.22 (0.57)	0.444	ns	ns	ns	ns
Total wake (%)	46	0.24 (0.58)	0.418	0.073	ns	ns	ns
WASO (min)	45	0	0.5	0.033	ns	ns	ns
AHI (events/h)	45	0	0.5	0.005	ns	–	ns
N1 (min)	46	0	0.5	ns	ns	ns	ns
N1 (%)	46	0	0.5	ns	ns	ns	ns
N2 (min)	46	0	0.5	ns	0.032	ns	ns
N2 (%)	46	0	0.5	0.006	ns	ns	0.026
SWS min	46	0.43 (0.26)	*0.058*	<0.0001	ns	ns	ns
SWS %	46	0.49 (0.24)	**0.030**	0.0007	ns	ns	ns
REM min	46	0.65 (0.44)	*0.052*	ns	ns	ns	ns
REM %	46	0.44 (0.30)	*0.069*	ns	ns	ns	ns
Delta power (μV^2)	48	0.50 (0.22)	**0.011**	0.0001	0.017	ns	ns
Theta power (μV^2)	48	0.93 (0.15)	**0.0001**§	ns	0.0004	ns	0.085
Alpha power (μV^2)	48	0.51 (0.41)	0.102	ns	0.027	ns	ns
Sigma power (μV^2)	48	0.71 (0.26)	**0.023**	ns	0.004	ns	ns

SEs are shown for all heritability estimates greater than zero. LRT *P* value: *P* values from LRT indicating whether the heritability estimated is significantly different from zero. Fixed-effects covariates *P* value: reported *P* values from LRT indicating whether the variable significantly impacted the heritability model. Age and sex were retained in the model regardless of their LRT *P* value, while OSA, BMI, and medication were dropped if $P > 0.1$ (reported in table as not significant [ns]) to result in a parsimonious model. Sleep stages are expressed as minutes as well as percent of total sleep time. Significantly different data appear in boldface type. Trends for statistical significance appear in italics. WASO, wake after sleep onset. §*P* values exceeding the Bonferroni corrected threshold for significance of $P = 0.003$ (corrected for the number of traits analyzed).

$$SE(\widehat{r_a}) \approx \sqrt{\frac{(1 - r_a r_P)^2 + (r_a - r_P)^2}{h^2_{P_1} h^2_{P_2} N^2 var(\Phi_{ij})}}$$

where r_P is the phenotypic correlation, $h^2_{P_1}$ and $h^2_{P_2}$ are the heritability estimates from the univariate analysis for the two phenotypes P_1 and P_2 respectively, N is the study sample size, and Φ_{ij} is the kinship coefficient.

RESULTS

The demographic characteristics of the 48 participants are given in Supplementary Table 1. Mean age (± SEM) was 45 ± 2 years. Participants were mostly overweight or obese and had comorbidities, including hypertension, dyslipidemia, coronary heart disease, and psychoaffective disorders. Nearly half of the participants had OSA. Five participants had type 2 diabetes, three of which were newly diagnosed during the current study.

Univariate and Bivariate Heritability of Sleep and Metabolic Phenotypes

We first calculated the heritability of height (h^2 = 0.74, SE = 0.27, P = 0.008) and BMI (h^2 = 0.81, SE = 0.42, P = 0.01) in our family sample, which yielded estimates within the expected previously reported range, indicating that the study was powered to examine highly heritable traits. We then determined the heritability of metabolic traits (Table 1). Figure 2 illustrates the heritability estimates of glucose, insulin, C-peptide, and ISR at each time point of the OGTT. For glucose, only the value at +180 min had significant heritability (h^2 = 0.57, SE = 0.31, P = 0.046). In contrast, the variables characterizing insulin secretion demonstrated significant heritability at baseline under fasting conditions and at 180 min when fasting conditions had nearly resumed. However, no appreciable heritability was detected at time points 30, 60, 90, and 120 min of the challenge test. Thus, insulin, C-peptide, and ISR demonstrated significant heritability, ranging between 0.60–0.90 at baseline and between 0.65–0.81 at 180 min. Additionally, HOMA-insulin resistance (IR), a marker of fasting insulin resistance, and QUICKI, a measure of fasting insulin sensitivity, were significantly heritable (Table 1). Heritability estimates were identical for HOMA-IR and QUICKI, as expected as these two indices are arithmetically related. Overall glycemic control, as assessed by HbA_{1c} and a surrogate measure of β-cell function during the fasting state (HOMA-β), did not show evidence of significant heritability (Table 1). Consistent with the lack of significant heritability of insulin, C-peptide, and ISR levels postglucose challenge, indices quantifying insulin release (insulinogenic index) and action (Matsuda Index) showed no evidence of heritability.

Next, we determined the univariate heritability for quantitative sleep traits (Table 2 and Fig. 3). We found significant heritability for the proportion of total sleep time spent in SWS (SWS %, h^2 = 0.49, SE = 0.24, P = 0.03), consistent with the findings of twin studies (40). Although heritability of SWS duration (SWS min, h^2 = 0.43, SE = 0.26, P = 0.058),

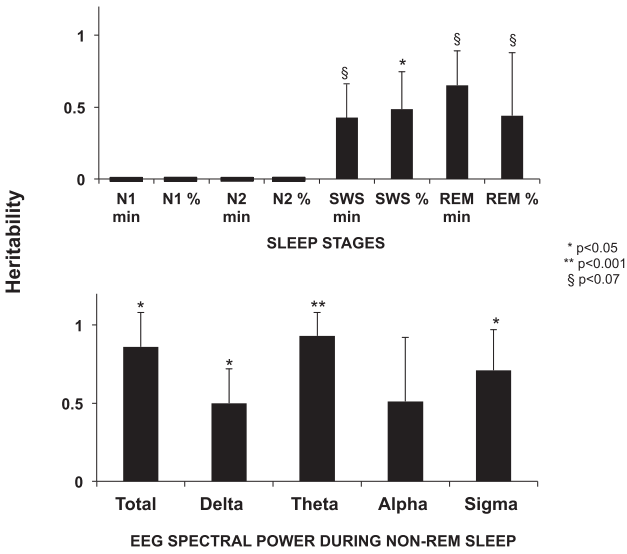

Figure 3—Heritability estimated for sleep variables (upper panel) and spectral analysis results (bottom panel). Sleep stages (N1, N2, SWS, and REM) are expressed as percentage of total sleep time. NREM total, delta, theta, alpha, and sigma power were computed in the first 6 h of sleep. Fixed covariates included age and sex. Additional covariates including BMI and psychotropic medications were included in the model if they significantly ($P < 0.1$) impacted the results based on a LRT comparing the models with and without these covariates. Error bars are shown for all estimates greater than zero.

duration of REM sleep (REM min, h^2 = 0.65, SE = 0.44, P = 0.052), and proportion of total sleep time spent in REM sleep (REM %, h^2 = 0.44, SE = 0.30, P = 0.069) failed to reach statistical significance at the $P < 0.05$ level, the point estimates were substantial. Additionally, we found significant heritability for variables obtained by spectral analysis of the EEG, including SWA or delta power (h^2 = 0.50, SE = 0.22, P = 0.011), theta power (h^2 = 0.93, SE = 0.15, P = 0.0001), and sigma power (h^2 = 0.71, SE = 0.26, P = 0.023). Heritability estimate for alpha power failed to reach significance (h^2 = 0.51, SE = 0.41, P = 0.102). None of the respiratory variables obtained from polysomnography (e.g., AHI, degree of oxygen desaturation) demonstrated significant heritability.

Only fasting C-peptide and theta power demonstrated univariate heritability estimates that exceeded a strict Bonferroni correction for the number of phenotypes tested. However, we note that many of the phenotypes (e.g., fasting HOMA-IR and insulin) are highly correlated (Supplementary Table 3), and therefore a strict Bonferroni correction is likely to be overly conservative. Nevertheless, our results should be interpreted with caution until replicated.

In bivariate analyses, we identified a significant genetic correlation between baseline ISR and delta power (rhoG = 1.0, SE = 0.46, P = 0.0062) in the first 6 h of sleep (Supplementary Table 2 and Fig. 4). Due to sample size limitations, SEs were sizable for the majority of the genetic correlations reported here, for example, the SE of the

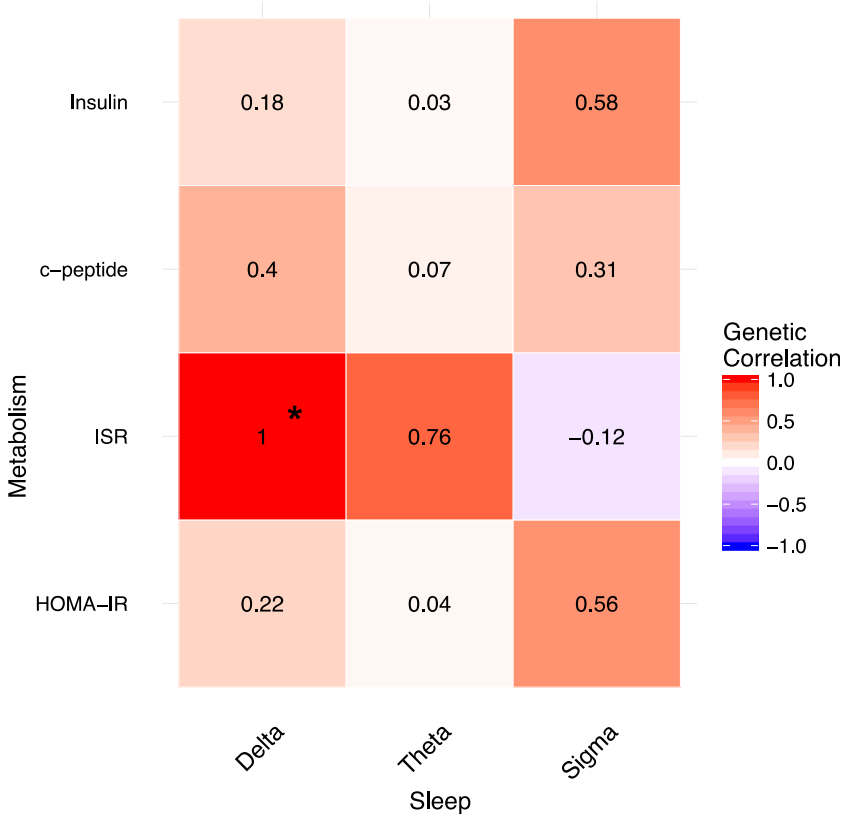

Figure 4—A heat map illustrating the genetic correlations calculated with SOLAR using the pedigree relationships. Blue indicates negative correlations and red indicates positive correlations. Although the point estimates are large for several trait pairs due to the small sample size, the SE are also sizeable and the only correlation reaching significance is delta power/ISR (rhoG = 1, SE = 0.46, $P = 0.0062$), indicated with an asterisk.

correlation between baseline ISR and delta power was 0.46, suggesting that the true point estimate of the genetic correlation falls between 0.54 and 1.0. None of the estimates of environmental correlation (rhoE) reached statistical significance ($P < 0.05$).

DISCUSSION

The current study combined in-depth concurrent phenotyping of sleep and metabolic traits using gold standard assessments in a large family of self-reported European descent to explore the novel hypothesis that common genetic pathways may underlie brain EEG activity during NREM sleep and glucose metabolism. The hypothesis of a pleiotropic control of sleep and glucose metabolism was suggested by a large body of evidence linking sleep disturbances and diabetes risk that has accumulated over the past 20 years. Our finding of a significant joint heritability between EEG spectral power in the delta range (i.e., SWA) and a measure of insulin secretion supports our hypothesis.

In addition to replicating significant heritability for metabolic traits (5,20–24), we provide novel family-based estimates of heritability for several quantitative sleep traits that are consistent with estimates from published twin studies (11,40). Our univariate heritability results are consistent with previous studies that focused on either diabetes risk or sleep traits. We indeed confirm and extend findings from previous heritability studies of insulin sensitivity (HOMA-IR and QUICKI) (23,24,41,42) and pancreatic β-cell function (fasting insulin and ISR) (20,22–24,43–45). Interestingly, heritability measures of insulin, HOMA-IR, and ISR became nonsignificant at intermediate time points of the OGTT and regained significance at 180 min. The loss and recovery of insulin trait heritability in our study was most likely due to changing phenotypic variance across the time points. For example, upon glucose challenge, variance in the insulin measures (shown as the SEM at each time point of the upper panels of Fig. 2) increased then decreased as glucose was metabolized and insulin levels returned to baseline. However, because genetic variance is stable, the resulting ratio of genetic to phenotypic variance (i.e., heritability) decreased during intermediate time points. The failure to detect significant heritability at intermediate time points of the OGTT could also relate to the limited sample size of our study. Although the literature on stability of the insulin heritability estimate across OGTT time points is sparse, a few studies with sample sizes in the hundreds have indeed reported significant heritability for OGTT measures at intermediate time points (22,23,46).

Although several genome-wide association studies of sleep traits have been published, few investigators have examined

the heritability of quantitative EEG variables derived from spectral analysis, and those that have exclusively examined twins. In these studies, significant genetic influence was reported for SWS (11,40) and REM sleep duration (11), as well as for the spectral composition of NREM sleep (11). Our results, which accounted for age, sex, BMI, and the use of psychoactive medications, show significance for the heritability of percent time spent in SWS and a strong trend for the heritability of the absolute duration of SWS and REM sleep, as well as for percent time spent in REM sleep. Consistent with existing evidence (11,40), we found no heritability for total wake time. Additionally, we found significant heritability for delta, theta, and sigma activities. It is noteworthy that during a normal night of sleep, the frequency of EEG slow waves overlaps, to some extent, the delta and theta frequency ranges and that sigma activity is in a strong inverse relationship with delta activity. Thus, our findings of high heritability estimates of delta, theta, and sigma activities support the hypothesis that the depth or intensity of NREM sleep, as reflected in the abundance and amplitude of EEG slow waves, is under genetic control.

Delta power is a stable individual trait that is highly heritable (10,12). This well-documented notion, taken together with our experimental observation that selective suppression of delta power causes a decrease in insulin sensitivity with no compensatory increase in insulin secretion, resulting in increased diabetes risk (13), led us to consider the possibility that the genetic regulation of these basic physiologic functions—sleep and glucose regulation—may

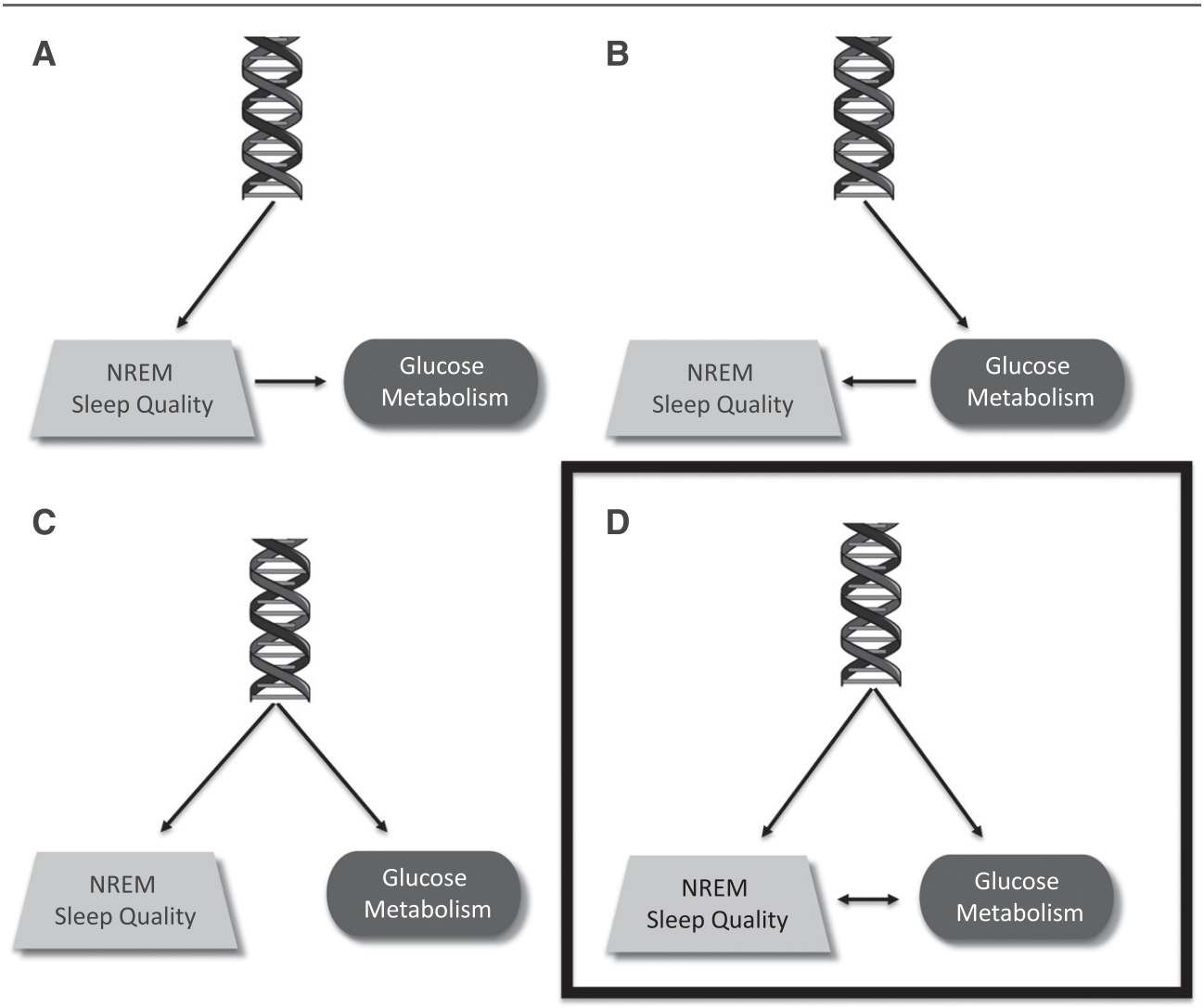

Figure 5—Four possible causal models underlying the observed relationship between glucose metabolism and NREM sleep quality. The panels illustrate the following models in which genetic effects govern NREM sleep quality that in turn influences glucose metabolism (*A*), genetic effects govern glucose metabolism which in turn influences NREM sleep quality (*B*), NREM sleep quality and glucose metabolism are coregulated through shared genetic effects (*C*), and NREM sleep quality and glucose metabolism are coregulated through shared genetic effects and influence each other (*D*). Our genetic correlation results rule out the models shown in panels *A* and *B*. Taken together with experimental findings, our data suggests the most likely model underlying the relationship between NREM sleep and glucose metabolism is shown in panel *D*.

be shared and that, in nonlaboratory settings, this shared biology contributes to the observed phenotypic and genetic correlation between poor sleep and risk for type 2 diabetes. The significant bivariate genetic correlation between a measure of β-cell function and delta activity (Fig. 4) detected in the current study is inconsistent with a strict causal model in which variance in one set of traits (sleep or metabolism) results in variance in the other set of traits (Fig. 5). Instead, our results (Fig. 4) suggest a model of joint heritability in which both insulin secretion and SWA are influenced by a partially overlapping set of genes. It is also likely that sleep quality and glucose metabolism influence each other (Fig. 5).

In conclusion, our analysis of highly accurate heritable phenotypes in a large three-generation family of European descent has provided novel evidence for pleiotropy in the genetic control of objectively assessed sleep traits and glucose homeostasis. Although the findings require replication and extension, they identify a putative mechanistic pathway linking sleep disturbances and the risk of type 2 diabetes, with important potential clinical implications. In particular, as sleep quality and β-cell function both deteriorate in the course of aging, our findings of a robust joint heritability of fasting ISR and intensity of deep NREM sleep suggest that a common genetic pathway may underlie the chronology of metabolic and sleep senescence.

Acknowledgments. The authors gratefully acknowledge the efforts made by all members of the family who participated in this study. The authors also acknowledge the invaluable help of the nursing staff of the General Clinical Research Center of The University of Chicago.

Funding. This work was partially funded by the National Institutes of Health (P01 AG-11412).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. L.L.M., E.R.G., E.T., E.V.C., and L.K.D. analyzed the data. L.L.M. and E.T. collected the data. L.L.M. and L.K.D. wrote the manuscript. E.T., N.J.C., E.V.C., and L.K.D. were responsible for the study design. E.V.C. identified the pedigree and obtained funding. All authors reviewed the draft prior to submission. L.K.D. 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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