Insomnia does not mediate or modify the association between *MTNR1B* risk variant rs10830963 and glucose levels

Hanna M Ollila^{1,2,3,4} · Erkki Kronholm⁵ · Johannes Kettunen^{1,6} · Kaisa Silander¹ · Markus Perola¹ · Tarja Porkka-Heiskanen² · Veikko Salomaa⁷ · Tiina Paunio^{1,3}

Received: 6 October 2015 / Accepted: 27 January 2016 / Published online: 24 February 2016
© Springer-Verlag Berlin Heidelberg 2016

Keywords Blood glucose · Genetic interaction · GxE · Insomnia · *MTNR1B* · Sleep · SNP · Type 2 diabetes

Abbreviations

DILGOM Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome
SNP Single nucleotide polymorphism

To the Editor: Short sleep and insomnia associate strongly with blood glucose levels and type-2 diabetes [1] and one of the possible mechanisms is that the same genetic variants regulate sleep and glucose metabolism. The single nucleotide polymorphism (SNP) rs10830963 in *MTNR1B* has been

associated with type 2 diabetes and blood glucose levels [2]. Traditionally, melatonin is thought to be a ‘dark hormone’ as its expression is controlled by light from the retina to the pineal gland. In the elderly, melatonin can be used to treat insomnia and improve sleep quality [3].

We tested whether rs10830963 associates with: (1) self-reported insomnia; or (2) short sleep. Finally, we studied whether sleep modifies the association of rs10830963 with blood glucose levels.

The association was examined in the Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) study [4], in which fasting blood glucose levels (10 h fasting, verified by interview), and self-reported insomnia and sleep duration were available. Blood glucose levels were measured from serum using the hexokinase method. Questionnaire-based sleep duration was assessed on the same day, and blood samples were taken, whereas insomnia was assessed with a questionnaire sent previously or, at the latest, the same day. RNA expression and genotyping were performed in 512 individuals from the Helsinki and Vantaa areas of southern Finland. The Health 2000 study (www.terveys2000.fi/doc/methodologyrep.pdf; accessed 1 December 2012) was used for replication.

The ethics committee of the Helsinki and Uusimaa Hospital District approved the study protocols. All participants provided written informed consent for the collection of samples and subsequent analysis.

Self-reported insomnia was queried in DILGOM with the question: ‘Do you suffer from insomnia?’ with answering options ‘often’, ‘sometimes’ and ‘never’. Self-reported insomnia in Health 2000 was queried with the question: ‘Have you had symptoms of insomnia or sleep disturbances during the past month?’ with answering options ranging from 1 = not at all to 5 = very much. Insomnia phenotypes were dichotomised comparing the ‘any insomnia symptoms’ group to those

✉ Tiina Paunio
Tiina.Paunio@thl.fi

¹ Department of Health, National Institute for Health and Welfare, Biomedicum, P.O. Box 104, 00251 Helsinki, Finland

² Institute of Biomedicine, Department of Physiology, University of Helsinki, Helsinki, Finland

³ Department of Psychiatry, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

⁴ Stanford University, Center for Sleep Sciences, Palo Alto, CA, USA

⁵ Department of Chronic Disease Prevention, Population Studies Unit, National Institute for Health and Welfare, Turku, Finland

⁶ NMR Metabolomics Laboratory, School of Pharmacy, University of Eastern Finland, Kuopio, Finland and Biocenter Oulu, University of Oulu, Finland and Computational Medicine, Center for Life Course Health Research, University of Oulu, Oulu, Finland

⁷ Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland

without symptoms. Sleep duration was evaluated with the question: ‘How many hours do you sleep per day?’. Use of depression medication and hypnotics during the past month was assessed with questionnaires.

DILGOM samples were genotyped using Cardio-MetaboChip (Illumina, San Diego, CA, USA). Individuals in the Health 2000 cohort were genotyped with Illumina 610K (Illumina) or by iPLEX Sequenom MassArray (Sequenom, San Diego, CA, USA) in the Health 2000 sample. The call rate was over 95% and Hardy–Weinberg equilibrium $p > 0.05$.

Glucose levels were normalised using inverse-normal transformation of ranks. Individuals deviating 3 SD or more from the mean were removed. An additive model was used adjusting for age, sex and BMI, and in RNA expression with time of blood sample. The gene–environment interaction model $Y = b_0 + b_1.ADD + b_2.COV1 + b_3.COV2 + b_4.ADD \times COV1 + b_5.ADD \times COV2 + e$ was conducted using PLINK v1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink/>) [5].

The association between rs10830963 and fasting blood glucose was confirmed at genome-wide significant level (Table 1). Nominally significant associations were observed with sleep traits and with interaction of self-reported insomnia on rs10830963 association with blood glucose levels (Table 1). The association with blood glucose levels was

replicated in Health 2000, whereas association with sleep traits or interaction of rs10830963 and self-reported insomnia on glucose levels was not (Table 1). No association between *MTNR1B* expression and rs10830963 was seen either in DILGOM ($p = 0.31$ $\beta = 0.0068$) or in the GTEx database (www.gtexportal.org/, accessed 1 November 2015).

The rs10830963 variant associated with glucose levels. Nominal association was seen with self-reported insomnia, and insomnia modified the genetic association of rs10830963 with glucose levels. However, the findings with insomnia were not replicated in Health 2000.

Notably, two earlier studies have evaluated the association of rs10830963 with sleep problems. Nominal associations of rs10830963 with insomnia and sleep maintenance and onset were reported [6]. Similarly, Tare et al showed a suggestive association with short sleep duration, although neither study saw interactions of short sleep or sleep problems on rs10830963 association with blood glucose levels or type 2 diabetes [6, 7]. These studies concluded that rs10830963 associates with glycaemic traits rather than sleep. Nevertheless, one challenge in the current and previous studies is that the sample sizes are underpowered to detect small effect interactions that would require 31,000 individuals to reach 80% power. Interestingly, another *MTNR1B* variant, rs10830964, was recently detected in a genome-wide association study on

Table 1 Association of *MTNR1B* risk variants with blood glucose levels and sleep phenotypes

Variable	<i>n</i>	β ($\pm$ SE)	OR (95% CI)	<i>p</i> value
Discovery data set				
Glucose	4691	0.189 $\pm$ 0.021	–	2.390 $\times$ 10 ⁻¹⁸
Short sleep	707 cases 2855 controls	–	1.118 (0.991, 1.261)	0.069
Insomnia	2579 cases 1973 controls	–	1.099 (1.000, 1.207)	0.049
Insomnia interaction	4691 with glucose 2579 cases 1973 controls for insomnia	0.120 $\pm$ 0.043	–	0.006 ^a
Replication data set				
Glucose	5981	0.045 $\pm$ 0.013	–	4.63 $\times$ 10 ⁻⁰⁴
Short sleep	1492 cases 3891 controls	–	0.989 (0.886, 1.105)	0.848
Insomnia	3577 cases 2240 controls	–	0.981 (0.906, 1.061)	0.625
Insomnia interaction	5981 with glucose 3577 cases 2240 controls for insomnia	0.013 $\pm$ 0.039	–	0.724 ^{a,b}

Both discovery and replication data sets are for CHR 11, rs10830963, minor allele G (frequency 0.36 in discovery data set)

DILGOM is partially (one third) overlapping with a previous study [9]

Average ($\pm$ SE) sleep duration was 7.30 $\pm$ 0.02 h, BMI 26.8 $\pm$ 0.07 kg/m² and fasting glucose 5.93 $\pm$ 0.01 mmol/l in the discovery data and 7.47 $\pm$ 0.03 h, BMI 27.3 $\pm$ 0.10 kg/m² and fasting glucose 5.36 $\pm$ 0.01 mmol/l in the replication data

^a *p* for interaction

^b $p = 0.410$, $\beta = -0.040$ when removing individuals using antidepressants or hypnotics during the previous month

caffeine-induced sleep disturbance that excluded general insomnia [8]. Based on 1000 Genomes data, rs10830964 and rs10830963 are not in linkage disequilibrium with each other (Centre d'Etude du Polymorphisme [Utah residents with northern and western European ancestry] [CEU] $r^2=0.017$, $D'=0.679$).

We conclude that meta-analyses in existing data sets and deeper sleep phenotyping are required to reliably estimate the modifying effects of sleep on rs10830963 association with blood glucose levels and type 2 diabetes.

Acknowledgements Some of the data were presented as an abstract at the 28th Annual Meeting of the Associated Professional Sleep Societies, LLC, in 2014.

Funding This study was supported by the Finnish Foundation for Cardiovascular Research, Emil Aaltonen Foundation, Paavo Nurmi Foundation, Finnish Brain Foundation and Finnish Cultural Foundation (for HMO) and Academy of Finland, no. 290039 (for TP).

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data. All authors participated in drafting the article and revising it critically for important intellectual content and all authors have approved the final version to be published. TP is responsible for the integrity of the work as a whole.

References

1. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA (2010) Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care* 33:414–420
2. Prokopenko I, Langenberg C, Florez JC et al (2009) Variants in MTNR1B influence fasting glucose levels. *Nat Genet* 41:77–81
3. Wade AG, Ford I, Crawford G et al (2007) Efficacy of prolonged release melatonin in insomnia patients aged 55–80 years: quality of sleep and next-day alertness outcomes. *Curr Med Res Opin* 23: 2597–2605
4. Inouye M, Kettunen J, Soininen P et al (2010) Metabonomic, transcriptomic, and genomic variation of a population cohort. *Mol Syst Biol* 6:441
5. Purcell S, Neale B, Todd-Brown K et al (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81:559–575
6. Olsson L, Pettersen E, Ahlbom A, Carlsson S, Midthjell K, Grill V (2011) No effect by the common gene variant rs10830963 of the melatonin receptor 1B on the association between sleep disturbances and type 2 diabetes: results from the Nord-Trøndelag Health Study. *Diabetologia* 54:1375–1378
7. Tare A, Lane JM, Cade BE et al (2014) Sleep duration does not mediate or modify association of common genetic variants with type 2 diabetes. *Diabetologia* 57:339–346
8. Byrne EM, Johnson J, McRae AF et al (2012) A genome-wide association study of caffeine-related sleep disturbance: confirmation of a role for a common variant in the adenosine receptor. *Sleep* 35:967–975
9. Dupuis J, Langenberg C, Prokopenko I et al (2010) New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* 42:105–116