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SHORT COMMUNICATION

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

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Abstract
Aims/hypothesis Genetic variation in the melatonin receptor 1B (*MTNR1B*) is associated with type 2 diabetes. Melatonin contributes to the regulation of sleep, and sleep problems are a documented risk factor for type 2 diabetes. The aim of this study was to investigate whether the *MTNR1B* gene variant rs10830963 is associated with sleep problems and whether this variant contributes to the association between sleep disturbances and type 2 diabetes.
Methods This was a case–control study nested within the population-based Nord-Trøndelag Health Study, including 1,322 prevalent cases of type 2 diabetes and 1,447 controls. In addition, prospective data were available for 838 incident cases and 1,133 controls. Genotyping was done by TaqMan single-nucleotide polymorphism allelic discrimination analysis. ORs and 95% CIs were calculated using logistic regression models.
Results Our findings confirm an association between sleep disturbances and type 2 diabetes (OR 1.69, 95% CI 1.22–2.33, p=0.0016) and between the risk allele of rs10830963 and type 2 diabetes (OR 1.12, 95% CI 1.00–1.27, p=0.0579). There was a tendency for an association between the risk allele and prevalence of sleep problems (specifically early awakening). However, the risk allele did not influence the association of sleep problems with diabetes, which was unaltered after adjustment for the *MTNR1B* risk allele (OR 1.69, 95% CI 1.23–2.34, p=0.0014). Results based on prospective data were similar, although non-significant.
Conclusions/interpretation Our findings do not support participation of the *MTNR1B* gene variant rs10830963 in the well documented association between sleep disturbances and type 2 diabetes.

Keywords Epidemiology · Melatonin · Norway · Sleep disorder · Type 2 diabetes

Abbreviations

GAD	Glutamic acid decarboxylase
HUNT	Nord-Trøndelag Health Study
SNP	Single-nucleotide polymorphism

Introduction

Disturbed sleep is associated with an increased risk of type 2 diabetes [1]. The possibility has been raised that this

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association might be explained in part by genetic variation in the melatonin receptor 1B (*MTNR1B*) gene [2]. Such an effect seems plausible since (1) *MTNR1B* encodes a receptor for melatonin and melatonin participates in the regulation of the circadian rhythm and the pattern of sleep [2], and (2) recent studies demonstrate that genetic variation in the *MTNR1B* gene is associated with type 2 diabetes [3–5]. The gene variant could influence both sleeping problems and the risk of type 2 diabetes [2]. Alternatively, disturbed sleep could be a mediator of the causal pathway between this gene variant and type 2 diabetes. An association between *MTNR1B* and sleep disturbances was tested for with negative results in one study [6]. However, whether *MTNR1B* influences the well documented association between sleep disturbances and type 2 diabetes has, to our knowledge, not been investigated.

Here we investigated whether the single-nucleotide polymorphism (SNP) rs10830963 in the *MTNRIB* gene plays a role in the association between sleep disturbances and type 2 diabetes. To this end, we used data from a case–control study nested within the Nord-Trøndelag Health Study (HUNT).

Methods

HUNT All inhabitants of the Norwegian county of Nord-Trøndelag who were aged ≥20 years were invited to take part in surveys during 1984–1986 (HUNT1) and 1995–1997 (HUNT2). The surveys featured clinical examinations as well as questionnaires on health and lifestyle. The response rate was 90.3% (n=76,885) in HUNT1 and 71.2% (n=66,140) in HUNT2.

The HUNT Study was approved by the Regional Medical Research Ethical Committee and the Norwegian Data Inspectorate. The participants gave informed consent.

Identification of cases Cases of known diabetes were identified by questionnaire. Participants aged ≥35 at diagnosis were classified as having type 2 diabetes if they were anti-glutamic acid decarboxylase (GAD) negative (assay described in detail by Olsson et al. [7]).

Study population This was a nested case–control study that included all cases of diabetes identified in HUNT2. We investigated 1,322 prevalent cases of type 2 diabetes together with 1,447 control participants without diabetes. The controls were selected randomly among the participants in HUNT2 and frequency matched by sex and age (in years, by decade) to the cases. In addition, prospective data were available for 838 cases of type 2 diabetes in HUNT2, who were free of diabetes at baseline, i.e. in HUNT1 (1984–1986), and 1,133 controls.

Genotyping DNA was extracted from blood samples (available from the HUNT2 clinical investigation), as described previously [8]. Genotyping for rs10830963 was done by TaqMan SNP allelic discrimination analysis (Applied Biosystems, Foster City, CA, USA). The genotype consistency rate among 95 duplicate samples was 100% and the average call frequency was 99%. The data were in agreement with Hardy–Weinberg equilibrium in the whole population and the p value threshold was 0.001. The minor allele frequency was 0.29 in the genotyped population.

Sleep disturbances Three questions about sleep disturbances were included in the HUNT2 questionnaire: (1) 'During the last month, have you woken too early and not been able to get back to sleep?' (Difficulties maintaining sleep); (2) 'Have you had difficulty falling asleep in the last month?' (Difficulties initiating sleep) (each with four response options, ranging from 'Never' to 'Every night'); and (3) 'How often do you suffer from insomnia' (with four response options ranging from 'Never or a few times a year' to 'More than once a week'). The HUNT1 questionnaire contained a single question on sleep disturbances: 'During the last month, have you had any problems falling asleep or sleep disorders?', with four response options ranging from 'Never' to 'Always'. For the analyses, the response options were dichotomised (category 1–2 vs category 3–4).

Statistical analysis ORs and 95% CIs were calculated using logistic regression models (Proc Logistic, SAS 9.2; SAS Institute, Cary, NC, USA). ORs were adjusted for age, sex and BMI, and an additive effect of the risk allele was assumed. Dominant and recessive models were used in additional analyses of associations with the *MTNR1B* gene variant. The same model was used for analyses of prospective data where we analysed the association between baseline measures of sleep problems (HUNT1) and risk of diabetes during follow-up (HUNT2).

Results

Characteristics of the study population Participants with diabetes were slightly older and heavier and had higher blood pressure compared with the controls (electronic supplementary material [ESM] Table 1). Among the cases, 51% were carriers of the risk *MTNR1B* gene variant rs10830963, as were 47% of the controls.

Variation in MTNR1B and type 2 diabetes By an additive model, type 2 diabetes tended to be more prevalent in carriers of the risk allele of rs10830963, with OR 1.12

Table 1 Odds ratio of self-reported sleep disturbances (cases and controls combined) associated with rs10830963 in the *MTNR1B* gene

Model	Difficulty maintaining sleep		Difficulty initiating sleep		Insomnia	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Additive	1.16 (0.98–1.36)	0.0802	0.98 (0.82–1.17)	0.8139	0.93 (0.75–1.14)	0.4701
Recessive	1.46 (1.03–2.06)	0.0352	0.96 (0.64–1.45)	0.8405	1.11 (0.70–1.76)	0.6663
Dominant	1.12 (0.91–1.39)	0.2822	0.98 (0.78–1.23)	0.8485	0.85 (0.65–1.11)	0.2378

Case–control data from the HUNT study, adjusted for age, sex and BMI

(95% CI 1.00–1.27, p=0.0579; ESM Table 2). By a dominant model, the OR for carriers of the risk allele when compared with non-carriers was 1.21 (95% CI 1.03–1.41, p=0.0190). Adjustment for sleep problems (difficulty maintaining sleep) did not appear to affect these results (OR 1.11, 95% CI 0.98–1.27, p=0.1111).

Variation in MTNR1B and sleep disturbances By an additive model, there was a tendency for an association between the *MTNR1B* rs10830963 variant and one of the sleep questions, i.e. difficulty maintaining sleep (OR 1.16, 95% CI 0.98–1.36, p=0.080; Table 1). The association was significant for the recessive model (OR 1.46, 95% CI 1.03–2.06, p=0.0352).

Sleep disturbances and type 2 diabetes in relation to variation in MTNR1B Type 2 diabetes was more prevalent among participants reporting disturbed sleep. Importantly, adjustment for the *MTNR1B* gene variant did not affect the strength of this association (Table 2). The OR of diabetes for the highest vs lowest sleep category, after adjustment for genotype, was 1.63 (95% CI 1.08–2.47, p=0.0206) for difficulty maintaining sleep, 1.47 (95% CI 1.03–2.09, p=0.0333) for difficulty initiating sleep and 1.50 (95% CI 0.99–2.26, p=0.0560) for insomnia. Insomnia was associated with type 2 diabetes in carriers of the *MTNR1B* gene variant (OR 1.79, 95% CI 1.08–2.95, p=0.0232) as well as in non-carriers (OR 1.67, 95% CI 1.09–2.56, p=0.0177). Similar results were seen for the questions on difficulties maintaining and initiating sleep. There was no significant interaction between *MTNR1B* and sleeping problems in relation to type 2 diabetes (p=0.7939, insomnia).

Prospective analyses Similar but non-significant results were seen when we analysed the risk of developing type 2 diabetes during the 11 year follow-up between HUNT1

Table 2 Association between sleep disturbances and prevalence/incidence of type 2 diabetes with and without adjustment for rs10830963 in the *MTNR1B* gene

Sleep disturbance	No. of cases/controls	Adjusted for age, sex and BMI		Adjusted for age, sex, BMI and *MTNR1B*	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value
Sleep disturbances in HUNT2 (1995–1997) and prevalence of type 2 diabetes					
Difficulty maintaining sleep					
Never or now and again	923/1,042	1		1	
Often or almost every night	188/166	1.23 (0.97–1.57)	0.0886	1.23 (0.96–1.56)	0.0978
Difficulty initiating sleep					
Never or now and again	956/1,068	1		1	
Often or almost every night	162/132	1.35 (1.04–1.75)	0.0261	1.36 (1.04–1.76)	0.0230
Insomnia					
Never or almost never	383/518	1		1	
Once a week or more	135/98	1.69 (1.22–2.33)	0.0016	1.69 (1.23–2.34)	0.0014
Sleep disturbances in HUNT1 (1984–1986) and incidence of type 2 diabetes (1984–1997)					
Insomnia					
Never or occasionally	761/1,044	1		1	
Frequently	77/89	1.35 (0.94–1.93)	0.1020	1.35 (0.94–1.94)	0.1010

Case–control data from the HUNT study

No. cases/controls differ as a result of internal non-response to the sleep questions

(1984–1986) and HUNT2 (1995–1997). After adjustment for genotype, the OR was 1.35 (95% CI 0.94–1.94, p= 0.101) for an association with baseline (HUNT1) sleep disturbances.

Discussion

Our main finding is a negative one, i.e. no apparent role of the genetic variant of melatonin receptor 1B in the association between sleep disturbances and type 2 diabetes. The strongest support for this conclusion is the association between sleep problems and type 2 diabetes seen in non-carriers of the risk allele. By our cross-sectional data we cannot assess the temporality of the associations nor exclude the possibility of reverse causality. Also, differential misclassification of sleeping problems among cases and controls could lead to an overestimation of the association However, prospective data, not subject to these limitations but based on smaller numbers, were consistent with results based on cross-sectional data.

There was a tendency for an association between *MTNR1B* and difficulty maintaining sleep. A selective effect on early awakening is compatible with the known effects of melatonin on sleep, since different times of peaking of this hormone during the night could lead to different types of sleep problem [9]. In addition, our finding is not necessarily discrepant with a previous negative finding [6] because the questions on sleep in that study were different from ours.

Low specificity of sleep information may have diluted our estimates regarding the association between *MTNR1B* and sleeping problems. On the other hand, it does not seem likely that crude information on sleep disturbances explains our main findings, i.e. an association between sleep and type 2 diabetes that is independent of variation in *MTNR1B*; crude information on sleep disturbances would actually dilute any association. Even so, our data do not suffice to rule out a minor sleep-dependent association between *MTNR1B* and type 2 diabetes, undetectable to us because of limited power.

As regards the diagnosis of diabetes by self-reporting, a validation study demonstrated >95% concurrence with healthcare records [10]. Our classification of participants as having type 2 diabetes on the basis of age ≥35 years at diagnosis and absence of anti-GAD does not exclude other forms of diabetes, but admixture of other forms should have been minor.

We confirm an association between rs10830963 in the *MTNR1B* gene and type 2 diabetes [5]. The gene is expressed in human beta cells [3] and melatonin inhibits glucose-stimulated insulin secretion [4]. Our results, indicating that the influence of *MTNR1B* on type 2 diabetes is not mediated by sleeping problems, offer indirect support for a primary role of the risk gene in beta cell function.

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

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