Accepted Manuscript

Associations between *REV-ERBα*, sleep duration and body mass index in European adolescents

Marcus Vinicius Nascimento Ferreira, Louisa Goumidi, Heráclito Barbosa Carvalho, Augusto César F. de Moraes, Alba Santaliestra-Pasías, Anthony Kafatos, Denes Molnar, Christina-Paulina Lambrinou, Stefaan De Henauw, Angel Gutierrez, Laura Censi, Ascensión Marcos, Kurt Widhalm, Frederic Gottrand, Marcela Gonzalez-Gross, Aline Meirhaeghe, Luis A. Moreno

PII: S1389-9457(18)30075-3

DOI: 10.1016/j.sleep.2018.01.014

Reference: SLEEP 3646

To appear in: *Sleep Medicine*

Received Date: 28 March 2017

Revised Date: 17 January 2018

Accepted Date: 18 January 2018

Please cite this article as: Nascimento Ferreira MV, Goumidi L, Carvalho HB, de Moraes ACF, Santaliestra-Pasías A, Kafatos A, Molnar D, Lambrinou C-P, De Henauw S, Gutierrez A, Censi L, Marcos A, Widhalm K, Gottrand F, Gonzalez-Gross M, Meirhaeghe A, Moreno LA, on behalf of the HELENA Study group, Associations between *REV-ERBα*, sleep duration and body mass index in European adolescents, *Sleep Medicine* (2018), doi: 10.1016/j.sleep.2018.01.014.

Associations between *REV-ERBα*, sleep duration and body mass index in European adolescents

Marcus Vinicius Nascimento Ferreira [a,b,*], Louisa Goumidi [c], Heráclito Barbosa Carvalho [a], Augusto César F. de Moraes [a,d], Alba Santaliestra-Pasías [b], Anthony Kafatos [e], Denes Molnar [f], Christina-Paulina Lambrinou [g], Stefaan De Henauw [h], Angel Gutierrez [i], Laura Censi [j], Ascensión Marcos [k], Kurt Widhalm [l], Frederic Gottrand [m], Marcela Gonzalez-Gross [n,o], Aline Meirhaeghe [c], Luis A. Moreno [b], on behalf of the HELENA Study group

[a] **Y**outh/**C**hild c**A**rdiovascular **R**isk and **E**nvironmental (YCARE) Research Group, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil

[b] **G**rowth, **E**xercise, **NU**trition and **D**evelopment (GENUD) Research Group, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria Aragón (IIS Aragón), Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn), Universidad de Zaragoza, Zaragoza, Spain

[c] INSERM U1167, Institut Pasteur de Lille, Universite Lille, Lille, France

[d] Bloomberg School of Public Health, Department of Epidemiology, John Hopkins University,

[e] Preventive Medicine and Nutrition Unit, University of Crete School of Medicine, Heraklion, Crete, Greece

[f] Department of Pediatrics, Medical Faculty, University of Pecs, Pecs, Hungary

[g] Department of Nutrition and Dietetics, Harokopio University, Athens, Greece

[h] Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University, Belgium

[i] Department of Physiology, School of Medicine, University of Granada, Granada, Spain

[j] CREA Research Centre for Food and Nutrition, Rome, Italy

[k] Immunonutrition Research Group, Department of Metabolism and Nutrition, Food Science and Technology and Nutrition Institute, Spanish National Research Council, Madrid, Spain

[l] Department of Pediatrics, Private Medical University Salzburg, Salzburg, Austria

[m] Inserm U995, Universite Lille 2, Lille, France

[n] Institut fur Ernahrungs-und Lebensmittelwissenschaften–Humanernahrung, Rheinische Friedrich-Wilhelms-Universitat Bonn, Bonn, Germany

[o] Department of Health and Human Performance, Faculty of Physical Activity and Sport-INEF, Technical University of Madrid, Madrid, Spain

*Corresponding author. Faculdade de Medicina da Universidade de São Paulo, avenida Doutor Arnaldo, 455 - Cerqueira César, São Paulo - SP, 01246-904, Brazil. Tel.: +55 11 3061 7074; fax: +55 11 3061 7074.

E-mail address: marcus1986@usp.br (Marcus Vinicius Nascimento Ferreira)

Abstract

Background/Objective: Although the *REV-ERBα* is considered an important regulator of both clock function and metabolism, its relationship with sleep duration and obesity is less clear. The objective of this study was to examine the association between the *REV-ERBα* clock gene and two outcomes – sleep duration and body mass index (BMI) – in European adolescents.

Methods: A sample of 831 adolescents (392 boys) aged 11.5–18.8 years from 10 European centers was used. The independent variables were *REV-ERBα* rs2071427 and rs2071570 SNPs, and their respective haplotypes. The outcomes were sleep duration and BMI.

Results: In girls, no significant association were found between rs2071427 or rs2071570 and the studied outcomes ($p \geq 0.43$). In boys, however, significant associations were found between rs2071570 and sleep duration (β: –0.32 hours/day for T minor allele carriers; $p=0.0017$), and rs2071427 and BMI (β: +0.72 kg/m^2 for A minor allele carriers; $p=0.016$). In the haplotype analysis, the TA haplotype (carrying the two minor alleles) was associated with both lower sleep duration (□□–0.38 hours/day; $p=0.05$) and higher BMI (□□+1.41 kg/m^2; $p=0.018$) in boys, when compared with the common CC haplotype.

Conclusions: The *REV-ERBα* rs2071427 and rs2071570 were associated with both sleep duration and BMI in boys. These findings confirmed the relevance of the *REV-ERBα* gene in human obesity, primarily in males, and also suggested that it has a potential role in affecting sleep duration.

Keywords:

Obesity

REV-ERBα clock gene

Sleep duration

Youth

Introduction

Based on the day-night cycle, the circadian rhythm provides appropriate timing efficiency in biological systems through the synchronization of behavior and physiology [1-3]. Metabolic homeostasis and the circadian clock (rhythm) are intimately linked in mammals [4]; moreover, misalignment of the circadian rhythm has been associated with metabolic abnormalities in humans [2] and in animal models [5,6]. In addition, it is currently accepted that these associations are modulated by clock genes (eg, *CLOCK*, *BMAL1*, *RORα*, *REV-ERBα*) [2,7-9].

Among clock genes, the *REV-ERBα* (also known as NR1D1) is considered an important regulator of both clock function [10] and metabolism [11,12]. In short, this gene modulates the fine-tuning of circadian rhythmicity, in that *BMAL1* activates *REV-ERBα* transcription and then *REV-ERBα* represses the transcription of *BMAL1* [7]. Specifically, studies on genetic variations of *REV-ERBα* have shown that rs2314339, rs2071427 and rs2071570 single-nucleotide polymorphisms (SNPs) are associated with obesity in adults [13,14] and youths [4]. Literature suggests a connection between the biological clock and obesity-related traits [14] and, more recently, it has been shown that *REV-ERBα* could modulate obesity in a sex-specific manner [13].

Although a link between *REV-ERBα* and obesity has been found [4,3,14], few studies have examined whether the association can vary depending on sex. Furthermore, it is believed that there are no studies that address the impact of *REV-ERBα* on sleep duration in humans. The current study examined the association between two *REV-*

ERBα SNPs (rs2071427 and rs2071570) and sleep duration, as well as BMI in European adolescents stratified by sex.

Methods

Subjects

The subjects were evaluated in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study, a cross-sectional study comprised of 3528 adolescents from 10 European cities (11.5–18.8 years) who were recruited between 2006 and 2007. One third of the study population was randomly selected for blood collection (n = 1155). After removing the participants who had missing values for *REV-ERB* SNPs, BMI, sleep duration and confounding factors, the sample used in the present study was set at 831 (47% boys). The study was performed following the ethical guidelines of the Declaration of Helsinki and was approved by the Research Ethics Committee of each city involved. Written, informed consent was obtained from each subject and their parent(s) or guardian(s) [15]. A detailed description of the methodology used in the HELENA study has been published elsewhere [16].

SNP selection and genotyping

The *REV-ERBα* rs2071427 and rs2071570 (also tagged as rs939347) SNPs were selected as independent variables based on previously published studies in which they were associated with health outcomes [4,10,13]. These SNPs are located in the intron 1 and the promoter of the NR1D1 gene, respectively. Genotyping was carried out through fasting blood samples collected by venipuncture after a 10-hour overnight fast. The

SNPs were genotyped using Illumina technology. The processes for obtaining and genotyping the blood samples have been described in detail elsewhere [4,17].

Outcomes

Sleep duration and body mass index (BMI) were considered dependent variables. Sleep duration was estimated using a questionnaire that included the following questions: "During weekdays: how many hours (and minutes) do you usually sleep?" and "During weekend days: how many hours (and minutes) do you usually sleep?" [18]. Self-reported sleep duration was calculated as:

[(mean sleep duration on weekdays x 5) + (mean sleep duration on days during the weekend x 2)]/7 [19].

The questionnaire was shown to be reliable, Cohen's weighted *k* statistic was 0.81 and 0.96 for weekdays and days during the weekend, respectively [18]. The adolescents' heights were measured to the nearest 0.1 cm, barefoot and in the Frankfort plane, with a telescopic stadiometer (SECA 225) and their weight were measured to the nearest 0.1 kg, in underwear and barefoot, with an electronic portable digital scale (SECA 861 type) [20]. BMI was calculated by dividing weight (kg) by height (m^2).

Potential confounders

Potential confounders were age (years), center (cities), maternal education and tobacco consumption. Maternal education was assessed using a self-reported questionnaire and classified into four levels: elementary education, lower secondary education, upper

secondary education and university degree. Tobacco consumption was defined as regular consumption over the week, varying between “no smoking” to “smoking every day”.

Statistical analysis

The Stata 12 (Stata Corp., College Station, TX, USA) program was used for statistical analysis. For the descriptive analyses, mean and 95% of confidence intervals (95% CI) were calculated for quantitative variables. Differences between the means were analyzed by Student’s *t*-test (two means) for unpaired samples. A linear trend test was performed to check the distribution of categorical variables. The significance level was set at $p \leq 0.05$.

Hardy-Weinberg equilibrium was tested using the Chi-squared test (1 degree of freedom). The significance level was set at $p \leq 0.05$. The linkage disequilibrium (LD) between the rs2071427 and rs2071570 SNPs was calculated based on the equations shown by Pritchard et al. [21]. Linear regression analyses were preformed to assess association between the two SNPs – rs2071427 and rs2071570 – and (i) sleep duration and (ii) BMI according to the additive genetic model [22], adjusted for potential confounders. Potential confounders with *p*-values of ≤0.20 were retained in the multivariate model. The major allele was used as the reference. Bonferroni correction was applied to control for multiple testing in the genetic analyses ($p \leq 0.05$ per two SNPs tested, resulting in $p \leq 0.025$) [22].

Interaction between sex and sleep duration or BMI was tested by adding an interaction term SNP*sex to the GLM model. Haplotype analysis was carried out using

the Thesias software [23], stratified by sex. The haplotype analyses were adjusted for potential confounders. The significance level was set at $p \leq 0.05$.

Results

The characteristics of the subjects are presented in Table 1. Sleep duration was higher in boys than in girls. BMI was similar between boys and girls. There were no differences in terms of maternal education and smoking habits between the sexes.

Table 2 shows the genotype distributions of the *REV-ERBα* rs2071427 and rs2071570 SNPs. The minor allele frequencies were 0.28 and 0.21, respectively, and the Hardy-Weinberg equilibrium was observed for each SNP (p=0.45 and p=0.76, respectively). A weak LD was found between *REV-ERBα* rs2071427 and rs2071570 SNPs (D' = 0.286 and r^2 = 0.048).

The associations between the *REV-ERBα* SNPs and sleep duration and BMI are presented in Table 3. Although no significant interaction between the SNPs and sex could be detected, the analyses were stratified by sex as (i) sleep duration varied for males and females in the HELENA study [19] and (ii) Ruano et al. [13] showed different associations between *REV-ERBα* SNPs and obesity in each sex. No significant association could be detected in girls; however, boys carrying the minor T allele of rs2071427 had a higher BMI than C allele carriers (□= +0.72 kg/m², p=0.018). In addition, boys carrying the minor A allele of rs2071570 had lower sleep duration than C allele carriers (□= –0.32 hours/day, p=0.0019). These associations remained significant after adjustment for potential confounders and were independent of each other.

In addition, haplotype analyses were performed, revealing that the rs2071427 and rs2071570 SNPs generate four haplotypes (frequency ranging 0.10–0.60). As for

individual SNP analyses, no significant association could be detected in girls (data not shown). In boys, despite a non-significant haplotype global effect on BMI, the TA haplotype (including the two minor alleles) was associated with higher BMI (+1.41 kg/m², p=0.018) compared with the reference CC haplotype (Table 4). This association was not modified by adjustment for sleep duration. With regards to sleep duration, there was a significant global haplotype effect, and both the TC and TA haplotypes were associated with lower sleep duration (–0.41, p=0.008 and –0.38 hours/day, p=0.05, respectively). These associations persisted after adjustment for BMI.

Discussion

A significant association was found between *REV-ERBα* rs2071427 and rs2071570 variants and both sleep duration and BMI, confirming previous findings of an association in humans between *REV-ERBα* and obesity phenotype [10,11,13,14] and adding evidence to the connection between *REV-ERBα* and sleep duration. Associations between *REV-ERBα* and sleep duration and BMI differed significantly by sex. Based on the data, the minor alleles of rs2071427 and rs2071570 were associated with higher BMI and lower sleep duration in boys, respectively. The haplotype carrying the two minor alleles was also associated with both higher BMI and lower sleep duration in boys, with effect sizes superior to those of the individual SNPs.

These findings suggest a potential link between *REV-ERBα* and sleep duration, with a possible specific role for each sex. Although the *REV-ERBα* gene circadian expression in peripheral tissues in both humans [8,11,24] and in animal models [6,7] is well documented, studies on *REV-ERBα* and sleep duration or circadian rhythms are

scarce. A recent study of humans showed associations between polymorphisms of this gene and circadian typologies (circadian preferences: evening type, intermediate, morning type) [10]. In knockout mice, Mang et al. provided important proof that *REV-ERBα* is involved in the sleep homeostatic phenotype [25]. Consistent with the current findings, lack of *REV-ERBα* may lead to deficits in engaging in waking behaviors such as exploratory behavior in mice [25]. In this sense, *REV-ERBα* could thus act as a sensor of the metabolic imbalance imposed at the neuronal level by periods of extended wakefulness, which is in keeping with the current proposal that clock genes not only set time of day, but in the cerebral cortex can also be used to keep track of and respond to time spent awake [26]. Another study, with animal models, showed that the absence of normal function in the *PER1* and *PER2* clock genes seemed to protect male mice from metabolic reprogramming, suggesting that the circadian timing system has a role in regulating the physiological effects of sleep disruption [5]. It could be a potential and partial explanation for the association between *REV-ERBα* rs2071570 SNP and sleep duration found exclusively in boys in the current study.

In addition, the current study found an association between the *REV-ERBα* rs2071427 polymorphism and BMI in boys. This result is consistent with previous studies showing associations between rs2071427 polymorphism and obesity phenotype (higher BMI) [13,14]. The current study also found an association between the TA haplotype (carrying the minor alleles for both rs2071427 and rs2071570 SNPs) and BMI. The *REV-ERBα* gene could favor a visceral accumulation of fat [7], especially in males [13], showing a different role of adipogenesis among males and females. In a study with animal models, Bugge et al. [7] showed the possible mechanisms of *REV-ERBα* influencing the clock function and mediating the interplay between circadian rhythms and metabolism. One of the most important mechanisms could be the hepatic

phosphatidylcholine regulated by the circadian clock through a BMAL1-REV-ERB*α*-CHK*α* axis, which suggests that an intact circadian timing system is important for the temporal coordination of phospholipid metabolism [27].

The current study showed results supporting the association between *REV-ERBα* with decreasing sleep duration (around 20 minutes per day) and increasing BMI (around 1 kg/m²) in boys. However, it remains uncertain whether specific metabolites that vary according to time of day and nutrient status (fasting vs feeding) may also affect the circadian function of energy-sensing neurons [2]. The mere perturbation of metabolic homeostasis with a high-fat diet is sufficient to alter the clock gene function [28]. Conversely, in mice, treatment of diet-induced obesity with *REV-ERB* agonist decreased obesity by reducing fat mass and markedly improving dyslipidemia and hyperglycemia, indicating *REV-ERB* as a potential pharmacological target for treatment of sleep disorders and metabolic diseases [29].

The major limiting factors in this study were: (i) its cross-sectional design, which does not allow for causal relationships to be observed; (ii) the use of a self-reported questionnaire to identify sleep duration; and (iii) although there is marked interest in the literature to elucidate the role of *REV-ERBα* rs2071427 and rs2071570 SNPs, the current subjects potentially affected by these minor alleles were 7% and 4%, respectively. Another limitation of this study was that analyses could not be adjusted for circadian typology (circadian preferences: evening type, intermediate, morning type), which is an outcome associated with *REV-ERBα* [10]. In addition, according to conceptual epidemiological framework [30], it was not possible to adjust the analysis of other factors potentially associated with BMI in either of the two models, such as physical activity and dietary patterns, because they are in the causative variables line.

In contrast, the present study had several strengths. Firstly, this was the first time that *REV-ERBα* has been studied in relation to sleep duration. Secondly, it confirmed the association between *REV-ERBα* and BMI through replication, a process by which genetic association results are validated [22]. This was also the first time that a *REV-ERBα* haplotype has been associated with both sleep duration and BMI. Additional studies in other populations are necessary to confirm the generalizability of these findings, and this study must be replicated in different age groups. Further genetic studies are needed to understand the *REV-ERBα* mechanisms in circadian clocks and circadian disorders.

Conclusions

REV-ERBα rs2071427 and rs2071570 were associated with sleep duration and BMI in boys. These findings confirm the relevance of *REV-ERBα* in human obesity, primarily in males, and also suggest that it has a potential role in affecting sleep duration. It is suggested that *REV-ERBα* be a target gene in the treatment of obesity.

Funding sources: The HELENA study received funding from the European Union's Sixth RTD Framework Program (Contract FOOD-CT-2005-007034). The GENUD Research Group was co-financed by the European Regional Development Fund (MICINN-FEDER). The work of Marcus Nascimento-Ferreira was supported by the São Paulo Research Foundation (FAPESP, process: 2016/18436-8). Heraclito Barbosa Carvalho received research funding from São Paulo Research Foundation (FAPSP, proc. 2014/11468-6). Augusto C. de Moraes was supported by the São Paulo Research

Foundation (FAPESP; process: 2015/14319-4 and 2014/25233-0, respectively). Luis Moreno was supported by FAPESP (process: 2015/11406-3). The funders had no role in study design, data collection and analysis, decision to submit, or production of the manuscript. The authors have indicated no financial conflicts of interest.

Acknowledgements: We gratefully acknowledge all participating adolescents and their parents for their collaboration. We are also grateful to the HELENA study group.

Conflict of interest: The authors declare no conflict of interest.

References

1. Shearman LP, Sriram S, Weaver DR, Maywood ES, Chaves I, Zheng B, et al. Interacting molecular loops in the mammalian circadian clock. Science 2000;288(5468):1013-9.

2. Huang W, Ramsey KM, Marcheva B, Bass J. Circadian rhythms, sleep, and metabolism. J Clin Invest 2011;121(6):2133-41.

3. Lall GS, Atkinson LA, Corlett SA, Broadbridge PJ, Bonsall DR. Circadian entrainment and its role in depression: a mechanistic review. J Neural Transm (Vienna) 2012;119(10):1085-96.

4. Goumidi L, Grechez A, Dumont J, Cottel D, Kafatos A, Moreno LA, et al. Impact of REV-ERB alpha gene polymorphisms on obesity phenotypes in adult and adolescent samples. Int J Obes (Lond) 2013;37(5):666-72.

5. Husse J, Hintze SC, Eichele G, Lehnert H, Oster H. Circadian clock genes Per1 and Per2 regulate the response of metabolism-associated transcripts to sleep disruption. PLoS One 2012;7(12):e52983.

6. Mavanji V, Billington CJ, Kotz CM, Teske JA. Sleep and obesity: a focus on animal models. Neurosci Biobehav Rev 2012;36(3):1015-29.

7. Bugge A, Feng D, Everett LJ, Briggs ER, Mullican SE, Wang F, et al. Rev-erbα and Rev-erbβ coordinately protect the circadian clock and normal metabolic function. Genes Dev 2012;26(7):657-67.

8. Amador A, Wang Y, Banerjee S, Kamenecka TM, Solt LA, Burris TP. Correction: Pharmacological and Genetic Modulation of REV-ERB Activity and Expression Affects Orexigenic Gene Expression. PLoS One 2016;11(5):e0156367.

9. Sookoian S, Gemma C, Gianotti TF, Burgueño A, Castaño G, Pirola CJ. Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. Am J Clin Nutr 2008;87(6):1606-15.

10. Kang JI, Park CI, Namkoong K, Kim SJ. Associations between polymorphisms in the NR1D1 gene encoding for nuclear receptor REV-ERBα and circadian typologies. Chronobiol Int 2015;32(4):568-72.

11. Solt LA, Kojetin DJ, Burris TP. The REV-ERBs and RORs: molecular links between circadian rhythms and lipid homeostasis. Future Med Chem 2011;3(5):623-38.

12. Yin L, Wu N, Curtin JC, Qatanani M, Szwergold NR, Reid RA, et al. Rev-erbalpha, a heme sensor that coordinates metabolic and circadian pathways. Science 2007;318(5857):1786-9.

13. Ruano EG, Canivell S, Vieira E. REV-ERB ALPHA polymorphism is associated with obesity in the Spanish obese male population. PLoS One 2014;9(8):e104065.

14. Garaulet M, Smith CE, Gomez-Abellán P, Ordovás-Montañés M, Lee YC, Parnell LD, et al. REV-ERB-ALPHA circadian gene variant associates with obesity in two independent populations: Mediterranean and North American. Mol Nutr Food Res 2014;58(4):821-9.

15. Béghin L, Castera M, Manios Y, Gilbert CC, Kersting M, De Henauw S, et al. Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. Int J Obes (Lond) 2008;32 Suppl 5:S12-8.

16. Moreno LA, De Henauw S, González-Gross M, Kersting M, Molnár D, Gottrand F, et al. Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int J Obes (Lond) 2008;32 Suppl 5:S4-11.

17. González-Gross M, Breidenassel C, Gómez-Martínez S, Ferrari M, Béghin L, Spinneker A, et al. Sampling and processing of fresh blood samples within a European multicenter nutritional study: evaluation of biomarker stability during transport and storage. Int J Obes (Lond) 2008;32 Suppl 5:S66-75.

18. Rey-López JP, de Carvalho HB, de Moraes AC, Ruiz JR, Sjöström M, Marcos A, et al. Sleep time and cardiovascular risk factors in adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study. Sleep Med 2014;15(1):104-10.

19. Garaulet M, Ortega FB, Ruiz JR, Rey-López JP, Béghin L, Manios Y, et al. Short sleep duration is associated with increased obesity markers in European adolescents: effect of physical activity and dietary habits. The HELENA study. Int J Obes (Lond) 2011;35(10):1308-17.

20. Nagy E, Vicente-Rodriguez G, Manios Y, Béghin L, Iliescu C, Censi L, et al. Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. Int J Obes (Lond) 2008;32 Suppl 5:S58-65.

21. Pritchard JK, Przeworski M. Linkage disequilibrium in humans: models and data. Am J Hum Genet 2001;69(1):1-14.

22. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. Nat Protoc 2011;6(2):121-33.

23. Tregouet DA, Garelle V. A new JAVA interface implementation of THESIAS: testing haplotype effects in association studies. Bioinformatics 2007;23(8):1038-9.

24. Yang X, Downes M, Yu RT, Bookout AL, He W, Straume M, et al. Nuclear receptor expression links the circadian clock to metabolism. Cell 2006;126(4):801-10.

25. Mang GM, La Spada F, Emmenegger Y, Chappuis S, Ripperger JA, Albrecht U, et al. Altered Sleep Homeostasis in Rev-erbα Knockout Mice. Sleep 2016;39(3):589-601.

26. Franken P. A role for clock genes in sleep homeostasis. Curr Opin Neurobiol 2013;23(5):864-72.

27. Gréchez-Cassiau A, Feillet C, Guérin S, Delaunay F. The hepatic circadian clock regulates the choline kinase α gene through the BMAL1-REV-ERBα axis. Chronobiol Int 2015;32(6):774-84.

28. Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab 2007;6(5):414-21.

29. Solt LA, Wang Y, Banerjee S, Hughes T, Kojetin DJ, Lundasen T, et al. Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature 2012;485(7396):62-8.

30. Szklo M, Nieto J. Epidemiology: beyond the basics. 3 ed. Burlington, MA: Jones & Bartlett Learning; 2014.

Table 1. Characteristics of the participating subjects in the HELENA study.

Variables	Mean ± SD or % (*n*)		
	Male (*n* = 392)	**Female (*n* = 439)**	***p*-value[Ŧ]**
Age, years	14.7 ± 1.5	14.6 ± 1.4	0.47
Sleep duration, hours/day	8.2 ± 1.2	8.0 ± 1.2	**0.0076**
BMI, kg/m^2	21.6 ± 4.1	21.4 ± 3.5	0.41
Maternal education, % (*n*)			0.24
Lower education	7.1 (28)	9.3 (41)	
Lower secondary education	27.3 (107)	26.4 (116)	
Higher secondary education	31.9 (125)	35.1 (154)	
University degree	33.7 (132)	29.2 (128)	
Tobacco consumption, % (*n*)			0.62
No smoking	82.9 (325)	82.0 (360)	
Less than once a week	5.4 (21)	3.9 (17)	
At least once a week, but not every day	3.6 (14)	3.4 (15)	
Every day	8.1 (32)	10.7 (47)	

Significant values are in **bold** ($p \leq 0.05$).

BMI, body mass index; SD, standard deviation.

[Ŧ] *p*-value for *t*-test.

[ŦŦ] *p*-value for linear trend test.

Table 2. Genotype distributions of the *REV-ERBα* SNPs in the participating subjects of the HELENA study.

REV-ERBα SNPs	*n*	Frequency	MAF	H-W *p*-value
rs2071427				
CC	431	0.52	0.28	0.45
CT	341	0.41		
TT	59	0.07		
Total	831			
rs2071570				
CC	514	0.62	0.21	0.76
CA	281	0.34		
AA	36	0.04		
Total	831			

H-W, Hardy-Weinberg; MAF, minor allele frequency; SNPs, single nucleotide polymorphisms.

Table 3. Associations between *REV-ERBα* SNPs and sleep duration and BMI by sex.

	Males (*n* = 392)				
	β coefficient	SE	p^a	p^b	p^c
rs2071427					
sleep duration (h/d)	–0.17	0.09	0.06	0.052	–
BMI (kg/m²)	0.72	0.30	**0.018**	–	**0.016**
rs2071570					
sleep duration (h/d)	–0.32	0.10	**0.0019**	**0.0017**	–
BMI (kg/m²)	0.60	0.35	0.09	–	0.07
	Females (*n* = 439)				
	β coefficient	SE	p^a	p^b	p^c
rs2071427					
sleep duration (h/d)	0.001	0.09	0.99	0.99	–
BMI (kg/m²)	0.03	0.27	0.90	–	0.90
rs2071570					
sleep duration (h/d)	–0.07	0.09	0.44	0.42	–
BMI (kg/m²)	–0.22	0.29	0.45	–	0.43

Significant values are in **bold** ($p \leq 0.025$).

BMI, body mass index; h/d, hours/day; SE, Standard error.

Beta coefficients are calculated with an additive model (minor allele effect).
[a] *p*-values adjusted for age, center, maternal education and tobacco consumption.
[b] *p*-values adjusted for age, center, maternal education, tobacco consumption and BMI.
[c] *p*-values adjusted for age, center, maternal education, tobacco consumption and sleep time.

Table 4. *REV-ERBα* haplotype frequencies and haplotype effects on BMI and sleep duration in boys (n = 392).

Haplotype rs2071427/rs2071570	Frequency	BMI (kg/m²)			Sleep duration (h/d)		
		Δ ± SE	Global effect (p-value)	Haplotype effect (p-value)	Δ ± SE	Global effect (p-value)	Haplotype effect (p-value)
CC	0.60	reference		–	reference		–
CA	0.19	0.24 ± 0.40	0.17	0.47	–0.17 ± 0.13	**0.005**	0.10
TC	0.11	0.04 ± 0.62		0.84	–0.41 ± 0.16		**0.008**
TA	0.10	1.41 ± 0.46		**0.018**	–0.38 ± 0.16		**0.05**

Significant values are in **bold** ($p \leq 0.05$).

BMI, body mass index; h/d, hours/day; SE, Standard error.

Data are difference in means ± SE compared with the CC reference haplotype.
Single nucleotide polymorphisms (SNPs) were used in the following order: rs2071427 and rs2071570.
p-values were adjusted for age, center, maternal education and tobacco consumption.

Highlights

- The *REV-ERBα* gene was associated with both sleep duration and body mass index in boys.
- The *REV-ERBα* gene was found to play an important role in human obesity, primarily in males.
- The *REV-ERBα* gene was found to have a potential role in sleep duration.