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MINI REVIEW

Association between genetic variants of the *clock* gene and obesity and sleep duration

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Abstract Obesity is a multifactorial disease caused by the interaction of genetic and environmental factors related to lifestyle aspects. It has been shown that reduced sleep is associated with increased body mass index (BMI). Circadian Locomotor Output Cycles Kaput (*CLOCK*) gene variants have also been associated with obesity. The objective of this mini-review was to discuss the available literature related to *CLOCK* gene variants associated with adiposity and sleep duration in humans. In total, 16 articles complied with the terms of the search that reported *CLOCK* variants associated with sleep duration, energy intake, and BMI. Overall, six *CLOCK* single nucleotide polymorphisms (SNPs) have been associated with sleep duration, and three variants have been associated with energy intake variables. Overall, the most studied area has been the association of *CLOCK* gene with obesity; close to eight common variants have been associated with obesity. The most studied *CLOCK* SNP in different populations is rs1801260, and most of these populations correspond to European populations. Collectively, identifying at risk *CLOCK* genotypes is a new area of research that may help identify individuals who are more susceptible to overeating and gaining weight when exposed to short sleep durations.

Keywords *CLOCK* polymorphism · Obesity · Sleep duration · Eating behavior

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Introduction

Obesity is a multifactorial disease caused by an interaction between genetic and environmental factors. Negative consequences of obesity have been linked to a variety of chronic diseases, including type 2 diabetes, hypertension, cardiovascular pathologies, and different forms of cancer [6]. Among the behavioral factors that can cause obesity, insufficient sleep (short sleep duration and/or poor sleep quality) depends on the light-dark cycle (circadian rhythm) and is gaining attention in the literature. Sleep disturbances can alter brain functions involved in the control of appetite, which can generate overeating in the current obesogenic environment [3–5]. Genetic studies on obesity have described more than 600 genes, genetic markers, or chromosomal regions that have been linked to obesity [24]. Of them, the Circadian Locomotor Output Cycles Kaput (*CLOCK*) gene participates in the regulation of circadian rhythms as a transcription factor [7]. There is extensive literature showing how sleep reduction is associated with the development of obesity [3, 5, 16]. However, studies

evaluating how genetics predisposes a person to obesity when sleep is reduced and how this modulates eating behavior are lacking.

The objective of this paper was to briefly review the studies examining the association between genetic variants of the *CLOCK* gene with obesity and sleep by focusing on aspects of adiposity, metabolic syndrome, eating behavior, and sleep duration. There is sufficient literature reviewing the associations among insufficient sleep, energy intake, and obesity. However, it remains unclear how a reduction in the hours of sleep affects homeostatic and non-homeostatic food intake and how it is associated with *CLOCK* polymorphisms and the susceptibility to obesity across different ethnic populations. We focused on cross-sectional studies that measured *CLOCK* polymorphisms associated with body mass index (BMI), weight loss interventions, and energy intake. The novel contribution of this paper is that it highlights the findings on the role of genetic factors in such relations and provides research avenues for future investigations.

Methods

The literature search was based on the following terms associated with the objective of this article: genetic variants or polymorphisms of the *CLOCK* gene (including the most studied variants), eating behavior, energy/food intake, obesity, BMI, weight, waist circumference, waist-to-hip ratio, body fat, adiposity, overweight, fat mass, metabolic syndrome, and sleep (including sleep duration, sleep deprivation, and insufficient sleep). We also considered mostly cross-sectional frequency studies from different populations (age, sex, and ethnicities) and sample sizes. Meta-analyses and *CLOCK* SNP (single nucleotide polymorphism) allele and genotype frequencies were analyzed. Studies that involved subjects with mental disorders and those that were performed under stressful conditions were not considered. The search was restricted to human studies. All of the studies reviewed had the full text, and the assessment of inclusion criteria in this review was independently performed by two co-authors of this study (MV and AMO).

Results

A total of 16 articles fully complied with the terms of the search (Table 1). The articles reported associations between *CLOCK* gene variants and (1) sleep duration, (2) BMI or metabolic syndrome, and (3) eating behavior, mostly related to energy intake. Given that sleep duration is related to factors associated with obesity, such as food intake, it is difficult to divide the latter into two variables, and in several articles, they are discussed together. Below, we present the most relevant aspects of the articles studying the abovementioned areas.

Associations between sleep duration and CLOCK gene variants

CLOCK gene variants have been reported to modify the association between sleep duration and energy intake in several studies. The meta-analysis by Allebrandt et al. [1] showed that sleep duration was lower in subjects carrying the A allele of the rs12649507 SNP. An additional significant interaction was observed between sleep duration and the rs12649507 *CLOCK* polymorphism on polyunsaturated fatty acids (PUFA) intake; the carriers of the A minor allele had a longer sleep duration with increased PUFA intake. The carriers with the minor allele (G) had a shorter sleep duration [4]. Additionally, sleep duration and rs6858749 were related to protein intake, with carriers of the minor allele (T) reporting lower protein intake with each additional hour of sleep [4]. Both studies suggested that more hours of sleep might act as a protective factor against the genetic predisposition to develop obesity from the *CLOCK* polymorphism [1, 4]. These two studies included an analysis of a large sample size to investigate the associations of *CLOCK* and its interactions with sleep duration and dietary intake. However, they only included a European population, and sleep was self-reported. Thus, including other populations and objective methods of measuring sleep is necessary for future studies. On the other hand, in European populations, the *CLOCK* variants rs11932595 and rs6843722 showed no association with sleep duration (self-reported) in three independent cohorts [19]; however, Allebrandt's study showed that carriers with a homozygous AA in the rs11932595 variant had reduced sleep duration.

Screening the entire coding *CLOCK* region in subjects with delayed sleep phase syndrome and non-24-h sleep-wake syndrome showed that *CLOCK* polymorphisms are unlikely to play an important role in the development of these syndromes [17]. This study is the only *CLOCK*-wide association study related with a disease. More studies are needed to amplify the coding

Table 1 Association between *CLOCK* gene variants, obesity, and energy intake in humans

Author/year	Population	Study design	CLOCK variant	Principal outcome	Main findings
Mishima et al. 2005 [21]	421 adult Japanese subjects	Cross-sectional study	rs1801260	Chronotype by questionnaire	Eveningness preference in CC genotype.
Monteleone et al. 2008 [22]	Normal (n=92), overweight/obese (n=192) Caucasian subjects	Cross-sectional study	rs1801260	Anthropometric variables	CC genotype higher BMI.
Sookoian et al. 2008 [26]	Lean (n=715), overweight, and obese (n=391) Argentine subjects	Cross-sectional study	rs1554483, rs11932595, rs4580704, rs6843722, rs6850524, and rs4864548	Anthropometric variables	The haplotypes CG or GA of rs1554483 and rs4864548 with obesity (1.8-fold risk).
Scott et al. 2008 [25]	North European subjects 25–57 years (n=537)	Cross-sectional study	rs4864548, rs3736544, and rs1801260	Anthropometric and biochemical analyses	CGC haplotype has lower WC, BMI, and leptin levels. The CAT haplotype associated with MetS.
Garaulet et al. 2009 [8]	US white population, mean age 48 years (n=1100)	Cross-sectional study	Tag SNPs	Anthropometric variables	Carriers of the CGA (rs3749474/rs45807/rs1801260) haplotype had lower BMI, WC, BP, and IR.
Allebrandt et al. 2010 [1]	Central Europe, Estonia, and South Tyrol subjects (n=77,000)	Meta-analysis	rs12649507 and rs11932595	Chronotype questionnaire	Haplotype GGAA of rs12649507/rs11932595 is associated with long sleep.
Garaulet et al. 2010 [10]	Overweight/obese southeastern Spain subjects 20–65 years (n=500)	Weight loss intervention	rs3749474, rs4864548, rs1464490, and rs1801260	Anthropometric, biochemical, and dietary intake variables	Carriers of the C allele (rs1801260) had higher risk for obesity and were less responsive to a weight loss intervention.
Garaulet et al. 2010 [9]	US white population European descent, mean age 48 years (n=1100)	Cross-sectional study	rs3749474, rs4864548, rs1464490, and rs4580704	Anthropometric and biochemical determinations, dietary intake by questionnaire	Association of rs3749474 with total energy intake.
Garaulet et al. 2011 [11]	Overweight/obese southeastern Spain subjects 20–65 years (n=1495)	Weight loss intervention	rs1801260	Biochemical and intake. Sleep and chronotype (questionnaire)	Allele C carriers had more difficulty losing weight and higher plasma ghrelin levels.
Garaulet et al. 2012 [12]	Overweight/obese southeastern Spain subjects 20–65 years (n=1465)	Weight loss intervention	rs1801260	Anthropometric, biochemical, and dietary intake variables	The C allele had a higher resistance to weight loss, higher intake of SF, and higher plasma ghrelin levels.
Lane et al. 2013 [19]	European ancestry 29–100 years (n=4987)	Cross-sectional study	rs11932595, rs6843722, and rs12649507	SD by self-reported and polysomnography	Not significantly associated with sleep.
Bandín et al. 2013 [2]	Overweight/obese subjects from southeastern Spain mean of BMI 28.6 (n=85)	Cross-sectional study	rs1801260	Anthropometric, chronotype, and biochemical variables	C carriers allele are less active and evening-type.
Giovaninni et al. 2014 [14]	Children from Sao Paulo (Brazil) 6–13 years (n=370)	Cross-sectional study	rs1801260	Anthropometric variables, hours of sleep by questionnaire	*CLOCK* genotype not associated with overweight or sleep duration.
García-Ríos et al. 2014 [13]	Subjects with MetS from Spain (n=475)	Intervention of low-fat diet	rs1801260, rs3749474, and rs4580704	HOMA-IR	TT of rs1801260 lower plasma insulin, IR and higher IS after 12 months of intervention.
Dashti et al. 2015 [4]	European descent (n=14,906)	Meta-analysis	9 SNPs	SD, BMI, MI	rs12649507 G allele: Higher PUFA intake and more sleep. rs6858749 T allele: Lower protein intake with each additional hour of sleep.
Gomez-Delgado et al. 2015 [15]	Patients with coronary heart disease from Spain (n=897)	Cross-sectional study under low-fat diet and Mediterranean diet	rs1801260, rs3749474, and rs4580704	Inflammation status and markers of lipid metabolism	C/C genotype displayed a greater decrease in high sensitivity C-reactive protein and a significant increase in HDL/apolipoprotein A1 ratio.

SNP single nucleotide polymorphism, *SD* sleep duration, *BMI* body mass index, *MI* macronutrient intake, *PUFA* polyunsaturated fatty acids, *WC* waist circumference, *BP* blood pressure, *IR* insulin resistance, *SF* saturated fats, *MetS* metabolic syndrome, *IS* insulin sensitivity

region of the *CLOCK* gene in different populations and to link it with other pathologies, such as obesity, involved with *CLOCK* functions. The Iwase et al. [17] study is interesting because it is the only one in which the entire *CLOCK* coding region has been analyzed to find new polymorphisms or mutations, and it should be an interesting study to replicate in populations of obese people.

Collectively, there is an absence of studies that measure the association between *CLOCK* gene variants and sleep duration in different populations, especially using an objective assessment of sleep, such as actigraphy. Additionally, it would be relevant to investigate the frequency of *CLOCK* gene variants in different populations and associate these frequencies with the amount of sleep in free-living conditions. It is also very important to investigate how *CLOCK* variants predispose a person to developing obesity when they are exposed to environmental conditions, such as reduced sleep. This concept is especially important in today's society in which many people are sleep deprived [20, 29].

Associations of *CLOCK* variants with BMI and metabolic syndrome

There are pathways that connect the central nervous system (CNS) and peripheral tissues, which allow the CNS to activate or silence peripheral tissues at different times of the day. Thus, an imbalance between the rhythms of different fat compartments differentially controlled by the autonomous nervous system and in turn by the CNS may be a possible cause of the metabolic syndrome [18]. Related to this, *CLOCK* polymorphisms have been described and associated with obesity or eating behavior traits. The variant 3111T>C (rs1801260) in conjunction with a specific haplotype (a combination of alleles at different loci on a chromosome that are transmitted together) has been associated with obesity and metabolic syndrome in different populations [8, 10, 11, 25]. It was determined that carriers of the C minor allele of the rs1801260 polymorphism were more resistant to weight loss than individuals with TT [11, 12]. Additionally, overweight/obese individuals

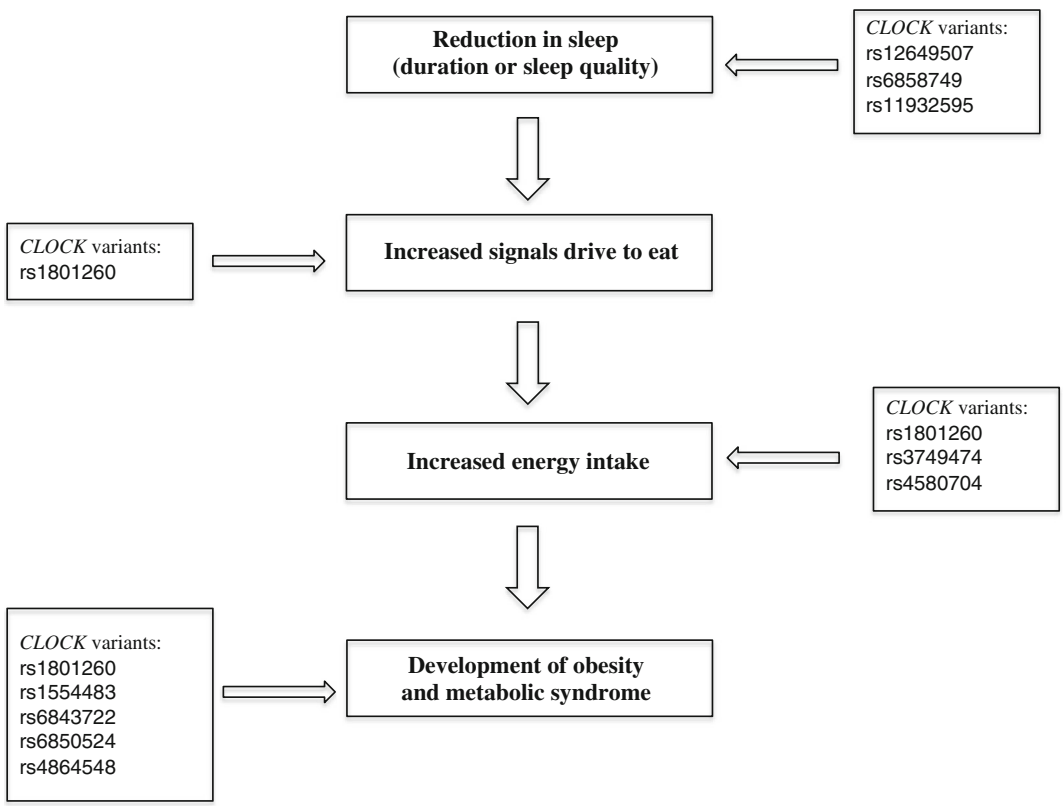

Fig. 1 Role of CLOCK genetic variants on the association of sleep reduction and obesity. *CLOCK* genetic variants may influence at diverse stages the association between sleep reduction and obesity. The figure shows different *CLOCK* genetic variants that have been described to be associated with reduction in sleep, energy intake, and development of obesity

carrying the CC rs1801260 genotype had significantly higher BMI values compared to those carrying the CT or TT genotypes [22]. Moreover, in the same study, obese class III individuals had a significantly higher frequency of both the CC genotype and the C allele compared to individuals with a BMI <40 kg/m^2. Additionally, the TT genotype of rs1801260 showed lower plasma insulin and insulin resistance, as well as higher insulin sensitivity, after 12 months of diet intervention [13]. Additionally, C carriers of rs1801260 were associated with an evening chronotype, which was also related to an obesity phenotype [2, 21]. However, the rs1801260 variant was not significantly associated with being overweight or with sleep duration in children [14].

In addition, the *CLOCK* polymorphisms rs1554483, rs6843722, rs6850524, and rs4864548 have been associated with overweight and obesity in an adult population [26], showing that carriers of haplotypes rs1554483-G and rs4864548-A have a 1.5 times (confidence interval (CI): 1.31, 2.54) greater risk of becoming overweight or obese. In another study, carriers of haplotype CGC (rs4864548, rs3736544, and rs1801260) were protected against the development of overweight and obesity, supporting the hypothesis that genetic variations of the *CLOCK* gene may play an important role in the development of metabolic syndrome, type 2 diabetes, and cardiovascular disease [25].

The observations found between the rs1801260 variant, obesity, and certain features of eating behavior can be explained by the fact that this polymorphism produces changes *in* the stability and half-life of mRNA, which influences mRNA translocation. Therefore, CLOCK protein levels affect circadian clock organization, sleep duration, and, consequently, eating behavior traits [17].

Eating behavior associated with genetic variants of *CLOCK* gene

One explanation of the relationship between *CLOCK* gene and obesity is that this gene regulates aspects of energy intake. In the case of the variant rs3749474, carriers of the minor T allele reported significantly higher energy intake than non-carrier subjects [8], which is concordant with a study by Garaulet et al. [9]. For rs4580704, the carriers of the minor C allele reported the lowest values of energy intake after adjusting for sex, age, family relationships, and BMI [8]. However, the authors did not achieve statistical significance with the rs1801260 SNP. Consistent with this, the homozygotes CC displayed a significant increase in HDL/apolipoprotein A1 ratio under a low fat diet [15]. These results show an association between SNP rs4580704 with energy intake aspects.

There is a definite lack of studies that associate *CLOCK* variants with different eating behavior traits, which could be assessed using validated questionnaires. For example, eating behavior traits measured with the Three-Factor Eating Questionnaire (TFEQ) have been previously associated with the *MC4R*, *MC3R*, *LEP*, and *LEPR* genes [23, 27, 28]. The role of *CLOCK* genetic variants on the association between insufficient sleep and obesity is summarized in Fig. 1.

Differences found in the same variant in different populations may be due to a linkage disequilibrium or the frequency of the genotypes in each population. Additionally, these differences may be due to the contribution of other variants of the same gene or other genes in different populations or a heterogeneity of phenotypes in which the number of subjects tested is not representative of the distribution of sleep duration.

Conclusion

There are some *CLOCK* variants that are related with obesity and short sleep duration. However, this type of research needs to be performed in other populations in which other genetic variants could be identified. Identifying at risk *CLOCK* genotypes is a new area of study with potential relevance for establishing sleep duration and eating behavior requirements to help with the prevention and treatment of obesity.

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References

1. Allebrandt KV, Teder-Laving M, Akyol M, Pichler I, Müller-Myhsok B, Pramstaller P et al (2010) CLOCK gene variants associate with sleep duration in two independent populations. Biol Psychiatry 67:1040–1047
2. Bandín C, Martinez-Nicolas A, Ordovás JM, Ros Lucas JA, Castell P, Silvente T et al (2013) Differences in circadian rhythmicity in CLOCK 3111T/C genetic variants in moderate obese women as assessed by thermometry, actimetry and body position. Int J Obes (Lond) 37:1044–1150

3. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA (2010) Sleep duration and all-cause mortality: a systematic review and meta-analysis of prospective studies. Sleep 33:585–592
4. Dashti HS, Follis JL, Smith CE, Tanaka T, Cade BE, Gottlieb DJ et al (2015) Habitual sleep duration is associated with BMI and macronutrient intake and may be modified by CLOCK genetic variants. Am J Clin Nutr 101:135–143
5. Dean E, Bloom A, Cirillo M, Hong Q, Jawl B, Jukes J, Nijjar M et al (2012) Association between habitual sleep duration and blood pressure and clinical implications: a systematic review. Blood Press 21:45–57
6. Friedman JM (2000) Obesity in the new millennium. Nature 404:632–634
7. Froy O (2010) Metabolism and circadian rhythms—implications for obesity. Endocr Rev 31:1–24
8. Garaulet M, Lee YC, Shen J, Parnell LD, Arnett DK, Tsai MY et al (2009) CLOCK genetic variation and metabolic syndrome risk: modulation by monounsaturated fatty acids. Am J Clin Nutr 90:1466–1475
9. Garaulet M, Lee YC, Shen J, Parnell LD, Arnett DK, Tsai MY et al (2010) Genetic variants in human CLOCK associate with total energy intake and cytokine sleep factors in overweight subjects (GOLDN population). Eur J Hum Genet 18:364–369
10. Garaulet M, Corbalán MD, Madrid JA, Morales E, Baraza JC, Lee YC et al (2010) CLOCK gene is implicated in weight reduction in obese patients participating in a dietary programme based on the Mediterranean diet. Int J Obes (Lond) 34:516–523
11. Garaulet M, Sánchez-Moreno C, Smith CE, Lee YC, Nicolás F, Ordovás JM (2011) Ghrelin, sleep reduction and evening preference: relationships to CLOCK 3111 T/C SNP and weight loss. PLoS One 6:1–7
12. Garaulet M, Tardido A, Lee YC, Smith CE, Parnell LD, Ordovás JM (2012) SIRT1 and CLOCK 3111T>C combined genotype is associated with evening preference and weight loss resistance in a behavioral therapy treatment for obesity. Int J Obes (Lond) 36:1436–1441
13. Garcia-Rios A, Gomez-Delgado FJ, Garaulet M, Alcala-Diaz JF, Delgado-Lista FJ, Marin C (2014) Beneficial effect of CLOCK gene polymorphism rs1801260 in combination with low-fat diet on insulin metabolism in the patients with metabolic syndrome. Chronobiol Int 31:401–408
14. Giovaninni NP, Fuly JT, Moraes LI, Coutinho TN, Trarbach EB, Jorge AA et al (2014) Study of the association between 3111T/C polymorphism of the CLOCK gene and the presence of overweight in schoolchildren. J Pediatr (Rio J) 90:500–505
15. Gomez-Delgado F, Garcia-Rios A, Alcala-Diaz JF, Rangel-Zuñiga O, Delgado-Lista J, Yubero-Serrano EM et al. (2015) Chronic consumption of a low-fat diet improves cardiometabolic risk factors according to the CLOCK gene in patients with coronary heart disease. Mol Nutr Food Res:1–9
16. Guo X, Zheng L, Wang J, Zhang X, Zhang X, Li J, Sun Y (2013) Epidemiological evidence for the link between sleep duration and high blood pressure: a systematic review and meta-analysis. Sleep Med 14:324–332
17. Iwase T, Kajimura N, Uchiyama M, Ebisawa T, Yoshimura K, Kamei Y et al (2002) Mutation screening of the human Clock gene in circadian rhythm sleep disorders. Psychiatry Res 109: 121–128
18. Kreier F, Kalsbeek A, Ruiter M, Yilmaz A, Romijn JA, Sauerwein HP et al (2003) Central nervous determination of food storage—a daily switch from conservation to expenditure: implications for the metabolic syndrome. Eur J Pharmacol 480:51–65
19. Lane JM, Tare A, Cade BE, Chen TH, Punjabi NM, Gottlieb DJ et al (2013) Common variants in CLOCK are not associated with measures of sleep duration in people of European ancestry from the Sleep Heart Health Study. Biol Psychiatry 15:33–35
20. Matricciani L, Olds T, Petkov J (2012) In search of lost sleep: secular trends in the sleep time of school-aged children and adolescents. Sleep Med Rev 16:203–211
21. Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y (2005) The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample. Am J Med Genet B Neuropsychiatr Genet 133:101–104
22. Monteleone P, Tortorella A, Docimo L, Maldonato MN, Canestrelli B, De Luca L, Maj M (2008) Investigation of 3111T/C polymorphism of the CLOCK gene in obese individuals with or without binge eating disorder: association with higher body mass index. Neurosci Lett 11:30–33
23. Obregón AM, Amador P, Valladares M, Weisstaub G, Burrows R, Santos JL (2010) Melanocortin-3 receptor gene variants: association with childhood obesity and eating behavior in Chilean families. Nutrition 26:760–765
24. Rankinen T, Bouchard C (2006) Genetics of food intake and eating behavior phenotypes in humans. Annu Rev Nutr 26: 413–434
25. Scott EM, Carter AM, Grant PJ (2008) Association between polymorphisms in the Clock gene, obesity and the metabolic syndrome in man. Int J Obes (Lond) 32:658–662
26. Sookoian S, Gemma C, Gianotti TF, Burgueño A, Castaño G, Pirola CJ (2008) Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. Am J Clin Nutr 87:1606–1615
27. Valladares M, Domínguez-Vásquez P, Obregón AM, Weisstaub G, Burrows R, Maiz A et al (2010) Melanocortin-4 receptor gene variants in Chilean families: association with childhood obesity and eating behavior. Nutr Neurosci 13:71–78
28. Valladares M, Obregón AM, Weisstaub G, Burrows R, Patiño A, Ho-Urriola J et al (2015) Association between feeding behavior, and genetic polymorphism of leptin and its receptor in obese Chilean children. Nutr Hosp 31:1044–1051
29. WB&A Market Research: National Sleep Foundation. Sleep in America poll. Washington DC: National Sleep Foundation 2002. Available via DIALOG. http://www.sleepfoundation.org/article/sleep-america-polls/2002-adult-sleep-habits. Accessed 10 June 2015