Chapter 8
Genetics in Chronobiology and Obesity

Marta Garaulet and Jose M. Ordovás

Abstract Genetics is behind our circadian machinery. Some of our chronobiological characteristics could be influenced by genes. Different psychological traits such as depression, bipolar disorders, anxiety and seasonal variations of mood are intrinsically connected to chronobiology through different genetic variants. Moreover, sleep disorders or short sleep duration, are both associated to several polymorphisms connected to obesity. In this regards, one of the most outstanding SNPs is the *CLOCK* 3111TC SNP which is significantly associated to short sleep duration, eveningness, several psychological traits and obesity. This SNP has been also related to a reduction in weight loss effectiveness in patients submitted to a behavioral treatment of obesity. Ghrelin, eveningness, and a lack of compliance to the Mediterranean diet habits, could be behind these results. Apart from *CLOCK* SNPs, others genetic variants in several clock genes such as *PERIOD* or *BMAL1* are also connected to obesity. The novel knowledge achieved in the circadian epigenome could give us new answers to the connections among genetics, circadian rhythmicity and obesity.

M. Garaulet, Ph.D. (✉)
Department of Physiology, University of Murcia, Murcia, Spain

J.M. Ordovás, Ph.D.
Functional Genomics Core, Nutrition and Genomics Laboratory, Jean Mayer USDA, HNRCA at Tufts University, 711 Washington Street, Boston, MA, 02111-1524, USA

The Department of Epidemiology and Population Genetics, Centro Nacional Investigaciones Cardiovasculares (CNIC), Instituto Madrileño de Estudios Avanzados en Alimentación (IMDEA-FOOD), Madrid, Spain
e-mail: jose.ordovas@tufts.edu

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Abbreviations

SNP	Single nucleotide polymorphism
CLOCK	Circadian locomotor output cycles kaput
PER	Period homolog 2 (Drosophila)
BMAL1 or ARNTL or MOP3	Aryl hydrocarbon receptor nuclear translocator-like
HGP	Human genome project
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
GH	Growth hormone
SCN	Suprachiasmatic nucleus
MetS	Metabolic syndrome
MD	Mood disorders
OMIM	Online Mendelian inheritance in man
CRY	Cryptochrome
REV-ERBα	Nuclear receptor Rev-ErbA-alpha
SIRT	Sirtuin
RORA or NR1D1	RAR-related orphan receptor A
VIP	Vasoactive intestinal polypeptide
ROR1	Receptor tyrosine kinase-like orphan receptor 1
PLCB1	Phospholipase C, beta 1
OSAS	Obstructive sleep apnea syndrome
MTNR1A	Melatonin receptor 1A
MTNR1B	Melatonin receptor 1B
GWAS	Genome-wide association studies
NFATC2	Nuclear factor of activated T cells 2
SCP2	Sterol carrier protein 2
CACNA1C	Calcium channel, voltage-dependent, L type, alpha 1C subunit
TCRA	T cell receptor alpha chain
POLE	Polymerase (DNA directed), epsilon
FAM3D	Family with sequence similarity 3, member D
ABCC9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
SUR2	Potential sterol desaturase similar to *S. cerevisiae*
HLA	Human leukocyte antigen
DQB1	Major histocompatibility complex, class II, DQ beta 1
PSD	Partial sleep deprivation
NPAS2	Neuronal PAS domain protein 2
APSS	Associated Professional Sleep Societies LLC
FTO	Fat mass and obesity associated
HOMA-IR	Homeostasis model assessment- insulin resistance

TMEM18	Transmembrane protein 18
NRXN3	Neurexin 3
BMI	Body mass index
GOLDN	Genetics of Lipids Lowering Drugs and Diet Network
FAs	Fatty acids
MUFA	Monounsaturated fatty acid
SFA	Saturated fatty acid
MCP1	Monocyte chemoattractant protein 1
IL-6	Interleukin 6
PTMs	Post translational modifications
HAT	Histone acetile transferase
BMI	Body mass index
SAT	Saturated fatty acids
MUFA	Monounsaturated fatty acids

The Human Genome Revolution: Feast or Famine?

The official presentation of the final Human Genome Project (HGP) was carried out in April, 2003 coinciding with the 50th anniversary of the seminal publication of the structure of the double helix of DNA for Watson and Crick. Though, already in the year 2001, the drafts of the human genome were published in two of the most prestigious scientific journals, Nature and Science. Shortly after, in 2007, the first two individual sequences were accomplished, one of them, belonging to James Watson was announced in Nature News on June 1 and the other, belonging to Craig Venter was published in PLOS Biology on September 4. Since then, thousands of genomes have been completed at incredibly faster speed and lower cost. The ultimate goal will be to use this information to develop new ways to treat, cure, or even prevent the thousands of diseases that afflict humankind.

However, the HGP has failed so far to produce the announced health revolution that some scientists, and other members of the society, had promised in a relatively short time. Indeed, the concept of "personalized medicine" that was supposed to provide with and make use of tests informing a person's risk for heart disease, cancer and other common illnesses, is demonstrating to be not as simple as some may have anticipated. Conversely, we are continuously learning about previously unknown levels of biological complexity, including not only the polygenic nature of all common diseases, but also the role of multiple epigenetic mechanisms that are heavily influenced by environmental factors.

Therefore, our current view shows that most common diseases arises from regulatory or structural dysfunctions of multiple genes that interact with a myriad of environmental and behavioral factors, including our own microbiota.

The Birth of New Genetic-Related Sciences: Nutrigenetics, Nutrigenomics and Epigenetics

The new perspective generated by the HGP and the ensuing research has fostered and intensified some areas of more specialized genetic research aimed to advance our knowledge and to translate this knowledge into practical applications

Among these new areas, we could highlight *Nutritional Genomic* which focuses on the relationship between human genome, nutrition and health that can be divided into two subspecialties:

(a) *Nutrigenomic* which studies the effect of nutrients on health through altering genome, proteome, metabolome and the resulting changes in physiology. For example, recently, it has been discovered that the health effects of food compounds are related mostly to specific interactions on molecular level, i.e., dietary constituents participate in the regulation of gene expression.
(b) *Nutrigenetic* which studies the effect of genetic variations on the interaction between diet and health with implications to susceptible subgroups. The genetic variation or SNPs (Single Nucleotide Polymorphisms) are changes in only one nucleotide that is the most frequent cause of the different responses to a diet or a drug. More specifically, Nutrigenetic studies how individual differences in genes influence the body's response to diet and nutrition. This necessitates the identification of gene variants associated with differential responses to nutrients and with higher susceptibility to diet-related diseases. The ultimate goal of nutrigenetics is to provide nutritional recommendations for individuals in what is known as personalized or individualized nutrition.

Another blossoming area relates to *Epigenetics* which is the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence—hence the name epi- (Greek: επ - over, above, outer) -genetics. It refers to functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence. Examples of such changes are DNA methylation and histone modification, both of which serve to regulate gene expression without altering the underlying DNA sequence. Moreover, as recently as 2011, it has been demonstrated that the methylation of mRNA has a critical role in human energy homeostasis, opening the related field of RNA epigenetics.

How Could Genetics Be Associated to Chronobiology? A Little Bit of History

The capacity to undergo rhythmic oscillations is a characteristic intrinsic to living matter. A fundamental statement of chronobiology states "many rhythms persist even in complete isolation from the major known environmental cycles." This concept supports that natural rhythms can exist independently of the periods defined by

geophysical cycles; this means that living matter has its own time, i.e., the "biological time." In this sense, it has been hypothesized the existence of a Chronome within the Genome [1].

Over the past two decades, biochemical, genetic, and molecular studies have been making substantial advances towards the elucidation of the molecular bases of rhythmicity in living things. Riding on the wave generated by the seminal studies in the 1970s focusing on in the circadian variability of hormones such as cortisol, melatonin or growth hormone (GH), or those related to the discovering and description of the physiological bases of the suprachiasmatic nucleus (SCN), current chronobiology has dramatically evolved thanks to the new genetic and molecular biology techniques.

A major stride in understanding the molecular basis of circadian rhythms was the identification by Konopa and Benzer in 1971 of a chromosomal region controlling the period of eclosion time in Drosophila, followed by the cloning of the first clock genes in *Drosophila melanogaster* in 1984. Today, thanks to these molecular techniques, we are able to study the expression of the known clock genes implicated in the circadian machinery. We already know that, in mammals, the core components of the clock molecular machinery operate in almost all cells of the body through a complex network of transcriptional-translation loops and modulate the expression of specific target genes and their products to oscillate in 24-h rhythm [2].

Nowadays, experimental models are allowing us to assess clock genes expression not only in the living animal but also outside of the body (in vitro techniques) and we are also able to analyze the 24 h fluctuations in gene expression and to assess the presence or absence of a peripheral clock in the different organs and tissues (see Chap. 2). Moreover, we can use experimental models to turn on and off specific components of the clock machinery to identify its effects on metabolic and disease phenotypes. From the genetic epidemiology point of view, the study of single nucleotide polymorphisms (SNPs), is contributing to the identification of the genetic background of chronotypes (morningness or eveningness), sleep alterations, or seasonal mood disorders.

More recently, epigenetic and nutrigenetic approaches have also been allowing us to study new interactions and layers of complexity that may have a significant impact on chronobiology as well as pathophysiology

Finally, the technological power of other "-omics" (i.e., metabolomics, proteomics) is becoming essential to our ability to "put-it-all-together" and we are fast learning about the timing of different metabolites such as aminoacids, lipids, xenobiotic, etc. in the liver in mice [3], and in plasma and saliva in humans [4], allowing us to achieve a more complete and refined knowledge of the circadian rhythm and its physiological effects. These advances have given to the science of chronobiology a renewed stimulus that makes this science increasingly robust and attractive.

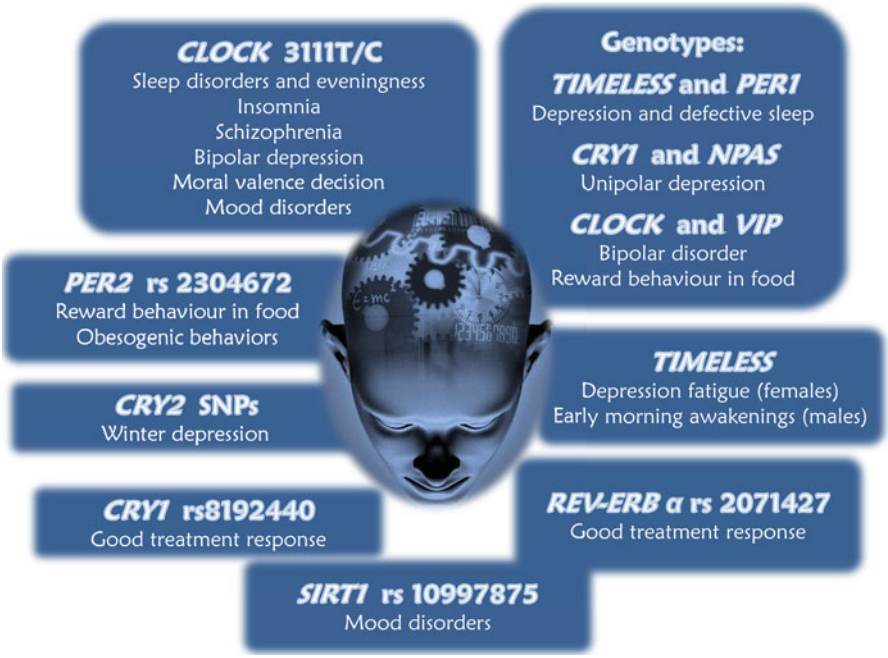

Fig. 8.1 Main genetic variants related to psychological traits

Genetic, Chronotype, and Psychological Traits

As shown in other chapters, there are individual differences in preferred times for activity and rest. The characteristics that differentiate "owls" from "larks" may reside primarily on their circadian systems, and presumably genetics prevail over environmental factors.

Initially, the emphasis of chronobiology genetics was placed on the study of the association between chronotype and different psychological illnesses such as depression, anxiety, or bipolar disorder. Other mood features that have been associated with clock genes are stress, seasonality, and personality traits related to the chronotype such as the morningness/eveningness profile.

Taking into account that obesity is related to behavior and also with personality traits, and considering that the chronotype is behind some of these relationships, an obvious step was to link these psychological-related SNPs with obesity-related traits. Proof of this hypothesis is the case of circadian locomotor output cycles kaput (*CLOCK)* and *PERIOD2* (*PER2)* SNPs that were firstly associated with mood disorders and then to obesity and Metabolic Syndrome (MetS) (Fig. 8.1).

The initial studies of clock genes and their association with psychological traits come from the observation that patients with Mood Disorders (MD) commonly show biological rhythm-related symptoms, such as characteristic disturbances in

the sleep/ wake cycle, diurnal mood changes, and a periodic pattern of symptom recurrence and remission [5]. In addition, alterations in the circadian pattern of core body temperature and neuroendocrine secretion have been also documented in psychological illnesses. [6] On the other hand, mood-stabilizing drug such lithium or antidepressants such as fluoxetin [7] are known to modulate circadian rhythms [8].

Probably the most studied of the circadian genes has been the *CLOCK* gene. The CLOCK locus is located on the long arm of chromosome 4q12 (Online Mendelian Inheritance in Man (OMIM) *601851, 25 exons in the genomic region spanning 115.138 kb). The interest of this gene is that its translation product is involved in the transcriptional regulation of many circadian output genes and in the core circadian clock.

A common 3111T→C SNP at the 3′-untranslated region of *CLOCK* gene contributes to our ability to classify people as "larks" or "owls." Thus, individuals carrying the 3111C allele tend to define themselves as nocturnal more often than homozygotes for 3111T allele.

Moreover, different psychological traits have been related to this SNP. For example, it has been found an interesting association between *CLOCK* 3111T/C and *attention deficit hyperactivity disorder* [9], psychological related *insomnia* [10], *Schizophrenia* [11] *bipolar depression* [12], *moral valence decision* [13] and *mood disorders* [14]. The association with all these psychological illnesses is related to the fact that minor allele carriers of *CLOCK* 3111TC display sleep disorders and eveningness [15], characteristics that, in addition, make these subjects susceptible to obesity.

Other clock genes have been also related to mood disorders or behaviors associated with obesity. This is the case of *PERIOD2* (*PER2).* A *PER2* SNP (rs2304672) has been shown to moderate *circadian-relevant reward circuitry* activity in adolescents. *Reward behavior* in animals is highly related to food intake and is influenced by circadian genes, including clock-pathway genes such as *PER2*. Several forms of psychiatric illness are associated with both altered reward function and disturbances in circadian function. Associations among circadian genes function in neural reward circuits, and circadian-influenced behavior could be important in obesity. Indeed, in a further work we have related this SNP with attrition during a weight loss treatment and with obesogenic behaviors such as stress with dieting, snacking, or eating when bored.

Cryptochrome genes (*CRY1 and CRY2*) code for the two cryptochrome proteins CRY1 and CRY2 act as light-independent inhibitors of CLOCK-BMAL1 components of the circadian clock and specifically, CRY2 participates in the regulation of the evening oscillator. This is of interest in mood disorders where a deficient switch from evening to morning oscillators has been postulated [5]. Indeed, in *depressed bipolar* patients, levels of *CRY2* mRNA are decreased and there is no response following sleep deprivation. These investigators have shown that genetic variation at the *CRY2* gene was significantly associated with winter depression in two independent population-based samples from Sweden and Finland [16].

Other clock genes have been also related to different behaviors or mood disorders. A functional polymorphism in the *REV-ERBα* (rs2071427) locus and a second variant in *CRY1* (rs8192440), both were nominally associated with positive response to psychological treatment [17]. Moreover, several recent investigations have impli-

cated *SIRT1* in the regulation of the circadian system in combination with the traditional circadian clock genes, and with the dopaminergic pathway. Therefore, the *SIRT1* locus has been added to the rank of candidate genes involved in mood disorders and this is supported by reported associations between the SIRT1 rs10997875 SNP and mood disorders [18].

Other authors have found significant associations between *TIMELESS* variants and depression with *fatigue* in *females*, and association to depression with *early morning awakening* in *males* [19]. Indeed, there was a significant interaction of gender and *TIMELESS*. Authors also obtained supported evidence for involvement of *TIMELESS* in sleeping problems in an independent set of control individuals with seasonal changes in mood, sleep duration, energy level and social activity in females and with early morning awakening or fatigue in males.

There was also some evidence of interaction between *TIMELESS* and *PER1* in females as well as between *TIMELESS* and *ARNTL*, *RORA*, or *NR1D1* in males. These findings support a connection between circadian genes and gender-dependent depression and defective sleep [19]. Other authors have found a differential association of circadian genes with mood disorders: *CRY1* and *NPAS2* are associated with *unipolar major depression* and *CLOCK* and *VIP* with *bipolar disorder* [5]. All these genetic variants could be important in the pathophysiology of obesity, taking into account that many of these alterations are related to food intake and emotions, both aspects highly associated to obesity and weight loss.

Genetics in Sleep Disorders

Other candidate SNPs connected to obesity could be those associated to sleep disorders. The contribution of genes, environment, and gene–environment interactions to sleep disorders is increasingly recognized. Well-documented familial and twin sleep disorder studies suggest an important influence of genetic factors. Most sleep disorders are complex in terms of their genetic susceptibility together with the variable manifestation of the phenotype even within the same family. Recent linkage, genome-wide and candidate gene association studies resulted in the identification of gene mutations, gene localizations, or evidence for susceptibility genes and/or loci in several sleep disorders [20].

One common sleep disorder in the current society is insomnia which is reported to chronically affect 10–15% of the adult population. However, very little is known about the genetics and metabolism of insomnia. A study performed in 10,038 Korean subjects [21] showed that about 16.5% reported insomnia and displayed distinct metabolic changes reflecting an increase in insulin secretion, a higher risk of diabetes, and disrupted calcium signaling. Insomnia-associated genotypic differences were highly concentrated within genes involved in neural function. The most significant SNPs resided in ROR1 and PLCB1 genes known to be involved in bipolar disorder and schizophrenia, respectively [21].

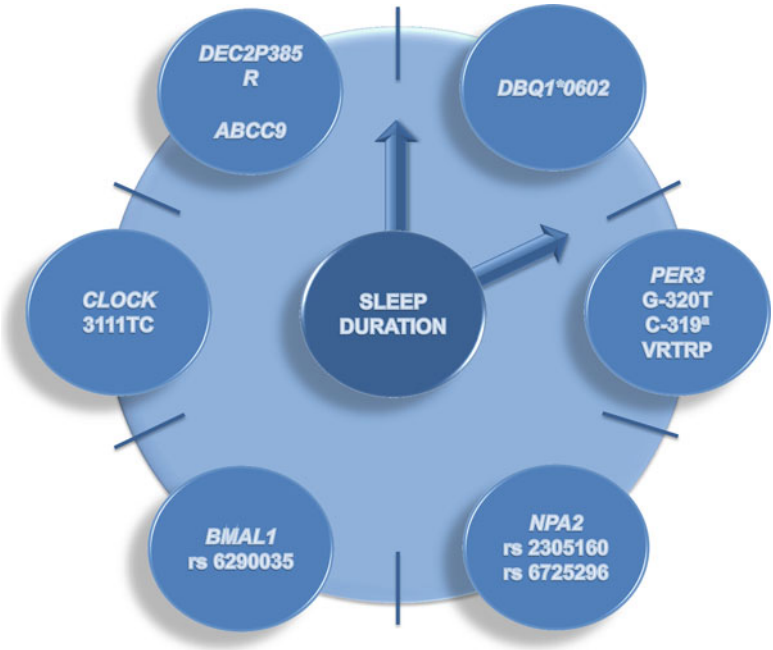

Fig. 8.2 Genetics of sleep duration

Another particular example of sleep disorder highly related to obesity is *Obstructive Sleep Apnea Syndrome* (*OSAS*), and it has been postulated that genetic variants in IL-6 could modify susceptibility to OSAS [22, 23]. They could also explain fatigue in different pathologies such as cancer [24] and could also link sleep with obesity and metabolic syndrome alterations, through inflammation. Others loci have been proposed in relation to sleep disorders; including serotonin receptors SNPs; β2-adrenergic receptor that are related with nocturnal asthma [25] and with nocturnal blood pressure dipping status [26] and the prepro-orexin gene polymorphism g1182C>T which associates with obstructive sleep apnea/hypopnea syndrome [27]. Particular relevant are the studies in SNPs in the promoter region of the melatonin receptor genes (*MTNR1A* and *MTNR1B*) [28] but their role in sleep disorders and its association with obesity and MetS still remain controversial [29].

Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness, cataplexy, and a pathological manifestation of rapid eye movement during sleep. Narcoleptic pathogenesis is triggered by both genetic and environmental factors. Genome-wide association studies (GWAS) have identified over 200 new genetic factors. Following replication in 222 narcoleptic patients and 380 controls, six genes, *NFATC2*, *SCP2*, *CACNA1C*, *TCRA*, *POLE*, and *FAM3D*, remained associated with narcolepsy [30]. One can speculate that some of these loci could contribute to our understanding of the relation between sleep disorders, obesity and other metabolic diseases.

Genetics in Sleep Duration

As has been referred in Chap. 7, sleep duration is related to obesity and genetic variability could be implicated in this relationship. Indeed, in the last years a number of SNPs have been related to sleep duration and some of them are being further studied in obesity (Fig. 8.2).

One of the most promising findings relates to the identification of a P385R mutation in a transcriptional repressor (hDEC2-P385R) that was associated with a human short sleep phenotype. The habitual self-reported total sleep time per 24-h day was much shorter in mutation carriers (average 6.25 h) compared with the noncarriers (average 8.06 h). Thus, they represent "natural short sleepers" who routinely sleep less than individuals with familial advanced sleep-phase syndrome (FASPS) or general controls. In order to proof the functionality of this variant, a wild-type or a P385R mDec2 construct was used in a luciferase assay, and the results indicated that the activity profiles and sleep recordings of transgenic mice carrying this mutation showed increased vigilance time and less sleep time than control mice in a zeitgeber time- and sleep deprivation-dependent manner [31]. However, although short sleep has been related to obesity, to our knowledge there is no such study has been carried for DEC2-P385R

One particular example that could link *sleep duration and obesity* is *ABCC9,* for which functional studies have shown that the protein plays a role in the pathogenesis of heart disease and diabetes and which also influences the duration of sleep in humans. The specific variant (rs11046205) was first discovered in a GWAS that investigated sleep patterns. More than 4,000 people from seven European populations, from countries as diverse as Estonia and Italy, took part in the project, and filled out a questionnaire designed to assess their sleeping habits. Analysis of the genetic and behavioral data revealed that individuals who had two copies of the minor ABCC9 rs11046205 A allele generally slept for a significantly shorter period (~5% less) in an undisturbed environment than did persons homozygotes for the common G allele [32]. The gene *ABCC9* codes for the protein SUR2, which forms the regulatory component of a potassium channel in the cell membrane. This ion channel acts as a sensor of energy metabolism in the cell.

Other authors have proposed the human leukocyte antigen *(HLA) DQB1*0602* allele may represent a genetic biomarker for predicting individual differences in both basal and sleep loss conditions. The influence of the *DQB1*0602* allele on sleep homeostatic and neurobehavioral responses has been examined in chronic partial sleep deprivation (PSD) [33] and in healthy subjects.

An interesting study that could further connect sleep with obesity is the one performed in *PERIOD3* (*PER3*) by Archer et al. [34] who screened the *PER3* promoter for polymorphisms and investigated the phenotypic associations of these polymorphisms with diurnal preference, delayed sleep phase disorder/syndrome and their effects on reporter gene expression. Authors demonstrated that SNPs G-320T, C-319A, occurred more frequently in sleep phase disorder subjects compared to morning or

evening type and that polymorphisms in the *PER3* promoter could affect its expression, leading to potential differences in the observed functions of *PER3* [34].

Another study, in which the population was stratified according to homozygosity for a variable-number (4 or 5) tandem-repeat polymorphism in the coding region of the clock gene *PER3,* indicated that this polymorphism conferred vulnerability to sleep loss and circadian misalignment through its effects on sleep homeostasis. Indeed, in the vulnerable genotype, activation in a posterior prefrontal area was already reduced when comparing the evening to the morning during a normal sleep–wake cycle. Furthermore, in the morning after a night of sleep loss, widespread reductions in activation in prefrontal, temporal, parietal and occipital areas were observed in this genotype [35]. It remains to be investigated whether this different vulnerability to sleep loss is also related to food intake, obesity or MetS characteristics.

Other clock genes are *NPAS2* and *BMAL 1* (or ARNTL) both important genes in the positive control of the clock machinery. In a study performed to assess seasonality and fertility in adults living in Finland [36] it has been concluded that *NPAS2* rs2305160 A allele carriers had lower Global Seasonality Scores, a sum score of six items, i.e., seasonal variation of sleep length, social activity, mood, weight, appetite, and energy level. Furthermore, carriers of the NPAS2 rs6725296 A allele had greater loadings on the metabolic factor (weight and appetite) of the global seasonality score, whereas individuals with ARNTL rs6290035 TT genotype experienced less seasonal variation of energy level. Considering these interesting results, further studies should get into these particular SNPs to assess their potential association with obesity.

Another obvious candidate gene potentially bridging sleep and obesity disorders is *CLOCK*. Along these lines, we have proposed that the association of the *CLOCK* 3111T/C SNP with obesity or weight loss could be mediated by sleep reduction and by ghrelin, connecting sleep, energy intake, and obesity genetics [15].

An association between variants of the human *CLOCK* gene and sleep duration has been reported in two independent populations [15]. In this study, sleep duration was assessed in Central Europe, Estonia, and South Tyrol ($n \sim 77{,}000$) with the Munich ChronoType Questionnaire. A follow-up association study was conducted with subjects from South Tyrol with short (<7 h) and long (>8.5 h) sleepers from Estonia (confirmation sample; $n = 1{,}011$). One hundred ninety-four SNPs covering 19 candidate clock genes were genotyped. From all these SNPs, single and multimarker associations were found within a CLOCK gene intronic region (rs12649507 and rs11932595). Moreover, in the meta-analysis between South Tyrol and Estonia association signals, rs12649507 remained significant.

Although there are multiple SNPs related to sleep that could consequently be associated with obesity, we need to consider that *sleeping less at night may increase the expression of genetic risks for obesity*, while getting enough sleep may suppress genetic influences on body weight [37]. Indeed, in this study, authors indicate that the heritability of BMI when sleep duration equaled 7 h was more than twice as large as the heritability of BMI when sleep duration equaled 9 h.

According to Nathaniel Watson, "there appears to be something about short sleep that creates a permissive environment for expression of obesity-related genes"[36]. Consistent with this notion, a recent work [38], has demonstrated in adolescents that

carriers of the TT allele (Risk allele) for one of the most important SNPs related to obesity the FTO SNP, exhibited associations between decreasing sleep duration and increasing BMI, waist circumference, visceral fat and Homeostasis model assessment-insulin resistance (HOMA-IR) (all $P<0.05$). Similar associations were observed in children with risk alleles (but not in those without risk alleles) for the *TMEM18* and *NRXN3* SNPs. On average, 2 h of sleep less per night was associated with an increase in body mass index (BMI) and with more waist circumference in genetically susceptible children.

Genetics in Chronobiology and Obesity

The great inter-individual differences observed in chronotype, responses to sleep curtailment, and association with obesity, point to an underlying genetic component, and some limited data suggest that common genetic polymorphisms involved in circadian regulation may underlie these large phenotypic differences. However, much more understanding is needed about the genetic basis of differential vulnerability in healthy subjects undergoing sleep deprivation, shift work, constant light exposure and snacking and the effects on obesity.

Animal Model

The current knowledge in the association between chronodisruption and obesity initially came from the studies in genetic mouse models of obesity which have demonstrated disrupted circadian sleep–wake patterns. Leptin-deficient ob/ob mouse and leptin-receptor db/db mouse show increased non-REM sleep time, decreased sleep consolidation, decreased locomotory activity, and a smaller compensatory rebound response to acute sleep deprivation [39, 40]. However, it was not until Turek et al. [41], study had been performed that the evidence of a molecular interaction between clock genes and obesity characteristics came out. This study revealed that mice with disruption of the Clock gene were prone to develop a phenotype resembling obesity and Mets. Previously, in 2004, Rudic et al. [42] already showed that mutations in *Clock* and *Bmal1* were associated with impaired glucose tolerance and more recently it has been also demonstrated that these mutant mice modified circadian variation in glucose and triglyceride [43].

A more recent article has shown that deficiency of, *Bmal1*, induces dyslipidemia and ectopic fat formation, indicating that *Bmal1* is involved in the utilization of fat as an energy source. Indeed, lack of *Bmal1* reduced the capacity of fat storage in adipose tissue, resulting in an increase in the levels of circulating fatty acids, including triglycerides, free fatty acids, and cholesterol. Elevation of the circulating fatty acids level induced the formation of ectopic fat in the liver and skeletal muscle [44].

Previous works have indicated that *Bmal*-deficient mice are characterized by having a greater amount of adipose tissue as compared to mice without this deficiency. However, there are conflicting studies suggesting that *Bmal1* plays a significant role in the regulation of adipose tissue differentiation, and also in lipogenesis of mature adipocytes.

Another interesting gene related to MetS is *Cry*. Indeed, transgenic mice overexpressing mutant *Cry1* develop symptoms of MetS, including polydipsia, polyuria, and hyperglycemia. A question that arises from these animal genetic models concerns whether the metabolic phenotypes are due to the disruption of the core clock mechanism itself, or whether they are secondary to altered feeding patterns present in these mice.

Human Genetics Studies

Given the above evidence in experimental models and the results of emerging epidemiological studies showing that alteration in circadian rhythmicity results in pathophysiological changes resembling MetS, there is active research examining the role of clock-related genes in human obesity and MetS alterations. So far, from the multiple genes within the clock machinery, *CLOCK* and *BMAL1* and *PERIOD* are the genes most frequently related to obesity (Table 8.1) and indication of the long way ahead.

CLOCK Gene and OBESITY

Since 2008, following the studies of Sookian et al. [45] in an Argentinean population, and those carried out by Scott's [46] group in an European population, it has been known that different *CLOCK* variants are associated with obesity and MetS, particularly with abdominal obesity [47].

Subsequently, our group has replicated these data in a North American white sample of 540 men and 560 women who participated in the Genetics of Lipids Lowering Drugs and Diet Network (GOLDN) [47]. In this population, *CLOCK* SNPs (rs3749474, rs4580704, and rs1801260 (3111TC)) were associated with body mass index (BMI), energy intake, and different variables related to obesity [48]. In fact, our results showed that individuals carrying the minor alleles ate more, slept less, ate more fat, and were more obese (Fig. 8.3). They particularly showed greater abdominal obesity, characterized as being the type of obesity with the greatest metabolic risk. Some of these associations may be functionally explained, such as the *CLOCK* polymorphism rs3749474, potentially leading to a change in mRNA structure that may affect its expression.

Table 8.1 Associations studies of Clock genes polymorphisms, obesity, and Metabolic Syndrome traits

Gene	SNP reference, genotype and Haplotype	Minor allele or Haplotype Frequency (%)	Population	Trait	Outcome	Reference
CLOCK	Haplotypes: (rs4864548/ rs3736544/rs1801260) CAT TGT CGC	CAT:31 TGT:33 CGC:28	537 men from White European population.	Metabolic syndrome.	No significant associations between any SNPs and the MetS subcomponents. The CGC haplotype may be protective for the development of obesity.	[46]
	rs1554483 C/G rs6843722 A/C rs6850524 G/C rs4864548 G/A Haplotype: rs1554483/rs4864548 CG/GA	G: 43 C: 41 C: 34 A: 43	Lean (n=715) and overweight or obese (n=391).	Overweight and obesity.	Four tSNP showed significant differences between lean and overweight/obese. No association was observed with MetS subcomponents. rs1554483G/ rs4864548A haplotype was associated with a 1.8-fold risk of obesity.	[45]
	rs1801260 3111T/C	C:53	284 Caucasian subjects including 92 normal weight and 192 over-weight/obese.	Binge eating disorders and BMI.	Genotype and allele frequencies did not significantly differ between normal weight and obese patients with and/or without BED but it seems to predispose obese individuals to a higher BMI.	[63]
	rs1801260 3111T/C	C:54	241 women including 90 healthy controls, 60 patients with anorexia and 91 patients with bulimia.	Anorexia and Bulimia and BMI.	3111T/C SNP does not play a major role in anorexia and bulimia, but it seems to predispose eating disorders patients to more severe lifetime body weight loss.	[64]

	rs1801260 3111T/C	C:47	500 patients attending to a weight loss program based in the Mediterranean diet.	Weight loss.	Carriers of the minor C allele were more resistant to weight loss than TT individuals.	[51]
	rs1801260 3111T/C	C:47	1,500 patients attending to a weight loss program based in the Mediterranean diet.	Weight loss, implications of ghrelin and eveningness.	Minor C allele carriers had: (1) shorter sleep duration, (2) higher plasma ghrelin concentrations, (3) delayed breakfast time, (4) evening preference, and (5) less compliance with a Mediterranean Diet pattern.	[15]
	rs3749474 T/C rs1801260 T/C rs4864548 A/G rs1464490 T/C rs4580704 C/G	C:0.40 C: 0.37 G:0.40 C: 0.41 C:0.41 G:0.33	*N* = 1,100 540 men and 560 women who participated in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study.	CLOCK genetic variation and metabolic syndrome risk: modulation by monounsatu-rated fatty acids.	For SNP rs4580704, minor allele carriers had a 46% lower risk of hypertension than did noncarriers. By dichotomizing MUFA intake significant gene–diet interactions were identified associated with MetS.	[47]
	rs3749474 T/C rs1801260 T/C rs4864548 A/G rs1464490 T/C rs4580704 C/G	C:0.40 C: 0.37 G:0.40 C: 0.41 C:0.41 G:0.33	*N* = 1,100; 540 men and 560 women who participated in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study.	Total energy intake.	Four of five CLOCK SNPs selected were significantly associated with total energy intake. For SNP rs3749474, the energy intake and total fat, protein, and carbohydrate intakes were higher in minor allele carriers than in non-carriers.	[48]
CLOCK/ SIRT1	Genotype combination SIRT1 (rs1467568) CLOCK (3111T/C, rs1801260)	Resistant genotype: *R*: 0.24	1,500 patients attending to a weight loss program based in the Mediterranean diet.	Weight loss.	Subjects carrying minor alleles at SIRT1 and CLOCK loci (R group) displayed a higher resistance to weight loss and a lower weekly weight loss rate as compared with homozygotes for both major alleles.	[56]

(continued)

Table 8.1 (continued)

Gene	SNP reference, genotype and Haplotype	Minor allele or Haplotype Frequency (%)	Population	Trait	Outcome	Reference
BMAL	59 SNPs from gene regions that showed preliminary evidence of associations with hypertension or Type 2 diabetes Haplotypes: rs7950226/ rs11022775 rs6486121/rs3789327/ rs969485.		1,304 individuals.	Hypertension and Type 2 diabetes.	After correcting for multiple testing (59 SNPs), none of the SNPs were significantly associated with hypertension or T2D. However, two haplotypes were associated with both pathologies.	[65]
PER2	rs2304672C>G and rs4663302C>T	G=0.06 T=0.34	500 individuals.	Abdominal obesity, psycho-behavioral factors, and attrition in the dietary treatment of obesity.	PER2 SNPs rs2304672C>G and rs4663302C>T were associated with abdominal obesity. Frequency of rs4663307 minor allele was greater in withdrawers. rs2304672 C>G minor allele carriers had a greater probability of dropping out, displaying extreme snacking, experiencing stress with dieting, eating when bored, and skipping breakfast than noncarriers.	[52]

BED binge eating disorders, *T2D* type 2 diabetes

CLOCK Interacts with Dietary Fat

A tantalizing finding from our research was the demonstration that these associations between the CLOCK gene and abdominal obesity or impaired glucose metabolism were only seen in individuals with dietary habits that included a high proportion of saturated fat (factory-made pastries, sausages, and so on) and a low proportion of monounsaturated fat (olive oil).

These results suggest that *CLOCK* polymorphisms interact with fatty acids to modulate MetS traits. Specifically, we identified significant gene–diet interactions associated to MetS at the CLOCK locus. By dichotomizing monounsaturated fatty acid (MUFA) intake, we found different effects across rs4580704 genotypes for glucose and insulin resistance. The protective effect of the minor allele on insulin sensitivity was only present when MUFA intake was high. Different effects were found across CLOCK 3111TNC genotypes for saturated fatty acid (SFA) intake. The deleterious effect of gene variants on waist circumference was only found with high SFA intakes [47]. These results suggest that dietary components (i.e., MUFA and SFA) are implicated in the relationship between alterations of circadian system and MetS.

CLOCK and Inflammation

As has been previously indicated, the quality and quantity of sleep could be associated with metabolic disruption and finally with obesity. Circadian clock genes are involved in sleep regulation [49, 50]. Therefore, the coordinated regulation of sleep or feeding behavior might be involved in the relationship between *CLOCK* SNPs and metabolic disorders. Along these lines, we have shown that the *CLOCK* locus was associated with plasma cytokine values, in particular with those that were involved in energy intake: MCP1, IL-6, and adiponectin [48]. Our data show strong associations between plasma IL-6 levels and the CLOCK rs3749474 SNP, which could be functionally related with changes in the *CLOCK* 3′-UTR structure (Fig. 8.3). Current data show that carriers of the minor allele, who reported high energy intake, also presented decreased plasma cytokine concentrations that could result, on the one hand, in a lower anorectic effect, and on the other, in decreased sleep

Moreover, we showed novel significant associations with individual MetS components such as waist, glucose metabolism-related variables and blood pressure. Carriers of the CGA (rs3749474/rs4580704/rs1801260 (3111T→C)) haplotype had lower BMI, waist circumference, blood pressure, and insulin resistance [46] (Fig. 8.3).

CLOCK and Weight Loss

Another study was performed in a Mediterranean population from south-east Spain, in a sample of 500 overweight/obese subjects, aged 20–65 years, who attended outpatient clinics specializing in obesity [51]. Consistent with a previous study, four

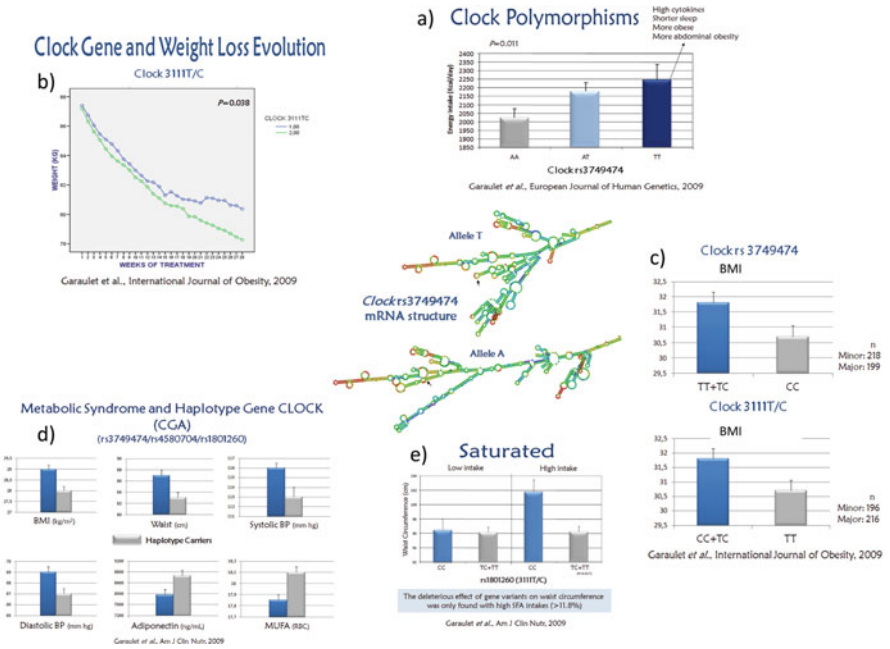

Fig. 8.3 *CLOCK* genetic variants and relationship with obesity and weight loss. (**a**) *CLOCK* rs 3749474and energy intake; (**b**) *CLOCK* 3111T/C and weight loss; (**c**) *CLOCK* SNPs and obesity; (**d**) Metabolic syndrome and haplotype *CLOCK* (CGA) (rs 3749474/rs4580704/rs1801260); (**e**) Nutrigenetics in *CLOCK*: interaction with saturated fat intake for obesity (adapted from [47, 48, 51]

out of five *CLOCK* SNPs selected were significantly associated with obesity variables. The genetic variation in the rs1801260 *CLOCK* was associated with obesity at baseline and also affected weight loss. Patients with the variant G allele lost significantly less weight compared with wild type patients.

Analysis of repeated measures showed that weight loss over time was significantly different between rs1801260 *CLOCK* variations. Carriers of the G allele displayed greater difficulty in losing weight than non-carriers. In this particular polymorphism, the frequency of short-time sleepers (≤6 h per day) was greater in minor G allele carriers than in non-carriers. *CLOCK* polymorphisms were also associated with significant differences in total plasma cholesterol at the completion of dietary treatment. It was concluded that the *CLOCK* rs1801260 SNP may predict the outcome of body weight reduction strategies based on low-energy diets. Furthermore, these results were replicated again in a bigger sample of 1,495 subjects, and this time we were also able to study different behaviors and metabolic variables in these subjects in order to elucidate which were the variables that were behind the association between this *CLOCK* SNP, weight loss and sleep (Fig. 8.4).

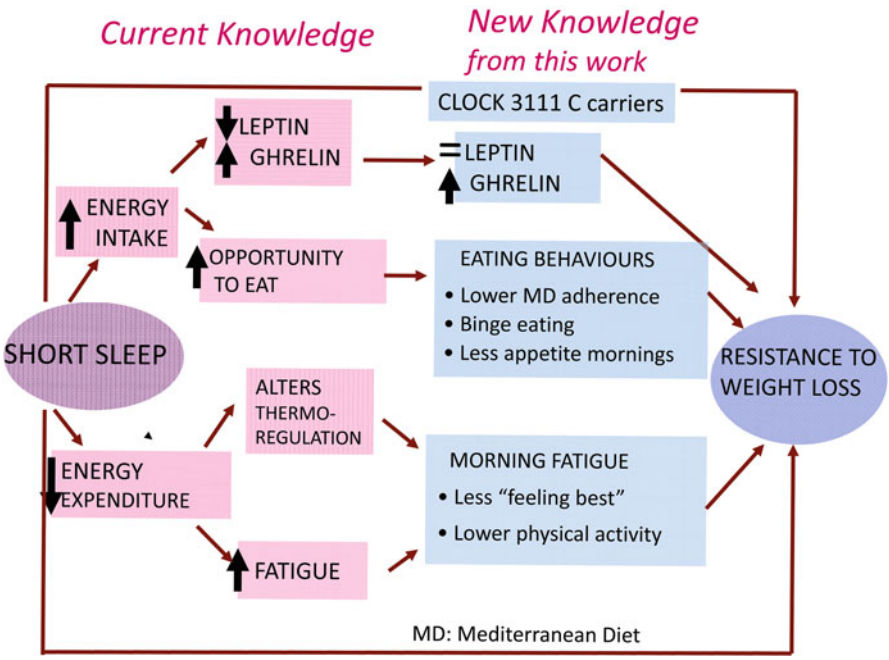

Fig. 8.4 Different behaviors and metabolic variables in CLOCK3111TC minor allele subjects (C carriers) behind the association between this SNP, weight loss, and sleep

PERIOD MetS and Obesogenic Behavior

As described above, various studies have shown that the presence of certain *PER2* gene polymorphisms such as (rs2304672C>G and rs4663302C>T) were associated with various psychological disturbances, particularly seasonal depression and bipolar disorder. This led us to consider whether a sample of overweight or obese patients would have emotional or psychological changes related to obesity and whether such changes would in turn be associated with *PER2* gene polymorphisms. Consistent with this idea, our results showed that people carrying the *PER2* gene variants were associated with abdominal obesity, and particularly *PER2* rs2304672 minor allele carriers (G) showed obesogenic behaviors, habits, and emotions, and greater rates of treatment discontinuation, nibbling, diet-induced stress, and food intake as an escape from boredom [52] (Fig. 8.5).

Another study performed in a population from Finland, also demonstrated the connection of *PER2* and MetS, in particular with high fasting blood glucose. *PER2* 10870 contributed to changes in glucose metabolism. *PER2*_10870 is an intronic mutation originally found by Spanagel et al. in 2005, when searching for the *PER2* SNPs modulating alcohol intake in mice. In this study performed by Englund et al. [53] *PER2*_10870 was associated with high fasting blood glucose. Another SNP in *PER2*

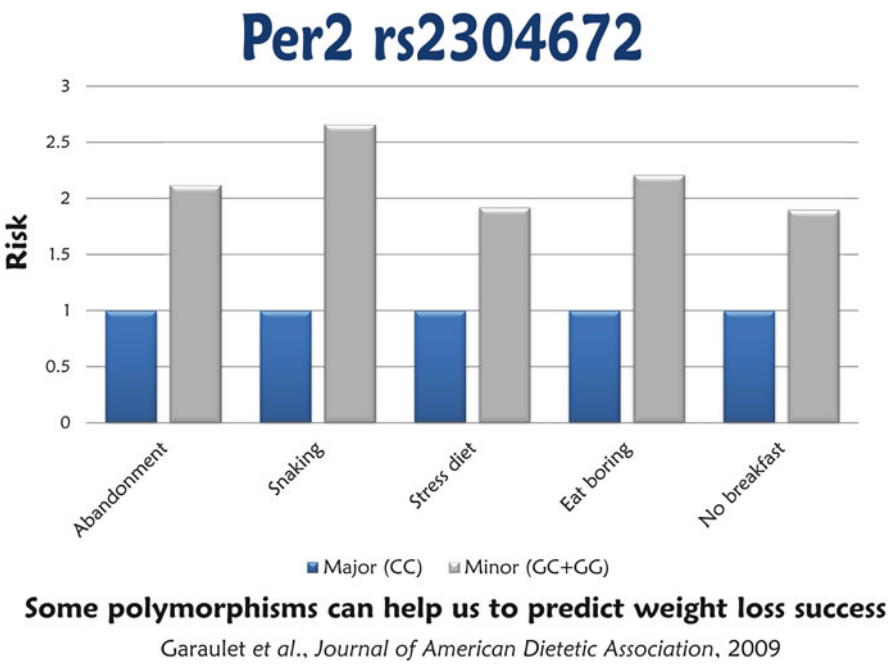

Fig. 8.5 *PER2* rs 2304672 and obesogenic behaviors (adapted from [51])

(*PER2* SNP rs934945) was also associated with waist circumference, and with MetS, although significance was lost after correcting for multiple comparisons [53].

BMAL1 and MetS

BMAL1 and *CLOCK* form a heterodimer and drive transcription from E-box elements found in the promoters of circadian-responsive genes. In addition to its roles in the control of circadian rhythms, *BMAL1* has been suggested to contribute to the metabolic regulation. Indeed, genome-wide profiling of *BMAL1* targets revealed their strict relationship with adipose tissue metabolism. Moreover, SNP analysis revealed that BMAL1 is associated with type II diabetes and hypertension [54]. However, replications are needed in different populations to determine the extension of this associations and the specific functional SNPs influencing MetS risk.

Gene × Gene Interactions in the Clock

Different gene by gene interactions between clock genes have been related to MetS, obesity or weight loss. This is the case of a significant interaction between the *5-HTTLPR* variant of the serotonin gene and the haplotype rs1554483–rs4864548 in *CLOCK* that was associated with diastolic and systolic blood pressure, arterial hypertension, plasma triglycerides and different MetS components [55]. In all these cases, the higher values were observed in rotating shift workers homozygous for the *SLC6A*4 S allele and carrying the haplotype composed by the *CLOCK* rs1554483 G and rs4864548 A variants. These data suggest a potential interaction (epistatic effect) of serotonin transporter and *CLOCK* gene variation on the genetic susceptibility to develop MS by rotating shift workers.

Other example of the gene × gene interaction is *SIRT1* and *CLOCK* 3111T > C combined genotype which is associated with evening preference and weight loss resistance in a behavioral therapy treatment for obesity. Our group has demonstrated that variants at both *SIRT1* and *CLOCK* have an additive effect on resistance to weight loss that could be related to the chronotype of the subject, higher plasma levels of ghrelin and less adherence to Mediterranean diet patterns [56].

Epigenetics and the Clock System

As indicated earlier, the world of epigenetics is revolutionizing genetics. Epigenetic research shows that we are not predetermined by our genome. What we eat, how much we sleep, if we exercise or even how we use our mind may change our epigenome, and our fate. Moreover, these changes are not restrained to us but can pass down to our children or even to our grant children. In other words, epigenetics does not change the DNA but decides how much or whether some genes are expressed in different cells in our bodies.

The molecular basis of epigenetics is complex. It involves modifications of the activation of certain genes, but not the core DNA structure. One way that gene expression is regulated is by the remodeling of chromatin (the complex of DNA and histones). Chromatin proteins associated with DNA may be activated or silenced. Histones can change how tightly or loosely the DNA wraps around them by modifying their amino acids. If the amino acids that are in the chain are changed, the shape of the histone sphere might be modified.

A second way of chromatin remodeling is the addition of methyl groups to the DNA, mostly at CpG sites, which are regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide. "CpG" is shorthand for "—C—phosphate—G—", that is, cytosine and guanine separated by only one phosphate. Methylation converts cytosine to 5-methylcytosine. Some areas of the genome are methylated more heavily than others, and highly methylated areas tend to be less transcriptionally active.

Epigenetic control can be exerted through a variety of mechanisms, including not only DNA methylation but also microRNA-mediated metabolic pathways, histone

variants and histone Post Translational Modifications or PTMs. Different studies point out to the association between epigenetics changes and several illnesses, for example cancer, in which methylation of CpG sites within the promoters of genes can lead to the silencing of tumor suppressor genes. In contrast, the hypomethylation of CpG sites has been associated with the over-expression of oncogenes within cancer cells.

In 2011, it was demonstrated for the first time that the methylation of mRNA had a critical role in human energy homeostasis. Obesity associated FTO gene was shown to be able to demethylate N6-methyladenosine in RNA. This opened the related field of RNA epigenetics and its relation to obesity.

Epigenetics and Circadian Rhythms

The connection between epigenetics and the clock machinery came out with the study of Crosio et al. [57], who demonstrated that chromatin remodeling was involved in circadian gene expression. These authors showed that a pulse of light, when applied to mice during the subjective night, induced histone phosphorylation in the SCN. This was an early effect which implicated an induction of *Per1* translation. Subsequently, it has been indicated that histone modifications at clock controlled genes promoters occur in a circadian manner.

It has been hypothesized that because the number of transcripts that oscillates in a circadian manner is high, there must be a widespread program of dynamic changes in chromatin remodeling that accompany circadian gene expression. This has been described as the "circadian epigenome" and probably includes cycles of chromatin transitions that allow a highly dynamic chromatin structure to be temporally permissive to transcription.

Histones can be modified at more than 30 sites within the N-terminal tails. There are several modifications in the histones such as acetylation and phosphorylation, among others. The finding that *CLOCK* has an intrinsic Histone Acetile Transferase (HAT) activity reveals one link between epigenetic control and the circadian clock. *CLOCK* may acetylate histones, particularly the lysine residues in the histones 3 and 4. Interestingly, *CLOCK* can also acetylate non-histone substrates. This is the case of *BMAL1* which is acetylated by *CLOCK* in a lysine residue, an event which is crucial for the circadian transcriptional program. Other substrate susceptible to be acetylated by *CLOCK* is the glucocorticoid receptor, whose function is regulated by this process. This acetylation activity by *CLOCK* has been demonstrated to be essential for circadian expression.

Another important player in the circadian epigenome, is sirtuin, particularly *SIRT1* and *SIRT6*. Sirtuins possess deacetylase activity and are implicated in the induction of gene silencing. Particularly *SIRT1* physically interacts with *CLOCK* and it has been defined as a circadian enzymatic rheostat [2], because it controls by different mechanisms the balance of acetylation and chromatin remodeling by the circadian clock.

Despite this conceptual knowledge, data about the connection between this circadian epigenome and obesity are still scarce. Nevertheless, recently our group has

demonstrated an association between the methylation status of CpG sites located in clock genes (CLOCK, BMAL1 and PER2) with obesity, Metabolic Syndrome and weight loss [58].

The Role of MicroRNAs in the Clock System

MicroRNAs are small (approximately 22 nucleotides), single-stranded, noncoding RNA species that act as potent gene silencers and are relevant players in different physiological and pathophysiological processes. They can be divided in three types depending o their characteristics: one type is the "*intergenic microRNA*" which are those microRNAs which are coded within introns and exons of noncoding RNA; the second type are the "*introgenic microRNA*" which are coded from introns of protein encoding genes, and the third type are the "*polycistronic microRNAs*" long RNA that contains multiple microRNAs in a cluster. The gene silencing function of microRNAs occurs via a reduction in mRNA translation efficiency or mRNA stability.

Considering that microRNAs appear to be involved in the regulation of >60% of human genes, it is not surprise that they have been identified as major players in the regulation of the circadian rhythm [59]. Therefore, they are emerging as new potential therapeutic targets for disorders of the circadian clock based on the following features:

(a) MicroRNAs *present in the SCN* can control the core clock molecular feedback by posttranscriptional-mediated control mechanisms [60].
(b) MicroRNAs *display circadian rhythmicity, in some cases regulated by light*, such as Arabidopsis miR167, miR168, miR171 and miR398, whereas in others, like the fly miR263b, the regulation is directly *coordinated by the circadian clock* [61].
(c) Some microRNAs have been related to *circadian alterations. In vivo* antisense silencing studies demonstrated that miR219 shortens the circadian period and that miR132 negatively regulates light-dependent resetting of the clock [60]. Both miR132 and miR219 affect *per1* expression, and thus influence the core circadian transcriptional loop [60, 62]. However, neither miR132 nor miR219 appears to directly target *per1*; thus, the precise mechanisms by which these microRNAs affect the clock are not known.
(d) In addition to rhythmic microRNA expression in the SCN clock, *peripheral oscillators* also exert circadian control over microRNA expression. For example, some microRNAs such as the miR192/194 cluster are highly expressed in the liver and kidneys (two oscillating organs). Others such as miR16, miR20a and miR141, oscillate in the intestine, and several microRNAs have been found to exhibit diurnal oscillations in the mouse retina [59, 62].

Given the importance of microRNAs it has been proposed that SNPs mapping with them may have functional consequences resulting in phenotypic variation. This is the case of a polymorphism in pre-miR182 which exhibits diurnal rhythmicity in the retina and has been significantly associated with major depression [63].

This promising preliminary evidence suggests that following a better understanding of microRNA biology; these may become a new therapeutic tool in the fight against obesity.

Conclusion

A number of genetic variants connect circadian rhythmicity and obesity. This new knowledge should be considered on the prevention and treatment of obesity. Moreover, it is important to expand the current nutrigenetic knowledge to guide the health professionals on the delivery of more tailored advice based on genetic variants.

Summary

1. Thanks to the drive infused by the completion of the HGP, a number of technologies and research approaches have evolved. Nowadays, new functional genomics areas to accomplish the goals started by the HGP. Nutrigenetics, nutrigenomics, and epigenetics are some examples.
2. The living matter has its own time, i.e., the "biological time." In this sense, we could assume that "a Chronome exists into the Genome":"
3. In the last years the study of genetics and its relationship to chronobiology has been focusing in the chronotype and different psychological illnesses associated to these chronotype such as depression, anxiety or bipolar disorder.
4. Some examples of genetic variants associated to psychological traits are *CLOCK* 3111T/C associated with evenningness, and different personality traits; *PERIOD2* (*PER2* rs2304672) polymorphism which moderates circadian-relevant reward circuitry; *CRY1* (rs8192440) related to psychological treatment effectiveness; *CRY2* associated with winter depression and S*IRT1* rs10997875 a good candidate gene for the pathophysiology for mood disorders.
5. Other candidate SNPs connected to obesity could be those associated to sleep disorders. Some examples are serotonin receptors, prepro-orexin or IL-6 SNPs which associate with obstructive sleep apnea syndrome. Others are SNPs residing in ROR1 and PLCB1which associated with insomnia.
6. One particular example that could link sleep duration and obesity is *ABCC9;* the leukocyte antigen *(HLA) DQB1*0602; PER3* (G-320T, C -319A) and *NPAS2* rs6725296 or *BMA1* rs6290035 TT which had greater loadings on the metabolic factor (weight and appetite) of the global seasonality score.
7. Sleep disorders or short sleep duration, are both associated to several polymorphisms connected to obesity. In this regard, one of the best studied *CLOCK* SNPs (3111TC) has been significantly associated to short sleep duration, eveningness, several psychological traits, weight loss and obesity.

8. *Epigenetics* is the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence. *CLOCK* and *SIRT* have both epigenetics effect in acetylation and deacetylation of histones, respectively. MicroRNAs may become novel therapeutic targets for disorders in the circadian clock. The knowledge achieved in the circadian epigenome could give us new answers to the connections among genetics, circadian rhythmicity, and obesity.

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