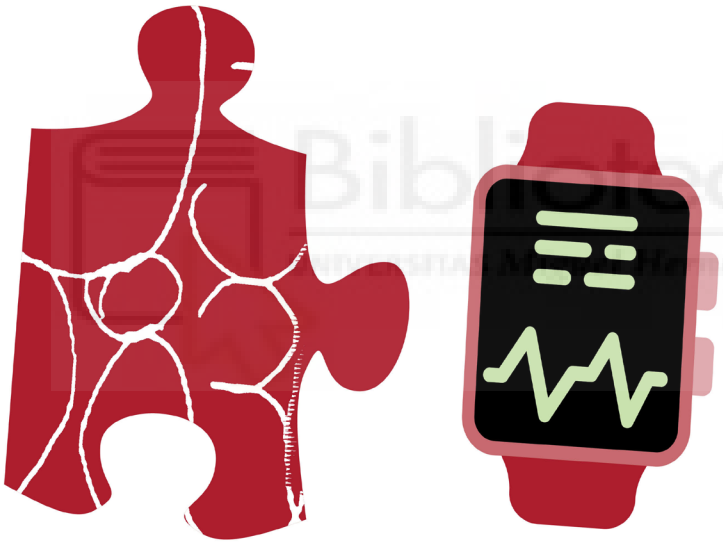
Monitorización Circadiana Ambulatoria y nuevos marcadores biomoleculares para el estudio del sueño en adultos con Trastorno del Espectro Autista (TEA) y discapacidad intelectual



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Tesis doctoral dirigida por:

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Ambulatory Circadian Monitoring and new biomarkers to study sleep in autistic adults with intellectual disability.

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Presentada por Dña. Pura Ballester Navarro para optar al grado de doctora Internacional por la Universidad Miguel Hernández de Elche (Alicante, España)

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HACE CONSTAR:

Que da su conformidad a la lectura de la Tesis Doctoral presentada por **PURA BALLESTER NAVARRO**, titulada **"Monitorización Circadiana Ambulatoria y nuevos marcadores biomoleculares para el estudio del sueño en adultos con Trastorno del Espectro Autista (TEA) y discapacidad intelectual"** considerando que cumple con los requisitos necesarios para ser presentada como Tesis Doctoral por compendio de publicaciones en la Universidad Miguel Hernández de Elche (Alicante).

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HACE CONSTAR:

Que da su conformidad a la lectura de la Tesis Doctoral presentada por **PURA BALLESTER NAVARRO**, titulada **"Monitorización Circadiana Ambulatoria y nuevos marcadores biomoleculares para el estudio del sueño en adultos con Trastorno del Espectro Autista (TEA) y discapacidad intelectual"** que se desarrolló dentro del Programa de Doctorado en Bioingeniería de este Instituto de Investigación bajo la dirección de la Profesora Dra. Ana María Peiró Peiró. Lo que firma a instancias de la interesada.

Elche, el 13 de marzo de 2019

Dr. José Manuel Pérez Pérez
Coordinador del programa de Doctorado de Bioingeniería
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A todos los que generosamente formáis parte del equipo humano del que hablan estos resultados.

No somos más que un débil saco

de sangre y huesos,

y un alfiler, verdad, puede matarnos;

pero corre en nosotros la semilla

que puede dejar fuera de nosotros

la mariposa única,

de luz sólo y de sombra sólo y sólo nuestras,

sin piel, red ni armadura,

ni posibilidad de ser cazada

por nada humano ni divino;

el ser invulnerable,

inmaterial, tan largo como el mundo,

que colma, libre, lo infinito

y se sale de él a lo imposible.

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INDEX

ABBREVIATIONS LIST

ACM: Ambulatory circadian monitoring

ASD: Autism spectrum disorder

***ASMT*:** Acetylserotonin o-methyltransferase

***BMAL1*:** Aryl hydrocarbon receptor nuclear translocator-like protein 1

CFI: Circadian function index

CI: Confidence interval

***CLOCK*:** Circadian locomotor output cycles kaput

CRSWD: Circadian rhythm sleep-wake disorder

CRY: Cryptochromes

CT: Clinical trial

DSM-5: Diagnostic and statistical manual of mental disorders-5

EudraCT: European clinical trials database

ICD-10: International classification of diseases

ICSD-3: International Classification of Sleep Disorders, 3rd Edition

ID: Intellectual disability

IQ: Intelligence quotient

IQR: Interquartile range

IS: Inter-daily stability

IV: Intraday variability

NPAS2*:* Neuronal PAS domain-containing protein 2

PER: Period

PSG: Polysomnography

RA: Relative amplitude

SD: Standard deviation

SE: Sleep efficiency

SEM: Standard error

SNP: Single nucleotide polymorphism

SoL: Sleep onset latency

TIB: Time in bed

TST: Total sleep time

(V)L5: 5 hour or (value) with less activity at night

(V)L10: 10 hour or (value) with less activity during the day

(V)M5: 5 hour or (value) with more activity at night

(V)M10: 10 hour or (value) with more activity during the day

WASO: Awake period duration after sleep onset duration

ABSTRACT

Introduction: Sleep problems are a common and permanent condition in autism spectrum disorder (ASD), also when intellectual disability (ID) is present. Causes remain unknown, but are likely multifactorial, which means that both genetic and environmental factors may play a role in their prevalence. Increasing knowledge of sleep assessment tools would help to test effectiveness of treatments for the most prevalent sleep problems in ASD: insomnia and Circadian Rhythm Sleep-Wake Disorders (CRSWDs).

Aims: This work aimed to evaluate the use of ambulatory circadian monitoring (ACM), in adults with ASD and ID, as an objective tool to report sleep problems, to test agomelatine effectiveness as a sleep treatment, and to analyse the influence of *circadian clock* and *melatonin pathway* genes as potential molecular biomarkers for sleep problems.

Methods: A prospective study (AGOTEA) was conducted using ACM (7 days, 24-hour recording) to analyse sleep-wake cycle in adults with ASD and ID (n=41) vs. controls (n=51). Then, participants with persistent sleep problems (n=23, insomnia and CRSWDs, according to International Classification of Sleep Disorders-Third Edition criteria), were included in a randomized, crossover, triple-blind, placebo-controlled clinical trial (AGOTEA-CT), to evaluate agomelatine (25mg/day, 3-months) effectiveness. Finally, an observational study (GENTEA, n=83), using ACM and Sequenom MassARRAY, analyzed genetic variants (*PER1, ASMT, NPAS2,* and *MTNR1A*) influence on sleep. Statistical data were analyzed by R 3.2.4 and Graph Pad Prism 5.0 software.

Results: Sleep parameters were only in normal range values in 16% of adults with ASD and ID. They showed significantly higher insomnia symptoms (low sleep efficiency,

prolonged sleep latency and increased number and length of night awakenings) and signs of an advanced sleep-wake phase disorder. Autistic adults had a pattern of daily sedentary behaviour and increased nocturnal activity. Agomelatine significantly increased a mean of 83 minutes the total sleep time at night, corrected the temperature phase and improved sleep rhythm stability. C*ircadian clock* gene *PER1* (rs885747, rs6416892) presented a different genotype distribution in autistic participants compared to controls. Rs6416892-G allele had better sleep pattern, together with a phase advancement of sleep and wrist temperature circadian rhythms.

Conclusions: ACM was effective and accurate in measuring sleep-wake cycle in autistic adults with ID. Agomelatine could be appropriate for the synchronization of circadian rhythms, improving sleep quantity and quality. There is a need for further clinical studies to confirm agomelatine effectiveness and the use of *circadian clock* and *melatonin* pathway genes as new molecular biomarkers for sleep problems in ASD.

RESUMEN

Introducción: Una persona con trastorno del espectro autista puede, de forma común y a lo largo de toda su vida, sufrir problemas de sueño, incluso cuando existe un diagnóstico de discapacidad intelectual asociado. Las causas de éstos son todavía desconocidas, pero probablemente sean multifactoriales, lo que significa que los factores genéticos y ambientales podrían jugar un papel en su prevalencia. Por tanto, investigar sobre las herramientas para evaluar el sueño ayudaría a valorar la eficacia de los tratamientos para los problemas de sueño más prevalentes en el trastorno del espectro autista: insomnio y trastornos del ritmo circadiano.

Objetivos: Evaluar en adultos con trastorno del espectro autista y discapacidad intelectual el uso de la monitorización circadiana ambulatoria como herramienta objetiva para determinar los problemas del sueño, la eficacia farmacológica de la agomelatina para mejorar el sueño, y la influencia de los genes *reloj* y de la *ruta de la melatonina* como posibles marcadores biomoleculares de los problemas de sueño.

Métodos: Se realizó un estudio prospectivo (AGOTEA) para analizar el ciclo de sueño-vigilia en adultos con trastorno del espectro autista y discapacidad intelectual (n=41) frente a controles (n=51) utilizando monitorización circadiana ambulatoria (7 días, registro continuo de 24 horas). Luego, aquellos participantes con problemas persistentes de sueño (n=23, insomnio y trastornos del ritmo circadiano, según criterios de la tercera Edición de la Clasificación Internacional de Trastornos del Sueño), se incluyeron en un ensayo clínico aleatorizado, cruzado, triple ciego, controlado con placebo (AGOTEA-CT), para evaluar la eficacia de la agomelatina (25mg/día, 3 meses). Finalmente, un estudio observacional (GENTEA, n=83), utilizando Sequenom MassARRAY y monitorización circadiana ambulatoria, analizó la influencia de las

variantes genéticas (*PER1, ASMT, NPAS2* y *MTNR1A*) en el sueño. El análisis estadístico se realizó con el software R 3.2.4 y Graph Pad Prism 5.0.

Resultados: Sólo el 16% de los adultos con autismo y discapacidad intelectual tuvo los parámetros de sueño dentro del rango normal. Tuvieron significativamente más síntomas de insomnio (baja eficiencia del sueño, latencia prolongada y aumento del número y la duración de los despertares nocturnos) e indicios de un adelanto en la fase del sueño. Además, eran más sedentarios durante el día y activos por la noche. La agomelatina aumentó significativamente el tiempo de sueño nocturno una media de 83 minutos, corrigió el ritmo de temperatura y mejoró la estabilidad del sueño. El gen *reloj circadiano PER1* (rs885747, rs6416892) presentó una distribución de genotipo diferente en los adultos con autismo comparada a la de los controles. El alelo G del rs6416892 tuvo un mejor patrón de sueño, así como un adelanto de fase del ritmo circadiano de sueño y temperatura periférica.

Conclusiones: Para la medición del ciclo de sueño-vigilia en adultos con trastorno del espectro autista y discapacidad intelectual, la monitorización circadiana ambulatoria fue efectiva y precisa. El uso de agomelatina podría ser apropiado para sincronizar los ritmos circadianos, e incrementar la calidad y cantidad de sueño. Para confirmar su eficacia y el uso de genes *reloj circadianos* y de la *ruta de la melatonina* como nuevos marcadores biomoleculares de los problemas de sueño en el trastorno del espectro autista, hay que llevar a cabo más estudios clínicos.

INTRODUCTION

1.1. – Autism spectrum disorder

Autism spectrum disorder (ASD) is a developmental disorder with a neurobiological aetiology. Although signs can appear at a young age, around 10 months, diagnosis confirmation happens around 24 months of age (Lai, Lombardo, & Baron-Cohen, 2014). Autistic condition is usually accompanied by an intellectual disability (ID), communication problem, epilepsy or other genetic disorder (Developmental Disabilities Monitoring Network Surveillance Year Principal Investigators, 2014). It is a matter of importance nowadays, for the society as well as for the health care system, due to the considerable and continuous growth in ASD diagnoses.

Up to date, the prevalence of this disorder is 17 per 1000 children, according to the US autism and developmental disabilities monitoring network (Traolach Brugha, 2018). The increase seen is somehow due to new diagnostic criteria (Fisch, 2012), the awareness and improved recognition for earlier diagnosis (Elsabbagh et al., 2012; Fombonne, 2009). Also, 30% of individuals with ASD have some level of ID, and up to 70% have some disability other than cognitive dysfunction (e.g., speech delays) (Mefford, Batshaw, & Hoffman, 2012). Even more, nearly two in five adults with moderate to profound ID had autism (Sparrow, 2011), a prevalence slightly higher than 30% expected (Cooper, Smiley, Morrison, Williamson, & Allan, 2007; Saemundsen et al., 2010).

An ID is a condition evidenced during the developmental period, that involves serious limitations in areas such as learning and reasoning, and severe functioning limitations in adaptive domains as conceptual, social, and practical. ID is arbitrarily diagnosed with average intellectual quotient (IQ) of 70 or below (Simms, 1992). That may be manifested as deficits in reasoning, problem-solving, planning, abstract thinking, judgment,

academic learning and learning from experience, and practical understanding confirmed by both clinical assessment and individualized, standardized intelligence testing (American Psychiatric Association, 2013; Rao, Raman, Thomas, & Ashok, 2015).

Besides the heterogeneity of ASD and ID, there is a potential biochemical overlap between them, and it is necessary to explore the effects that those co-occurring conditions may cause (Cervantes & Matson, 2015). That necessity is covered with a growing interest in identifying candidate genes that may be involved in ASD or ID or both. Thus, increasing genetic knowledge may estimate the risk of co-occurrence, the molecular and cellular nature of the pathobiology and potential pharmacological approaches (Srivastava & Schwartz, 2014).

Diagnostic criteria

ASD was broken down into different autism subtypes based on severity of symptoms. In 2012, the 10th revision of the International Classification of Diseases (ICD-10), referred to autism as a pervasive developmental disorder, and emphasized the early onset of a triad of features: (i) impairments in social interaction; (ii) impairments in communication; and (iii) restricted, repetitive and stereotyped behaviour, interests and activities (WHO, 1993).

These subtypes were eliminated in 2014 from the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5), and their diagnoses have all been absorbed into the "ASD" without a definition of subtypes. That turned the triad mentioned into a dyad: difficulties in social communication and social interaction; and restricted and repetitive behaviours, interests or activities. Different language developments were removed from

the criteria, and now is considered as a co-occurring condition (American Psychiatric Association, 2013) (Table 1).

Table 1. Diagnostic and Statistical Manual of Mental Disorders-5 (DMS-5) diagnostic criteria for autism spectrum disorder (ASD).

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history (examples are illustrative, not exhaustive, see text):
1. Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interests, emotions, or affect; to failure to initiate or respond to social interactions. 2. Deficits in nonverbal communicative behaviours used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expressions and nonverbal communication. 3. Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behaviour to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.
B. Restricted, repetitive patterns of behaviour, interests, or activities, as manifested by at least two of the following, currently or by history (examples are illustrative, not exhaustive; see text):
1. Stereotyped or repetitive motor movements, use of objects, or speech (e.g., simple motor stereotypies, lining up toys or flipping objects, echolalia, idiosyncratic phrases). 2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns or verbal nonverbal behaviour (e.g., extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat food every day). 3. Highly restricted, fixated interests that are abnormal in intensity or focus (e.g, strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interest). 4. Hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment (e.g., apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement). C. Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities or may be masked by learned strategies in later life).
D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.
E. These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum disorder and intellectual disability, social communication should be below that expected for general developmental level.

1.2. – Sleep problems in autism spectrum disorder

ASD has prevalent co-occurring medical conditions, including a wide range from sleep problems, gastrointestinal disorders (constipation or feeding difficulties), attention deficit/hyperactivity disorders, epilepsy, anxiety or disruptive behaviours. All those

conditions add complexity to the ASD diagnose and disturb the finding of an appropriate treatment and intervention (Jacob et al., 2019). For example, recent studies suggest that up to 80% of young people with ASD also have difficulty falling and/or staying asleep at night (Souders et al., 2017). These problems make it harder for them to pay attention, reduce their ability to function, and lead to poor behaviour. In addition, parents of children with ASD and sleep problems tend to report greater family stress and poorer overall health among themselves (Valicenti-McDermott et al., 2015).

There is a high incidence rate of sleep problems in autistic adults with ID, according to data published up to 45% present a sleep problem (J. L. Matson, Wilkins, & Ancona, 2008). Lack of sleep can exacerbate some of the behavioural characteristics of ASD, such as hyperactivity, aggression, and lack of concentration. As a result, people with ASD who have a hard time sleeping may struggle at work or in their classroom. A good intervention in sleep problems at any point may improve their behaviour and functioning, as well as relieve stress (Carol Bagnall, 2012).

Sleep problems or disorders are any condition that affects, disrupts, or involves sleep and include nocturnal/diurnal manifestations. Thus, this is a condition that frequently affects one's ability to get enough quantity or appropriate quality of sleep and is presumed to arise when there is a disruption in the sleep-wake cycle (Edinger et al., 2004).

- Insomnia

The International Classification of Sleep Disorders-Third Edition (ICSD-3) defines insomnia disorder as a "persistent difficulty with sleep initiation, duration, consolidation or quality that occurs despite adequate opportunity and circumstances for sleep, and

results in some form of daytime impairment"(American Academy of Sleep Medicine, 2014).

- Circadian rhythm sleep-wake disorders (CRSWDs)

Circadian rhythms, as the sleep-wake cycle, are physiologic or behavioural cycles with a recurring periodicity of approximately 24 hours, and can be measured as daily oscillations of hormones (melatonin, cortisol), core body temperature, rest-activity cycles or transcriptome patterns. The circadian system orchestrates endogenous rhythms with clocks present in most organ systems, which are synchronized by the central pacemaker in the suprachiasmatic nucleus and by the light/dark cycle (Bhadra, Thakkar, Das, & Pal Bhadra, 2017).

In mammals, the circadian clock network controls circadian rhythms and orchestrates the expression of a range of downstream genes, allowing the organism to anticipate and adapt to environmental changes (Figure 1). Beyond their role in circadian rhythms, several studies have highlighted that *circadian clock* genes in autism may have a widespread physiological effect on cognition and synapsis formation during critical brain developmental periods (Bourgeron, 2007; Geoffray, Nicolas, Speranza, & Georgieff, 2016). The cascade effects from mutations in *circadian clock* genes are unknown. So far, they could participate in sleep problems, psychiatric symptoms related to brain development timing, interfere in neuroendocrine or body temperature rhythms. That may impair how the individual integrates his/her internal and external rhythms (Charrier, Olliac, Roubertoux, & Tordjman, 2017).

Figure 1. Schematic representation of sleep status in a normal phase (green) according to circadian alerting (e.g., light exposure in this case in yellow).

CRSWDs arise when the circadian system and sleep-wake cycle become desynchronized. This category of disorders includes conditions in which sleep timing is out of alignment with social, work, and sleep normal patterns across the 24-hour day. In other medical fields, the study of the status of the circadian system is becoming relevant in terms of adapting medical decisions (e.g., timing of dose administration), increasing knowledge in this field in autism may benefit this population (Ortiz-Tudela, Innominato, Rol, Lévi, & Madrid, 2016).

These circadian disorders are characterized by the inability to sleep at desired times, difficulties initiating and/or maintaining sleep, early awakening and impairments in daytime functioning. They require symptoms to be present for at least three months and confirmed with fourteen days of actigraphy (American Academy of Sleep Medicine, 2014). ICSD-3 categorizes into seven different disorders, however, the most important for autism are those with the circadian phase shifted to an earlier time (phase advance), or later time (phase delay) (Buhr & Takahashi, 2013; Burgess & Emens, 2018).

Sleep problem in ASD prevalence

Most of the literature related to sleep problems in ASD is developed in children, as sleep is important in the early stages of life. There is scarce research data from adults on the spectrum, even less, when ID is present. Nevertheless, ASD is a lifelong condition, and recent research proved a continuance of sleeping difficulties. Sleep problems are particularly common in autistic children with prevalence rates ranging from 50% up to 86% compared to 9-50% prevalence rates, in aged-matched typically developing children. Further, the rate of sleep problems for children with ASD was also higher than for children with non-ASD developmental disabilities (Richdale & Schreck, 2009; Schreck & Mulick, 2000; Souders et al., 2017; Wiggs, 2001). Parental reports confirmed the permanence of sleep problems in up to 63% of their children over a long period of time (Robinson & Richdale, 2004), and a longitudinal study reported that sleep problems worsened in frequency and type into adolescence (Hodge, Carollo, Lewin, Hoffman, & Sweeney, 2014).

The first study carried out in autistic adults was in 1993, when Simblett and Wilson described three cases of young autistic adults (without ID), and they stated for one case, “sleep disturbance with early morning waking” (Simblett & Wilson, 1993). After that, a large sample study described the prevalence in autistic adults that presented a sleep disorder as a 18% (n=1.507) compared with 10% (n=15.070) of typically developing adults (Croen et al., 2015). One year later, that prevalence increased up to 41% in a longitudinal follow up study with a sample of 92 autistic participants (Jones et al., 2016). These data continued increasing and a decade ago, 45% of autistic adults with severe ID had sleep problems according to the results of the DASH II questionnaire (J. L. Matson et al., 2008).

On one hand, cognitively able autistic adults had increased insomnia symptoms (longer sleep latency, poor sleep efficiency, shorter night sleep) (E. K. Baker & Richdale, 2015; Godbout, Bergeron, Limoges, Stip, & Mottron, 2000; S. Goldman et al., 2017; Tani et al., 2005) and advanced or delayed CRSWD (E. K. Baker & Richdale, 2017; Dougal Julian Hare, Steven Jones, & Kate Evershed, 2006a; Limoges, Mottron, Bolduc, Berthiaume, & Godbout, 2005).

On the other hand, when only ID is present, disruptions of sleep and the sleep–wake rhythm maintenance are common (Espie, 2000). A review reported that up to 34% of adults with ID presented frequent night awakenings and significant sleep difficulties (van de Wouw, Evenhuis, & Echteld, 2012). While using actigraphy in a large sample of adults with ID (n=301; 50-92 years old) at least one sleep disturbance was found in 72% of participants (van de Wouw, Evenhuis, & Echteld, 2013).

When ASD and ID are present, six studies in adults have reported poor sleep conditions (reduced total sleep time (TST), and sleep efficiency (SE) and prolonged sleep onset latency (SoL)) (Bradley, Summers, Wood, & Bryson, 2004; Diomedi et al., 1999; Dougal Julian Hare, S Jones, & Kate Evershed, 2006b; Kaufmann et al., 2017; Johnny L Matson, Ancona, & Wilkins, 2008; Øyane & Bjorvatn, 2005). However, some of them include adolescents (Diomedi et al., 1999; Kaufmann et al., 2017; Øyane & Bjorvatn, 2005); have small samples sizes (Bradley et al., 2004; Diomedi et al., 1999; Hare et al., 2006b); and/or the limitation of questionnaires or sleep-wake diaries completed by carers (Kaufmann et al., 2017; Johnny L Matson et al., 2008; Øyane & Bjorvatn, 2005). According to the data published, there is a need to develop sleep studies in autistic adults with ID using objective assessment tools and with bigger sample sizes.

Throughout this time, the work of Hare et al. (2006b) presented some contradictory findings reporting that sleep measures in adults with autism and ID did not differ from adults with ID alone. Thus, further studies in autistic adults, with and without ID, compared to typically developing controls should be done in order to know how both conditions might be influencing on sleep problems.

1.2.1. – Aetiology of sleep problems

Sleep problems aetiology in ASD remains unknown. It is likely to be multifactorial, which means that genetic, environmental, and other factors may play a role in their prevalence (Figure 2).

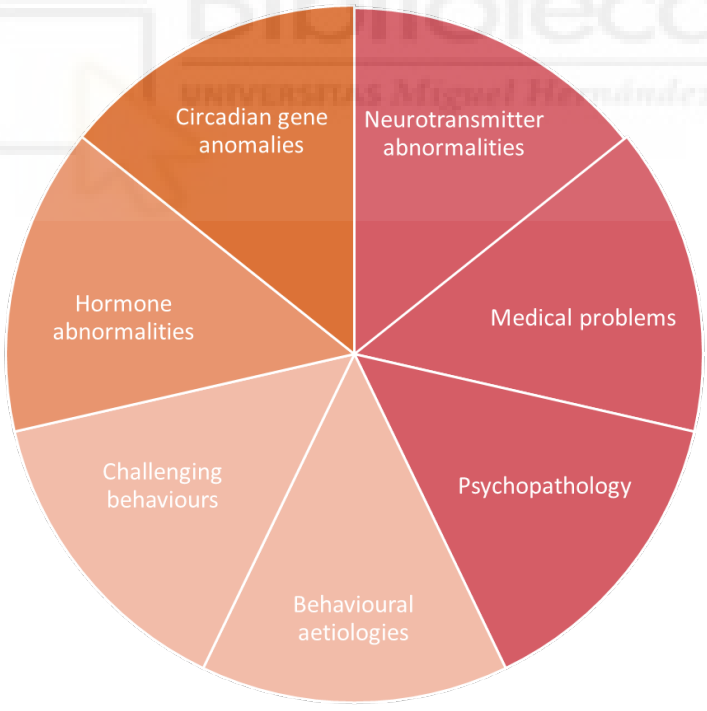

Figure 2. Multicausal theory of sleep problems in autism spectrum disorder.

To the best of our knowledge, these are potential causes of sleep problems in ASD:

- Medical problems like gastrointestinal disorders (Klukowski, Wasilewska, & Lebensztejn, 2015), epilepsy (Kaleyias et al., 2008) or anxiety/depression (Hollocks, Lerh, Magiati, Meiser-Stedman, & Brugha, 2018; Nadeau et al., 2015; Richdale, Baker, Short, & Gradisar, 2014).
- Behavioural component such as poor sleep habits (B. A. Malow et al., 2012; Reynolds & Malow, 2011), consolidated sleep routine (e.g., co-sleeping, needing to wear a particular item of sleepwear, (Henderson, Barry, Bader, & Jordan, 2011)) or challenging and aggressive behaviours (Johnny L Matson et al., 2008).
- Social component that may involve employment status and social interaction (E. K. Baker, Richdale, & Hazi, 2018), living out of phase with our inner clock, mostly related with social cues (McKenna, van der Horst, Reiss, & Martin, 2018) or with an inappropriate light exposure patterns (Fenton & Penney, 1985). Also, social-communication difficulties occurring in ASD, that may result in misinterpretation or failure to understand social cues associated with sleep (Markram & Markram, 2010).
- Alterations in molecular biomarkers related to sleep-wake transition, like neurotransmitters (e.g., increased levels of serotonin (Anderson & Lombroso, 2002; Malow et al., 2006)), hormones (e.g., melatonin and cortisol profiles (Tordjman et al., 2012) or their timing peaks (E. K. Baker, Richdale, Hazi, & Prendergast, 2017)). Also, anomalies in *circadian clock* and *melatonin pathway* genes (Cécile Pagan et al., 2017; Yang et al., 2016) that may be related to autistic condition through problems in developmental brain timing (Geoffray et al., 2016).

1.2.1.1 – Genetic variants influence

It has been a while since authors have linked the role of *circadian clock* genes with the impaired timing in ASD (Wimpory, Nicholas, & Nash, 2002), that may be affecting critical brain developmental periods, and therefore contributing to ASD aetiology (Geoffray et al., 2016). Apart from that, *circadian clock* genes are closely related to melatonin, serotonin and GABA, molecules that play a role in the sleep wake transition (Bourgeron, 2007). Thus, insomnia and CRSWDs symptomatology reported in autistic individuals could be in part due to variations in genes that control melatonin and biological clocks rhythms (Fukuya et al., 2007). Gene polymorphisms including: (i) *circadian clock* genes, *Period family (PER1*, *PER2*, and *PER3)* and *Neuronal PAS domain-containing protein 2* (*NPAS2)* (Nicholas et al., 2007; Yang et al., 2016); and/or (ii) *melatonin pathway* genes, *Acetyl serotonin methyl transferase* (*ASMT)* and melatonin receptor (*MTNR1A*) (Chaste et al., 2010; Jonsson et al., 2010; Melke et al., 2008; Cécile Pagan et al., 2017; Yang et al., 2016).

- *Circadian clock* genes

Circadian rhythm disruption can be caused by genetic variations in *circadian clock* genes (McKenna et al., 2018). In mammals, the nuclear molecular clock model is formed by genes that operate as a coordinated system of transcription/translation auto regulatory feedback loops (Looby & Loudon, 2005). *Circadian Locomotor Output Cycles Kaput (CLOCK)* and *Aryl hydrocarbon receptor nuclear translocator-like protein 1* (*BMAL1*) transcription factors are the initiation of the clock. This complex (*CLOCK*, *BMAL1*) bounds

to DNA and activates *PER* and *Cryptochromes (CRY1, CRY2)* transcription. They are regulated and turned off when the transcription levels of *CRY* and *PER1* increase. This forms a feedback loop of rising *CLOCK* and *BMAL1* that are then inhibited by rising levels of *CRY* and *PER*. The proper oscillation of the system takes a period of ~24-h, and implies molecular migrations of proteins and mRNA, respectively, to and from the nucleus, together with the protein turnover time (Machicao et al., 2016). *NPAS2* is a paralog of *CLOCK* that dimerizes with *BMAL1* and forms active transcriptional complexes (Landgraf, Wang, Diemer, & Welsh, 2016) (Figure 3).

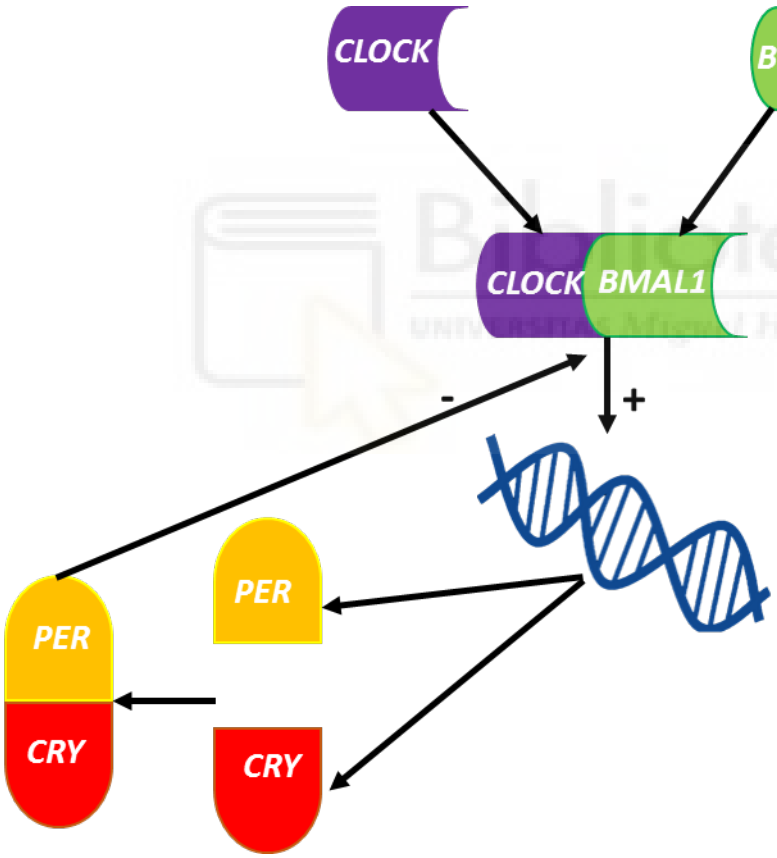

Figure 3. Schematic representation of the regulation of genes believed to be involved in the circadian clock. *[CLOCK] Circadian Locomotor Output Cycles Kaput, [BMAL1] Aryl hydrocarbon receptor nuclear translocator-like protein 1, [PER] Period family and [CRY] Cryptochromes.*

In terms of sleep, *PER family* is involved in the negative feedback loop of the clock (Crane & Young, 2014; Lowrey & Takahashi, 2011), and their inactivation proved to decrease TST in an animal model (Cirelli, 2009). On the other hand, *NPAS2* affects the length of the rest-activity cycle when regulating *PER* levels (Ciarleglio, Resuehr, & McMahon, 2011; Vitaterna et al., 1994), and is also a transcriptional regulator of non-rapid eye movement sleep in mice (Franken et al., 2006). The above mentioned is important, as some authors have described the disturbed sleep architecture in autism (Elia et al., 2000; Limoges et al., 2005).

- *Melatonin pathway* genes

Additionally, when sleep initiation is delayed, some authors have pointed the importance of *MTNR1A* expression (Simonneaux & Ribelayga, 2003). But little is known in humans, there is only a manuscript that described polymorphisms in this gene that altered the surface of the receptor and avoid any melatonin binding in autistic participants (Chaste et al., 2010) (Figure 4).

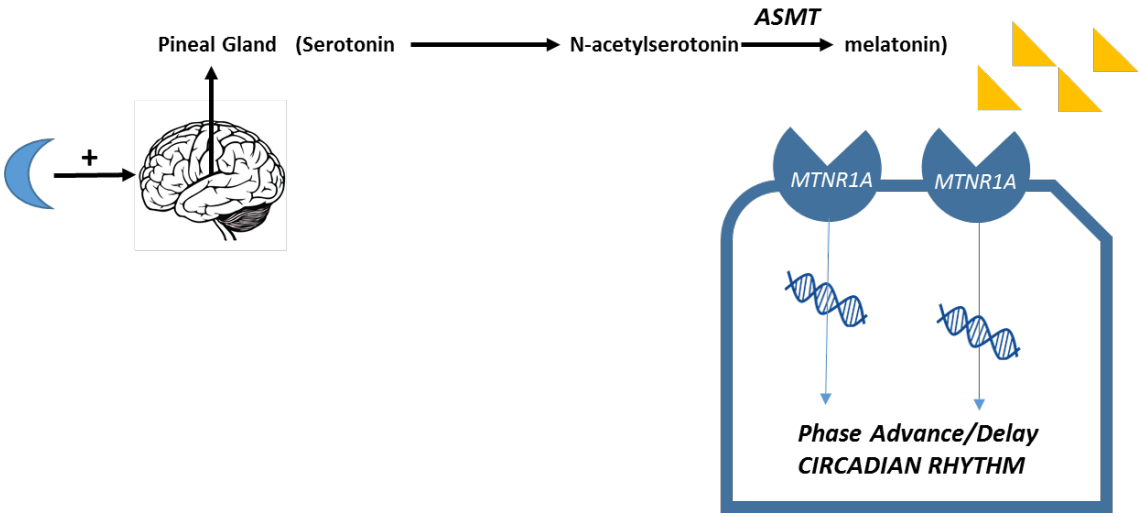

Figure 4. Schematic representation of the melatonin synthesis, secretion and signaling mechanism in charge of maintaining circadian rhythm within the cell. [ASMT] acetyl serotonin methyl transferase.

The last enzyme in the synthesis of melatonin from serotonin (ASMT*)* has been evaluated as a genetic biomarker for autism, and also its implication in sleep problems in autistic individuals (Ackermann & Stehle, 2006; Jonsson et al., 2010; Maronde et al., 2011; C Pagan et al., 2014; Cécile Pagan et al., 2017; Toma et al., 2007; Veatch et al., 2015). Furthermore, *PER1* gene and melatonin interact, as the hormone levels can modify its expression (Agez, Laurent, Pevet, Masson-Pévet, & Gauer, 2007), worsening sleep disturbances. The effects in sleep-wake cycle of *circadian clock* and *melatonin pathway* genes are poorly understood. So far, they could play a role in sleep problems of autistic adults with ID, especially when the regulation of circadian rhythms is impaired and insomnia symptoms are present.

1.2.2. – Diagnosis of sleep problems

According to the frequency and permanence of sleep problems in autistic individuals across the lifespan, it is a matter of importance to properly assess them during clinical visits. Usually, this is done through subjective measures like interviews, parent/caregiver-report questionnaires or a sleep diary record. In contrast, objective measures of sleep rely less on information from others, and instead directly measure aspects of sleep. Figure 5 illustrates some of the most common subjective and objective approaches used to assess sleep in ASD (Moore, Evans, Hanvey, & Johnson, 2017).

Figure 5. Graphic representation from subjective to objective tools available to assess sleep problems in participants.

In this work, Ambulatory Circadian Monitoring (ACM) devices were used since they are wearable, generally well-tolerated, and able to estimate participants' sleep in the home environment (Anders, Iosif, Schwichtenberg, Tang, & Goodlin-Jones, 2011; Jean-Louis, Kripke, Cole, Assmus, & Langer, 2001). Furthermore, it has been reported that values obtained with ACM are closer to polysomnography (PSG) sleep estimations than actigraphy records (Ortiz-Tudela et al., 2014). It can thus provide sleep information that may be otherwise not easily available in individuals with ASD and ID. In addition, ACM is considered more reliable than sleep diaries that are currently accepted (Ancoli-Israel et al., 2003; Tsuchiyama, Nagayama, Kudo, Kojima, & Yamada, 2003).

Recent literature has pointed activity monitoring as a good outcome that could replace complex, expensive and in most cases restricted to the hospital environment measurements like PSG or core body temperature in order to evaluate sleep and the status of the circadian system in humans (Carvalho Bos, Waterhouse, Edwards, Simons, & Reilly, 2003). Furthermore, ACM presents two extra variables, the body position and TAP, when compared to any of the standard devices used to measure sleep ambulatory (Pigeon et al., 2018). Body position defines the rest-activity periods with more accuracy,

and that is a matter of interest in order to detect a potential sedentary lifestyle that may influence sleep. Furthermore, TAP variable is a combination of peripheral temperature, motor activity and body position, and that allows the correction of mistakes attributable for the interpretation of single variables (Ortiz-Tudela, Martinez-Nicolas, Campos, Rol, & Madrid, 2010). Every variable may introduce when treated individually some artefacts as it is influenced by several external signals (Waterhouse et al., 2005).

Even though ACM has not been used in ASD before, is therefore a reliable, non-invasive and continuously assessing tool for circadian status (e.g., information provided by the integrated variable TAP). This variable provides useful insights about the phase, stability and fragmentation of the rhythm. It can be used in intervention to evaluate and preserve the function of the circadian system (Ortiz-Tudela et al., 2016).

1.2.3. – Pharmacological treatment of sleep problems

Given the involvement of neurotransmitters and hormones described above in the regulation of the sleep-wake cycle, and the evidences of their dysregulation in at least some individuals with autism, pharmacological treatments may be useful in treating their insomnia symptoms and CRSWDs. However, there are no practice guidelines for sleep problems in adolescents and adults on the autism spectrum (Frazier et al., 2011). Therefore, behavioural therapy is usually the first-line treatment for patients with poor sleep, and on top pharmacological therapies to improve their performance in daily activities (Glickman, 2010; Ridderinkhof, de Bruin, Blom, & Bögels, 2018). But, there are only two drugs: aripiprazole (Marcus et al., 2009; Owen et al., 2009) and risperidone (Scahill et al., 2006; Shea et al., 2004) that have US Food and Drug Administration

approval for their use in ASD, to treat irritability and disruptive behavioural symptoms, respectively.

Thus, there is a diverse range of off-label pharmacotherapeutic options for sleep problems in ASD, without effectiveness proved in a clinical trial. That increases combined prescriptions that may induce polypharmacy (understood as 4 or more active prescriptions during at least 6 months) in this population without clear evidence of effectiveness. The off-label use of some drugs to treat sleep problems could increase the risk of developing side effects. Those that are concerning include metabolic disturbances, weight gain, extrapyramidal symptoms and hyperprolactinemia, which should be kept in mind when prescribing antipsychotics (LeClerc & Easley, 2015).

Even more, most failures of pharmacological treatments may be due to lack of matching treatment to the underlying cause of the sleep problem.

- Melatonin receptor agonists

As mentioned earlier, one of the causes of sleep problems in autism may have something to do with melatonin levels and/or peak times. Thus, nowadays, one of the most promising molecules in treating sleep onset insomnia in ASD are melatonin receptor agonist drugs. Melatonin circadian rhythm is a phase marker of the sleep-wake rhythm, and the evening rise in melatonin (dim light melatonin onset) is one of the best markers of circadian rhythmicity (Saper, Scammell, & Lu, 2005). Its release from the pineal gland follows a cyclical 24-hour pattern, with low levels during the day and elevated levels at night (Ellis, Lemmens, & Parkes, 1996). Lower urinary 6-sulphatoxymelatonin (Tordjman, Anderson, Pichard, Charbuy, & Touitou, 2005) and

plasma melatonin (Melke et al., 2008) levels have been found in ASD individuals in some studies, indicating some abnormalities in this pathway.

Insomnia and CRSWD can be treated with melatonin to reduce SoL and increase TST (Braam et al., 2009; B. Malow et al., 2012) or move sleep phase (Souders et al., 2017; Zee, Attarian, & Videnovic, 2013). It has been shown to be effective, with minimal adverse events (Giannotti, Cortesi, Cerquiglini, & Bernabei, 2006). A reduction of wakes after sleep onset (WASO) may be achieved using prolonged release forms, that have already proved safety in a trial up to 2-years (Maras et al., 2018).

Melatonin, together with behavioural interventions (e.g., functional analysis of the problem or stimulus control) (Wiggs & Stores, 2001) and sleep hygiene (reducing naps, increasing morning exercise, good morning light exposure), have been used to treat sleep problems in ASD (Zisapel, 2018). However, results reported from clinical trials with melatonin vary considerably depending on the age range studied (Gringras, Nir, Breddy, Frydman-Marom, & Findling, 2017), the diagnostic tools employed (Allik, Larsson, & Smedje, 2008; Johnson & Malow, 2008; McArthur & Budden, 1998), the length of the study (Jan & O'Donnell, 1996; Paavonen, Nieminen-von Wendt, Vanhala, Aronen, & von Wendt, 2003), the different doses used (Andersen, Kaczmarska, McGrew, & Malow, 2008; Jan & O'Donnell, 1996; Wasdell et al., 2008), and the sleep parameters which improve after treatment (Rossignol & Frye, 2011).

There is a small study of six adults that proved melatonin effectiveness in decreasing SoL time (Galli-Carminati, Deriaz, & Bertschy, 2009). Thus, there is a lack of clinical trials to test the effectiveness of pharmacological treatments for sleep problems, generally in autistic adults, particularly in autistic adults with ID.

- Agomelatine

Agomelatine (Valdoxan®) was first reported in 1992, acting as an agonist for melatonin receptors (MT_1 and MT_2) and as an antagonist on serotonin 5HT2c receptors. One of the most important pharmacological properties of agomelatine is its pro-chronobiological effect, accelerating the restoration of circadian rhythms. The binding affinity of agomelatine for MT1 and MT2 is similar to melatonin, but its half-life is longer (De Berardis et al., 2015; Stahl, 2014) (Figure 6).

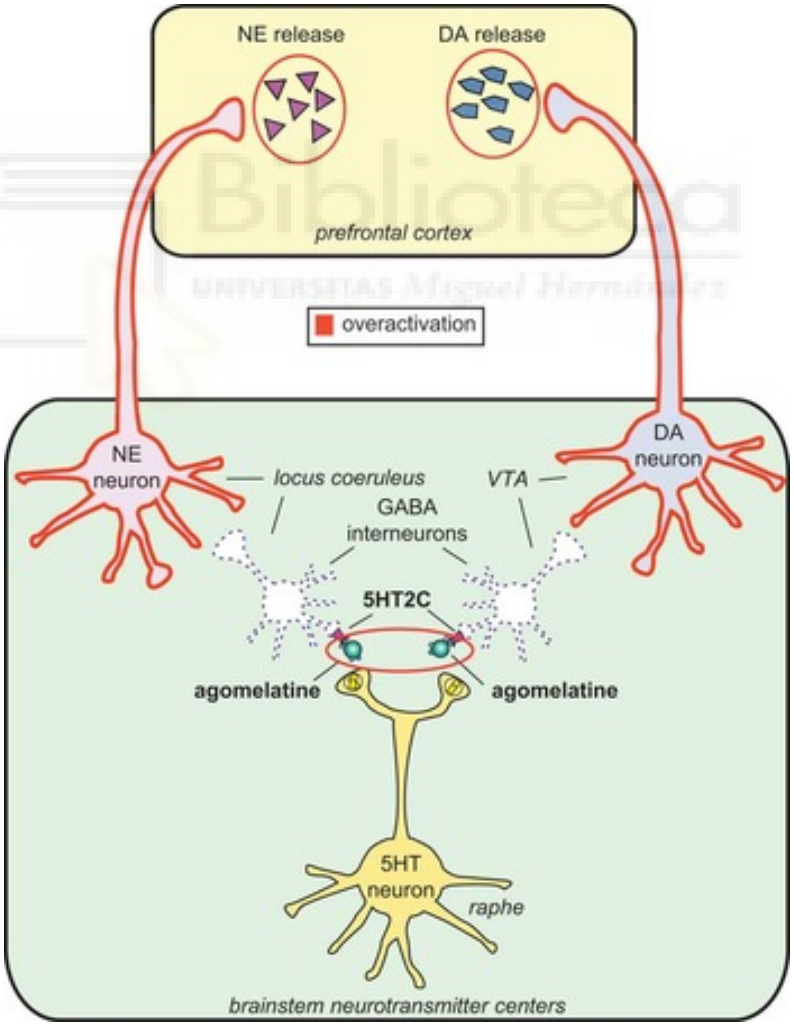

Figure 6. A schematic representation of agomelatine mechanism of action. Adapted from Stahl (2014). [NE]Noradrenaline, and [DA] Dopamine.

- Other drugs

Apart from the above mentioned, some drugs that prompt sleep (antipsychotics, antidepressants and stimulants) are being prescribed to children, young people and adults with ASD (Aman, Lam, & Van Bourgondien, 2005; Esbensen, Greenberg, Seltzer, & Aman, 2009; Mandell et al., 2008; Oswald & Sonenklar, 2007; Williams et al., 2012). Longitudinal studies proved that there is an increase in prescriptions of psychotropic medication across the time, from 31% in children to 45% in adults with autism (Aman et al., 2005). Also, recent studies reported an increment of psychotropic prescriptions up to 73%, in young autistic adults, with ages from 18 to 21 years old (Mandell et al., 2008). The above mentioned occurs even though there is limited evidence of pharmacological effectiveness derived from clinical trials with ASD participants.

The rationale behind this work is that autistic condition often coexists with others, such as ID and sleep problems. When ASD and ID are present, sleep problems can appear in up to 45% of adults. However, studies are generally developed in children or cognitively able adults, and use small samples or subjective sleep measures. Apart from that, causes of sleep abnormalities remain unknown, but are likely to be multifactorial, involving *circadian clock* and *melatonin pathway* genes. In addition, there is a lack of clinical trials, in autistic adults with ID, to test the effectiveness of sleep pharmacological treatments. Therefore, ACM is a promising device to evaluate sleep problems, the effectiveness of agomelatine and the role of *circadian clock* and *melatonin pathway* genes as potential sleep biomarkers in this vulnerable population. Increasing knowledge in this field is important to guarantee a proper and complete evaluation of sleep and to test interventions in ASD which could have a significant impact on their health.

HYPOTHESIS AND OBJECTIVES

2.1. – Hypothesis

It has been observed that autistic adults with ID associated may present sleep problems, insomnia and CRSWDs, as co-occurring symptoms with high impact on their health. It is considered that recording sleep-wake cycle with an objective tool could help to better characterize circadian rhythms and sleep parameters. ACM would confirm in our sample those sleep problems described as highly prevalent across the literature. Furthermore, in case that sleep problems were present in our population, ACM may help to assess the effectiveness of a new pharmacological treatment, agomelatine, in a randomized clinical trial, and also the role of *circadian clock* and *melatonin pathway* genes as potential biomarkers for sleep-wake cycle.

2.2. – Objectives

Main objective:

The main objective of our study was to evaluate the use of ACM recordings to provide information about the sleep-wake cycle, and possible sleep problems in autistic participants with ID compared to controls.

Secondary objectives

1- To assess the effectiveness and tolerability of agomelatine, a selective agonist of melatonin receptors (MT_1 and MT_2), as a pharmacological treatment for sleep problems in autistic adults with ID.

2- To analyse the presence and influence of *circadian clock* (*PER1 and NPAS2*) and *melatonin pathway* (*ASMT* and *MTNR1A*) gene polymorphisms in sleep parameters and circadian rhythms in autistic adults with ID.

PARTICIPANTS AND METHODS

3.1. – Ethics

Hospital Ethics Review Board approved the two studies. All participants, or their legal guardians received information about the design and purpose of each project, and participants or legal guardians' informed consent was obtained. All was performed in accordance with the principles of Helsinki Declaration.

1- **"Effectiveness of agomelatine in sleep problems in Autism Spectrum Disorder (ASD)"**// "Eficacia de agomelatina en la alteración del sueño en el Trastorno del Espectro Autista (TEA)" **AGOTEA and AGOTEA Clinical Trial (CT) code (EudraCT: 2011-003313-42)**

Hospital Ethics Review Board approval was in February the 29th, 2012. *Appendix 1.*

Promoter and principal investigator: Dr. Ana M. Peiró Peiró in Alicante's General Hospital. Pura Ballester Navarro was a research collaborator.

Funded by Servier Laboratories and Pura's contribution was supported by a predoctoral grant from Fundación La Caixa.

2- **"Synchronized actimetry with the FITBIT Flex System: assessment of sleep problems measured by actigraphy and its application to autism spectrum disorder"** // "Actimetría sincronizada con el Sistema FITBIT Flex: evaluación de la alteración del sueño medido por actigrafía y su aplicación al trastorno del espectro autista" **GENTEA code.** We present only the data of the genetic substudy.

Hospital Ethics Review Board approval was in October the 1st, 2014. *Appendix 1.*

Promoter and principal investigator: Dr. Ana M. Peiró Peiró in Alicante's General Hospital. Pura Ballester Navarro was a research collaborator.

Funded by Alicia Koplowitz Foundation 2014 (UGP-14-011) and Pura's contribution was supported by a predoctoral grant from Fundación La Caixa.

3.2. – Participant Recruitment and Inclusion Criteria

- Autistic group

Most autistic participants were residents across four Spanish autism institutions for adults located in Alicante and Valencia (Centro Infanta Leonor, EDUCATEA, Centro Ángel Rivière-APNAV and Centro San Rafael). They were recruited after researchers met with parents and carers in the institutions, from clinics specializing in adults on the spectrum (Alicante General Hospital, Spain) and via social media.

Inclusion criteria for the adults with ASD were age from 18 to 65 years; agreement to one or more clinical visits, and a previous diagnosis of ASD substantiated using the DSM-5 (American Psychiatric Association, 2013) criteria for ASD. Diagnosis based on DSM-5 criteria was confirmed by a clinician from the individual's residential facility and by a psychiatrist from our research team. ID (intelligence quotient (IQ) <70) for the adults with ASD was confirmed from medical records of professionals from the Spanish social services. Sleep problems were not required (AGOTEA and GENTEA), or they were compulsory (AGOTEA CT) to participate.

Adults on the autism spectrum continued to take their regular medications during the study. Sleep medication or melatonin were not allowed in any study, and individuals taking CYP 1A2 inhibitors were excluded from AGOTEA CT.

- Control groups

The inclusion criteria for both control groups were: same geographical area, and agreement to an initial clinical visit (AGOTEA only) and that their data would be included in this study. No participant in the control groups had a clinically significant medical or psychiatric condition, or was taking any psychotropic medications.

The control group in AGOTEA was composed of typically developing adults recruited from the same geographical area, who were also participating in a longitudinal study being conducted by the Chronobiology group at Murcia University.

The control group in GENTEA was formed by typically developing adults all healthy and from the same geographical area. Samples came from the Genotyping National Centre sample collection (CeGen, Santiago de Compostela, Spain), integrated within the Resources Network Platform (PRB2) sponsored by Carlos III Health Institute (Madrid, Spain, CEGEN-PRB2-ISCIII).

All participants could withdraw from the studies at any time, and those with any medical condition that was incompatible with the study conditions were excluded.

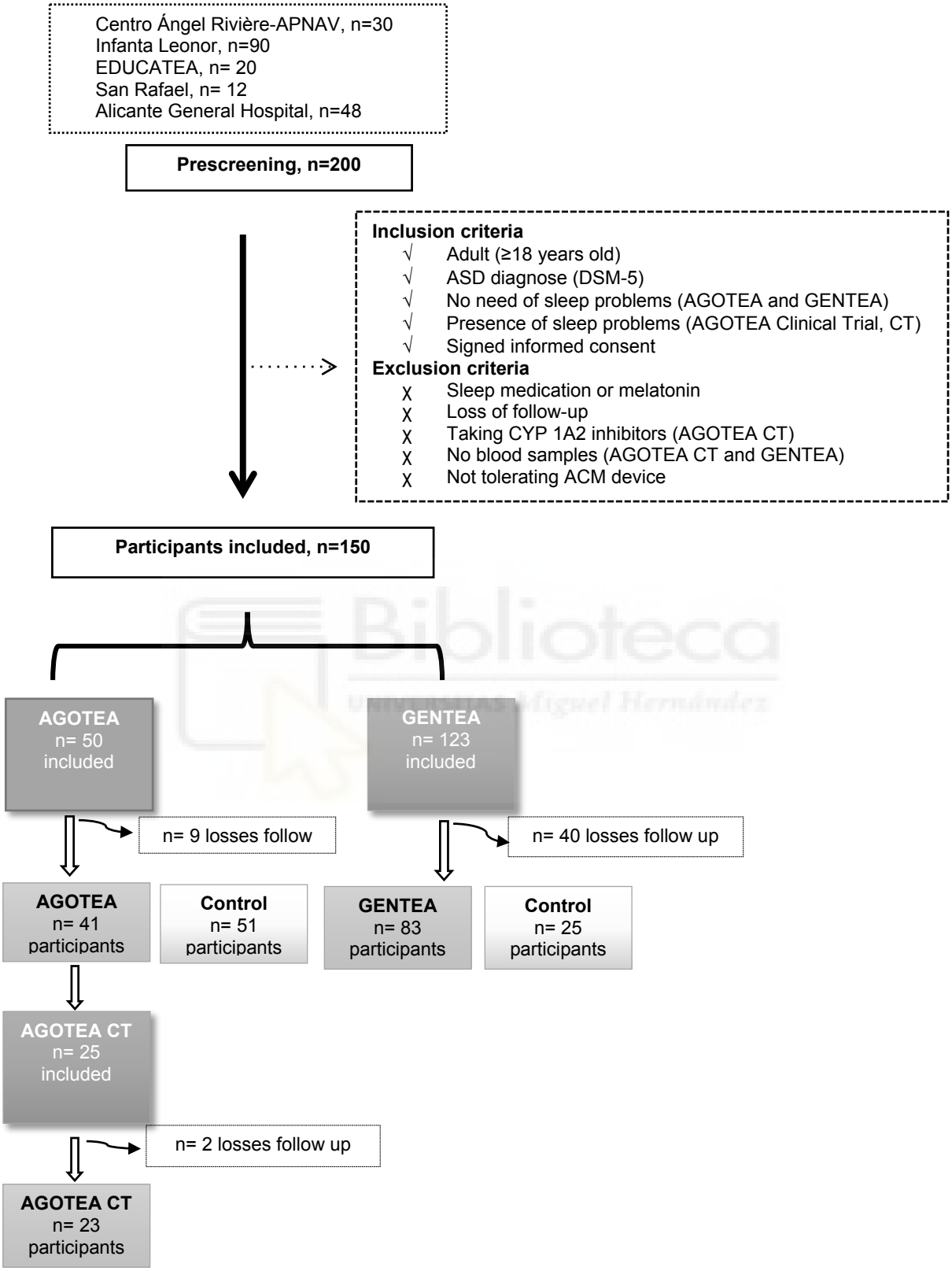

Figure 7. Flow chart of ASD sample selection. [DSM-5]: Diagnostic and Statistical Manual of Mental Disorders 5th Edition, [ACM]: Ambulatory Circadian Monitoring, and [CT]: clinical trial.

3.3. – Methods

Both studies, AGOTEA and GENTEA, analyzed sleep parameters and circadian rhythms with ACM device. Therefore, this device is explained in this section. The particularities of each study will be further developed in Results section.

Ambulatory Circadian Monitoring (ACM)

As it was the first time that this device was used in a sample of autistic adults with ID, we designed a protocol of habituation to the device. The first phase, one week, consisted in anticipation to ACM device using visual support (e.g., photos and pictograms). When concluded, the second phase began and participants wore fake watches and bands to simulate the ACM (this phase had different duration, from one to three weeks).

After the training was completed and participants tolerated the device, they started the measurement week. All participants were asked to follow their usual routines and wore the ACM device on the wrist of their non-dominant arm for seven days. The ACM was removed during showering or any other activity where the ACM device might get wet; data were filtered in order to eliminate erroneous measurements produced by its temporary removal. (Figure 8).

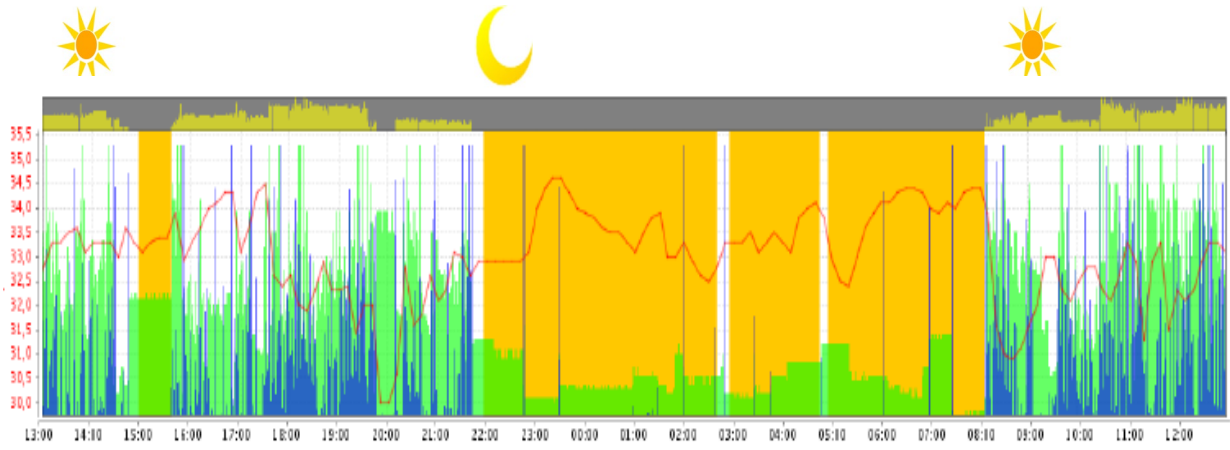

Figure 8. Sample of 24h recordings with ACM, wrist temperature [red], Motor Activity [blue], Body Position [green], Light intensity [light yellow at the top] and sleep [dark yellow].

Control participants, completed a 7-day sleep-wake diary (mornings and evenings). In case of the adults with ASD, parent or caregiver was responsible for completing participant's sleep-wake diary, which were used as a backup for the ACM recordings if needed.

Sensors registration

The ACM device (Kronowise) has three different sensors: (i) The first measures wrist temperature, Thermochron iButton DS1921H, ± 1 °C accuracy, and sampling every 10 min; (Carrier & Monk, 1997). (ii) The second is an actimeter that estimates motor activity using degrees of change in position; and body position using the angle between the X-axis of the actimeter (90º verticality) and the horizontal plane (0º maximum horizontality), HOBO Pendant G Acceleration Data Logger UA-004-64, three-channel logger (X-, Y-, Z- axis) with 8-bit resolution, programmed to record data every minute; (Edgar & Dement, 1991; Mormont et al., 2000). Actimeter information was analyzed defining two variables: motor activity and body position (Bonmati-Carrion et al., 2015;

Carvalho Bos et al., 2003). (iii) The third sensor measures light intensity, HOBO Data Logger UA-002-64, it has a measurement range of 0–320 000 lux, measured in 30-second intervals (Martinez-Nicolas, Ortiz-Tudela, Madrid, & Rol, 2011).

Sleep parameters and circadian sleep-wake rhythm calculation

Sleep parameters and circadian sleep-wake rhythm indexes were calculated from the data recordings. The information stored in the Thermochron iButton, the actimeter and light sensor was transferred to a personal computer using the software provided by the manufacturer through an adapter (DS1402D-DR8; IDC, Spain) or an optical USB Base Station (MAN-BASE-U-4, HOBO). The Circadianware software implemented in the Kronowizard platform (https://kronowizard.um.es/) calculated the single integrated variable, TAP, from the integration of wrist temperature (inverted), motor activity and body position where maximum values should occur at the same time of the day and indicate a high level of activation (values near 1) or complete rest and sleep (around 0) (Lopez, Jaussent, & Dauvilliers, 2014; Ortiz-Tudela et al., 2014). For computing TAP values, motor activity and body position data were added up and averaged, respectively, in 10-minute intervals (matching wrist temperature sampling rate). Sleep was inferred from TAP and converted into a binary code, with 1 corresponding to a resting period and 0 to an active period (Ortiz-Tudela et al., 2014; Sarabia, Rol, Mendiola, & Madrid, 2008).

Sleep parameters

The sleep parameters (Table 2) calculated were: (1) TST (normal value from 420 to 540 minutes), defined as the number of minutes registered as sleep between sleep onset

and sleep offset during the night (similar to PSG sleep period time); (2) time in bed (TIB, normal value from 420 to 569 minutes), total minutes in bed until sleep offset; (3) SoL (normal value <30 minutes) the time until sleep onset at night; (4) number of awakenings after sleep onset (normal value 0-1 number), during the TST interval; (5) awake period duration after sleep onset duration (WASO, normal value <20 minutes), the awake period length in minutes during the TST interval; and (6) SE (normal value ≥85%), calculated as TST/TIB ratio multiplied by 100.

Table 2. Sleep parameters definitions and normality ranges for adults.

Sleep parameters	
Total sleep time (min) % in normal range (420-540 min)	Defined as the number of minutes registered as sleep between sleep onset and sleep offset during the night
Time in bed (min) % in normal range (420-569 min)	Total minutes in bed until sleep offset
Sleep onset latency (min) % in normal range (<30 min)	The time until sleep onset at night
Number of awakenings (nº) % in normal range (0-1 nº)	Number of awakenings during the TST interval
Wake after sleep onset (min) % in normal range (<20 min)	The awake period length in minutes during the TST interval
Sleep efficiency (%) % in normal range (≥85 %)	Calculated as the ratio of TST/TIB multiplied by 100

The results of the sleep parameters from all participants were classified as normal or not, according to population normality ranges (Carskadon & Rechtschaffen, 2000; Natale, Plazzi, & Martoni, 2009; Watson et al., 2015; Zhang et al., 2011).

Circadian sleep-wake rhythm indexes

Non-parametric circadian rhythm analysis is a method for extracting circadian characteristics from the rest–activity cycle (Van Someren & Riemersma-Van Der Lek, 2007; Weitzman et al., 1981). Of major interest is the relative amplitude (RA), since it shows how activity is distributed throughout the day compared with night: the higher the RA, the better the consolidation of daytime activity and night-time sleep. The RA is calculated from the ratio of the most active 10-hour period (M10) to the least active 5-hour period (L5) across the averaged 24-h profile. A second characteristic is the inter-daily stability (IS), which quantifies the invariability day by day, that is, how well the sleep–wake cycle is synchronized to supposedly stable environmental cues. Thirdly, intraday variability (IV) gives an indication of the fragmentation of the rhythm. Timing and phase information comes from determining the onset of the 5 hours with least activity (L5) and of the 10 hours with most activity (M10). Finally, the circadian function index (CFI) assesses circadian rhythmicity status and was calculated as (IS+ (2-IV) +RA)/3. CFI has proved to be very sensitive to changes in circadian consistency (Ortiz-Tudela et al., 2010; Witting, Kwa, Eikelenboom, Mirmiran, & Swaab, 1990).

All the circadian rhythms are measured during a week; every day generates a wave per rhythm. According to the waves of different days or their fluctuations within a day we can describe a rhythm with indexes such as inter-daily stability (Figure 9a), intraday variability (Figure 9b); and phase markers or relative amplitude (Figure 9c).

a)

b)

c)

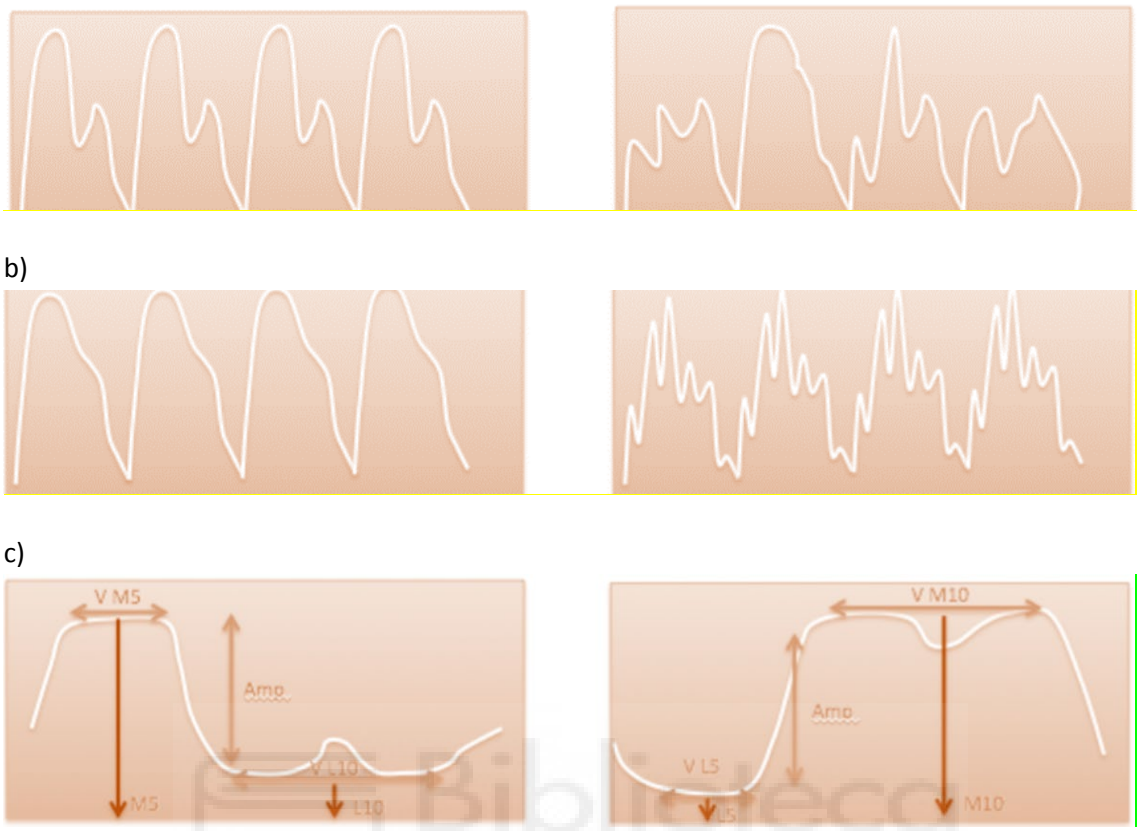

Figure 9. Graphic representation of 4 days of a) more (value close to 1, left) and less (value close to 0, right) inter-daily stability, b) more (value close to 0, left) and less (value close to 2, right) intraday variability, and c) a day with its relative amplitude and phase markers in a rhythm with higher (left, e.g., wrist temperature) and lower (right, e.g., motor activity) values at night.

3.4. – Statistical Analyses

The Shapiro-Wilk normality test was used as the basis for selection of parametric or non-parametric statistical tests. Continuous variables are presented as mean ± standard deviation (SD), mean ± standard error (SEM) or median and interquartile range (IQR, $P_{25,}$ P_{75}) according to assessed normality of the variable. Categorical variables are expressed as percentages. All statistical analyses were performed with R 3.2.4 software and Graph Pad Prism 5.0. P-values of <0.05 were considered to indicate significance for all analyses.

Summary

The three manuscripts included in this work shared the ACM device to calculate sleep parameters (TST, TIB, SoL, Num. awakenings, WASO, and SE), their percentages of normality, and circadian sleep-wake rhythm indexes (IS, IV, RA, CFI and phase markers). Also, the participants from the three manuscripts were all autistic adults with ID.

Ballester, P., Martínez, M. J., Javaloyes, A., Inda, M. D. M., Fernández, N., Gázquez, P., Aguilar V., Pérez A., Hernández L., Richdale A.L. Peiró, A. M. (2018). Sleep Problems in Adults with Autism Spectrum Disorder and Intellectual Disability. Autism Research.

4.1. – Main objective

Introduction

Sleep problems are common comorbid symptoms in individuals with ASD and can impair social behaviour, cognitive daytime performance, and quality of life. Sleep problems can be life-long, with prevalence in childhood ranging from 50% up to 86%, and a 45% in autistic adults with severe ID. The aim of our study was to compare circadian rhythms and sleep patterns in adults with ASD and ID with typically developing adults using ACM.

Methods

Procedure

The study was conducted from February 2012 to December 2013, excluding summer (June-August) because weather could affect the accuracy of the temperature sensor. During the first visit, demographic information (age, sex, body weight, height, and job status) and current medications were ascertained.

Participants

Participant Recruitment and Inclusion Criteria

The adults on the autism spectrum were recruited from four Spanish autism associations after researchers met with parents and carers (93%), from clinics specializing in adults on the spectrum (5%) and via social media (2%); all participants were resident across three institutions for adults with ID. The study information provided to participants emphasized that no sleep problem was required to participate in this sleep study.

For further details about recruitment process and inclusion criteria for the adults with ASD and ID, and control participants see section 3.2. - Participant Recruitment and Inclusion Criteria.

Statistical Analyses

For shared statistical analyses, please refer to Methods section. A t-test for independent samples or a Mann Whitney U test was used to assess group differences; effect sizes (η^2, r respectively) and 95% confidence intervals (CIs) are also reported. Frequencies were compared using the Chi-Square test, with Yate's continuity correction as appropriate and X^2 (df, n) were reported.

As treatments used by adults on the autism spectrum for their comorbidities (antipsychotics, anticonvulsants, antidepressants or anxiolytics) could have potential effects on sleep, comparison analyses of sleep parameters between typically developing controls and adults with ASD with or without comorbidity treatments were conducted using the Student t-test or Mann Whitney U-test. Acknowledging that the categories of antipsychotics, anticonvulsants, antidepressants or anxiolytics could embrace several different molecules that could affect sleep in different ways, further intragroup analysis

classifying the molecules according to how they affect sleep (positively, negatively or neutral (Brunton, Chabner, & Knollman, 2011)) were conducted by Kruskal Wallis test.

Results

Forty-one individuals with ASD and ID (78% male, BMI 24.4 ± 1 Kg/m^2) between the ages of 27 and 39 years old, and 51 adults with normal intellectual functioning and no diagnosis of mental or physical health problems (41% male, BMI 23.2 ± 0.6 Kg/m^2) with ages from 28 to 38 years old were included in this study (Figure 10).

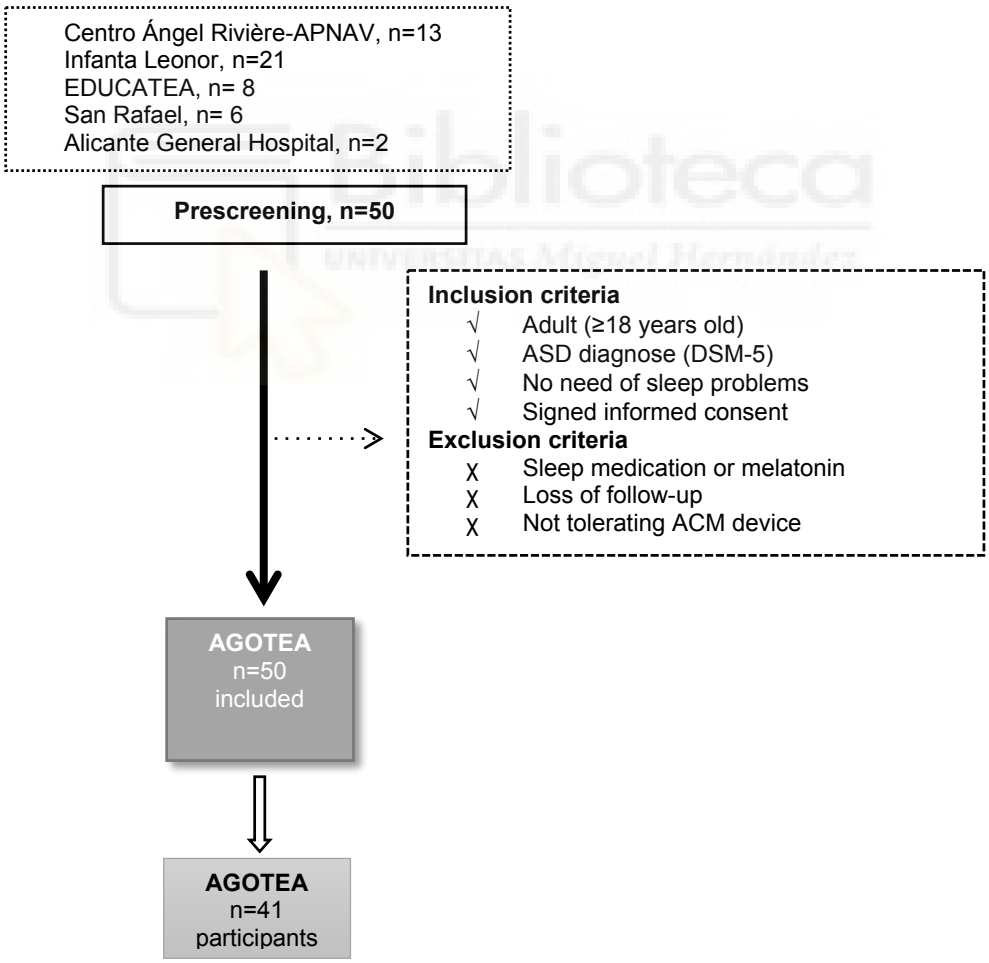

Figure 10. Flow chart of AGOTEA sample selection [DSM-5] Diagnostic and Statistical Manual of Mental Disorders 5th Edition, and [ACM] Ambulatory Circadian Monitoring.

A total of thirty-three (80%) participants with ASD reported various comorbid clinical diagnoses (epilepsy, depression and aggressive behaviour) and were taking medications associated with them. Demographic data are summarized in Table 3.

Table 3. Participants' demographic information.

	ASD n=41	**Control n=51**
Sex, Men/Women (n)	31/10	21/30
Age, mean ± SD (years)	33±6	33±5
BMI, mean ± SD (Kg/m^2)	24.4±1	23.2±0.6

Data is expressed as mean ± SD or count. [ASD] Autism Spectrum Disorder, and [BMI] Body Max Index.

The ASD group had a median of three comorbidities (IQR_{25-75}: 1-4, 38% epilepsy) and five prescribed medications associated with these comorbidities (IQR_{25-75}: 2-6, 61% antipsychotics, 51% anticonvulsants, 22% antidepressants and 32% anxiolytic, 20% without treatment). Figure 11 presents the different medications with potential effects on sleep (increasing sleep or somnolence) taken by ASD participants. Other drugs taken by ASD participants, but without potential effects on sleep were: proton pump inhibitors (17%), anticholinergic agents (22%), ß-agonists (2%) and/or ß-blockers (2%).

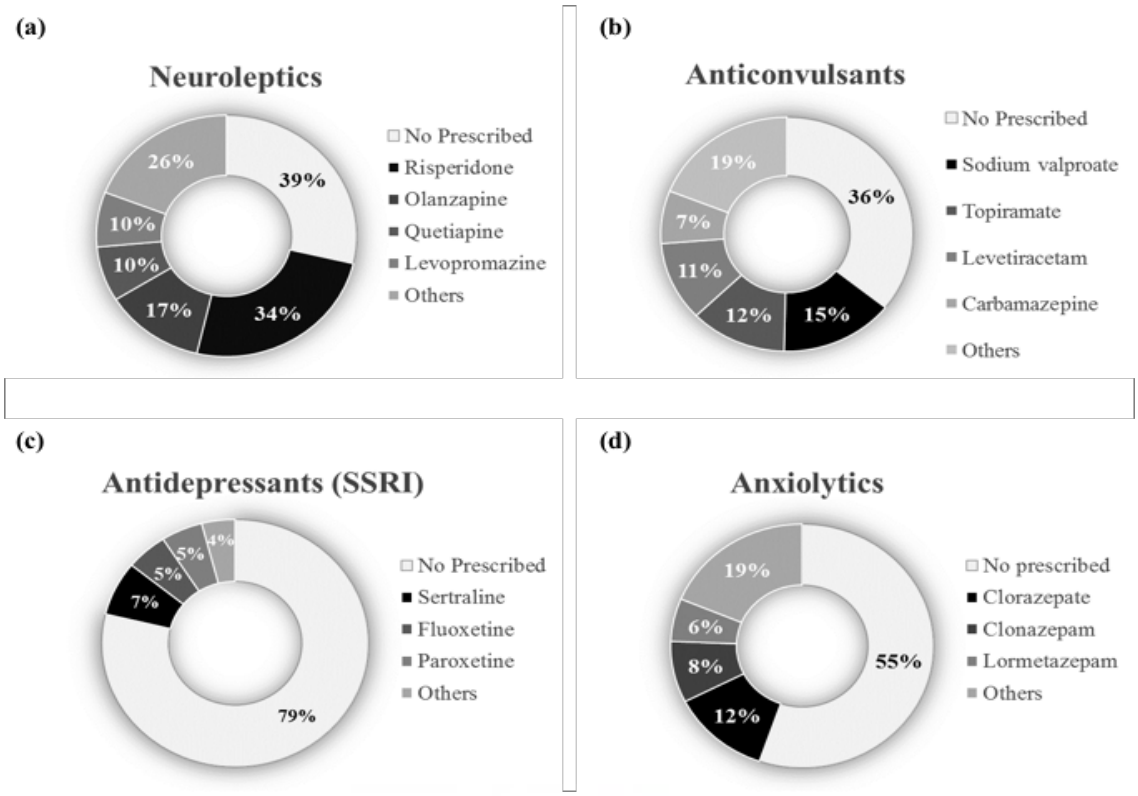

Figure 11. Categorization of medications prescribed to ASD participants with potential effects on sleep: antipsychotics (a), anticonvulsants (b), antidepressants (c), and anxiolytics (d).

There were no significant differences between ASD and control groups with respect to age, but as may be expected, there were significantly more males in the ASD group ($p=0.002$; X^2 (1, 92) = 13.27). A comparison analysis showed significant differences for ASD participants with and without anxiolytic treatment only for the number of awakenings only ($p=0.006$; 95% CI -2.812 - -0.5072; $r=0.196$). No significant differences for any sleep parameter were found when drug categories (antipsychotics, anticonvulsants, antidepressants or anxiolytics) were analyzed by their potential effects on sleep (positive, negative or neutral).

Ambulatory Circadian Monitoring

Sleep parameters

Comparison of sleep parameter data between ASD and control groups obtained from ACM recordings is presented in Table 4. Significant differences between ASD and control groups were found for all sleep parameters, except TST. The ASD group showed significantly increased TIB, SoL, number of awakenings and WASO than controls and significantly lower SE. Overall, with the exception of WASO, significantly fewer individuals with ASD had sleep parameters in the normal range compared to those in the control group. In spite of the difference in gender distribution observed between ASD and control groups, differences in sleep parameters were maintained when analyzed by sex ($p \geq 0.05$ only for TST). No differences between males and females within each group were observed.

Table 4. Sleep parameters comparison obtained from Ambulatory Circadian Monitoring recordings between adults with Autism Spectrum Disorder and Intellectual Disability and control participants.

Sleep parameters	**ASD**	**Control**	**p value**	**R or η^2**	**X^2**
Total sleep time (min)	478±140	457±77	0.064	0.19	
% in normal range (420-540 min)	5	72	**<0.001**		40.01
Time in bed (min)	657±182	540±72	**<0.001**	2.74	
% in normal range (420-569 min)	16	75	**<0.001**		31.53
Sleep onset latency (min)	60±48	14±9	**<0.001**	4.85	
% in normal range (<30 min)	16	95	**<0.001**		59.92
Number of awakenings (nº)	3 (2-5)	2 (1-3)	**<0.001**	0.1746	
% in normal range (0-1 nº)	3	33	**<0.001**		12.57
Wake after sleep onset (min)	106±74	50±35	**<0.001**	2.13	
% in normal range (<20 min)	0	10	0.073		3.77
Sleep efficiency (%)	70	85	**<0.001**	2.48	
% in normal range (≥85 %)	5	67	**<0.001**		34.44

Data is expressed as mean ± SD. Statistically significant differences were found by Mann Whitney or Chi Square test $p<0.05$ are highlighted in bold. For all parameters X^2 (df, n) equals to (1, 92). [Min] minutes.

Circadian sleep-wake rhythm indexes

Analysis of the circadian rhythms (wrist temperature, motor activity, body position, TAP, sleep, and light intensity) showed significant differences between the ASD and control groups (Figure 12).

The ASD group had higher wrist temperature values with a lower amplitude and a wake maintenance zone (time interval of minimal sleep probability, minimal daily distal temperatures) that appeared earlier than in controls (ASD: 17:00-18:00 h versus controls: 20:00-21:00 h) (Figure 12a). Lower overall motor activity with less difference between the least (L5) and most active (M10) phases was observed in the ASD group (Figure 12b).

Furthermore, the ASD group showed higher motor activity values between 3:00 to 6:00 h (Figure 12b) and body position at 5:00 h (Figure 12c). Together, these results point to sedentary daytime behaviour in the ASD adults, with nocturnal activation (Figures 12b and 12c). As expected, TAP and sleep values were consistent with the results for motor activity and body position (Figures 12d and 12e). Differences in light intensity between both groups were also found. In the morning, individuals with ASD were exposed to higher light levels than control subjects, and from 15:00 to 7:00h, they experienced lower light levels (Figure 12f).

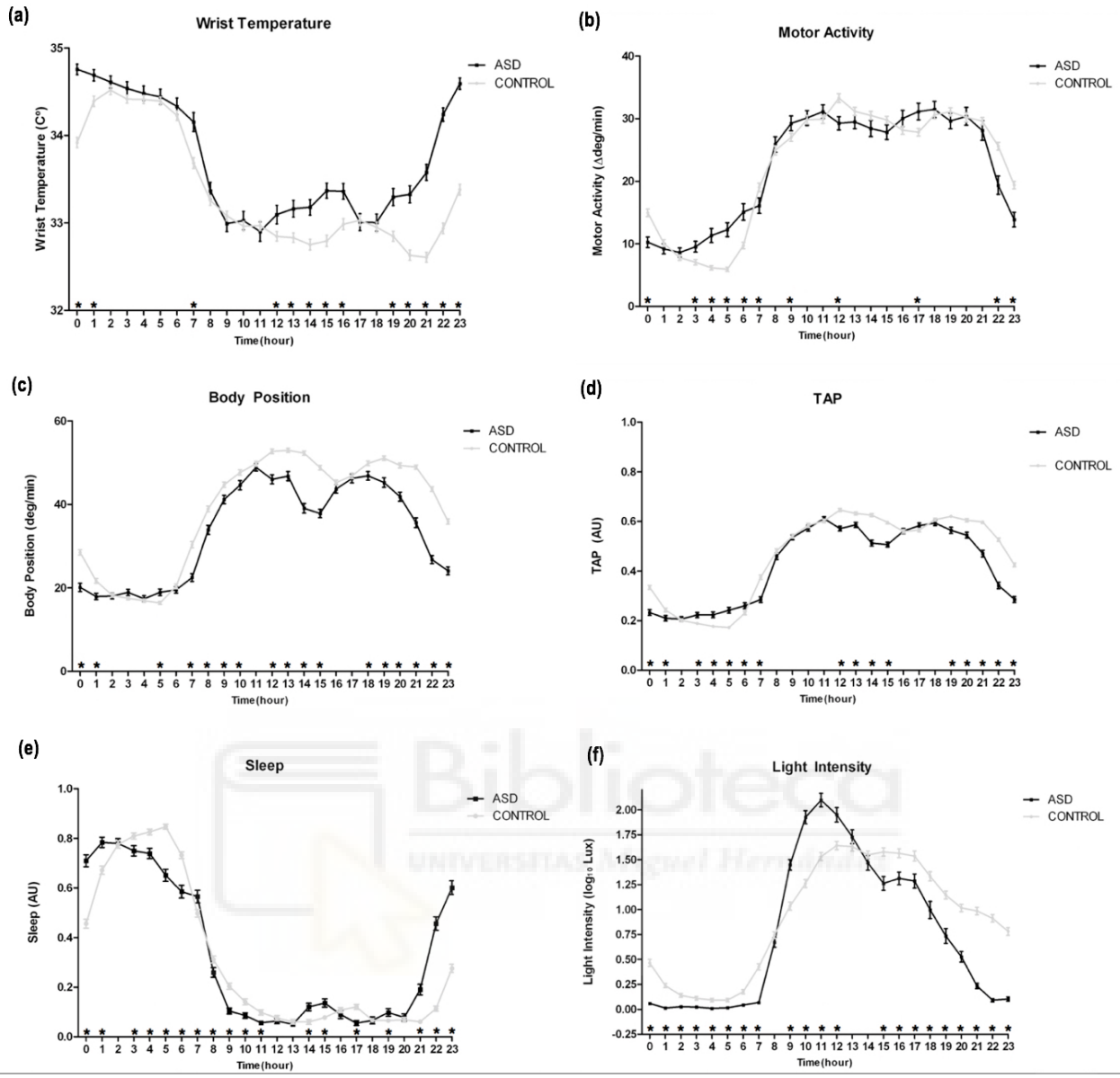

Figure 12. Circadian rhythms from wrist temperature (a), motor activity (b), body position (c), TAP (d), sleep (e), and light intensity (f) in ASD and control subjects. [ASD] Autism Spectrum Disorder.

Overall, circadian phase advance in the ASD group was suggested by the higher values for wrist temperature and sleep and the lower motor activity and body position during the late afternoon and the first part of the night when compared to controls.

Table 5 shows the non-parametric indexes for the circadian rhythms plotted in Figures 12a to 12f. The circadian characteristics of the sleep-wake rhythm showed a consistent phase advance in ASD subjects as evidenced by the four phase markers (L5, L10, M5 and

M10). Significant group differences were present in all circadian rhythms studied (wrist temperature, motor activity, body position, TAP, sleep and light intensity) when compared with controls (Table 5). The autistic group showed significantly higher inter-daily stability in wrist temperature and light intensity, and lower inter-daily stability in motor activity. Intraday variability results were consistent with those found for inter-daily stability. Lower amplitude was found in sleep records for the autistic group in contrast with light intensity, indicating poor sleep conditions in adults with ASD and ID.

Table 5. Analysis of non-parametric circadian rhythms along 24-hour period in adults with Autism Spectrum Disorder and Intellectual Disability compared to typical developing controls.

Wrist temperature					Motor activity					Body position				
	ASD	Control	p value	r		ASD	Control	p value	r		ASD	Control	p value	r
IS	0.44±0.04	0.38±0.02	**0.048**	0.31	IS	0.29±0.02	0.25±0.02	**0.002**	0.69	IS	0.42±0.03	0.41±0.02	0.231	0.04
IV	0.14±0.01	0.21±0.01	**0.001**	0.79	IV	1.02±0.04	1.04±0.02	0.369	0.01	IV	0.42±0.06	0.42±0.02	0.091	0.14
RA	0.28±0.03	0.29±0.02	0.384	0.01	RA	0.72±0.03	0.71±0.02	0.252	0.04	RA	0.49±0.03	0.51±0.02	0.447	0.00
M5	1:07±0:40	4:32±0:38	**<0.001**	1.27	L5	2:37±0:24	3:54±0:11	**0.001**	0.76	L5	2:26±0:39	4:20±0:08	**0.003**	0.61
VM5	34.75±0.16	34.6±0.10	0.208	0.06	VL5	5.42±0.85	5.97±0.55	0.087	0.15	VL5	15.78±1.44	16.92±0.77	**0.035**	0.26
L10	14:35±0:22	18:04±0:31	**<0.001**	0.01	M10	15:58±0:41	15:55±0:30	0.495	0.00	M10	15:12±00:17	15:55±0:13	**0.017**	0.36
VL10	32.79±0.17	32.66±0.13	0.326	0.02	VM10	28.09±2.17	31.56±1.09	**0.024**	0.33	VM10	44.70±1.94	51.52±0.88	**0.001**	0.82
CFI	0.55±0.02	0.52±0.02	0.070	0.18	CFI	0.5±0.02	0.48±0.01	0.128	0.20	CFI	0.58±0.02	0.57±0.01	0.278	0.03
TAP					Sleep					Light intensity				

	ASD	Control	p value	r		ASD	Control	p value	r		ASD	Control	p value	r
IS	0.52±0.03	0.49±0.02	0.133	0.10	IS	0.59±0.03	0.59±0.02	0.352	0.01	IS	0.62±0.06	0.45±0.02	**<0.001**	0.95
IV	0.43±0.04	0.36±0.02	0.139	0.10	IV	0.36±0.03	0.28±0.02	**0.005**	0.54	IV	0.18±0.02	0.29±0.02	**<0.001**	1.64
RA	0.54±0.02	0.56±0.02	0.346	0.01	RA	0.82±0.03	0.89±0.02	**0.028**	0.31	RA	0.97±0.03	0.93±0.02	**0.007**	0.46
L5	2:54±0:19	4:13±0:09	**<0.001**	0.90	M5	00:59±0:55	3:49±0:16	**<0.001**	0.05	L5	2:23±0:13	4:04±0:18	**<0.001**	1.40
VL5	0.18±0.01	0.18±0.01	0.140	0.09	VM5	0.79±0.04	0.85±0.02	0.195	0.31	VL5	0.02±0.01	0.14±0.05	**<0.001**	1.55
M10	14:59±0:20	15:56±0:17	**0.013**	0.40	L10	13:40±0:23	14:56±0:21	0.361	0.12	M10	14:04±0:23	15:25±0:19	**<0.001**	1.57
VM10	0.56±0.02	0.63±0.01	**<0.001**	1.01	VL10	0.12±0.03	0.05±0.01	**0.027**	0.06	VM10	1.43±0.11	1.55±0.09	0.098	0.13
CFI	0.61±0.02	0.63±0.01	0.369	0.00	CFI	0.72±0.03	0.78±0.01	0.051	0.22	CFI	0.80±0.02	0.74±0.01	**<0.001**	1.3

Non-parametric circadian rhythm analysis values expressed as mean ± SEM. [IS] stands for inter-daily stability, [IV] for intraday variability, [RA] for relative amplitude, phase markers: [M5] and [M10], [L5] and [L10], indicate central values of consecutive 5- and 10- hour period of maximum and minimum values respectively, and [CFI] corresponds to the circadian function index. Statistically significant differences were found by Mann Whitney test. $p<0.05$ are highlighted in bold. For all parameters r refers to size effect.

Ballester, P., Martínez, M. J., Inda, M. D. M., Javaloyes, A., Richdale A.L., Muriel J., Belda C., Toral N., Moral D., Fernández E., Peiró, A. M. (2018). ***Evaluation of agomelatine for the treatment of sleep problems in adults with Autism Spectrum Disorder and co-morbid intellectual disability.*** [Under review: Journal of Psychopharmacology]

4.2. – Objective 1

Introduction

Based on the regular practice using melatonin to treat sleep problems in individuals on the spectrum, and reports of its effectiveness in a short study with autistic adults, we hypothesized that the treatment of current sleep problems with an agonist of melatonin receptors, agomelatine, would be effective at improving insomnia symptoms in adults with ASD and ID. We also hypothesized that it would be safe, as melatonin, according to safety data published.

Methods

Participants

Participant Recruitment and Inclusion Criteria

Participants were recruited from three Spanish Autism associations (Figure 14), after researchers met with their parents and carers, and from a clinic specializing in adults on the autism spectrum.

For details about participant recruitment process and inclusion criteria see section 3.2. - Participant Recruitment and Inclusion Criteria. The exception is that the participants of this study had a sleep problem with a chronic development, (present more than 6 months), and still present for at least one month following the introduction of good sleep hygiene habits (reducing naps, increasing morning exercise, good morning light exposure) as described previously (Malow, MacDonald, Fawkes, Alder, & Katz, 2016).

Procedure

The clinical trial (EudraCT: 2011-003313-42) was conducted from February 2012 to March 2016, excluding summer (June-August) because Spanish summer temperatures could affect the accuracy of the temperature sensor in the ACM device.

Pre-trial sleep-wake diary data were only used to determine participants' inclusion in the study and no other analyses were performed with these data. The sleep problem was reported by carers via a sleep-wake diary and had to meet the ICSD 3rd Edition criteria (Sateia, 2014) for insomnia (includes difficulty falling asleep, difficulty staying asleep, or poor quality sleep, and impaired daytime functioning over the past 3 or more

months) or a CRSWD (a misalignment between the endogenous circadian rhythm and exogenous factors such as light that affect sleep timing and duration) (Thorpy, 2017).

All adults presented insomnia, with either longer SoL, abnormal TST and/or increased number of night awakenings. Individuals taking potent CYP1A2 inhibitors, behavioural or pharmacological treatment for poor sleep, and/or melatonin were not eligible to participate in the study; those with any medical condition (e.g., epilepsy) that was incompatible with the study requirements also were excluded. Even though ID (Intelligence quotient (IQ) scores <70) was not an inclusion criterion, all participants had an associated ID. Participants and their legal guardians' agreement to attend seven clinical visits was required, and participants could withdraw from the study at any time. All participants were included in the study without changing their daily routines. In the three residential facilities, the wake up (≈ 8:00 h), bed (≈ 22:00 h), workshops (≈ 10:00 h and 17:00 h) and meal times (≈ 9:00 h, 14:00 h, and 21:00 h) are pretty constant, regardless week or weekend days, so sleep could not be affected by that. Evening and trial medications were administered at meal time, and there were no bedtime medications. Participants engaged into different morning or evening activities (e.g., walks and workshops), but they remained the same during the study period. All participants shared their bedrooms, but the roommate did not change all over the study. The study was designed as a crossover, triple-blind (investigators, participants and carers), randomized, placebo-controlled clinical trial with two periods of 3-months, starting either with agomelatine or placebo, with a washout of 2-weeks in between each treatment period (Figure 13). Given the high variability in sleep and ongoing medications reported among adults with ASD, this design also represents a strength compared to parallel designs because every participant acts as their own control.

Participants began the study following pre-trial entry screening (age; ASD diagnosis; insomnia and/or CRSWD present for at least 6 months; failure to respond to 1-month sleep hygiene intervention). At this visit, demographic information (age, sex, body weight and height) and current medications were obtained, and sleep diaries and response to the sleep hygiene intervention were reviewed. Participants were randomly assigned for the first period, to one of the two treatment sequences, agomelatine (25 mg/day) followed by placebo (A-B) or placebo followed by agomelatine (B-A), using a computer-generated random number sequence.

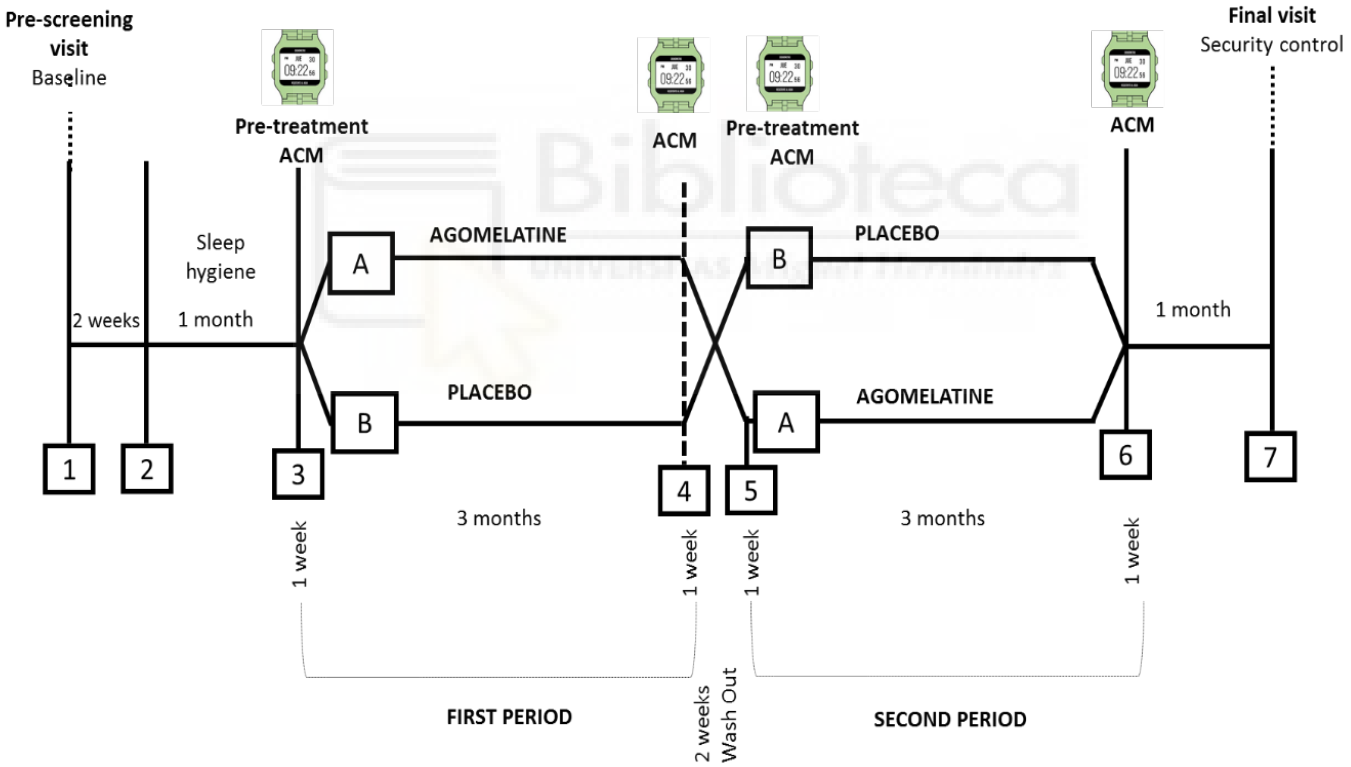

Figure 13. AGOTEA CT study design. [ACM] Ambulatory Circadian Monitoring, [A] agomelatine, and [B] placebo.

Both placebo and agomelatine were formulated as tablets and their appearance was indistinguishable the one from the other. Agomelatine or placebo was given daily, 1

hour before bedtime, over 12 weeks. A washout period of 2 weeks followed between periods one and two in the crossover design. Patients' sleep was assessed using ACM and sleep diaries at pre- (week 1) and post-treatment (week 12, the last week of treatment) for each treatment sequence; data for both pre- and post-treatment periods were available. All participants continued taking their regular medications during both periods of the clinical trial. Researchers were notified of any changes in a participant's ongoing medication during the trial period and these participants ceased to take part in the study. The participants and investigators in charge of data evaluation and analysis were blinded to the randomization, and did not know the patients' drug regimen. Adverse events were recorded and assessed by a physician across the whole treatment period, and hepatic function was assessed in blood samples initially, and then every 3-weeks to test tolerability.

Adherence was controlled by carers from the residential facilities, and then checked by the research team and the Pharmacy Service in Alicante General Hospital (Spain).

Statistical Analyses

T-test for paired samples test was used to assess group differences, period and factor were taken into account to eliminate their potent interaction with the treatment effect; effect sizes (Cohen's *d*) and 95% confidence intervals (CIs) are also reported. Frequencies were compared using the Chi-Square test, with Yate's continuity correction as appropriate and X^2 (df, n) are reported. The effect of treatments associated with patients' comorbidities and bed/wake up times were analyzed with a two-way ANOVA

and effect size (η^2) is provided. Possible carry-over and order effects of the treatment were analyzed using the Mann-Whitney-U test.

Results

Participants from AGOTEA were pre-screened at their ASD Centres, 36 of whom met inclusion criteria, and informed consent was obtained for 25 of these individuals. Two participants (8%) were later withdrawn from the study, one due to an increase in hepatic enzymes and the other due to a change in his medication. Twenty-three participants (35 ± 12 years-old, 83% males, BMI 25 ± 1 Kg/m^2) thus completed the study and their data were available for analysis (Figure 14).

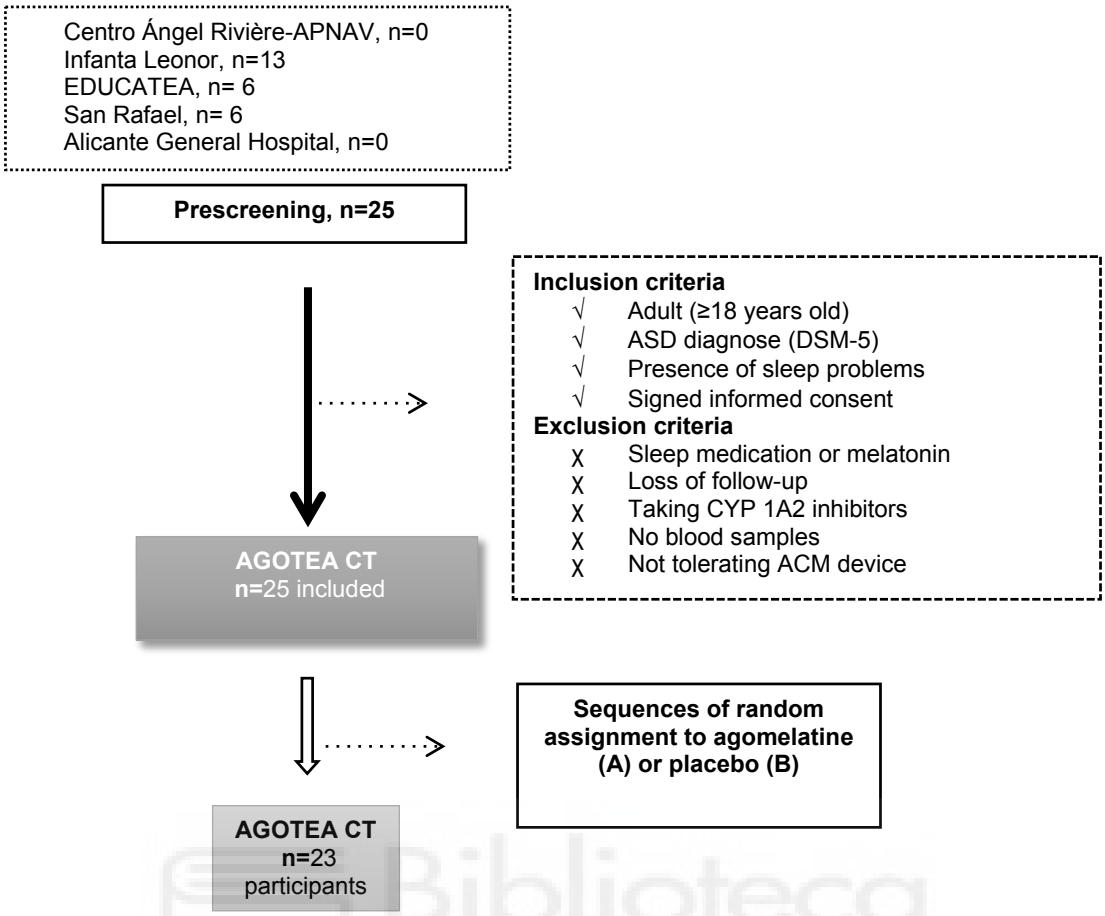

Figure 14. Flowchart of AGOTEA CT sample selection. [DSM-5] Diagnostic and Statistical Manual of Mental Disorders 5th Edition, [ACM] Ambulatory Circadian Monitoring, [CT] clinical trial.

Central nervous system comorbid conditions were reported in 87% of the cases (median of 3 comorbidities; IQR: 1-4); mood disorders (58%), followed by aggressive behaviour (12%), and anxiety (5%) were the most prevalent comorbidities. Participants were prescribed a median of five (IQR: 2-7) drugs (Figure 15), associated with their comorbid conditions (mood disorders, aggressive behaviour, anxiety and mood fluctuations); antipsychotics were most frequently prescribed (71%), followed by mood stabilizers (63%) anxiolytics (38%) and antidepressants (17%). Only 8% of participants were unmedicated.

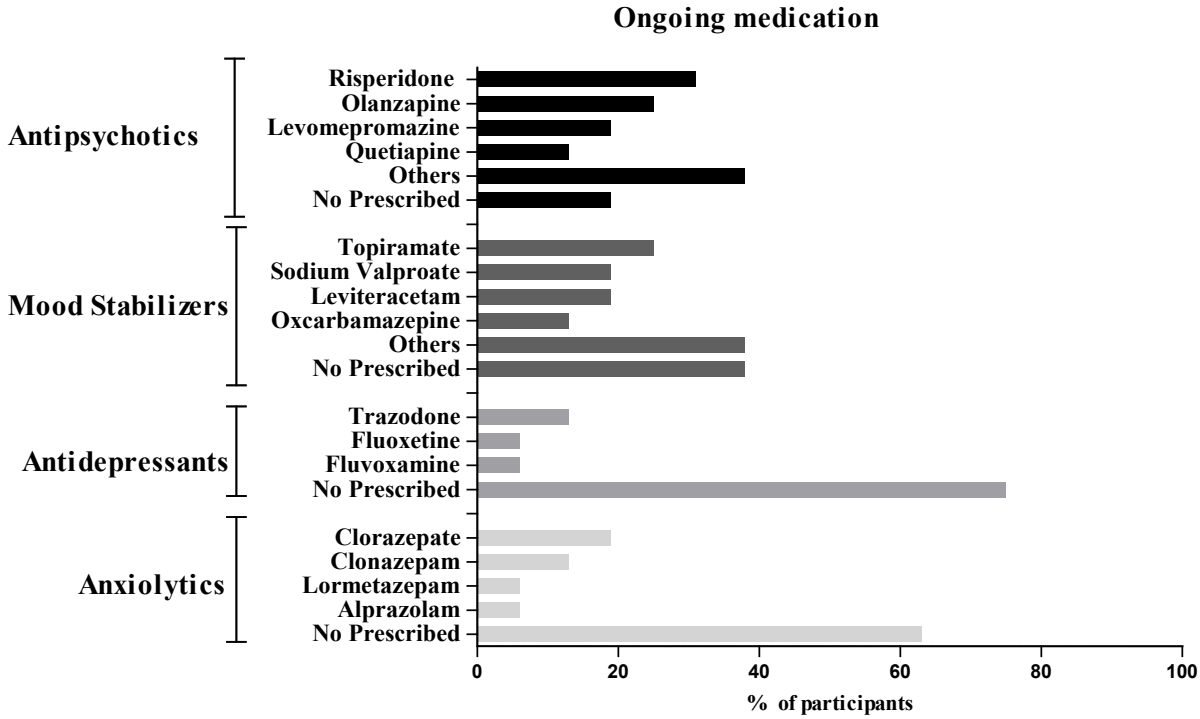

Figure 15. Consumption of medications with potential effects on sleep by the ASD participants during the study, antipsychotics (a), mood stabilizers (b), antidepressants (c) and anxiolytics (d). Results are expressed in %.

Initially, 52% of the participants were randomized to agomelatine treatment and 48% to placebo. There was only one adverse event associated with agomelatine, which was a case of aminotransferase elevation. However, this person had basal levels of aminotransferase already near the upper limit of the normal range. This adverse event was notified to the Spanish pharmacovigilance system. There were no adverse events associated with placebo.

Ambulatory Circadian Monitoring

Sleep parameters

Comparison of sleep parameter values obtained by ACM for agomelatine and placebo after three-month treatment and pre-treatment are presented in Table 6. All

participants at pre-screening visit, according to carers 'reports, presented insomnia with either longer SoL, abnormal TST and/or increased number of awakenings. Pre-treatment values were not significantly different across the two groups. Prior to agomelatine treatment, all the participants presented insomnia with abnormal SoL and number of awakenings (100%), and 50% had also abnormal TST. Prior to placebo treatment (pre-placebo), 71% of the individuals presented abnormal SoL and number of awakenings, and 60% abnormal TST. At pre-treatment, all participants in both arms had either SoL, night waking and/or TST within the abnormal range.

Following agomelatine treatment, 91% of participants still had abnormal SoL and increased awakenings, but only 16% had abnormal TST, and 8% of participants had SoL, num. of awakenings and TST in the normal range. Following placebo treatment 83% of participants had abnormal SoL and number of awakenings, and 67% had abnormal TST, and 8% had all three parameters in the normal range. Agomelatine treatment significantly increased TST at post-treatment compared with pre-treatment (532 ± 121 vs. 449 ± 177 min; p=0.016; d=0.55 and 95% CI 455-609 vs. 337-561 min) no other significant effects of agomelatine on sleep parameters were found. Placebo treatment did not significantly alter any sleep parameters. Any differences between both pre-agomelatine and pre-placebo sleep parameters were assessed using t-tests for paired samples and no significant differences were found (all p>0.05) for any parameters reported in Table 6. No carry-over effects and/or possible order effects of the treatment were detected (all p>0.05) (Tables 8 and 9).

Analysis of the effect of patients' medications (antipsychotics, mood stabilizers, anxiolytics and antidepressants) on their sleep parameters demonstrated that only antidepressants influenced sleep, affecting SoL (p=0.027; η^2=1.51) and the number of

awakenings (p=0.045; η^2=0.321), with those on antidepressants having significantly increased values for both sleep parameters. This influence was similar in both the agomelatine and placebo treatment phases, and thus did not differentially affect these sleep parameters (Table 6).

No significant differences were found in bed (22:02 ± 00:34; 21:31 ± 00:24; 22:10 ± 00:37 h; p=0.094; η2=0.16) and wake up (8:06 ± 00:50; 8:12 ± 00:29; 7:45 ± 00:35 h; p=0.334; η2=0.08) times among the three centres.

Table 6. Comparison of sleep parameters between pre-treatment vs. agomelatine or placebo obtained from Ambulatory Circadian Monitoring recordings.

Sleep parameters	Agomelatine (n=23)					Placebo (n=23)				
	Pre-treatment	**Post-treatment**	**95% CI**	**p-value**	**Cohen's *d***	**Pre-treatment**	**Post-treatment**	**95% CI**	**p-value**	**Cohen's *d***
Total Sleep Time (min)	449±177	532±121	**455-609**	**0.016**	0.55	542±106	574±67	531-616	0.148	0.36
Time in bed (min)	704±152	711±116	637-785	0.270	0.06	687±72	733±76	684-781	0.234	0.62
Sleep Onset Latency (min)	55±23	54±22	40-67	0.399	0.04	51±24	53±23	38-67	0.264	0.08
Number of awakenings	3.7±1.9	4.3±2.3	3-6	0.336	0.28	3.1±1.7	3.6±1.6	3-5	0.207	0.30
Wake After Sleep Onset (min)	134±112	116±63	76-156	0.278	0.20	91±35	90±64	49-131	0.469	0.02
Sleep Efficiency (%)	64	75	68-81	0.062	0.18	78	79	73-84	0.399	0.56

Data is expressed as mean ± SD or as percentage. Statistically significant differences $p<0.05$ are highlighted in bold. For all parameters Cohen's d refers to size effect. [Min] minutes.

Circadian sleep-wake rhythm indexes

During recruitment, only 30% of participants had a suspected CRSWD based on their sleep diaries. At pre-treatment, after ACM pre-trial determination (i.e., before randomization to intervention), 66% of the participants had a CRSWD reflected mainly their phase marker values for wrist temperature, TAP and sleep. While, the phase movement that agomelatine generated in the five hours of central sleep (1:38 ± 1:16 vs. 22:32 ± 3:11 h; p=0.082; *d*=0.51 and 95% CI 00:47-2:30 h vs. 21:43-3:55 h) is not significantly different from the placebo condition, the effect size is moderate. No participant had a free running cycle. Comparison of the circadian sleep-wake rhythm indexes for the agomelatine and placebo treatments, and for pre-treatment, are presented in Table 7 and Supplementary Tables 10-12.

Peripheral temperature phase advancement CRSWD values during the ACM pre-treatment week were: 58% with a CRSWD at pre-agomelatine, and 75% with a CRSWD at pre-placebo. CRSWD values during the ACM week following treatment were: post-agomelatine 23% with a CRSWD, and post-placebo 67% with a CRSWD. Treatment with agomelatine resulted in a significant improvement in rhythm stability (IS, 0.52 ± 0.18 vs. 0.43 ± 0.29; *p*=0.007; *d*=0.35 and 95% CI 0.41-0.63 vs. 0.25-0.62) and a significant phase correction (2h delay) in the wrist temperature rhythm (3:15 ± 2:20 vs. 1:45 ± 2:28 h; *p*=0.037; *d*=0.62 and 95% CI 1:51-4:40 h vs. 00:11-3:19 h), with significantly higher temperature values at night (35.09 ± 0.82 vs. 34.58 ± 1.31ºC; *p*=0.027; *d*=0.47 and 95% CI 34.6-35.6 ºC vs. 33.70-35.46 ºC) (Table 7). Agomelatine also reduced the variability of the sleep-wake rhythm during the post-treatment week (0.67 ± 0.09 vs. 0.55 ± 0.27; *p*=0.037; *d*=0.60 and 95% CI 0.61-0.72 vs. 0.37-0.72) compared with the pre-treatment

week (Table 7) and the period of major motor activity appeared significantly earlier in the day (14:45 ± 1:57 vs. 18:16 ± 5:53 h; p=0.024; d=0.81 and 95% CI 13:26-16:04 h vs. 14:32-21:00 h) compared to the pre-treatment condition (Table 10). The differences between both pre-agomelatine and pre-placebo were assessed with paired samples t-tests and no significant differences were found (all p>0.05) in any of the parameters analyzed. No carry-over effects and/or possible order effects of the treatment on circadian parameters were detected (all p>0.05) (Tables 8 and 9).

Table 7. Circadian sleep-wake rhythm indexes comparison for wrist temperature and sleep circadian rhythm in agomelatine and placebo treatments.

	Wrist Temperature								Sleep							
	Agomelatine				Placebo				Agomelatine				Placebo			
	Pre-treatment	**Post-treatment**	**p-value**	**Cohen's *d***	**Pre-treatment**	**Post-treatment**	**p-value**	**Cohen's *d***	**Pre-treatment**	**Post-treatment**	**p-value**	**Cohen's *d***	**Pre-treatment**	**Post-treatment**	**p-value**	**Cohen's *d***
IS	0.43±0.29	0.52±0.18	**0.007**	0.37	0.43±0.23	0.38±0.20	0.319	0.23	0.55±0.27	0.67±0.09	**0.037**	0.6	0.63±0.19	0.68±0.16	0.385	0.28
IV	0.14±0.07	0.15 ±0.05	0.313	0.16	0.11±0.06	0.12±0.10	0.396	0.12	0.38±0.17	0.40±0.13	0.180	0.13	0.30±0.13	0.34±0.12	0.053	0.32
RA	0.28±0.17	0.30 ±0.09	0.116	0.15	0.30±0.15	0.31±0.18	0.369	0.06	0.79±0.24	0.87±0.12	0.371	0.42	0.82±0.27	0.84±0.13	0.311	0.09
M5	1:45±2:28	3:15±2:20	**0.037**	0.62	00:45±3:18	2:24±2:09	0.111	0.59	00:32±3:11	1:38±1:16	0.082	0.51	2:11±1:26	2:18±1:46	0.134	0.07
VM5	34.58±1.31	35.09±0.82	**0.027**	0.47	34.62 ±0.82	34.59±0.95	0.500	0.03	0.72±0.29	0.84±0.14	0.406	0.53	0.91±0.06	0.90±0.09	0.278	0.13
L10	14:12±1:11	14:00±2:41	0.500	0.09	13:57±2:09	13:42±3:47	0.296	0.08	13:34±1:32	14:09±1:38	0.312	0.37	14:16±1:10	14:36±2:08	0.323	0.19
VL10	32.74±0.76	33.07±0.96	0.248	0.38	32.64±0.67	32.49±0.94	0.285	0.18	0.11±0.17	0.06±0.06	0.500	0.39	0.11±0.20	0.08±0.07	0.313	0.2
CFI	0.50±0.15	0.50±0.12	0.367	0	0.55±0.12	0.51±0.17	0.248	0.27	0.72±0.10	0.78±0.05	0.116	0.76	0.77±0.14	0.79±0.08	0.461	0.17

Non-parametric circadian rhythm analysis values expressed as mean ± SD. [IS] stands for inter-daily stability, [IV] for intraday variability, [RA] for relative amplitude, phase markers: [M5] and [M10], [L5] and [L10], indicate central values of consecutive 5- and 10- hour period of maximum and minimum values respectively, and [CFI] corresponds to the circadian function index. Statistically significant differences $p<0.05$ are highlighted in bold. For all parameters Cohen's d refers to size effect.

Table 8. Comparison of sleep parameters in the pre-treatment attending to order sequence A/B vs. B/A for agomelatine or placebo obtained from Ambulatory Circadian Monitoring recordings.

Sleep parameters	Agomelatine – Pre-treatment (n=23)				Placebo – Pre-treatment (n=23)			
	A/B	B/A	p-value	Cohen's *d*	A/B	B/A	p-value	Cohen's *d*
Total Sleep Time (min)	459±202	420±84	0.473	0.25	551±76	535±136	0.628	0.15
Time in bed (min)	734±164	616±64	0.955	0.94	704±54	675±90	0.475	0.39
Sleep Onset Latency (min)	54±22	60±30	0.853	0.23	43±14	57±30	0.629	0.60
Number of awakenings	4±2	3±1	0.183	0.63	4±1	3±2	0.368	0.63
Wake After Sleep Onset (min)	148±129	94±13	0.824	0.59	91±8	90±50	1	0.03
Sleep Efficiency (%)	62	68	0.128	0.32	78	79	1	0.13

Data is expressed as mean ± standard deviation or as percentage. P values correspond to the comparison among individuals in sequence A/B vs B/A. Cohen's d refers to size effect. [A] Agomelatine, [B] Placebo, and [Min] minutes.

Table 9. Comparison of sleep parameters in the post-treatment attending to order sequence A/B vs. B/A for agomelatine or placebo obtained from Ambulatory Circadian Monitoring recordings.

Sleep parameters	Agomelatine – Post-treatment (n=23)				Placebo – Post-treatment (n=23)			
	A/B	B/A	p-value	Cohen's *d*	A/B	B/A	p-value	Cohen's *d*
Total Sleep Time (min)	512±135	561±106	1	0.4	611±60	521±61	0.087	1.49
Time in bed (min)	696±140	732±84	0.639	0.31	761±66	694±79	0.639	0.92
Sleep Onset Latency (min)	61±17	43±25	0.104	0.84	51±22	55±27	0.806	0.16
Number of awakenings	4±2	5±3	0.804	0.39	4±1	3±2	0.452	0.63
Wake After Sleep Onset (min)	106±56	130±75	0.569	0.36	84±44	98±91	0.935	0.2
Sleep Efficiency (%)	73	77	0.626	0.35	80	76	0.512	0.41

Data is expressed as mean ± standard deviation or as percentage. P values correspond to the comparison among individuals in sequence A/B vs B/A. Cohen's d refers to size effect. [A] Agomelatine, [B] Placebo, and [Min] minutes.

Table 10. Circadian sleep-wake rhythm indexes comparison for the motor activity rhythm in agomelatine and placebo treatments.

	Agomelatine				Placebo			
	Pre-treatment	**Post-treatment**	**p-value**	**Cohen's *d***	**Pre-treatment**	**Post-treatment**	**p-value**	**Cohen's *d***
IS	0.27±0.11	0.33±0.11	0.188	0.54	0.34±0.11	0.36±0.23	0.285	0.11
IV	1.05±0.22	1.09±0.12	0.500	0.23	0.94±0.34	0.98±0.11	0.285	0.16
RA	0.71±0.17	0.67±0.20	0.461	0.22	0.79±0.15	0.81±0.13	0.284	0.14
M10	18:16±5:53	14:45±1:57	**0.024**	0.80	14:43±1:36	15:26±2:08	0.180	0.38
VM10	27.54±16.43	31.44±11.28	0.216	0.28	29.21±14.98	26.78±11.11	0.285	0.18
L5	2:21±1:50	1:19±2:01	0.161	0.54	2:40±1:50	2:10±1:48	0.065	0.28
VL5	6.23±7.14	6.41±4.91	0.461	0.03	3.86±3.14	2.41±1.42	**0.037**	0.60
CFI	0.48±0.07	0.49±0.09	0.188	0.12	0.55±0.13	0.56±0.11	0.500	0.08

Non-parametric circadian rhythm analysis values expressed as mean ± SD. [IS] stands for inter-daily stability, [IV] for intraday variability, [RA] for relative amplitude, phase markers: [M5] and [M10], [L5] and [L10], indicate central values of consecutive 5- and 10- hour period of maximum and minimum values respectively, and [CFI] corresponds to the circadian function index. Statistically significant differences $p<0.05$ are highlighted in bold. For all Cohen's d refers to size effect.

Table 11. Circadian sleep-wake rhythm indexes comparison for the body position rhythm in agomelatine and placebo.

	Agomelatine				Placebo			
	Pre-treatment	**Post-treatment**	**p-value**	**Cohen's *d***	**Pre-treatment**	**Post-treatment**	**p-value**	**Cohen's *d***
IS	0.36±0.17	0.48±0.15	0.213	0.75	0.42±0.20	0.57±0.21	**0.039**	0.73
IV	0.38±0.24	0.44±0.14	0.285	0.31	0.63±0.56	0.34±0.12	**0.008**	0.72
RA	0.42±0.18	0.44±0.17	0.252	0.11	0.52±0.08	0.58±0.13	0.078	0.55
M10	15:21±2:30	14:50±1:08	0.422	0.27	15:01±1:01	15:37±1:32	0.337	0.46
VM10	42.43±14.36	41.58±7.71	0.064	0.07	46.29±6.39	44.97±6.45	0.422	0.21
L5	23:26±4:38	1:57±2:13	0.074	0.69	2:54±2:18	2:21±2:31	0.200	0.23
VL5	19.02±13.00	16.54±6.79	0.180	0.24	15.05±4.39	12.17±4.74	0.156	0.63
CFI	0.53±0.11	0.57±0.11	0.180	0.36	0.59±0.08	0.66±0.09	**0.031**	0.822

Non-parametric circadian rhythm analysis values expressed as mean ± SD. [IS] stands for inter-daily stability, [IV] for intraday variability, [RA] for relative amplitude, phase markers: [M5] and [M10], [L5] and [L10], indicate central values of consecutive 5- and 10- hour period of maximum and minimum values respectively, and [CFI] corresponds to the circadian function index. Statistically significant differences $p<0.05$ are highlighted in bold. For all parameters Cohen's d refers to size effect.

Table 12. Circadian sleep-wake rhythm indexes comparison for TAP rhythm in agomelatine and placebo treatments.

	Agomelatine				Placebo			
	Pre-treatment	**Post-treatment**	**p-value**	**Cohen's *d***	**Pre-treatment**	**Post-treatment**	**p-value**	**Cohen's *d***
IS	0.49±0.24	0.55±0.13	0.406	0.32	0.55±0.1010	0.62±0.20	0.371	0.44
IV	0.48±0.32	0.47±0.11	0.289	0.04	0.52±0.26	0.39±0.19	0.231	0.57
RA	0.47±0.17	0.52±0.13	0.156	0.33	0.59±0.06	0.65±0.11	0.05	0.67
M10	15:46±2:17	14:51±1:26	0.242	0.31	15:11±1:14	15:06±2:03	0.10	0.05
VM10	0.60±0.18	0.56±0.06	0.125	0.30	0.56±0.09	0.56±0.09	0.473	0
L5	23:36±7:39	1:33±1:45	0.469	0.35	2:25±1:12	2:10±1:57	0.472	0.15
VL5	0.21±0.13	0.18±0.07	**0.039**	0.29	0.15±0.04	0.12±0.05	0.074	0.66
CFI	0.57±0.14	0.61±0.09	0.074	0.34	0.63±0.08	0.69±0.10	0.273	0.67

Non-parametric circadian rhythm analysis values expressed as mean ± SD. [IS] stands for inter-daily stability, [IV] for intraday variability, [RA] for relative amplitude, phase markers: [M5] and [M10], [L5] and [L10], indicate central values of consecutive 5- and 10- hour period of maximum and minimum values respectively, and [CFI] corresponds to the circadian function index. Statistically significant differences $p<0.05$ are highlighted in bold. For all parameters Cohen's d refers to size effect.

Figure 16 summarizes the 24-hour registry of the sleep circadian rhythm analyzed for both agomelatine (Figure 16a) and placebo (Figure 16b). Only agomelatine resulted in significant differences, showing higher sleep rhythm amplitude values during the night time; at 1:00 am (0.87 ± 0.19 vs. 0.70 ± 0.27; p=0.011; d=0.73 and 95% CI 0.76-0.98 vs. 0.53-0.87) and 2:00 am (0.84 ± 0.23 vs. 0.65 ± 0.26; p=0.015; d=0.77 and 95% CI 0.70-0.97 vs. 0.49-0.82) amplitudes were significantly higher when compared with pre-treatment. Furthermore, sleep values with agomelatine were increased across the whole night compared to pre-treatment conditions, which reflects an increase in the amplitude of the sleep rhythm following agomelatine treatment, but not after placebo.

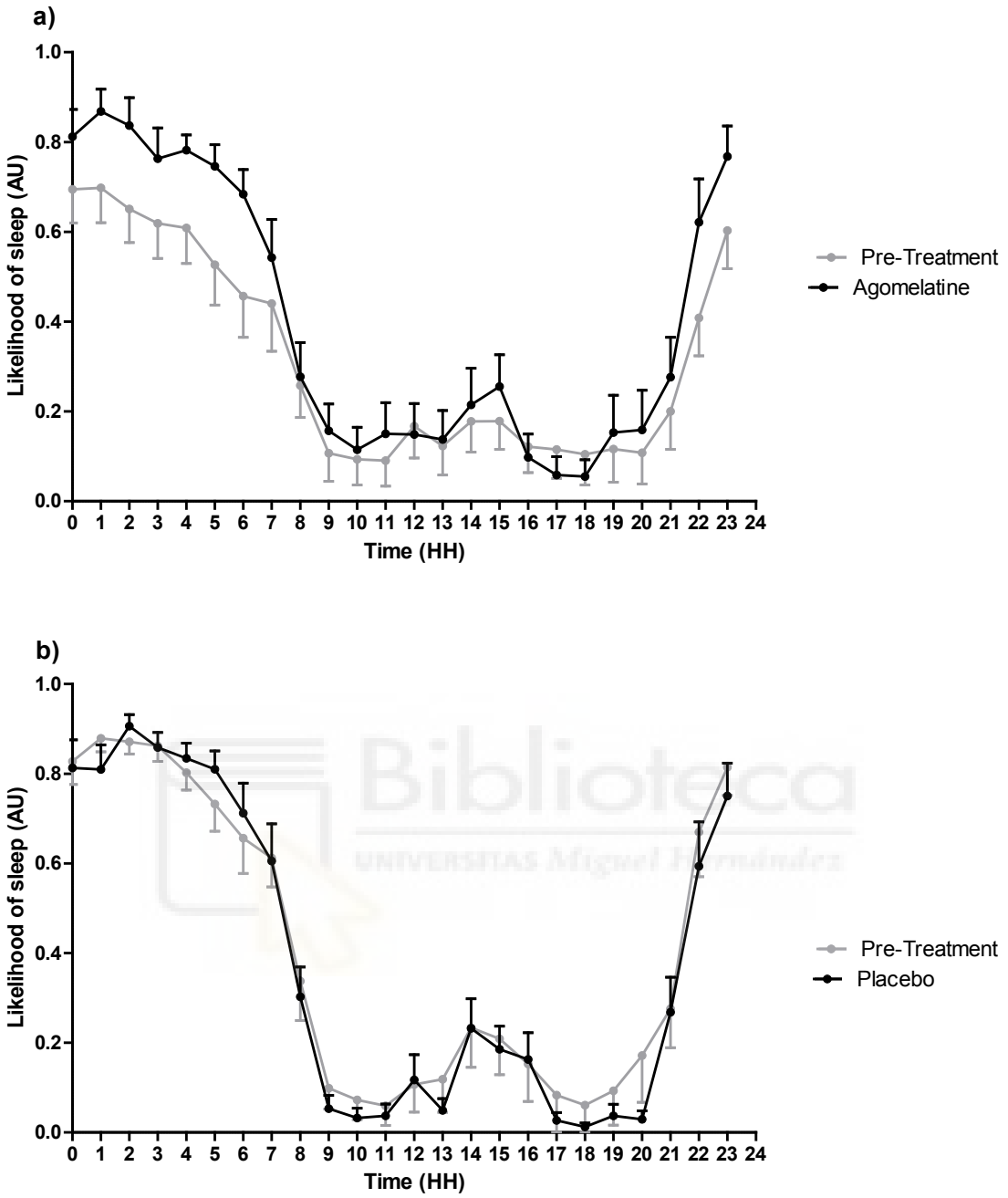

Figure 16. Sleep circadian rhythms for agomelatine (a) or placebo (b) period treatment in adults with ASD and ID. Sleep calculated by ACM does not have standardized measure that is why is expressed as [AU] arbitrary units, and [HH] hours.

Ballester, P., Martínez, M. J., Javaloyes, A., Belda C., Aguilar, V., Inda, M. D. M., Richdale A.L., Muriel J., Fernández E., Peiró, A. M. (2019).

Interplay of circadian and melatonin genetic variants in adults with autism, intellectual disability and sleep problems.

[Under review: Journal of Autism and Developmental Disorders]

4.3. – Objective 2

Introduction

In this study we analyzed the role of *circadian clock* (*PER1* and *NPAS2*) and *melatonin pathway* (*ASMT* and *MTNR1A*) genes on sleep parameters and circadian rhythms in individuals with autism and ID.

Methods

Procedure

After screening was successfully completed, the autistic adults began the study. During their clinical visit, demographic information (age, sex, body mass index) and regular prescribed medications were recorded. Blood samples for genotyping were taken during a routine blood test in the following 6-months of ACM week recording. All participants were asked to follow their regular habits, and sleep-wake diaries were completed by carers. Data from sleep diaries were used as backup for ACM records.

Participants

Participant Recruitment and Inclusion Criteria

All participants were residents in Spanish autism institutions and were recruited there or in clinics specializing in ASD after researchers met with parents and carers. Over 200 participants were pre-screened (Figure 17), due to the experience of the research team the participation increased in this study, and informed consent was obtained from 123 adults with ASD. Complete data recorded by ambulatory circadian monitoring (ACM) and genotyping were finally obtained from 83 participants. Sleep problems were not a condition to participate in this study.

For further details about recruitment process and inclusion criteria for autistic adults with ID, and control participants see section 3.2. - Participant Recruitment and Inclusion Criteria.

Genotyping

Following the routine blood test, mononuclear cells were isolated, DNA was extracted using the QIAamp® DNA Blood kit and concentrations were measured with a Nanodrop 2000 spectrophotometer. DNA from control population was obtained from the National Spanish Gene Bank. Genotyping of *PER1* (rs885747 G>C, rs6416892 T>G), *ASMT* (rs4446909 G>A, rs5989681 G>C), *NPAS2* (rs1811399 T>G), and *MTNR1A* (rs28383652 NA, rs7654853 NA) was performed at CEGEN-PRB2-ISCIII. The monomorphic SNPs in MTNR1A were removed during the quality control of this study.

In order to detect single nucleotide polymorphisms (SNPs), target regions were initially amplified using multiplex PCR, subsequently hybridized to custom-designed primers, and after that, subjected to a single base extension reaction using single mass-modified

nucleotides. Spotting of the products onto a matrix chip and subsequent ionization enables real-time detection of nucleotides by Sequenom MassARRAY mass spectrometry. Genotypic and allelic frequencies were calculated and the results were compared for both populations to test if the presence of any of the SNPs studied was linked to having an ASD diagnosis (Gibbs et al., 2003).

Statistical Analyses

Sleep parameters and non-parametric circadian indexes values were compared to normal ranges and categorized [in range (✓), higher (⇑), lower (⇓)] to indicate how participants' sleep varied from expected values. Frequencies (alleles and genotypes) were compared using the Chi-Square test, with Yate's continuity correction as appropriate or Odds Ratio, and X^2 (df, n) or (IC 95%) are reported.

The distributions of observed allelic and genotypic frequencies were tested for Hardy-Weinberg equilibrium. The influence of genotype on the circadian rhythm indexes and sleep parameters was analyzed using multiple regression analyses for codominant, dominant or recessive models, t-test for independent samples, or a Mann Whitney U test to assess group differences, and correction for multiple testing was performed (Bonferroni or Dunns).

The rest, alpha-values <0.05 were considered to indicate significance for all analyses. Effect sizes were calculated and reported using Cohen's d (range small (0.20), medium (0.50) or large (0.80)); or R squared ([negative or positive]: no linear correlation (0), weak (0.10), average (0.50), considerable (0.75), very strong (0.95) or perfect (1)).

Results

While informed consent was obtained from 123 participants, blood samples were possible only from 92 participants, and from those 83 tolerated the ACM device. Thus, 83 participants with ASD and ID (age range 29- to 40-years, 78% males, body mass index 26.6 ± 6.4 Kg/m^2) completed the ACM week and were genotyped (Figure 17).

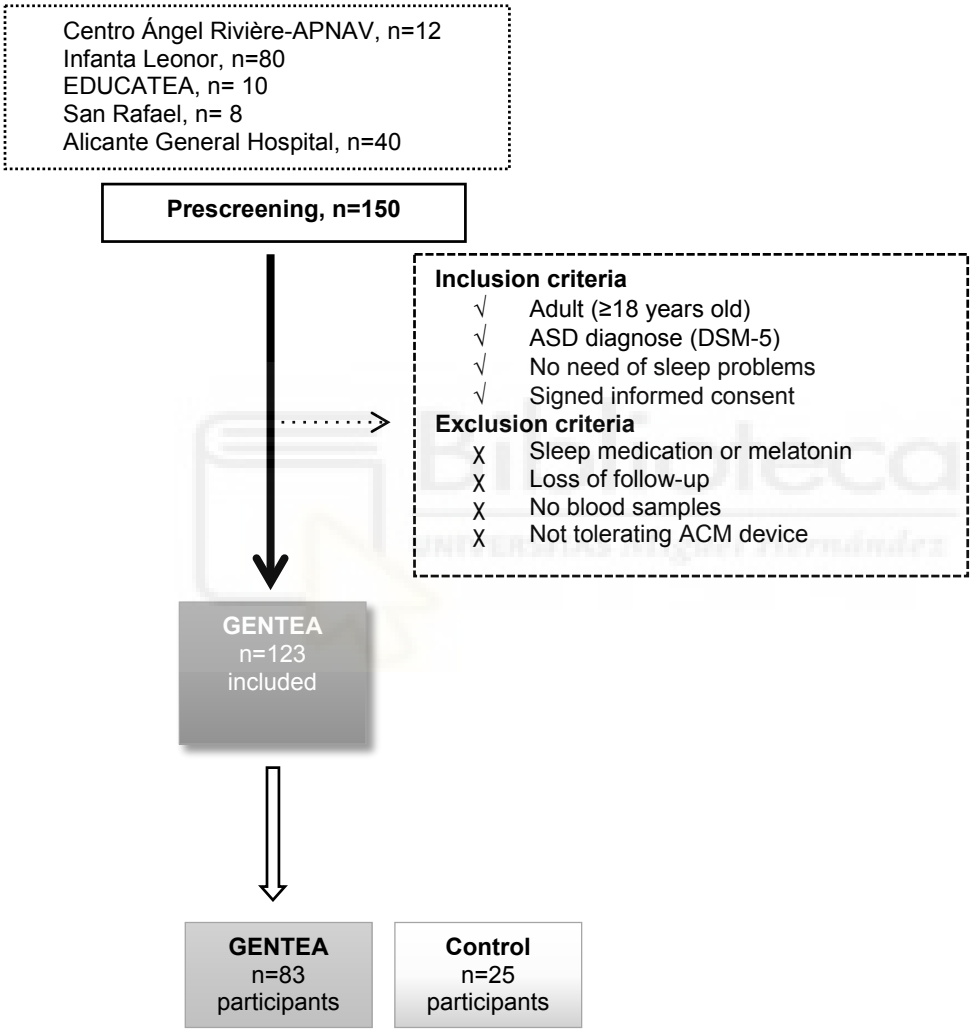

Figure 17. Flow chart of GENTEA sample selection. [DSM-5] Diagnostic and Statistical Manual of Mental Disorders 5th Edition, and [ACM] Ambulatory Circadian Monitoring.

Participants' demographical data are summarized in Table 13. Cases presented a median of three comorbidities per person (IQR25-75: 1-4, e.g., epilepsy, aggressive behaviour,

or mood disorders) and three pharmacological prescriptions (IQR25-75: 2-5). Drugs more often prescribed were antipsychotics (86%), mood stabilizers (61%), anxiolytics (43%), and antidepressants (40%). All controls were older than 18 years old, date of birth was unknown, and only geographical data, matched with cases, was available at the National Spanish Gene Bank (n=25).

Table 13. Demographic data for all subjects with Autism Spectrum Disorder included, genotyped and analyzed by ambulatory circadian monitoring.

	Included (n=123)	**Genotyped (n=92)**	**Genotyped+ACM (n=83)**
Sex, Men/Women (n)	98/25	74/18	65/18
Age, mean ± SD (years)	29±11	30±10	29±11
BMI, mean ± SD (Kg/m^2)	26.5±6.4	26.2±6.2	26.6±6.4

[ACM] stands for Ambulatory Circadian Monitoring, and [BMI] Body Max Index.

Ambulatory Circadian Monitoring

Sleep parameters

Sleep parameters values for the adults with ASD and ID are presented in Table 14.

All participants (100%) presented with insomnia symptoms, either reflected in a SoL duration that exceeded the normal value (46 ± 30 min, 62% outside normal values); higher values for TIB (667 ± 107 min, 81% outside normal values), or difficulty maintaining sleep at night (WASO) reflected in both the number (3 ± 2 num., 80%

outside normal values) and duration (122 ± 87 min, 89% outside normal values) of night awakenings. While the average and SD for TST was within the normal range, only 49% of the ASD subjects actually had a TST value in the normal range. These sleep alterations were confirmed with SE, 66% of autistic participants had abnormal SE value.

Table 14. Sleep parameters obtained by ambulatory circadian monitoring recordings in ASD participants.

Sleep parameters mean ± SD	**ASD (n=83)**	**Normal values**	**ASD in normal range n (%)**	**Tendency**
Total Sleep Time (TST, min)	521±121	420-540	39 (49)	✓
Time in bed (TIB, min)	667±107	420-569	15 (19)	⇑
Sleep Onset Latency (SoL, min)	46±30	30	30 (38)	⇑
Number of awakenings (median, IRQ)	3±2	1	16 (20)	⇑
Wake After Sleep Onset (WASO, min)	122±87	20	9 (11)	⇑
Sleep Efficiency (SE, %)	77	>85	27 (34)	⇓

Normal values came from Watson et al. (2015): ✓ (in range), ⇑ (higher), ⇓ (lower), and [Min] minutes.

Circadian sleep-wake rhythm indexes

Non-parametric circadian sleep indexes for the adults with ASD and ID are presented in Table 15. A total of 79% of participants presented with a phase advanced sleep rhythm (based on central value of consecutive 5- hour period of maximum sleep, M5). Up to 75% of the participants had abnormal circadian function index rhythmicity, with low stability (61% outside normal range), or high fragmentation (100% outside normal

range). In summary, non-parametric circadian values indicate poor quality rhythm, and suggest a phase advanced sleep disorder.

Table 15. Non-parametric circadian indexes values for sleep circadian rhythm.

	Sleep	Normal values	ASD in normal range n (%)	Significance
IS	0.59±0.26	>0.70	32 (39)	⇓
IV	0.34±0.18	<1	0 (0)	⇑
RA	0.77±0.17	>0.80	52 (63)	⇓
M5	1:57±2:35	3:00-5:00	22 (26)	⇓
VM5	0.84±0.18	>0.90	40 (48)	⇓
L10	14:17±2:18	14:00-17:00	27 (32)	✓
VL10	0.12±0.20	<0.25	70 (84)	✓
CFI	0.73±0.17	>0.85	21 (25)	⇓

Non-parametric circadian rhythm analysis values expressed as mean ± SD. [IS] stands for inter-daily stability, [IV] for intraday variability, [RA] for relative amplitude, phase markers: [M5] and [M10], [L5] and [L10], indicate central values of consecutive 5- and 10- hour period of maximum and minimum values respectively, and [CFI] corresponds to the circadian function index. Normal values came from Ortiz-Tudela et al. (2010): ✓ (in range), ⇑ (higher), and ⇓ (lower).

Genotype influence

All polymorphisms analyzed were in Hardy-Weinberg equilibrium, except for MTNR1A (rs28383652 NA, rs7654853 NA) which were monomorphic in all our cases, and thus, excluded from the analysis. Genotypic and allelic frequencies are shown in Table 16. All frequencies found in individuals with ASD and ID were similar to the healthy control population, except the ones for *PER1* gene. Autistic participants presented a significantly higher prevalence of rs885747-CG ($p=0.0003$; X2 (df,n), 16.14 (2, 117)) and rs6416892-TT ($p<0.0001$; X2 (df,n), 20.53 (2, 117)) genotypes when compared to controls.

Table 16. Genotypic and allelic distribution for *NPAS2* (rs1811399), *PER1* (rs885747 and rs6416892) and *ASMT* (rs4446909 and rs5989681) genes. Allelic and genotypic frequencies were calculated and expressed as percentages.

Gene	ASD %	Control %	P value X2 (df,n) or OR (IC 95%)
***NPAS2* (rs1811399, G>T)**			
GG	2	0	0.362
GT	42	50	2.03 (2,117)
TT	56	50	
Allele G	23	25	0.869
Allele T	77	75	0.90 (0.47 to 1.72)
***PER1* (rs885747, C>G)**			
CC	16	23	**0.0003**
CG	47	65	**16.14 (2, 117)**
GG	37	12	
Allele C	40	55	**0.047**
Allele G	60	45	**0.55 (0.31 to 0.96)**
***PER1* (rs6416892, G>T)**			
GG	17	21	**<0.0001**
GT	46	69	**20.53 (2, 117)**
TT	37	10	
Allele G	40	58	**0.018**
Allele T	60	44	**0.51 (0.29 to 0.89)**
***ASMT* (rs4446909, A>G)**			
AA	11	12	0.777
AG	34	38	0.504 (2, 117)
GG	55	50	
Allele A	28	31	0.757
Allele G	72	69	0.87 (0.47 to 1.59)
***ASMT* (rs5989681, C>G)**			
CC	17	19	0.639
CG	38	42	0.90 (2, 117)
GG	45	38	
Allele C	36	40	0.564
Allele G	64	60	0.84 (0.48 to 1.50)

Statistically significant differences were found by Chi Square [X2] or Odds Ratio [OR] $p<0.05$ are highlighted in bold. For all parameters X2 (df,n) or OR (IC 95%) refers to size effect.

Sleep parameters

Figure 18-19 show that genotype significantly influenced sleep parameters in the autistic participants. PER1 rs6416892-GG (17%) needed less time to accomplish the transition from

full wakefulness to sleep as evidenced by a higher percentage of SoL values inside the normal range (p= 0.012; OR 0.2, 95% CI 0.06 to 0.70; Figure 18). These individuals needed approximately 10 min less to fall asleep (TT and GT 48 ± 30 vs. GG 36 ± 27 min; p=0.033; d=0.4). However, a significantly higher number of night awakenings were associated with the PER1 rs6416892-TT (2 ± 1 GG, 3 ± 2 GT, 4 ± 3 TT num.; p=0.047; R2 =0.04) and the ASMT rs5989681-CC/GG (3 ± 2 CC, 2 ± 2 CG, 3 ± 2 GG num.; p=0.049; R2 =0.09) genotypes, but both correlations were weak, see Figure 19. No other significant differences were found for the remaining sleep parameters (TST, TIB, WASO and SE) in relation to the polymorphisms analyzed.

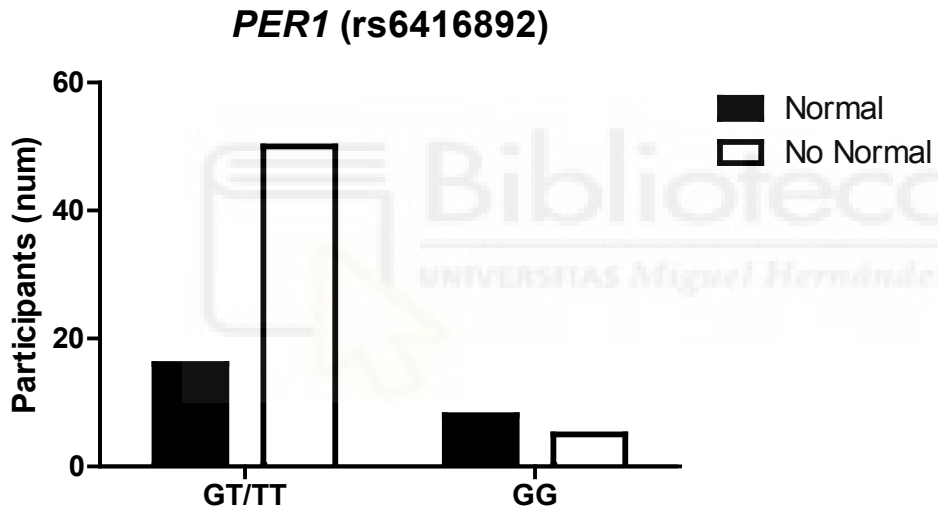

Figure 18. PER1 rs6416892 *g*enotype influence on SoL sleep parameter. Data are expressed as % of normal/abnormal values of SoL duration according to genotype.

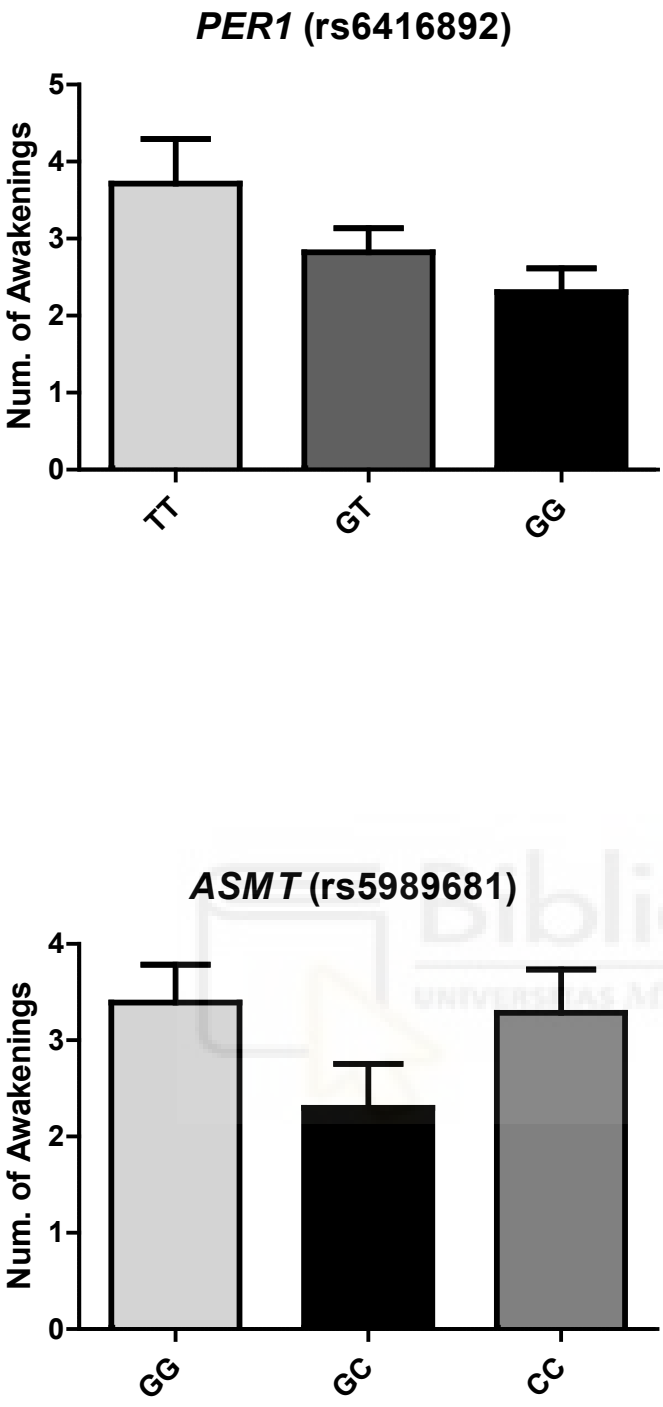

Figure 19. Genetic influence on Comparison of genotypes using a codominant model of the number of awakenings for *PER1* and *ASMT* genotypes.

Circadian sleep-wake rhythm indexes

Figures 20 and 21 show significant genotype influences on autistic individuals' sleep circadian rhythm. While the primary focus of the study is sleep, the ACM device also measures wrist (skin) temperature. Night sleep should occur on the falling phase of the core body temperature rhythm, and this is facilitated by a rise in skin temperature, especially at the extremities, thus the sleep-wake and temperature rhythms are expected to be in phase. Thus, any significant results related to the skin temperature rhythm were also examined.

When comparing the 24h wave of the sleep circadian rhythm by genotype, a sleep phase advancement was observed for the *PER1* gene rs6416892-GG genotype with increased sleep values at 22:00h (0.84 ± 0.21 vs. 0.51 ± 0.30; p=0.0001; *d*=0.70) and 1:00h (0.88 ± 0.2 vs. 0.81 ± 0.19; p=0.032; *d*=0.36), for the major nocturnal sleep values (M5 index normal value: 3:00-5:00 h, Figure 20). Overall, lower sleep values and a tendency to phase advancement was found for the *ASMT* rs5989681–GG genotype. Significantly decreased sleep values were found at 3:00h (0.72 ± 0.22 vs. 0.85 ± 0.18; p=0.015; *d*=0.65), which moved the major sleep circadian rhythm index forward (M5; 1:30 ± 3:05 h vs. 2:24 ± 1:25 h; p=0.044; *d*=0.42; Figure 21).

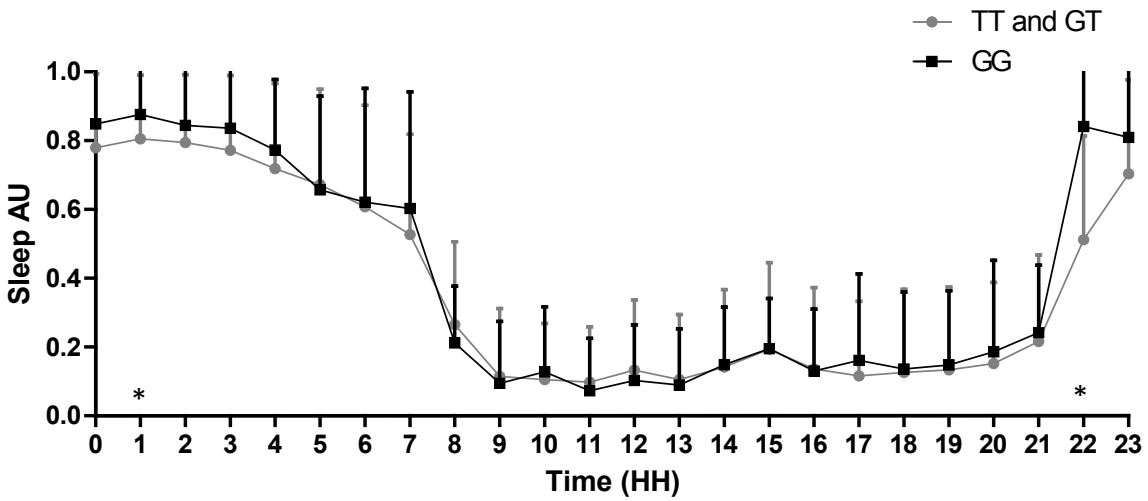

Figure 20. Sleep circadian rhythm for *PER1* rs6416892 genotypes in autistic adults. Sleep calculated by ACM does not have standardized measure that is why is expressed as [AU] arbitrary units, and [HH] hours.

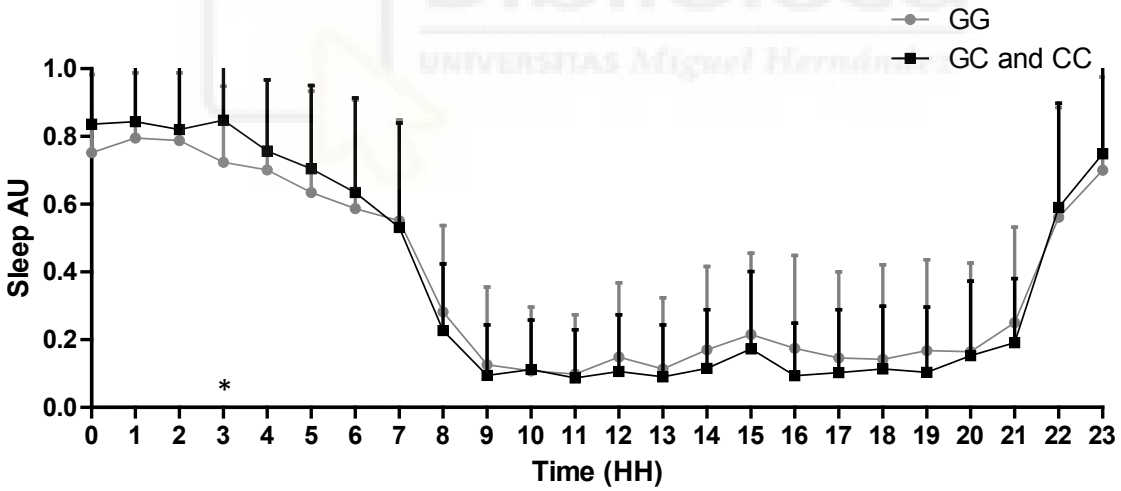

Figure 21. Sleep circadian rhythm for *ASMT* rs5989681 genotypes in autistic adults. Sleep calculated by ACM does not have standardized measure that is why is expressed as [AU] arbitrary units, and [HH] hours.

As temperature is an important rhythm in order to assess the status of the circadian system, significant results for *PER1*, *ASMT* and *NPAS2* are discussed below. The same *PER1* rs6416892-GG genotype showed also a temperature phase advancement across the day: for the major

nocturnal values M5 (00:15 ± 3:49GG, 2:07 ± 2:40GT/TT h; p=0.045; *d*=0.59) and diurnal rhythms L10 (12:49 ± 2:21GG; 14:17 ± 2:06GT/TT h; p=0.023; *d*=0.64). The ASMT rs5989681–GG genotype was associated with a higher fragmentation of the wrist temperature rhythm (IV, 0.14 ± 0.08GG, 0.11 ± 0.05GC/CC; p=0.033; d=0.57) with major temperature values during the day (VL10, 32.46 ± 1.67GG, 32.26 ± 1.07GC/CC ºC; p=0.049; d=0.11). NPAS2 rs1811399-GG individuals showed higher temperature circadian rhythm values during the day (VL10, 32.46 ± 0.01GG, 32.27 ± 1.43GT/TT ºC; p=0.017; d=0.69) There were no further influences of these polymorphisms on circadian sleep-wake or temperature rhythm indexes.

DISCUSSION

ACM device was effective and accurate in measuring sleep-wake cycle in autistic adults with ID, where insomnia symptoms and a phase advancement in all circadian rhythms analyzed were highly prevalent. Only 16% of participants had sleep parameters in normal value range. In addition, agomelatine increased TST and corrected wrist temperature circadian rhythm, thus, could be appropriate for synchronization of endogenous rhythms, improving sleep quality. In this population, *PER1* rs6416892-G allele had more chances of a better sleep pattern, and a phase advancement of sleep and/or wrist temperature rhythms, *ASMT* rs5989681 *and NPAS2* rs1811399 polymorphisms also influenced insomnia and CRSWD.

This work presents the biggest sample of autistic adults with ID assessing sleep-wake cycle using an objective tool (Hare et al., 2006b; Rosbergen, Jansen, Rosbergen-De, Roke, & Otten, 2017), and the first time that ACM device is used in autistic participants with ID. Neither adults with ASD with or without ID, have received much attention in the literature (E. K. Baker & Richdale, 2017; Hare et al., 2006a; Hare et al., 2006b; Limoges et al., 2005; Johnny L Matson et al., 2008; Wiggs & Stores, 2004).

5.1. – Diagnosis of insomnia

Results confirmed the higher prevalence of sleep problems in adults with ASD and ID, when compared to a healthy control population, with only 16% of autistic participants with sleep parameters in normal range. Data is consistent with a higher vulnerability to sleep problems in the ASD population from early childhood through to middle age (S. E. Goldman, Richdale, Clemons, & Malow, 2012; Tani et al., 2004). Overall, the findings indicate that adults with ASD and ID have insomnia symptoms (low SE, prolonged latency, and increased frequency and length of night awakenings), compared with neurotypical adults. In fact, sleep disturbances

described for this sample of adults with ASD and ID are worse to those already described for adults without ID, children and adolescents on the spectrum, evidencing that are a life-long condition (E. Baker, Richdale, Short, & Gradisar, 2013; E. K. Baker & Richdale, 2015; Souders et al., 2017).

Previously, some studies reported an increased SoL and WASO, more frequent nocturnal awakenings, decreased total sleep and lower sleep efficiency in cognitively able adolescents and adults on the autism spectrum (E. K. Baker & Richdale, 2015, 2017; S. Goldman et al., 2017; Hare et al., 2006a; Limoges et al., 2005; Tani et al., 2003). However, the percentages of normality in all sleep parameters in our ASD and ID participants are smaller than those previously described (Øyane & Bjorvatn, 2005). Only 5% of individuals on the spectrum had a total sleep time within the normal range for adults, which differed from 72% of the controls. This impaired sleep in adults with ASD and ID can be associated to severe challenging behaviours (Johnny L Matson et al., 2008).

The percentage of participants in the control group with normal number and duration of awakenings after sleep onset was low. Although it is striking, values are similar to other studies in healthy individuals (Carrier, Monk, Buysse, & Kupfer, 1997; Chakar et al., 2017; Ohayon, Carskadon, Guilleminault, & Vitiello, 2004), and could be related to other factors such as occupational status (Kim & Lee, 2015) or raising a child (Reader, Teti, & Cleveland, 2017). Also, although some authors did not find a link between different residential conditions and sleep problems (Cohen et al., 2017), longer SoL and TIB and shorter SE described for the adults on the spectrum could be a result of prescribed schedules in their residences, that do not allow flexibility that fits with individual preferences and circadian differences. Even more, anxiety, high physiological arousal (Hundley, Shui, & Malow, 2016) and problematic daytime behaviour (Johnny L Matson et al., 2008) may influence SoL length.

As this could be relevant, we analyzed those adults with ASD taking anxiolytic medication for co-morbid anxiety disorders and only differed from other adults in their number of awakenings.

In summary, the findings support that autistic adults with ID sleep worse than typically developing adults, and only a 5% of them reached TST normal duration, even though it was the only mean sleep parameter value that was not significantly different. Next step will be to study how these results registered by ACM could influence on life quality, commorbidities or diurnal functionality.

5.2. – Diagnosis of circadian rhythm sleep-wake disorders

To the best of our knowledge, the study AGOTEA is the largest study examining objective circadian sleep-wake rhythm indexes in ASD adults with ID. Similar to previous research from childhood to middle age, our data showed significant differences, a worse sleep and circadian system regulation with a tendency for a phase advancement in all circadian rhythms analyzed (E. K. Baker & Richdale, 2015; S. Goldman et al., 2017; Souders et al., 2017), between adults with ASD and comorbid ID and an age-matched, healthy, adult population. They reflected a sedentary pattern of lifestyle, together with higher levels of day light intensity and night deprivation. Daily life impact of these results is not yet defined in this population.

When assessing the circadian system, the most common marker rhythms are core body temperature, motor activity, light intensity, cortisol and melatonin (Klerman, Lee, Czeisler, & Kronauer, 1999; Van Someren, 2000). Wrist (skin) temperature is also a consolidated, non-invasive, robust and reliable tool for assessing circadian system function (Blazquez, Martinez-Nicolas, Salazar, Rol, & Madrid, 2012; Bonmati-Carrion et al., 2014). Circadian rhythms need

to be continuously synchronized with the environment, and the most powerful *zeitgeber* (environment time cue that entrains circadian rhythmicity) is the 24-h light/dark cycle (Wright Jr et al., 2013), but non-photic *zeitgebers* such as meal timing and social contacts can also be influential (Kalsbeek, Merrow, Roenneberg, & Foster, 2012).

The phase advance found in this study (M5 and L5 at ~ 01:00 h) affected 60% of individuals with ASD which is higher than in other studies that reported this in around 10% of participants (E. K. Baker & Richdale, 2017; Hare et al., 2006a). This was previously reported in ASD children (Tatsumi et al., 2015) and in a small number of ASD adults (E. K. Baker & Richdale, 2017; Hare et al., 2006a; Limoges et al., 2005).

Our data was consistent along all the circadian rhythms analyzed, similar to previous results (E. K. Baker & Richdale, 2017; Hare et al., 2006a; Limoges et al., 2005; Tatsumi et al., 2015). Normal timing of M5 and L5 takes place around 04:00 h (Ortiz-Tudela et al., 2010), during rapid eye movement sleep with minimum muscle tone (Madrid & de Lama, 2006), and when melatonin reaches its nocturnal peak (Claustrat, Brun, & Chazot, 2005). However, as light intensity is a synchronizer of melatonin secretion (Morin & Allen, 2006), melatonin peaks in the adults with ASD from our sample could be happening before the usually described times (Arendt, 1994).

- Wrist temperature circadian rhythm

This rhythm reflects endogenous and exogenous circadian influences, thus presenting considerable advantages for evaluating the effects of synchronizing agents, such as light exposure, on circadian function. Peripheral temperature, as reflected by the wrist temperature, affects sleep-regulating areas in the brain, and increases before sleep onset, remaining high overnight until waking and remaining low during the rest of the day (Benloucif et al., 2005; Sarabia et al., 2008). The time of minimal sleep probability known as ‘‘wake

maintenance zone'', is usually between 20:00 and 22:00h in healthy adults, and matches with the lowest value of daily peripheral temperature (Ortiz-Tudela et al., 2010), and the dim light melatonin onset (Lewy, Cutler, & Sack, 1999). Thus, it can be used to assess advanced or delayed circadian system timing due to its relation with the intrinsic circadian period (Wright Jr, Gronfier, Duffy, & Czeisler, 2005).

In our ASD group, the wrist temperature 24h rhythm showed an advanced wake maintenance zone (17:00-18:00 h) when compared with the control group (20:00-21:00 h); the phase markers M5 and L10 supported this phase advancement. Misalignment of the wrist temperature rhythm could contribute to the sleep problems described in our adults (Shekleton et al., 2013). Furthermore, adults on the spectrum in this study displayed an overall rhythm of increased wrist temperature, that could be related to reduced activation of noradrenergic vasoconstrictor tone resulting in a slower sleep onset (Blazquez et al., 2012) and melatonin secretion is strongly influenced by the temperature rhythm (Kräuchi, Cajochen, & Wirz-Justice, 1998).

- Motor activity circadian rhythm

Motor activity 24h wave and non-parametric circadian rhythm values from adults on the spectrum are higher during the night (21:00-01:00 h) and lower during the day (12:00 h). Those elevated night values could be due to certain sleep problems such as restless legs syndrome or epilepsy. However, the actimeter should be worn on the leg to detect the first (Cippà et al., 2013), and this is complicated in our sample. Only one participant suffered two epileptic seizures (one of them, during the day) at ACM week data collection. Thus the results together with the L5 value of motor activity suggest an earlier night-wake activation, as previously described for cases of phase advancement (Limoges et al., 2005). The higher IS and the VM10 values in ASD suggest a stability in physical activity tasks (Iosa et al., 2014) and sedentary lifestyle (MacDonald, Esposito, & Ulrich, 2011; Orsmond & Kuo, 2011).

Motor activity, together with wrist temperature and light intensity, provide a better evaluation of chronobiologic disorders than temperature alone (Klerman et al., 1999), and have been described as a substitute for PSG to test circadian rhythms status in humans (Carvalho Bos et al., 2003).

- Body position circadian rhythm

A lower body position values are described for our sample during the day. As described, body position together with motor activity help to define rest-activity spans properly (Blazquez et al., 2012). Phase advance in our sample with ASD is also evidenced with body position phase markers L5 and M10, and lower TAP values registered from midday until midnight.

- TAP and Light intensity circadian rhythms

Our TAP rhythm in ASD matched that described for circadian rhythm alterations in mild cognitive impairment (Ortiz-Tudela et al., 2014). The light intensity values recorded in the ASD sample were significantly higher in the morning and more robust during the week, according to the greater CFI value found. According to the literature wrist temperature is a marker of circadian phase, and light applied after night arousal has a phase-advancing effect (Pavlova, 2017) so higher morning values in ASD could be moving the rhythm forward.

Apart from the melatonin peak and light intensity received, differences may be due to the employment conditions in our sample. All participants with ASD in our study were unemployed, and unemployment has been related to the presence of a sleep disorder in high-functioning ASD (E. K. Baker & Richdale, 2017). However, most unemployed individuals in their study met criteria for delayed sleep wake rhythm disorder (E. K. Baker et al., 2018). Nevertheless, adults with ASD in these studies did not have an ID and were not institutionalized (E. K. Baker & Richdale, 2017; Hare et al., 2006a), and thus presumably were able to self-select bed and wake times.

Overall, there is a consistent phase advancement in all the circadian rhythms analyzed by ACM in autistic adults with ID when compared to healthy controls. There is also a sedentary lifestyle in autistic adults with ID together with an intense light exposure in the morning, which effects are yet unknown in this vulnerable population.

5.3. – Pharmacological treatment of sleep problems

The study AGOTEA CT has the largest sample of autistic adults with ID enrolled in a randomized clinical trial, where an objective tool as ACM assesses the effectiveness of a

pharmacological treatment for sleep problems. There is only a clinical trial that has a bigger sample size than this with twenty-seven participants. However, the sample contained different mental pathologies, so there were no conclusions about sleep improvement only in autistic participants (Ishizaki, Sugama, & Takeuchi, 1999).

In autistic children and adolescents, melatonin is prescribed for sleep problems in most pharmacological interventions, but agomelatine is not allowed according to the summary of product characteristics. The current study is the first clinical trial in adults with ASD and ID that uses a robust design (a crossover clinical trial, randomized, placebo-controlled) to examine effectiveness and safety of a new pharmacological treatment, agomelatine, for their presenting sleep problems in ASD adults.

Very few data are available in regards to the safety of agomelatine in long-term use. A large clinical trial (n=711 participants) reported that seven individuals suffered an elevation of transaminase levels during the 8-week treatment, from those six recovered normal values without leaving the trial. In autism, there is no evidence about long-term side effects of agomelatine (San & Arranz, 2008). Despite our sample was already medicated with a median of five other drugs, the tolerability of agomelatine was good, with only one adverse event (4%), and the effects of antidepressant drugs on participants' sleep parameters were constant for both placebo and agomelatine treatment periods.

5.3.1. – Agomelatine in insomnia

The results of the randomized clinical trial indicated that for these individuals, agomelatine was both effective in increasing the average TST and safe. The increment in TST and circadian sleep-wake rhythm indexes found with agomelatine, an agonist of melatonin receptors, are consistent with previous outcomes published for melatonin in children with ASD (Wasdell et

al., 2008; Wirojanan et al., 2009). Melatonin has been the main pharmacological treatment for sleep problems in autism, and has been shown as effective in reducing insomnia symptoms including: reducing SoL (Cortesi, Giannotti, Sebastiani, Panunzi, & Valente, 2012; B. Malow et al., 2012; Wright et al., 2011), increasing sleep efficiency (Cortesi et al., 2012), reducing number of awakenings (Garstang & Wallis, 2006), increasing TST (Wirojanan et al., 2009; Wright et al., 2011) and also improving CRSWD (Wasdell et al., 2008; Wirojanan et al., 2009). Only two studies reporting that melatonin was effective at treating insomnia included either young adults (Ishizaki et al., 1999) or six adults (Galli-Carminati et al., 2009). Melatonin is reported as safe and effective on its own (Maras et al., 2018) or in combination with other psychotropic treatments (Andersen et al., 2008) in children with autism. Apart from melatonin, donezepil, an acetylcholinesterase inhibitor, has been studied to treat sleep pathology in autism during early childhood in an open label study tested by polysomnography (Buckley et al., 2011), and an increase in TST and normalization of Rapid Eye Movement (REM) sleep values were shown. Additionally, the use of other pharmacological treatments (e.g., risperidone) for autistic symptoms has indirectly improved participants' sleep performance (Gvozdjáková et al., 2014; Kent, Hough, Singh, Karcher, & Pandina, 2013). Sleep improvements shown here are also consistent with agomelatine studies in depression (Poluéktov & Levin, 2013; Pribytkov, Panova, Popova, & Emtsov, 2013). The average increase of TST resulting from agomelatine treatment, according to ACM recordings, has been described in adults with major depressive disorder using PSG (Poluéktov & Levin, 2013; Pribytkov et al., 2013). TST was also increased following agomelatine treatment in a larger study (Quera-Salva et al., 2011) that compared agomelatine against escitalopram using PSG. In the results from an open label study (Salva et al., 2007) similar increase in TST, and also improved sleep efficiency and an increase in non-rapid eye movement stage 3 were found with the same dose as used in our study.

Other studies have found significant differences in other sleep parameters (e.g., day time sleepiness, shorter sleep onset latency) using questionnaires (Urade, Mahakalkar, & Tiple, 2015), but as our adults with ASD and ID are nonverbal, questionnaires are not validated for our population. Studies in participants with mood disorders or anxiety that used subjective sleep measures also have reported an increase in TST following agomelatine treatment (Hale et al., 2010; Stein, Ahokas, & de Bodinat, 2008) and a reduction in insomnia symptoms has been observed upon agomelatine treatment (Lemoine, Guilleminault, & Alvarez, 2007). However, some authors have described no effects on sleep after agomelatine treatment (Calandre et al., 2014; Englisch et al., 2016).

5.3.2. Agomelatine in circadian rhythm sleep-wake disorders

Apart from that, agomelatine, but not placebo, resulted in a phase correction (2h delay) in the skin temperature rhythm, a reduction in the variability of the sleep-wake rhythm, and an advance in the motor activity rhythm in the morning. Additionally, the amplitude of the sleep rhythm increased. These findings support that agomelatine treatment also results in improvements of the circadian sleep-wake rhythm, indicated by the increase in daily functionality of individuals following agomelatine treatment according to motor activity.

Following agomelatine treatment, there was a phase correction of the peripheral temperature rhythm. As temperature is a marker of circadian system status (Sarabia et al., 2008), that indicates an improvement of CRSWD, and has been described already (Laux & Group, 2012). Furthermore, an open label study described a phase advancement of two hours in the temperature rhythm in older men using a larger dose of agomelatine than used in this work (Leproult, Van Onderbergen, L'Hermite-Balériaux, Van Cauter, & Copinschi, 2005). Moreover, another study showed a phase correction of the mid-range temperature with both

5 mg and 100 mg of agomelatine (Krauchi, Cajochen, Mori, Graw, & Wirz-Justice, 1997). All is consistent with our findings, and may be due to the dual effect of agomelatine upon melatonin and serotonin receptors. Higher amplitude values of sleep at night in the 24 h wave rhythm, together with the greater stability of the sleep circadian rhythm in the agomelatine condition is consistent with what has been described by other authors using a sleep screening questionnaire (Pjrek et al., 2007) to assess CRSWD.

It is well known that when targeting CRSWD with melatonin agonists the timing of administration as well as the pharmacokinetic parameters are important. To produce a phase advancement in individuals with a phase delay administration must occur several hours' prior bedtime. On the other hand, in individuals with phase advancement some guidelines have recommended a low dose of melatonin (Auger et al., 2015), while several authors pointed the need for further research about the effectiveness of melatonin or its agonists in treating CRSWD (Auger et al., 2015; Mundey, Benloucif, Harsanyi, Dubocovich, & Zee, 2005; Williams III, McLin III, Dressman, & Neubauer, 2016).

We do not know if our results can be extended to adults with ASD and no ID, or to children with ASD and sleep problems, or whether agomelatine may also improve the daytime sleepiness, shorter sleep onset latency, or increased night waking also found in individuals with ASD.

5.4. – Genetic variants influence on sleep problems

Our results show that *PER1* rs6416892 and *ASMT* rs5989681 polymorphisms affect both sleep parameters and circadian rhythms in ASD, as indicated by sleep and wrist temperature phase marker indexes. In addition, an isolated significant effect was found for the *gene NPAS2* rs1811399 on diurnal wrist temperature values. Furthermore, in our population, the SNPs

located in the melatonin receptor were monomorphic, so no analysis or comparison with previous studies could be performed (Chaste et al., 2010).

We found that specific polymorphisms on the *PER1 gene* (rs885747 G>C with a frequency of 60% of allele G; rs6416892 T>G with a frequency of 60% of allele T) appeared in a higher frequency in autistic individuals, than in the control group. This result is supported by a previous study in a sample of trios, formed by autistic progeny and parents, where they found similar allelic distribution to ours for rs6416892 and rs885747, and linked those frequencies with the autistic condition (Nicholas et al., 2007). The frequencies of polymorphisms described for autistic individuals in this study and previously (Nicholas et al., 2007) could be linked to an abnormal expression of *PER1* gene in brain areas that affect sleep wake transition, e.g., in the suprachiasmatic nuclei, a brain structure deeply involved in sleep process (Charrier et al., 2017; Sun et al., 1997). The suprachiasmatic nuclei is the principal circadian pacemaker. It controls circadian rhythms in rest and activity, and several cyclical processes, such as: core body temperature or neuroendocrine function (i.e., hormone release) (Sun et al., 1997). Some authors have related the allelic distribution of *circadian clock* genes in autistic participants with a rhythm dysregulation that could affect brain development periods and therefore cause the ASD condition (Bourgeron, 2007; Geoffray et al., 2016; Wimpory et al., 2002).

5.4.1. – Genetic influence on insomnia

In our sample, those participants with a *PER1* rs6416892-GG genotype needed less time to accomplish the transition from wakefulness to sleep and had fewer night awakenings. Little is known about how *PER1* rs6416892 affects sleep in autism, but studies in animal models

(rodents) have described that when there is a lack of this gene there is a reduction in total sleep time during the day (Cirelli, 2009).

The *ASMT* rs5989681–GC genotype is also related to fewer night awakenings. Some authors have described that rs5989681-G allele is associated with a dramatic decrease of acetylserotonin O-methyltransferase (*ASMT*) activity, the last enzyme in melatonin production. They also associated that with the lower melatonin levels described in autism (Melke et al., 2008; Cécile Pagan et al., 2017; Toma et al., 2007). As the analyses in our study were made by genotype instead of allele, the C-allele could be protecting G-allele carriers and therefore positively influencing the number of awakenings per night, as the presence of both alleles is associated with a reduction in the mean number of nocturnal awakenings.

5.4.2. – Genetic influence on circadian rhythm sleep-wake disorders

PER1 helps to reset the biological clock (Crane & Young, 2014; Jonsson et al., 2010; Lowrey & Takahashi, 2011). Only a study with a small sample size has previously described polymorphisms in this gene to be more frequent in autistic individuals, and relate them with sleep problems (Yang et al., 2016). Related to circadian rhythm, this variation evidenced sleep and temperature phase advancement in general population, and these findings are consistent with early morning awakening found in ASD (Utge et al., 2010). Furthermore, some authors have described that when silencing *PER1* expression, there is an alteration of sleep-wake circadian rhythm (Cirelli, 2009; Nagel, Clijsters, & Agami, 2009). In addition, disruptions in the complex with *CRY1* and *CRY2*, are related with an early morning preference and the inability to reset the clock in response to environmental cues such as daylight (Berson, Dunn, & Takao, 2002; Katzenberg et al., 1998).

According to our results, adults on the spectrum with a GG genotype in *ASMT* rs5989681, presented a phase advancement in the sleep circadian rhythm. The G allele is associated with abnormal activity of *ASMT* the last enzyme in melatonin production (Melke et al., 2008; Cécile Pagan et al., 2017; Toma et al., 2007). That may result in abnormal levels of melatonin, apart from that, the phase advancement could be also related to an earlier time of melatonin release, as suggested before (Lewy et al., 1998). This timing release alterations may be related with the role that *PER1* plays in sustain the clock adequate oscillatory function (Oster, van der Horst, & Albrecht, 2003).

Autistic adults on the spectrum with *PER1* rs6416892-GG, *ASMT* rs5989681–GG and *NPAS2* rs1811399-GG genotypes presented major temperature values during the day. Many previous studies in humans indicate that sleep is strongly linked to thermoregulation. Core body temperature, which also cycles together with the sleep-wake rhythm, decreases during the nocturnal sleep phase and increases during the wake phase across the 24-hour circadian rhythm (Kryger & Sheldon, 2005).

This phase relationship with temperature is important for maintaining sleep. In this work, *PER1* rs6416892-GG was related to a phase advancement in the wrist temperature rhythm, and it has been described how temperature influences sleep timing (Ciarleglio et al., 2011).

Although individuals taking sleep medications were excluded, the influence of potential drug side effects on insomnia and circadian rhythm sleep-wake disorders and *circadian clock* gene expression cannot be excluded. There is some evidence that selective serotonin reuptake inhibitors (e.g., fluoxetine) alter the expression of the *PER1* gene in brain areas related to sleep (Ammon, Mayer, Riechert, Tischmeyer, & Höllt, 2003; Uz et al., 2005).

Summary

This work recruited the largest sample of autistic adults with ID enrolled in a sleep study using an objective tool, ACM, also first-time used in this population. Results showed significant insomnia symptoms together with a phase advancement in all rhythms explored, when compared to a sample of healthy controls. In addition, a sedentary pattern of lifestyle according to motor activity, body position and TAP rhythms, and an intense exposure to light during the first part of the morning were detected in autistic participants. The consequences of these results are not yet defined. Thus, future studies are required to understand their meaning and the impact on the health of these subjects.

From those subjects who had persistent sleep problems and agreed to participate in the randomized clinical trial, agomelatine was effective increasing night TST average value, and also improving the CRSWD, as demonstrated through improvements in peripheral temperature, motor activity and sleep circadian rhythms. Furthermore, there were minimal adverse events, that demonstrates a good short-term safety profile for agomelatine. Finally, some relations were found between *PER1, ASMT* or *NPAS2* genotypes and sleep problems (insomnia symptoms and a phase advancement of sleep and wrist temperature rhythms). There is a need for randomized clinical trials to confirm agomelatine long-term use effectiveness, and the role of *circadian clock* and *melatonin* genes as new molecular biomarkers for sleep problems in ASD.

- Limitations related to the autistic group:
    - Diagnosis: In this work, two clinicians confirmed the diagnosis according to DSM-5 criteria, using different assessment tools or questionnaires will be considered in future studies.
    - Selection bias (AGOTEA and GENTEA): In both, study information emphasized that no sleep problem was required to participate. However, it is possible that parents or legal guardians of autistic adults with disturbed sleep were more likely to participate.
    - Polypharmacy and potential drug-drug interaction (AGOTEA CT): Trying to control them, all concomitant treatments remained constant in both arms (treatment and placebo). Furthermore, all prescriptions were stable before the trial a minimum of 6 months.
    - Sample size: Although the samples could be considered relatively small, were in line with published manuscripts using similar design and methodology. Further studies with larger number of participants should be performed to confirm our results.
- Limitations related to control group:
    - Demographical variables (AGOTEA): Both groups (case and control) only matched on age, but not on sex, IQ, employment status or living conditions (e.g., forced bedtime schedules or evening light exposure). We acknowledge that all these factors could affect our results. Although, we are unable to modify living conditions in those ASD institutions, these factors should be considered in the interpretation of present results.

- Health status: The control participants were healthy, non-medicated adults, but most adults in the ASD group were medicated, and polypharmacy was common.
- Demographical variables (GENTEA): It would have been interesting to know further details about control samples used, however, knowing that they were healthy and from the same geographical area as our autistic participants was sufficient to test outcomes for the present work.
- Sample size (GENTEA): We acknowledge that the number of individuals is small to such study, so results must be confirmed using a larger sample size.

- Limitation related to melatonin:
  - We could not obtain enough saliva volume to measure melatonin levels in our population as was planned in the protocol. That happened because all autistic participants were severely handicapped and cotton swabs did not remain enough time in their mouth to get wet.

- In order to know how autism and ID may be influencing sleep problems, it would be useful to compare sleep-wake cycles from autistic adults, with and without ID, and typically developing controls.
- Validate a new objective device in order to measure sleep-wake cycle in our population reducing losses of information ACTRUST PROJECT code, (approved by Ethics Committee the 1st of October, 2014, current phase: data analysis).
- Create a pharmacovigilance monitoring system (Centro San Rafael and Psychiatry Unit in Alicante General Hospital) to study suspicions of adverse events related to drugs. The experience generated in this project will be used in other patients with off-label pharmacological treatments or prevalent sleep problems.
    - Analyze the impact of polypharmacy according to their *CYP2D6* metabolizing phenotype, and explore the causes of the adverse events detected in our participants VIGITEA PROJECT code (approved by Ethics Committee the 21st of January, 2016, current phase: retrospective phase data analysis).
- Potentially organize, with the knowledge generated in this work, homogenous groups of autistic participants with a similar sleep phenotype, and then design an individualized treatment per group.
    - Develop treatment guidelines for autistic adults in order to improve sleep problems and therefore impact on their life quality and daily functionality. VIGITEA PROJECT code (approved by Ethics Committee the 21st of January, 2016, current phase: prospective phase development).

1. ACM device was an objective, effective and accurate tool, measuring sleep-wake cycle in autistic adults with ID. All participants presented insomnia symptoms, and 16% was the highest normality percentage reached in sleep parameters.
2. Autistic adults with ID presented CRSWD according to a consistent phase advancement in all rhythms analyzed. They reflected a sedentary pattern of lifestyle together with higher levels of day light exposition and night deprivation. Daily life impact of these results is unknown.
3. Agomelatine treatment (25mg/day, 3-months) was effective and safe improving insomnia symptoms, through an average increase of 83 minutes of sleep at night, correcting temperature circadian rhythm disorder, and increasing sleep rhythm stability. Agomelatine long-term use effects are not yet defined in this population.
4. C*ircadian clock* (*PER1* rs885747 and rs6416892) genotype distribution was different between autistic adults and controls. Polymorphisms in *PER1* and *ASMT* genes influenced on insomnia symptoms: increasing SoL normality percentages (*PER1* rs6416892) and reducing number of awakenings (*PER1* rs6416892 and *ASMT*, rs5989681).
5. Polymorphisms in *circadian clock* (*PER1* rs6416892 and *NPAS2* rs1811399) and *melatonin pathway* (*ASMT* rs5989681) genes influenced on the phase of sleep and temperature rhythms.
6. There is a need for randomized clinical trials in autistic adults with ID to confirm agomelatine effectiveness, together with further studies evaluating the use of *circadian clock* and *melatonin pathway* genes as genetic biomarkers for sleep problems. Both actions would increase the understanding of sleep problems in this population, and choose wiser the pharmacological therapy.

CONCLUSIONES

1. El dispositivo de monitorización circadiana ambulatoria fue una herramienta objetiva, efectiva y precisa para medir el ritmo de sueño-vigilia en adultos con autismo y discapacidad intelectual. Todos los participantes tenían síntomas de insomnio según los valores de los parámetros de sueño, y 16% fue el mayor porcentaje de normalidad registrado.

2. Los adultos con autismo y DI presentaron trastornos del ritmo circadiano, con un adelanto de fase consistente en todos los ritmos analizados. Además de un estilo de vida más sedentario, mayores niveles de luz durante el día y oscuridad durante la noche. Se desconoce el impacto de estos resultados en la vida de los participantes.

3. El tratamiento con agomelatina (25mg/día, 3 meses) fue efectivo y seguro, mejorando síntomas de insomnio a través de un incremento medio de 83 minutos de sueño nocturno, corrigiendo el ritmo circadiano de temperatura e incrementando la estabilidad del ritmo de sueño. Los efectos del tratamiento a largo plazo con agomelatina no han sido definidos en esta población.

4. Hubo diferencias en la distribución genotípica del gen *reloj circadiano* (*PER1* rs885747 y rs6416892) entre adultos con autismo y controles. Polimorfismos en los genes *PER1* y *ASM* influyeron en los síntomas de insomnio: incrementando los porcentajes de normalidad de latencia de sueño (*PER1* rs6416892) y reduciendo el número de despertares (*PER1* rs6416892; *ASMT*, rs5989681).

5. Polimorfismos en los genes *reloj circadianos* (*PER1* rs6416892 y *NPAS2* rs1811399) y de la *ruta de la melatonina* (*ASMT* rs5989681) influyeron en la fase del sueño y en el ritmo circadiano de temperatura.

6. Se precisan futuros ensayos clínicos aleatorizados en adultos con autismo y discapacidad intelectual para confirmar la eficacia de la agomelatina, y estudios que evalúen el uso de genes *reloj circadianos* y de la *ruta de la melatonina* como biomarcadores genéticos

de problemas de sueño. Llevas a cabo estas acciones incrementará el conocimiento sobre los problemas de sueño en esta población, y ayudará a elegir mejor el tratamiento farmacológico.

Appendix

Appendix 1

GENERALITAT VALENCIANA
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HOSPITAL GENERAL UNIVERSITARIO DE ALICANTE
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DICTAMEN DEL CEIC DE REFERENCIA A PROTOCOLO DE ENSAYO CLÍNICO

Dña. Elena López Navarro, Secretaria del Comité Ético de Investigación Clínica del Hospital General Universitario de Alicante.

CERTIFICA

El Comité Ético de Investigación Clínica del Hospital General Universitario de Alicante, en su reunión de día 29 de Febrero de 2012, evaluó el ensayo clínico:

TÍTULO	Eficacia de Agomelatina en la alteración del sueño en el trastorno del espectro autista (TEA)
Nº EUDRACT	2011-003313-42
PROMOTOR	Dra. Ana Mª Peiró (Sección de Farmacología Clínica, Hospital General Universitario de Alicante)
INVESTIGADOR PRINCIPAL	Dra. Ana Mª Peiró (Sección de Farmacología Clínica)
CÓDIGO DEL PROTOCOLO	AGO-TEA
VERSIÓN DEL PROTOCOLO	Versión 4
FECHA DEL PROTOCOLO	18 de Enero de 2012
HOJA DE INFORMACIÓN AL PACIENTE (Versión y fecha)	Versión 4 de 18 de Enero de 2012

Y tomando en consideración las siguientes cuestiones:

-La pertinencia del estudio, teniendo en cuenta el conocimiento disponible, así como los requisitos del Real Decreto 223/2004, de 6 de febrero y las normas que lo desarrollan.

-Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto, teniendo en cuenta los beneficios esperados.

-El seguro o la garantía financiera previstos son adecuados.

-El procedimiento para obtener el consentimiento informado, incluyendo la hoja de información para los sujetos versión 4 de 18 de Enero de 2012, y el plan de reclutamiento de sujetos previstos son adecuados, así como las compensaciones previstas para los sujetos por daños que pudieran derivarse de su participación en el ensayo.

-La capacidad del investigador y sus colaboradores son apropiados para llevar a cabo el estudio.

Departamento de Salud de Alicante
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-Las instalaciones y medios disponibles son apropiados para llevar a cabo el estudio.

-El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.

Por tanto, este Comité como Comité de Referencia, emite Dictamen Favorable para la realización de dicho ensayo en España, en el centro siguiente por el investigador principal que se relaciona a continuación:

- ***Hospital General Universitario de Alicante*** por la ***Dra. Ana Mª Peiró Peiró*** como investigador principal.

Que el Comité tanto en su composición con en los PNT cumple con las normas de BPC (CPMP/ICH/135/95) y con el Real Decreto 223/2004, y su composición actual es la siguiente:

- **Presidente:** Dr. José Francisco Horga de la Parte (Jefe de Sección de Farmacología Clínica)
- **Vicepresidente:** Dr. Víctor Soriano Gómis (Médico Adjunto de Alergia)
- **Secretaria:** Dª. Elena López Navarro (Técnico de la Función Administrativa-Servicio de Farmacia)
- **Vocales:**

Dr. Juan Antonio Marqués Espí (Gerente del Departamento Hospital General).
Dr. Bartomeu Massuti Sureda (Jefe de Servicio de Oncología).
Dra. Monserrat Mauri Dot (Médico Adjunto de Análisis Clínicos).
Dra. Amparo Burgos San José (Farmacéutica Adjunta del Servicio de Farmacia).
Dª. Mª Teresa Domenech Barón (Auxiliar Administrativo del CEIC).
Dr. Vicente Boix Martínez (Médico Adjunto de la Unidad de Enfermedades Infecciosas).
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Dr. Mariano Esteban García-Fontecha (Médico Adjunto de la UCI).
Dr. Fernando Quirce Andrés (Médico de Atención Primaria del C.S. Florida).
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Dª. María Gazapo Martínez (Lda. en Psicología, Miembro lego ajeno al centro).
D. Óscar Fuentes Coso (Jurista).
Dr. Julián Megías Garrigós (Médico Adjunto del Servicio de Urología).
Dra. Caridad Tapia Collados (Servicio de Pediatría).
Dra. Ana Mª Peiró Peiró (Unidad de Farmacología Clínica).
Dr. Fabian Tarin Rodrigo (Servicio de Hematología)

Lo que firmo en Alicante, a 29 de Febrero de 2012

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LA SECRETARIA DEL CEIC,

Fdo.: Dña. Elena López Navarro.

GENERALITAT VALENCIANA
CONSELLERIA DE SANITAT

ALACANT HOSPITAL GENERAL
DEPARTAMENT DE SALUT

(Ref. CEIC PI2014/12)

INFORME DEL COMITE ETICO DE INVESTIGACION CLINICA

Reunidos los miembros del Comité Ético de Investigación Clínica del Hospital General Universitario de Alicante, en su sesión del día 24 de Septiembre de 2014, y una vez estudiada la documentación presentada por la **Dra. Ana Mª Peiró Peiró,** Médico Adjunto de la Unidad de Farmacología Clínica del Hospital General Universitario de Alicante, tiene bien a informar que el proyecto de investigación titulado **"Actimetría sincronizada con el sistema FITBIT Flex: evaluación de la alteración del sueño medido por actigrafía y su aplicación al trastorno del espectro autista",** se ajusta a las normas deontológicas establecidas para tales casos.

Y para que conste, lo firma en Alicante con fecha uno de Octubre de dos mil catorce.

Fdo. Mayte Domenech Varón
Secretaría del CEIC

Avda. Pintor Baeza, 12 · 03010 ALICANTE · Tfno.: 965 933 000 · Fax 965 245 971

Manuscript Published in Autism Research

Title: Sleep problems in Adults with Autism Spectrum Disorder and intellectual disability.

Short running title: Autism sleep problems in adulthood.

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CONFLICT OF INTERESTS

Authors declare no conflict of interest.

LAY ABSTRACT

Sleep problems are very frequent in autism from childhood to adulthood. We recorded sleep with a watch-like device in adults with autism and intellectual disability and compared sleep patterns with non-autistic volunteers. Results showed poorer sleep conditions in adults with autism (increased sleep latency and number/length of night awakenings) that resulted in decreased sleep efficiency. Increasing knowledge of the sleep problems in adults on the autism spectrum will allow improving their and their families' quality of life

ABSTRACT

Sleep problems are recognized as a common comorbid condition in autism spectrum disorder (ASD) and can influence core autism symptoms and mental and physical health. Sleep problems can be life-long and it has been reported that adults on the autistic spectrum with and without intellectual disability (ID) present sleep problems (longer sleep latency, frequent night awakenings, and circadian rhythm sleep-wake disorders).

A prospective, objective sleep study was conducted in 41 adults with ASD (33 ± 6 years-old) and intellectual disability and 51 typically developing adults (33 ± 5 years-old) using ambulatory circadian monitoring (ACM) recording wrist temperature, motor activity, body position, sleep and light intensity.

The findings indicated that individuals with ASD presented sleep difficulties including low sleep efficiency, prolonged sleep latency and increased number and length of night awakenings, together with daily sedentary behavior and increased nocturnal activity. Furthermore, indications of an advanced sleep-wake phase disorder were found in these autistic adults.

Examining sleep and markers of the circadian system showed significant differences between adults with ASD and ID and an age-matched, healthy adult population. The sleep disturbances described for this sample of adults with ASD and ID are similar to those already described for adults with ASD without ID; their relationship with intellectual ability should be further studied. Improving knowledge of sleep patterns in ASD adults with ID might help to designed targeted interventions to improve their functioning and reduce family stress.

Keywords: Autism spectrum disorder, intellectual disability, sleep problems, circadian rhythm, circadian rhythm sleep-wake disorder.

INTRODUCTION

Sleep problems are common comorbid symptoms in individuals with autism spectrum disorder (ASD) and can impair social behavior [Tani et al., 2004; Couturier et al., 2005; Matson et al., 2008; Cortesi et al., 2010; Hollway et al., 2013; Buck et al., 2014], cognitive daytime performance [Limoges et al., 2013], and quality of life [Adkins et al., 2012; May et al., 2015; Kuhlthau et al., 2017]. Sleep problems can be life-long with a prevalence in childhood ranging up to 86% [Souders et al., 2017], while 44.7% of 168 autistic adults with severe intellectual disability (ID) were reported to have sleep problems using the DASH II questionnaire [Matson et al., 2008]. However, Hare et al. (2006) reported that sleep measures in a small group of adults with autism and ID did not differ from adults with ID alone. Cognitively able autistic adults similarly are reported to have increased insomnia symptoms, including increased sleep latency, poor sleep efficiency, shorter night sleep and advanced or delayed circadian sleep-wake rhythms [Tani et al., 2003; Limoges et al., 2005; Hare et al., 2006; Baker & Richdale, 2015; Baker & Richdale, 2017; Goldman et al., 2017]. Causes for sleep abnormalities are likely to be multifactorial, such as neurotransmitter abnormalities (e.g., serotonin) [Anderson & Lombroso, 2002; Malow et al., 2006], medical problems (e.g., gastrointestinal disorders [Klukowski et al., 2015], epilepsy [Kaleyias et al., 2008]), psychopathology [Richdale et al., 2014; Nadeau et al., 2015] or behavioral etiologies (e.g., poor sleep habits; [Reynolds & Malow, 2011; Malow et al., 2012]). In children with ASD, sleep problems can also be a marker of parental stress and one of the main reasons for pharmacological intervention [Schwichtenberg et al., 2013; Valicenti-McDermott et al., 2015]. However, comparisons of sleep patterns between individuals with ASD and the general population have been performed in only a few studies [Limoges et al., 2005; Baker et al., 2013; Baker & Richdale, 2015], and studies of sleep in adults with ASD and comorbid ID are lacking.

The sleep-wake cycle is a circadian rhythm. Circadian rhythms are physiologic or behavioral cycles with a recurring periodicity of approximately 24 hours and can be measured as daily oscillations of hormones (melatonin, cortisol), core body temperature, rest-activity cycles or transcriptome patterns [Bhadra et al., 2017]. There is good evidence that circadian rhythm sleep-wake disorders (CRSWD) play a role in many psychiatric illnesses such as depression, posttraumatic stress disorder, and eating disorders, which are often comorbid with ASD [Schuch et al., 2017]. These CRSWD are characterized by blunted amplitude and altered circadian phase [Boivin, 2000; Wirz-Justice et al., 2009]. To date, information concerning circadian sleep–wake cycles in individuals with ASD is scarce. A small number of children with autism are reported to have sleep problems suggestive of a CRSWD [Wiggs & Stores, 2004; Souders et al., 2009], and in adults circadian sleep-wake disturbances also have been indicated in a small number of individuals [Limoges et al., 2005; Hare et al., 2006]. Most recently, Baker & Richdale (2017) reported that 44.4% of cognitively able autistic adults met criteria for a CRSWD, primarily delayed sleep wake rhythm disorder, but nothing is known about adults with ASD and comorbid ID.

Sleep can be assessed using subjective (e.g., questionnaires or sleep diaries) or objective measures (e.g., actigraphy, ambulatory circadian monitoring (ACM), or polysomnography (PSG)). In this study, ACM devices were used since they are wearable, generally well-tolerated, and able to estimate participants' sleep in the home environment [Jean-Louis et al., 2001; Anders et al., 2011]. Furthermore, it has been reported that values obtained with ACM are closer to PSG sleep estimations than actigraphy records [Ortiz-Tudela et al., 2014]. In addition, ACM is considered more reliable than sleep diaries and is currently widely accepted [Ancoli-Israel et al., 2003; Tsuchiyama et al., 2003]. It can thus provide sleep information that may be otherwise not easily available in individuals with ASD and ID.

Thus, the aim of our study was to compare circadian rhythms and sleep patterns in adults with ASD and ID with those of typically developing adults using ACM. The results from this study will contribute to a better understanding of sleep problems in adults with ASD across the spectrum, and therefore help to prevent or treat sleep difficulties in this population.

METHODS

Participants

Forty-one individuals with ASD and ID (78% male, BMI 24.4 ± 1 Kg/m^2) between the ages of 27 and 39 years old and 51 adults with normal intellectual functioning and no diagnosis of mental or physical health problems (41% male, BMI 23.2 ± 0.6 Kg/m^2) with ages from 28 to 38 years old were included in this study. Demographic data are summarized in Table 1.

Procedure

Following Hospital Ethics Review Board approval, all participants, or their legal guardians received information about the design and purpose of the study, and participants or legal guardians' informed consent was obtained. The study was performed in accordance with the principles of the Helsinki Declaration.

Participant Recruitment and Inclusion Criteria

The adults on the autism spectrum were recruited from Spanish autism associations after researchers met with parents and carers (93%), from clinics specializing in adults on the spectrum (5%) and via social media (2%); all participants were resident across three institutions for adults with ID. The control group was composed of typically developing adults recruited from the same geographical area who were also participating in a longitudinal study being conducted by the Chronobiology group at Murcia University. The study information

provided to participants emphasized that no sleep problem was required to participate in this sleep study.

Inclusion criteria for the adults with ASD (Figure 1) were age from 18 to 45 years; agreement to an initial clinic visit, and a previous diagnosis of ASD substantiated using the 5th Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [American Psychiatric Association, 2013] criteria for ASD. Diagnosis based on DSM-5 criteria was confirmed by a clinician from the individual's residential facility and by a psychiatrist from our research team. ID (intelligence quotient (IQ) <70) for the adults with ASD was confirmed from medical records of professionals from the Spanish social services. Adults on the autism spectrum continued to take their regular medications during the study, but individuals taking any sleep medication or melatonin were excluded from the study. No participant in the control group had a clinically significant medical or psychiatric condition, or was taking any psychotropic medications. The inclusion criteria for the control group were: age ≥18 to 45 years, agreement to an initial clinical visit and that their data would be included in this study.

Participants in both groups could withdraw from the study at any time. In both groups, those with any medical condition that was incompatible with the study conditions were excluded.

The study was conducted from February 2012 to December 2013, excluding summer (June-August) because weather could affect the accuracy of the temperature sensor. During the first visit, demographic information (age, sex, body weight, height, and job status) and current medications were ascertained. Thirty-three (80%) participants with ASD reported various comorbid clinical diagnoses (epilepsy, depression and aggressive behavior) and were taking medications associated with them. Figure 2 presents the different medications with potential effects on sleep (increasing sleep or somnolence) taken by ASD participants. Other drugs taken by ASD participants, but without potential effects on sleep were: proton pump

inhibitors (17%), anticholinergic agents (22%), ß-agonists (2%) and/or ß-blockers (2%). No participant was receiving cognitive behavioral therapy, so sleep was not influenced by this factor.

Ambulatory Circadian Monitoring (ACM)

The ACM device (Kronowise) has three different sensors. The first measures wrist temperature (Thermochron iButton DS1921H, ± 1 °C accuracy, and sampling every 10 min; [Carrier & Monk, 1997]. The second is an actimeter that estimates motor activity using degrees of change in position; and body position using the angle between the X-axis of the actimeter (90º verticality) and the horizontal plane (0º maximum horizontality), (HOBO Pendant G Acceleration Data Logger UA- 004-64, three-channel logger (X-, Y-, Z- axis) with 8-bit resolution, programmed to record data every minute; [Edgar & Dement, 1991; Mormont et al., 2000]. The actimeter information was analyzed defining two variables: motor activity and body position [Carvalho Bos et al., 2003; Bonmati-Carrion et al., 2015]. The third sensor measures light intensity (HOBO Data Logger UA-002 64, it has a measurement range of 0–320 000 lux, measured in 30-second intervals; [Martinez-Nicolas et al., 2011].

Van Someren non-parametric indexes and sleep parameters were calculated from the data recordings. The information stored in the Thermochron iButton, the actimeter and light sensor was transferred to a personal computer using the software provided by the manufacturer through an adapter (DS1402D-DR8; IDC, Spain) or an optical USB Base Station (MAN-BASE-U-4, HOBO). The Circadianware software implemented in the Kronowizard platform (https://kronowizard.um.es/) calculated the single integrated variable, TAP, from the integration of wrist temperature (inverted), motor activity and body position where maximum values should occur at the same time of the day and indicate a high level of activation (values near 1) or complete rest and sleep (around 0) [Lopez et al., 2014; Ortiz-

Tudela et al., 2014]. For computing TAP values, motor activity and body position data were added up and averaged, respectively, in 10-minute intervals (matching wrist temperature sampling rate). Sleep was inferred from TAP and converted into a binary code, with 1 corresponding to a resting period and 0 to an active period [Scheer et al., 2007; Sarabia et al., 2008; Ortiz-Tudela et al., 2014].

All participants were asked to follow their usual routines and wore the ACM device on the wrist of their non-dominant arm for seven days. The ACM was removed during showering or any other activity where the ACM might get wet; data were filtered in order to eliminate erroneous measurements produced by its temporary removal. Additionally, control participants, completed a 7-day, sleep-wake diary (mornings and evenings) and in case of the adults with ASD, a parent or caregiver was responsible for completing each participant's sleep diary. Sleep diaries were used as a backup for the ACM recordings if needed.

The sleep parameters calculated were: (1) total sleep time (TST, normal value between 420 and 540 minutes), defined as the number of minutes registered as sleep between sleep onset and sleep offset during the night (similar to PSG sleep period time); (2) time in bed (TIB, normal value from 420 to 569 minutes), total minutes in bed until sleep offset; (3) sleep onset latency (SoL, normal value <30 minutes) the time until sleep onset at night; (4) number of awakenings after sleep onset (normal value 0-1 number), number of awakenings during the TST interval; (5) awake period duration after sleep onset duration (WASO, normal value <20 minutes), the awake period length in minutes during the TST interval; and (6) sleep efficiency (SE, normal value ≥85%), calculated as the ratio of TST/TIB multiplied by 100. The results of the sleep parameters from all participants were classified as normal or abnormal values according to ranges previously described for the general population [Carskadon & Rechtschaffen, 2000; Natale et al., 2009; Zhang et al., 2011]. In addition, the time that the

person was still in bed after the last sleep episode ("residual TIB") was calculated by subtracting TST, SoL and WASO from the TIB.

Circadian sleep-wake rhythm disorders determination

Non-parametric circadian rhythm analysis is a method for extracting circadian characteristics from the rest–activity cycle [Weitzman et al., 1981; Van Someren & Riemersma-Van Der Lek, 2007]. Of major interest is the relative amplitude (RA), since it shows how activity is distributed throughout the day compared with night: the higher the RA, the better the consolidation of daytime activity and nighttime sleep. The RA is calculated from the ratio of the most active 10-hour period (M10) to the least active 5-hour period (L5) across the averaged 24-h profile. A second characteristic is the inter-daily stability (IS), which quantifies the invariability day by day, that is, how well the sleep–wake cycle is synchronized to supposedly stable environmental cues. Thirdly, intraday variability (IV) gives an indication of the fragmentation of the rhythm. Timing information comes from determining the onset of the 5 hours with least activity (L5 onset) and onset of the 10 hours with most activity (M10 onset). Finally, the circadian function index (CFI), which assesses circadian rhythmicity status, was calculated as (IS+(2-IV) +RA)/3. CFI has proved to be very sensitive to changes in circadian robustness [Witting et al., 1990; Ortiz-Tudela et al., 2010].

Statistical Analyses

The Shapiro-Wilk normality test was used as the basis for selection of parametric or non-parametric statistical tests. Continuous variables are presented as mean ± standard deviation (SD), mean ± standard error (SEM) or median and interquartile range (IQR, P_{25}, P_{75}) according to assessed normality of the variable. Categorical variables are expressed as percentages. A t-test for independent samples or a Mann Whitney U test was used to assess group differences; effect sizes (η^2, r respectively) and 95% confidence intervals (CIs) are also

reported. Frequencies were compared using the Chi-Square test, with Yate's continuity correction as appropriate and X^2 (df, n) were reported. All statistical analyses were performed with R 3.2.4 software and Graph Pad Prism 5.0. P-values of <0.05 were considered to indicate significance for all analyses.

As treatments used by adults on the autism spectrum for their comorbidities (neuroleptics, anticonvulsants, antidepressants or anxiolytics) could have potential effects on sleep, comparison analyses of sleep parameters between typically developing controls and adults with ASD with or without comorbidity treatments were conducted using the Student t-test or Mann Whitney U-test. Acknowledging that the categories of neuroleptics, anticonvulsants, antidepressants or anxiolytics could embrace several different molecules that could affect sleep in different ways, further intragroup analysis classifying the molecules according to how they affect sleep (positively, negatively or neutral [Brunton et al., 2011]) were conducted by Kruskal Wallis test.

RESULTS

The ASD group had a median of three comorbidities (IQR_{25-75}: 1-4, 38% epilepsy) and five prescribed medications associated with these comorbidities (IQR_{25-75}: 2-6, 61% neuroleptics, 51% anticonvulsants, 22% antidepressants and 32% anxiolytic, 20% without treatment, Figures 2). Control group participants were not medicated. There were no significant differences between ASD and control groups with respect to age, but as may be expected, there were significantly more males in the ASD group (X^2 (1, 92) = 13.27, p=0.002). A comparison analysis showed significant differences for ASD participants with and without anxiolytic treatment only for the number of awakenings only (p=0.006, 95% CI -2.812 - -0.5072, r=0.196). No significant differences for any sleep parameter were found when drug

categories (neuroleptics, anticonvulsants, antidepressants or anxiolytics) were analyzed by their potential effects on sleep (positive, negative or neutral).

Sleep parameters determination

Comparison of sleep parameter data between ASD and control groups obtained from ACM recordings is presented in Table 2. Significant differences between ASD and control groups were found for all sleep parameters, except TST. The ASD group showed significantly increased TIB, SoL, number of awakenings and WASO than controls and significantly lower SE. Overall, with the exception of WASO, significantly fewer individuals with ASD had sleep parameters in the normal range compared to those in the control group. In spite of the difference in gender distribution observed between ASD and control groups, differences in sleep parameters were maintained when analyzed by sex ($p \geq 0.05$ only for TST). No differences between males and females within each group were observed ($p \geq 0.05$).

Circadian sleep-wake rhythm disorders determination

Analysis of the circadian rhythms (wrist temperature, motor activity, body position, TAP, sleep, and light intensity) showed significant differences between the ASD and control groups (Figure 3). The ASD group had higher wrist temperature values with a lower amplitude and a wake maintenance zone (time interval of minimal sleep probability, minimal daily distal temperatures) that appeared earlier than in controls (ASD: 17:00-18:00 h versus controls: 20:00-21:00 h) (Figure 3a). Lower overall motor activity with less difference between the least (L5) and most active (M10) phases was observed in the ASD group (Figure 3b). Furthermore, the ASD group showed higher motor activity values between 3:00 to 6:00 h (Figure 3b) and body position at 5:00 h (Figure 3c). Together, these results point to sedentary daytime behavior in the ASD adults, with nocturnal activation (Figures 3b and 3c). As expected, TAP and sleep values were consistent with the results for motor activity and body position (Figures

3d and 3e). Differences in light intensity between both groups were also found. In the morning, individuals with ASD were exposed to higher light levels than control subjects, and from 15:00 to 7:00 h they experienced lower light levels (Figure 3f).

Overall, circadian phase advance in the ASD group was suggested by the higher values for wrist temperature and sleep and the lower motor activity and body position during the late afternoon and the first part of the night when compared to controls.

Table 3 shows the non-parametric indexes for the circadian rhythms plotted in Figures 3a to 3f. The circadian characteristics of the sleep-wake rhythm showed a consistent phase advance in ASD subjects as evidenced by the four phase markers (L5, L10, M5 and M10). Significant group differences were present in all circadian rhythms studied (wrist temperature, motor activity, body position, TAP, sleep and light intensity) when compared with controls (Table 3). The ASD group showed significantly higher inter-daily stability in wrist temperature and light intensity and lower inter-daily stability in motor activity. Intraday variability results were consistent with those found for inter-daily stability. Lower amplitude was found in sleep records for the ASD group in contrast with light intensity, indicating poor sleep conditions in adults with ASD and ID. Residual TIB significantly correlated with central sleep hour, phase marker M5, ($r= -0.35$, $p=0.026$) indicating that the time the ASD participants rested awake in bed after sleep influenced their central sleep hour.

DISCUSSION

Similar to previous cross-sectional research from childhood to middle age [Souders et al., 2009; Baker & Richdale, 2015; Goldman et al., 2017], we found significantly poorer sleep quantity and quality in our ASD participants with ID compared to controls, together with daytime sedentary behavior, nocturnal activation and a consistent phase advance in circadian rhythms. The latter has been previously reported in children [Tatsumi et al., 2015] and in a

small number of ASD adults [Limoges et al., 2005; Hare et al., 2006; Baker & Richdale, 2017]. These are consistent with a higher vulnerability to sleep problems in the ASD population from early childhood through to middle age [Tani et al., 2004; Goldman et al., 2012]; neither adults with ASD and no ID, nor adults with ASD and ID have received much attention in the literature [Wiggs & Stores, 2004; Limoges et al., 2005; Hare et al., 2006; Hare et al., 2006; Matson et al., 2008; Baker & Richdale, 2017]. To our knowledge, our study is the largest study examining objective sleep parameters and circadian markers in ASD adults with ID.

Overall, the findings indicate that adults with ASD and ID have low sleep efficiency, prolonged latency, and increased frequency and length of night awakenings, compared with neurotypical adults. While they did not show less total sleep on average than controls, total night sleep was highly variable across the ASD group and only 5% of individuals on the spectrum had a total sleep time within the normal range for adults, which differed from controls where 72% were within the normal range. Our results support previous studies in individuals with ASD. Increased SoL and WASO, more frequent nocturnal awakenings, decreased total sleep and lower sleep efficiency are variously reported in cognitively able adolescents and adults on the autism spectrum [Tani et al., 2003; Limoges et al., 2005; Hare et al., 2006; Baker & Richdale, 2015; Baker & Richdale, 2017; Goldman et al., 2017]. Impaired sleep in adults with ASD and ID also is reported as related to severe challenging behaviors [Matson et al., 2008].

The longer SoL and TIB and shorter SE described for the adults on the spectrum could be a result of prescribed schedules in their group residences that do not allow flexibility that fits with individual preferences and circadian differences, however some authors did not find a link between different residential conditions and sleep problems in children with ASD and ID [Cohen et al., 2017]. Anxiety, high physiological arousal [Hundley et al., 2016] and problematic

daytime behavior [Matson et al., 2008] may influence SoL length. However, our results showed that those adults with ASD taking anxiolytic medication for co-morbid anxiety disorders only differed from other adults in their number of awakenings.

In our ASD group, the wrist temperature 24h rhythm showed an advanced wake maintenance zone (17:00-18:00 h) when compared with the control group (20:00-21:00 h); this phase advancement was supported by the phase markers M5 and L10. Misalignment of the wrist temperature rhythm could contribute to the sleep problems described in our adults [Shekleton et al., 2013]. Furthermore, adults on the spectrum in this study displayed an overall rhythm of increased wrist temperature, that could be related to reduced activation of noradrenergic vasoconstrictor tone resulting in a slower sleep onset [Blazquez et al., 2012] and melatonin secretion is strongly influenced by the temperature rhythm [Kräuchi et al., 1998].

Vulnerability to sleep problems can be associated with the functioning of the circadian system. When assessing the circadian system, the most common marker rhythms are core body temperature, motor activity, light intensity, cortisol and melatonin [Klerman et al., 1999; Van Someren, 2000]. Wrist (skin) temperature is also a consolidated, non-invasive, robust and reliable tool for assessing circadian system function [Blazquez et al., 2012; Bonmati-Carrion et al., 2014]. Circadian rhythms need to be continuously synchronized with the environment, and the most powerful zeitgeber (environment time cue that entrains circadian rhythmicity) is the 24-h light/dark cycle [Wright et al., 2013], but non-photic zeitgebers such as meal timing and social contacts can also be influential [Kalsbeek et al., 2012]. The wrist temperature rhythm reflects endogenous and exogenous circadian influences, thus presenting considerable advantages for evaluating the effects of synchronizing agents, such as light exposure, on circadian function. Peripheral temperature, as reflected by the wrist

temperature, affects sleep-regulating areas in the brain, and increases before sleep onset, remaining high overnight until waking and remaining low during the rest of the day [Benloucif et al., 2005; Sarabia et al., 2008]. The time of minimal sleep probability known as ‘‘wake maintenance zone’’, is usually between 20:00 and 22:00h in healthy adults, and matches with the lowest value of daily peripheral temperature [Ortiz-Tudela et al., 2010], and the dim light melatonin onset [Lewy et al., 1999]. Thus it can be used to assess advanced or delayed circadian system timing due to its relation with the intrinsic circadian period [Wright et al., 2005].

The motor activity 24 h wave and non-parametric circadian rhythm values from adults on the spectrum are higher during the night (21:00-01:00 h) and lower during the day (12:00 h). Those elevated night values could be due to certain sleep problems such as restless legs syndrome or epilepsy, but authors have described that to detect this, the actimeter should be worn on the leg [Cippà et al., 2013], and there was only one participant who suffered two epileptic seizures during ACM data collection and one of those was during the day. Thus the results together with the L5 value of motor activity suggest an earlier night-wake activation as previously described for cases of phase advancement [Limoges et al., 2005]. The higher IS and the VM10 values in ASD suggest a stability in physical activity tasks [Iosa et al., 2014] and sedentary lifestyle [MacDonald et al., 2011; Orsmond & Kuo, 2011].

Motor activity, together with wrist temperature and light intensity, provide a better evaluation of chronobiologic disorders than temperature alone [Klerman et al., 1999] and have been described as a substitute for PSG to test circadian rhythms status in humans [Carvalho Bos et al., 2003]. The body position added information to motor activity records. Lower body position values are described for our sample during the day. As described, body position together with motor activity help to define rest-activity spans properly [Blazquez et

al., 2012]. Phase advance in our sample with ASD is also evidenced with phase markers L5 and M10 and lower TAP values registered from midday until midnight. Our TAP rhythm in ASD matched that described for circadian rhythm alterations in mild cognitive impairment [Ortiz-Tudela et al., 2014]. The light intensity values recorded in the ASD sample were significantly higher in the morning and more robust during the week, according to the greater CFI value found. According to the literature wrist temperature is a marker of circadian phase, and light applied after night arousal has a phase-advancing effect [Pavlova, 2017] so higher morning values in ASD could be moving the rhythm forward.

The phase advance in adults with autism and ID detected (M5 and L5 at ~ 01:00 h AM) was consistent along all the circadian rhythms analyzed, similar to previous results [Limoges et al., 2005; Hare et al., 2006; Tatsumi et al., 2015; Baker & Richdale, 2017]. Normal timing of M5 and L5 [Ortiz-Tudela et al., 2010] takes place around 04:00 h, during REM sleep with minimum muscle tone [Madrid & de Lama, 2006] and melatonin reaches its nocturnal peak then [Claustrat et al., 2005]. However, as light intensity is a synchronizer of melatonin secretion [Morin & Allen, 2006], melatonin peaks in the adults with ASD from our sample could be happening before the usually described times [Arendt, 1994].

The criteria to diagnose a CRSWD, according to the International Classification of Sleep Disorders (3rd edition, 2014), require symptoms to be present for at least three months and confirmed with 14 days of actigraphy. We have only one week of ACM data and thus cannot confirm the presence of a CRSWD. However, the results presented in our paper provide enough information to evaluate the circadian system and its disruptions [Focan, 1995; Cornelissen et al., 1999; Lewy, 1999; Corbalán-Tutau et al., 2011], and strongly infer the presence of a CRSWD, primarily advanced sleep wake rhythm disorder, in adults with autism and ID.

The phase advance found in this study affected 60% of individuals with ASD which is higher than in other studies [Hare et al., 2006; Baker & Richdale, 2017] who reported this advanced sleep wake rhythm disorder in 10% of their ASD participants. This could be due to reasons other than light exposure. All participants with ASD in our study were unemployed, and unemployment has been related to the presence of a sleep disorder in high-functioning ASD [Baker & Richdale, 2017]. However most unemployed individuals in their study met criteria for delayed sleep wake rhythm disorder [Baker et al., in press]. Nevertheless, adults with ASD in these studies did not have an ID and were not institutionalized [Hare et al., 2006; Baker & Richdale, 2017], and thus presumably were able to self-select bed and wake times.

Our study presents several limitations. First, as a neurodevelopmental disorder, we acknowledge that the most life critical phase for symptom manifestation of sleep problems in ASD is in childhood [Humphreys et al., 2014], but they have been documented as life-long conditions [Matson et al., 2010; Hollway et al., 2013; Baker & Richdale, 2015]. Studying the course of sleep problem development across the lifespan is crucial for developing supports and assessing the efficacy of appropriate interventions [Bangerter et al., 2017]. Second, even though, the study information emphasized that no sleep problem was required to participate, it is possible that parents or legal guardians of adults with ASD and ID with disturbed sleep were more likely to participate. Third, ACM has not been validated in ASD to study sleep and this is the first study that has used this device to assess sleep in autistic individuals; however ACM has been shown to give rise to more accurate results than actigraphy [Ortiz-Tudela et al., 2014]. Furthermore, this device has shown its effectiveness for assessing sleep in individuals with Parkinson [Madrid-Navarro et al., 2018], mild cognitive impairment [Ortiz-Tudela et al., 2014], or critically ill confined participants [J Madrid-Navarro et al., 2015]. Furthermore, the algorithms used by actigraphy devices to predict sleep periods are

sometimes designed for specific populations, which could impair the proper detection of sleep in groups with different conditions [Sadeh & Acebo, 2002; Insana et al., 2010]; however, the TAP variable predicts rest-activity periods with high accuracy and independently from conditions that participants may have [Ortiz-Tudela et al., 2010]. Fourth, the comparison group was healthy, non-medicated adults, but most adults in the ASD group were medicated and polypharmacy was common. As it is well established that some medications can alter levels of neurotransmitters (e.g., serotonin) and change sleep architecture by decreasing arousals or redistributing the REM stage [Esbensen et al., 2009; Seda et al., 2014] such effects could bias the results in the ASD group. Future research in regard to these effects should consider the study of sleep EEG rhythms [Vakalopoulos, 2014]. However, even though the polypharmacy in our ASD group was defined as "medication not matching diagnosis" [Logan et al., 2015], only anxiolytic medication affected sleep significantly (i.e., number of awakenings). Nevertheless, the impact of polypharmacy is being broadly analyzed in an ongoing study. Although the sample could be considered relatively small, effect sizes were moderate/large and the number of participants was similar to other published studies that used actigraphy to assess insomnia symptoms and CRSWD in adults on the spectrum [Hare et al., 2006; Hare et al., 2006; Baker & Richdale, 2015; Goldman et al., 2017]; however, further studies with larger numbers of participants should be performed to validate our results. Additionally, the percentage of participants in the control group with normal number and duration of awakenings after sleep onset was low. However, our data are similar to other studies in healthy individuals [Chakar et al., 2017], and could be related to other factors such as occupational status [Kim & Lee, 2015] or raising a child [Reader et al., 2017]. Finally, the group with ASD was only matched on age with the control group, but not on sex, IQ, employment status or living conditions. However, when we examined sex, no differences were found for sleep. Nevertheless, as other authors have described [Knutsson, 2004; Tudor

et al., 2012], IQ, employment and living conditions (e.g., bedtime schedule, light exposure) may impact negatively on some of the sleep parameter values found in this study, but these conditions are the reality for adults on the spectrum with associated ID. We acknowledge that all these factors, including forced bedtime schedules or evening light exposure, could affect our results; however, we are unable to modify living conditions of ASD individuals in those institutions.

In conclusion, examining sleep and markers of the circadian system showed significant differences between adults with ASD and comorbid ID and an age-matched, healthy adult population. In the adults on the autism spectrum, there was a high prevalence of sleep problems and a predisposition to phase advanced circadian rhythms. The sleep disturbances described for this sample of adults with ASD and ID are similar to those already described for adults with ASD without intellectual disability, and in children and adolescents on the spectrum, evidencing that sleep problems are a life-long condition; their relationship with intellectual ability should be further studied. The ACM device used here provided important information about the status of the circadian system and could be used to achieve a better understanding of sleep in ASD, and to examine response to treatment of sleep difficulties.

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Table 1. Participants' demographic information.

	ASD n=41	Control n=51
Gender (n)		
Male	31	21
Female	10	30
Age (years old)	33 ± 6	33 ± 5
BMI (Kg/m^2)	24.4 ± 1	23.2 ± 0.6

ASD: Autism Spectrum Disorder; BMI: Body Max Index. Data is expressed as mean ± SD or as counts.

Table 2. Sleep parameters comparison obtained from ACM recordings between adults with ASD and ID and control participants.

Sleep parameters	ASD	Control	p value	**R or η^2**	95% CI
Total sleep time (min)	478±140	457±77	0.064	0.19	
Time in bed (min)	657±182	540±72	<0.001	2.74	
Sleep onset latency (min)	60± 48	14± 9	<0.001	4.85	
Number of awakenings (nº)	3 (2-5)	2 (1-3)	<0.001	0.1746	0.71-1.9
Wake after sleep onset (min)	106±74	50±35	<0.001	2.13	
Sleep efficiency (%)	70	85	<0.001	2.48	

% From normal population range	ASD	Control	p value	**X^2**
Total sleep time (420-540 min)	5	72	<0.001	40.01
Time in bed (420-569 min)	16	75	<0.001	31.53
Sleep onset latency (<30 min)	16	95	<0.001	59.92
Number of awakenings (0-1 nº)	3	33	<0.001	12.57
Wake after sleep onset (<20 min)	0	10	0.073	3.77
Sleep efficiency (≥85 %)	5	67	<0.001	34.44

Sleep parameters derived from ACM recordings in participants expressed as mean ± SD. Statistically significant differences were found by Mann Whitney or Chi Square test $p<0.05$ are highlighted in bold. For all parameters X^2 (df, n) equals to (1, 92).

Table 3. Analysis of non-parametric circadian rhythms along 24-hour period in adults with ASD and ID compared to typical developing controls.

Wrist temperature					Motor activity					Body position				
	ASD	Control	p value	r		ASD	Control	p value	r		ASD	Control	p value	r
IS	0.44±0.04	0.38±0.02	0.048	0.31	IS	0.29±0.02	0.25±0.02	0.002	0.69	IS	0.42±0.03	0.41±0.02	0.231	0.04
IV	0.14±0.01	0.21±0.01	0.001	0.79	IV	1.02±0.04	1.04±0.02	0.369	0.01	IV	0.42±0.06	0.42±0.02	0.091	0.14
RA	0.28±0.03	0.29±0.02	0.384	0.01	RA	0.72±0.03	0.71±0.02	0.252	0.04	RA	0.49±0.03	0.51±0.02	0.447	0.00
M5	1:07±0:40	4:32±0:38	<0.001	1.27	L5	2:37±0:24	3:54±0:11	0.001	0.76	L5	2:26±0:39	4:20±0:08	0.003	0.61
VM5	34.75±0.16	34.6±0.10	0.208	0.06	VL5	5.42±0.85	5.97±0.55	0.087	0.15	VL5	15.78±1.44	16.92±0.77	0.035	0.26
L10	14:35±0:22	18:04±0:31	<0.001	0.01	M10	15:58±0:41	15:55±0:30	0.495	0.00	M10	15:12±00:17	15:55±0:13	0.017	0.36
VL10	32.79±0.17	32.66±0.13	0.326	0.02	VM10	28.09±2.17	31.56±1.09	0.024	0.33	VM10	44.70±1.94	51.52±0.88	0.001	0.82
CFI	0.55±0.02	0.52±0.02	0.070	0.18	CFI	0.5±0.02	0.48±0.01	0.128	0.20	CFI	0.58±0.02	0.57±0.01	0.278	0.03
TAP					Sleep					Light intensity				
	ASD	Control	p value	r		ASD	Control	p value	r		ASD	Control	p value	r
IS	0.52±0.03	0.49±0.02	0.133	0.10	IS	0.59±0.03	0.59±0.02	0.352	0.01	IS	0.62±0.06	0.45±0.02	<0.001	0.95

IV	0.43±0.04	0.36±0.02	0.139	0.10	IV	0.36±0.03	0.28±0.02	0.005	0.54	IV	0.18±0.02	0.29±0.02	<0.001	1.64
RA	0.54±0.02	0.56±0.02	0.346	0.01	RA	0.82±0.03	0.89±0.02	0.028	0.31	RA	0.97±0.03	0.93±0.02	0.007	0.46
L5	2:54±0:19	4:13±0:09	<0.001	0.90	M5	00:59±0:55	3:49±0:16	<0.001	0.05	L5	2:23±0:13	4:04±0:18	<0.001	1.40
VL5	0.18±0.01	0.18±0.01	0.140	0.09	VM5	0.79±0.04	0.85±0.02	0.195	0.31	VL5	0.02±0.01	0.14±0.05	<0.001	1.55
M10	14:59±0:20	15:56±0:17	0.013	0.40	L10	13:40±0:23	14:56±0:21	0.361	0.12	M10	14:04±0:23	15:25±0:19	<0.001	1.57
VM10	0.56±0.02	0.63±0.01	<0.001	1.01	VL10	0.12±0.03	0.05±0.01	0.027	0.06	VM10	1.43±0.11	1.55±0.09	0.098	0.13
CFI	0.61±0.02	0.63±0.01	0.369	0.00	CFI	0.72±0.03	0.78±0.01	0.051	0.22	CFI	0.80±0.02	0.74±0.01	<0.001	1.3

IS stands for inter-daily stability; IV for intraday variability; RA for relative amplitude; phase markers: M5 and M10, L5 and L10, indicate consecutive 10- and 5-hour period of maximum and minimum values respectively; and CFI corresponds to the circadian function index. Non-parametric circadian rhythm analysis values expressed as mean ± SEM. Statistically significant differences were found by Mann Whitney test. p<0.05 are highlighted in bold. For all parameters r refers to size effect.

Figure 1. Flow chart of ASD sample selection.

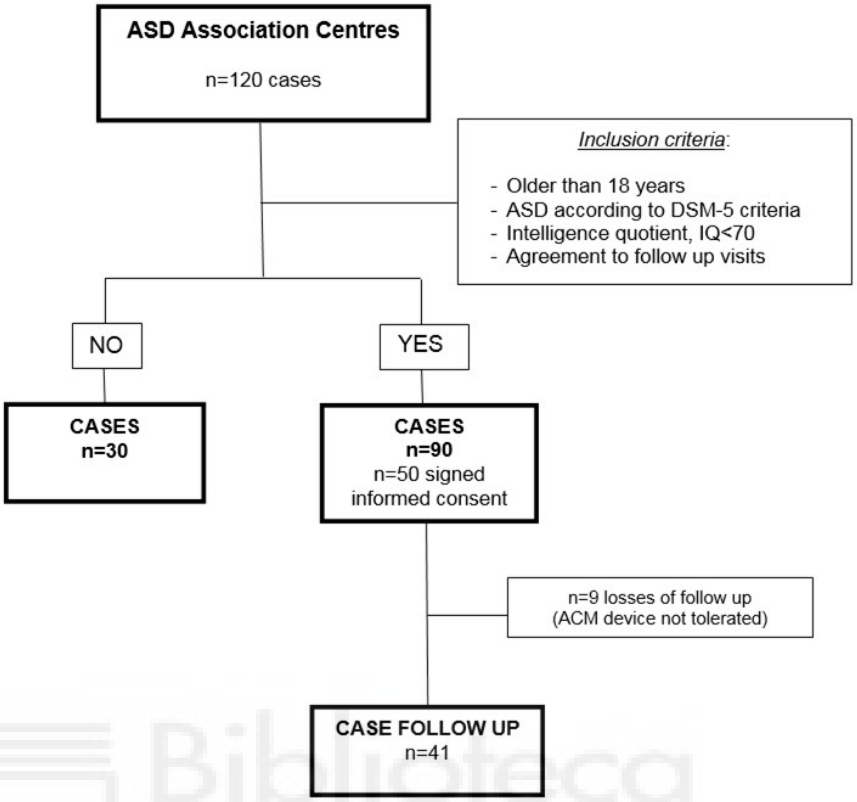


Figure 2. Categorization of medications prescribed to ASD participants with potential effects on sleep: neuroleptics (a), anticonvulsants (b), selective serotonin reuptake inhibitor (SSRI) antidepressants (c), and anxiolytics (d).

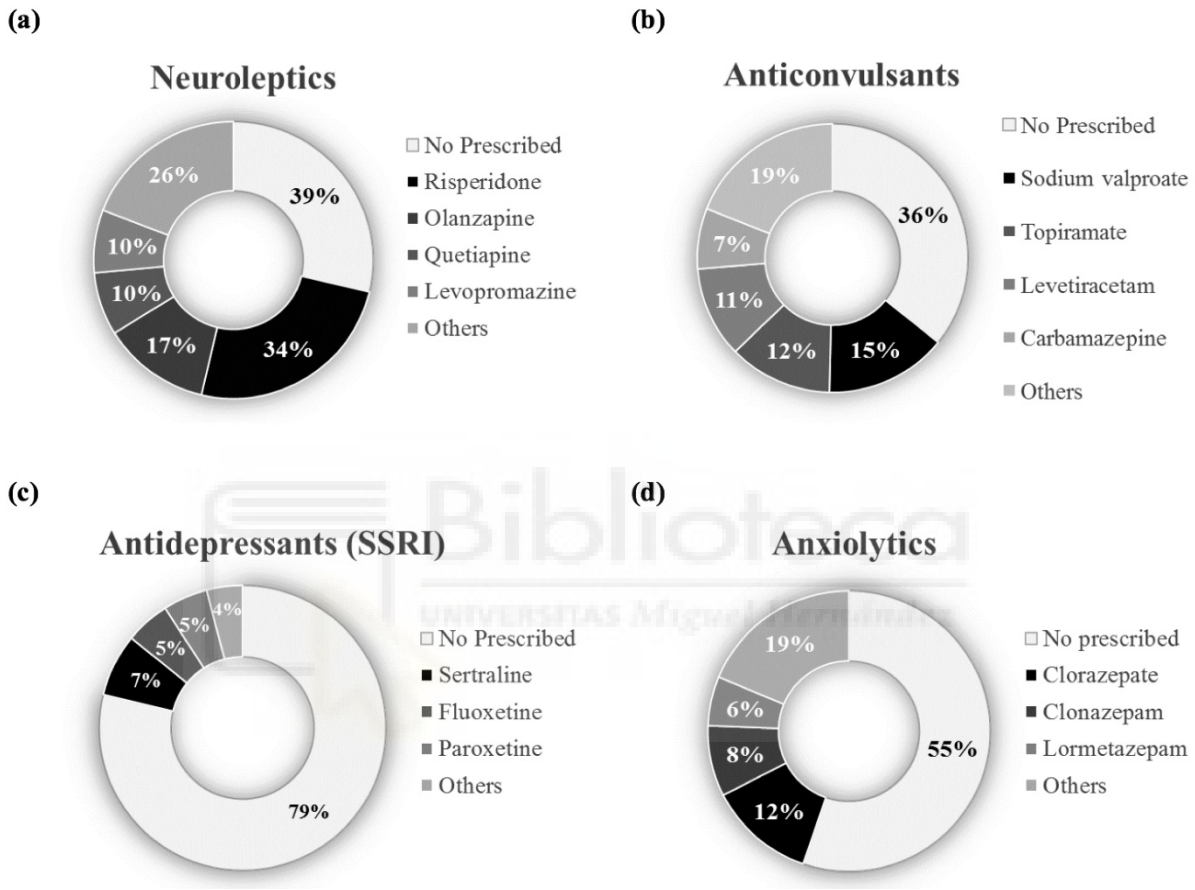
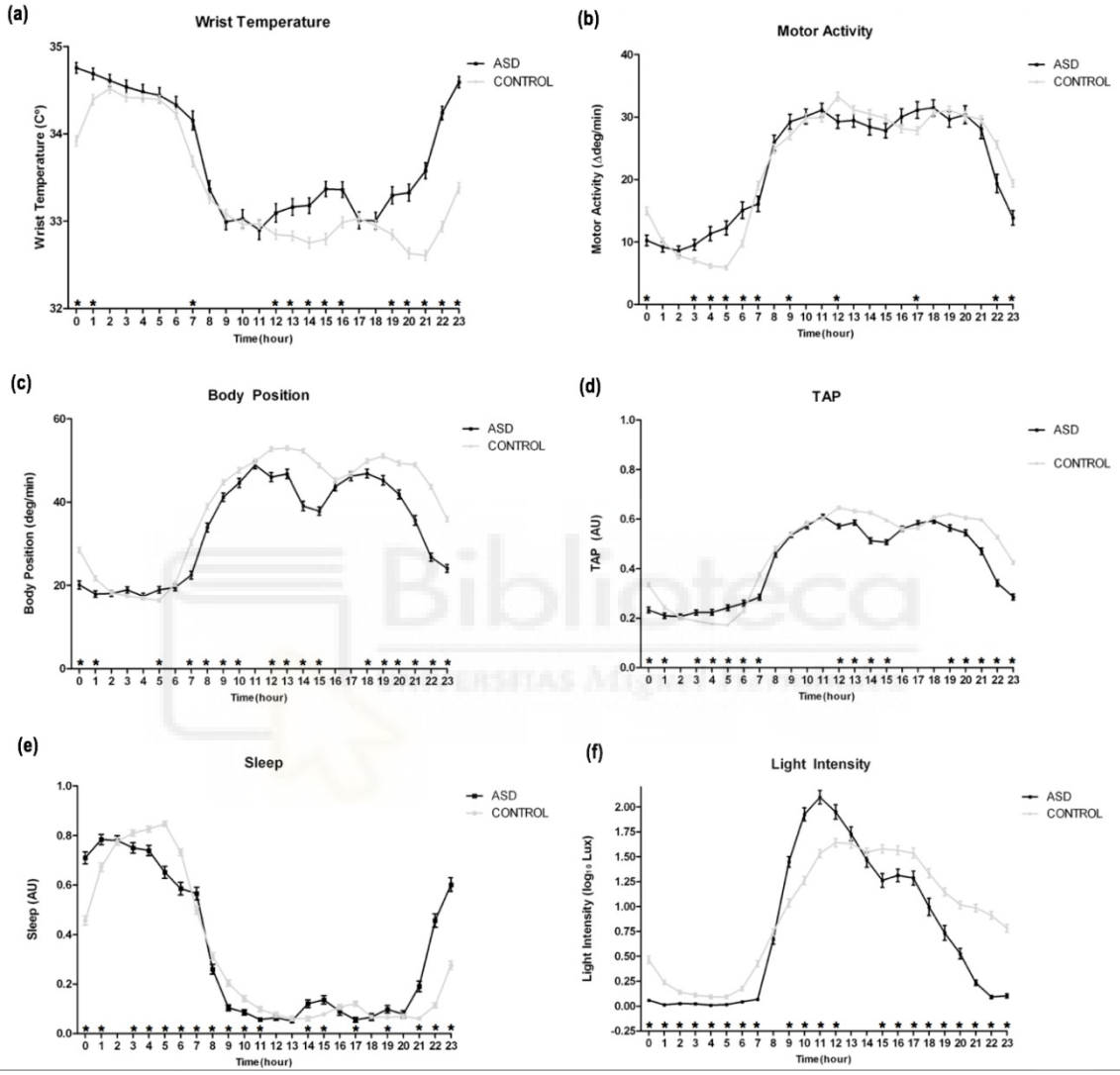


Figure 3. Circadian rhythms from wrist temperature (a), motor activity (b), body position (c), TAP (d), sleep (e), and light intensity (f) in ASD and control subjects.



Manuscript under review in Journal of Psychopharmacology

Evaluation of agomelatine for the treatment of sleep problems in adults with Autism Spectrum Disorder and co-morbid intellectual disability.

Agomelatine for sleep problems in autism

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CONFLICT OF INTERESTS

Authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Conception and design, or analysis and interpretation of data: P Ballester, MJ Martínez, MM Inda, A Javaloyes, C Belda, N Toral, D Morales, J. Muriel, E. Fernández, AM Peiro

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ABSTRACT

Background: Intellectual disability (ID) and Autism Spectrum Disorder (ASD) are common, co-occurring developmental disorders and are frequently associated with sleep problems. **Aim**: To assess the effectiveness and tolerability of agomelatine as a pharmacotherapy for sleep problems in ASD adults with ID. **Experimental Approach**: A randomized, crossover, triple-blind, placebo-controlled clinical trial, with two periods of 3-months of treatment starting either with agomelatine or placebo and washout period of 2-weeks. Ambulatory circadian monitoring (24 hours/7 days) evaluated total sleep time (TST) as the primary outcome variable. **Key Results:** Participants (n=23, 35 ± 12 years-old, 83% males) had a median of three (IQR: 1-4) comorbidities and five (IQR: 2-7) prescribed drugs. Before agomelatine or placebo treatment, all subjects presented with insomnia including longer sleep latency (55±23 min) or shorter TST (449±177 min) and 66% had circadian rhythm sleep-wake abnormalities with rhythm phase advancements according to the M5 sleep phase marker values. During 3-month agomelatine treatment, night TST significantly increased a mean of 83 min (532±121 min), together with a phase correction (M5 1:45±2:28h vs. 3:15±2:20h), improving sleep stability in wrist temperature rhythm (0.43±0.29 vs. 0.52±0.18 AU). Adverse events were mild and transient. **Conclusions and Implications**: Agomelatine was effective and well tolerated for treating insomnia and circadian rhythm sleep problems present in adults with ASD and ID. **Trial registry:** AGOTEA, EudraCT: 2011-003313-42.

Keywords: Autism spectrum disorder, ambulatory circadian monitoring, sleep problems, agomelatine, circadian rhythm.

ABBREVIATIONS LIST

ACM: Ambulatory circadian monitoring

ASD: Autism spectrum disorder

CFI: Circadian function index

CRSWD: Circadian rhythm sleep-wake disorder

ID: Intellectual disability

IQ: Intelligence quotient

IQR: Interquartile range

IS: Inter-daily stability

IV: Intraday variability

(V)L5: 5 hour or (value) with less activity

(V)L10: 10 hour or (value) with less activity

(V)M5: 5 hour or (value) with more activity

(V)M10: 10 hour or (value) with more activity

RA: Relative amplitude

REM: Rapid eyes movement

SE: Sleep efficiency

SoL: Sleep onset latency

TIB: Time in bed

TST: Total sleep time

WASO: Awake period duration after sleep onset duration

INTRODUCTION

Sleep problems are a commonly reported complaint for individuals with autism spectrum disorder (ASD) including those with comorbid intellectual disability (ID), with prevalence (50-80%) generally exceeding that reported for individuals in the general population (Richdale and Schreck, 2009). These sleep problems, primarily insomnia symptoms, influence daytime behaviour (Fadini et al., 2015) and may contribute to the development of depression and anxiety (Aronen et al., 2000). The basis of sleep problems in ASD is poorly understood and it has been suggested that they are linked to poorly modulated circadian rhythmicity, and may be closely related to an altered melatonin profile (Baker et al., 2017).

Circadian rhythms regulate behavioural and physiological processes (Geoffray et al., 2017) and are driven by a central clock mechanism located in the suprachiasmatic nucleus of the hypothalamus. The pineal gland produces melatonin, a hormone that regulates sleep-wake cycles (de Faria Poloni et al., 2011). Melatonin release from the pineal- follows a cyclical, 24-hour pattern with low levels during the day and elevated levels at night (Ellis et al., 1996). Melatonin circadian rhythm is a marker of the circadian phase of the sleep-wake rhythm, and the evening rise in melatonin (dim light melatonin onset) is one of the best markers of circadian rhythmicity (Saper et al., 2005). Lower urinary 6-sulphatoxymelatonin (Tordjman et al., 2005) and plasma levels of melatonin have been found in ASD individuals in some studies (Melke et al., 2008), together with normal levels in others (Goldman et al., 2014). Melatonin has been used to treat sleep problems in ASD patients (Zisapel, 2018), however, results reported from clinical trials vary considerably depending on the dose of melatonin used (Andersen et al., 2008; Jan

and O'Donnell, 1996; Wasdell et al., 2008), the diagnostic tools employed (Allik et al., 2008; Johnson and Malow, 2008; McArthur and Budden, 1998), the length of the study (Jan and O'Donnell, 1996; Paavonen et al., 2003) and the sleep parameters, which improve after treatment (Rossignol and Frye, 2011). Furthermore, most trials have been with children with ASD and poor sleep (Gringras et al., 2017).

Given the absence of clinical trials to test the effectiveness of pharmacological treatments for sleep problems in adults with ASD and associated ID, the aim of the current study was to analyse the effectiveness and tolerability of agomelatine, a selective agonist of melatonin MT1 and MT2 receptors and antagonist at 5HT2C receptors, in treating insomnia symptoms in these population. The conduct of an agomelatine randomized clinical trial (RCT) will generate valuable knowledge about additional pharmacological treatment options for sleep problems in individuals with ASD.

Based on the regular practice using melatonin to treat sleep problems in individuals on the autism spectrum and reports of its effectiveness in children and adolescents with ASD (Gringras et al., 2017), we hypothesized that the treatment of current sleep problems with an agonist of melatonin receptors would be effective at improving insomnia symptoms in adults with ASD. We also hypothesized it would be safe, as melatonin is a naturally occurring neurohormone produced by the pineal gland, and a recent clinical trial in children with ASD (age 2-17.5 years) showed no adverse effects up to 1-year later (Maras et al., 2018).

METHODS

Participants

Participants were recruited from four Spanish Autism associations, Figure 1a, after researchers met with their parents and carers, and from a clinic specializing in adults on the autism spectrum. Inclusion criteria were: (i) age from 18 to 65 years; (ii) psychiatric diagnosis of ASD using 5th Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013) criteria; (iii) a sleep problem with a chronic development (present more than 6 months), and still present for at least one month following the introduction of good sleep hygiene habits (reducing naps, increasing morning exercise, good morning light exposure) as described previously by Malow and colleagues (Malow et al., 2016b). The sleep problem was reported by carers via a sleep diary and had to meet the International Classification of Sleep Disorders 3rd Edition (ICSD; Sateia, 2014) for insomnia (includes difficulty falling asleep, difficulty staying asleep, or poor quality sleep, and impaired daytime functioning over the past 3 or more months), or a suspected Circadian Rhythm Sleep-Wake disorder (CRSWD; a misalignment between the endogenous circadian rhythm and exogenous factors such as light that affect sleep timing and duration) (Thorpy, 2017). Pre-trial sleep diary data were only used to determine participants' inclusion in the study and no other analyses were performed with these data.

Study design and Ethics

The screening, recruitment processes and study design are described in Figures 1 (a and b respectively). The study was designed as a crossover, triple-blind (investigators, participants and carers), randomized, placebo-controlled clinical trial with two periods of 3-months, starting either with agomelatine or placebo, with a washout of 2-weeks in

between each treatment period, Figure 1b. Following Alicante General Hospital Ethics Committee approval, all participants or their legal guardians received information about the design and purpose of the study, and participants' or legal guardians' informed consent was obtained. The study was performed in accordance with the principles of the Helsinki Declaration.

The clinical trial (EudraCT: 2011-003313-42) was conducted from February 2012 to March 2016, excluding summer (June-August) because Spanish summer temperatures could affect the accuracy of the temperature sensor in the Ambulatory Circadian Monitoring (ACM) device (see below).

Procedures

Participants began the study following pre-trial entry screening (age; ASD diagnosis; insomnia and/or CRSWD present for at least 6 months; failure to respond to 1-month sleep hygiene intervention). At this visit demographic information (age, sex, body weight and height) and current medications were obtained, and sleep diaries and response to the sleep hygiene intervention were reviewed. All adults presented with insomnia, with either longer SoL, abnormal TST and/or increased number of night awakenings. Individuals taking potent CYP1A2 inhibitors, behavioural or pharmacological treatment for poor sleep, and/or melatonin were not eligible to participate in the study; those with any medical condition (e.g., epilepsy) that was incompatible with the study requirements also were excluded. Even though ID (Intelligence quotient (IQ) scores <70) was not an inclusion criterion, all participants had an associated ID. Participants and their legal guardians' agreement to attend seven clinical visits was required, and participants could withdraw from the study at any time.

All participants who were included in the study were resident in one or other of three institutional centres. Their daily routines within their centre did not change during the study. In participants' residential institutions, wake-up (≈ 8:00h), bedtime (≈ 22:00h), workshops (≈ 10:00h and 17:00h) and mealtimes (≈ 9:00h, 14:00h, and 20:30h) are generally constant, regardless of the day of the week, so sleep could not be affected by changes to participants' schedules. Evening and trial medication were administered at evening mealtime, and there were no bedtime medications. Participants engaged in different morning or evening activities (e.g., walks, workshops) but these remained the same during the study period. All participants shared their bedrooms, but the roommate did not change across the study.

Participants were randomly assigned for the first period, to one of the two treatment sequences, agomelatine (25 mg/day) followed by placebo (A-B) or placebo followed by agomelatine (B-A), using a computer-generated random number sequence (Figure 1b). Both placebo and agomelatine were formulated as tablets and their appearance was indistinguishable the one from the other. Agomelatine or placebo was given daily, 1 hour before bedtime, over 12 weeks. A washout period of 2 weeks followed between periods one and two in the crossover design. Patients' sleep was assessed using ACM and sleep diaries at pre- (week 1) and post-treatment (week 12, the last week of treatment) for each treatment sequence; data for both pre- and post-treatment periods were available. All participants continued taking their regular medications during both periods of the clinical trial. Researchers were notified of any changes in a participant's ongoing medication during the trial period and these participants ceased to take part in the clinical trial. The participants and investigators in charge of data evaluation and analysis were blinded to the randomization, and did not know the patients' drug regimen.

Adverse events were recorded and assessed by a physician across the whole treatment period, and hepatic function was assessed in blood samples initially, and then every 3-weeks to test tolerability. Adherence was complete; it was recorded initially by carers from the residential facilities and then checked by the research team and the Hospital Pharmacy Department.

Ambulatory Circadian Monitoring (ACM)

The ACM apparatus (Kronowise), is a watch-like device, which monitors wrist temperature, motor activity and body position through two different sensors. Wrist temperature is measured with a Thermochron iButton DS1921H sensor, with an accuracy of ± 1 °C, with sampling every 10 min. Motor activity and body position are assessed using a second sensor, an actimeter (HOBO Pendant G Acceleration Data Logger UA- 004-64, three-channel logger [X-, Y-, Z- axis]) with 8-bit resolution, programmed to record data every minute (Ortiz-Tudela et al., 2010). This sensor estimates motor activity, according to the change in degrees of its position, and body position using the angle between the Z- and X-axis of the sensor (0º for maximum horizontality and 90º for maximum verticality, Martinez-Nicolas et al., 2011).

The single integrated variable TAP (where 1 indicates a high level of activation, and 0 is a sign of complete rest and sleep) uses the inversion of the wrist temperature values and the values for motor activity and body position. For computing TAP values, motor activity and body position data, respectively, were added and averaged in 10-minute intervals (matching wrist temperature sampling rate). The Circadianware software available in the Kronowizard platform (https://kronowizard.um.es/) infers the hours of

sleep from the TAP values, and converts the records into a binary code, with 1 corresponding to a resting period and 0 to an active period (Ortiz-Tudela et al., 2014).

In order to evaluate the rhythms described and estimate sleep, all participants wore the ACM on their non-dominant wrist for a week. Data from the ACM device were transferred to a personal computer through an adapter (DS1402D-DR8; IDC, Spain) or an optical USB Base Station (MAN-BASE-U-4, HOBO) using the software provided by the manufacturer. The ACM device was removed during showering or any other activity where it might get wet and data were filtered in order to eliminate erroneous measurements produced by its temporary removal. In addition, during the ACM carers completed a 7-day sleep-wake diary (mornings and evenings). Sleep diaries were used only as a backup for the ACM recordings in case of need; they were completed by carers and may not be completely accurate because during the night shift, residential facility staff numbers are reduced. Furthermore, sleep diaries did not include any subjective report of sleep improvements, as participants did not assist in their completion. Recordings from the ACM were used to calculate sleep parameters and circadian sleep-wake rhythm indexes for each participant across the four different phases of the clinical trial (Figure 1). A total of four ACM assessments (two for each treatment sequence) per each participant were performed. ACM assessments were performed one week prior to start of treatment (pre-treatment) and one week prior to end of treatment (post-treatment) for each treatment sequence (agomelatine or placebo), Figure 1b. The primary variables of interest calculated by the ACM device were wrist temperature and sleep variables.

Sleep parameters

Total sleep time (TST) was the primary outcome variable used to evaluate the effectiveness of agomelatine treatment. Treatment was considered effective if an increase of 30 min or more in TST was observed and TST remained in the normal range. TST was defined as the total number of minutes between nighttime sleep onset and sleep offset registered as sleep (normal range 420-540 min). In addition, the following sleep parameters were also analyzed: (1) time in bed (TIB, normal value 420-569 min), total minutes in bed at night until sleep offset; (2) sleep onset latency (SoL, normal value <30 min) the time until sleep onset at night; (3) number of awakenings (normal value 0-1 awakenings) during the TIB interval; and (4) wake after sleep onset (WASO, normal value <20 min) in minutes during the TIB interval; and (5) sleep efficiency (SE, normal value ≥85%) calculated as the ratio of TST/TIB multiplied by 100. Normal values for sleep parameters were obtained from previous studies (Watson et al., 2015; Carskadon and Dement, 2005). Sleep diaries were used only as a backup for ACM; wake up and bed times were calculated using the 24h wave of body position of each participant.

Circadian sleep-wake rhythm indexes

The rest-activity cycle is characterized by the information provided by non-parametric circadian rhythm analysis (Van Someren and Riemersma-Van Der Lek, 2007). One of the key indexes is the relative amplitude (RA), since it shows how activity is distributed throughout the day compared with the night: higher RA means better consolidation of daytime activity and night-time sleep. A second index is the inter-daily stability (IS), which quantifies how well the sleep–wake cycle is synchronized to supposedly stable environmental cues. Thirdly, intraday variability (IV) gives an indication of the fragmentation of the rhythm. Timing information comes from determining the onset of

the 5 hours with least activity (L5 onset) and onset of the 10 hours with most activity (M10 onset). Finally, the circadian function index (CFI) assesses circadian rhythmicity status and is calculated via the formula (IS+ (2-IV) +RA)/3 using the values estimated from the ACM software. Participants' data were explored for advanced, delayed or free running phase cycles.

Statistical Analyses

The Shapiro-Wilk test was used to assess normality in order to select parametric or non-parametric statistical analysis. Continuous variables are presented as mean ± standard deviation (SD) or median and inter-quartile range (IQR) according to normality tests. Categorical variables are expressed as percentages. T-test for paired samples test was used to assess group differences, period and factor were taken into account to eliminate their potent interaction with the treatment effect; effect sizes (Cohen's *d*) and 95% confidence intervals (CIs) are also reported. Frequencies were compared using the Chi-Square test, with Yate's continuity correction as appropriate and X^2 (df, n) are reported. The effect of treatments associated with patients' comorbidities and bedtime or wake-up time were analyzed with a two-way ANOVA and effect size (η^2) is provided. Carry-over effects and possible order effects of the treatment were analyzed using the Mann-Whitney-U test. All statistical analyses were performed with R 3.2.4 software and Graph Pad Prism 5.0. P-values of <0.05 were considered to indicate significance for all analyses.

RESULTS

Participants

Fifty individuals on the autism spectrum were pre-screened at their ASD Centres, 46 of whom met inclusion criteria, and informed consent was obtained for 25 of these individuals (Figure 1). Two participants (8%) were later excluded from the study, one due to an increase in hepatic enzymes and the other due to a change in his medication. Twenty-three participants (35 ± 12 years-old, 83% males, BMI 25 ± 1 Kg/m^2) thus completed the study and their data were available for analysis. Central nervous system comorbid conditions were reported in 87% of the cases (median of 3 comorbidities; IQR: 1-4); mood disorders (58%) were the most prevalent comorbidity, followed by aggressive behaviour (12%), and anxiety (5%). Participants were prescribed a median of five (IQR: 2-7) drugs (Figure 2) associated with their comorbid conditions (mood disorders, aggressive behaviour, anxiety and mood fluctuations); drugs for psychosis were most frequently prescribed (71%), followed by drugs for relapse prevention (63%) drugs for anxiety (38%) and drugs for depression (17%). Only 8% of participants were unmedicated.

Initially, 52% of the participants were randomized to agomelatine treatment and 48% to placebo. There was only one adverse event associated with agomelatine, which was a case of aminotransferase elevation. However, this person had basal levels of aminotransferase already near the upper limit of the normal range. This adverse event was notified to the Spanish Pharmacovigilance system. There were no adverse events associated with placebo.

Ambulatory Circadian Monitoring (ACM)

Sleep parameters

Comparison of sleep parameter values obtained by ACM for agomelatine and placebo after three months treatment and pre-treatment are presented in Table 1. All participants at pre-screening visit, presented with insomnia with either longer SoL, abnormal TST and/or increased number of awakenings according to care-givers' reports. Pre-treatment values were not significantly different across the two groups. Prior to agomelatine treatment, all the participants (100%) presented with the insomnia symptoms (abnormal SoL and number of awakenings); 50% also had abnormal TST. Prior to placebo treatment, 71% of the individuals presented abnormal SoL and number of awakenings and 60% had abnormal TST. At pre-treatment, all participants in both arms had either SoL, night waking and/or TST within the abnormal range.

Following agomelatine treatment, 91% of participants still had abnormal SoL and increased awakenings, but only 16% had abnormal TST, and 8% of participants had SoL, awakening and TST in the normal range. Following placebo treatment 83% of participants had abnormal SoL and number of awakenings, 67% had abnormal TST, and 8% had all three parameters in the normal range. Agomelatine treatment significantly increased night TST at post-treatment compared with pre-treatment (532 ± 121 [95%CI 455-609] min vs. 449 ± 177 [95%CI 337-561] min; p=0.016; d=0.55) which is an average increase of 83 minutes at night, with a shift from 50% of individuals with abnormal TST pre-agomelatine treatment to 16% post- treatment. No other significant effects of agomelatine on sleep parameters were found. Placebo treatment did not significantly alter any sleep parameters. Any differences between both pre-agomelatine and pre-placebo sleep parameters were assessed using t-tests for paired samples and no significant differences were found (all p>0.05) for any parameters reported in Table 1.

No carry-over effects and/or possible order effects of the treatment were detected (all p>0.05) (Supplementary tables 1 and 2).

Analysis of the effect of patients' medications (drugs for psychosis, relapse prevention, anxiety and depression) on their sleep parameters demonstrated that only drugs for depression influenced sleep, affecting SoL (p=0.027, η^2=1.51) and the number of awakenings (p=0.045, η^2=0.321), with those on drugs for depression (serotonin reuptake inhibitors) having significantly increased values, that is poorer sleep, for both sleep parameters. This influence was similar in both the agomelatine and placebo treatment phases and thus did not differentially affect these sleep parameters (Table 1).

No significant differences were found for bedtime (22:02±00:34h; 21:31±00:24h; 22:10±00:37h; p=0.094, η^2=0.16) and wake-up time (8:06±00:50h; 8:12±00:29h; 7:45±00:35h; p=0.334, η^2=0.08) across centres.

Circadian sleep-wake rhythm indexes

Comparison of the circadian sleep-wake rhythm indexes for the agomelatine and placebo treatments, and for pre-treatment, are presented in Table 2 and Supplementary Tables 3-5. During recruitment, only 30% of participants had a suspected CRSWD based on their sleep diaries. At pre-treatment, after ACM pre-trial determination (i.e., before randomization to intervention), 66% of the participants had a CRSWD reflected mainly their phase marker values for wrist temperature, TAP and sleep; no participant had a free running cycle. While, the phase movement that agomelatine generated in the five hours of central sleep (1:38±1:16 vs. 22:32±3:11 p=0.082 d=0.51 and 95% CI 00:47-2:30 vs.21:43-3:55) is not significantly different from the placebo condition, the effect size is moderate. No participant had a free running cycle.

Peripheral temperature phase advancement CRSWD values during the ACM pre-treatment week were: 58% with a CRSWD at pre-agomelatine, and 75% with a CRSWD at pre-placebo. CRSWD values during the ACM week following treatment were: post-agomelatine 23% with a CRSWD, and post-placebo 67% with a CRSWD. Treatment with agomelatine resulted in a significant improvement in rhythm stability (IS, 0.52 ± 0.18 vs. 0.43 ± 0.29; *p*=0.007; *d*=0.35 and 95% CI 0.41-0.63 vs. 0.25-0.62) and a significant phase delay in the wrist temperature rhythm (3:15 ± 2:20 h vs. 1:45 ± 2:28 h; *p*=0.037; *d*=0.62 and 95% CI 1:51-4:40 vs. 00:11-3:19), with significantly higher temperature values at night (35.09 ± 0.82ºC vs. 34.58 ± 1.31ºC; *p*=0.027; *d*=0.47 and 95% CI 34.6-35.6 vs. 33.70 vs. 35.46). Agomelatine also reduced the variability of the sleep-wake rhythm during the post-treatment week (0.67 ± 0.09 vs. 0.55 ± 0.27; *p*=0.037; *d*=0.60 and 95% CI 0.61-0.72 vs. 0.37-0.72) compared with the pre-treatment week and the period of major motor activity appeared significantly earlier in the day (14:45 ± 1:57 h vs. 18:16 ± 5:53 h; *p*=0.024; *d*=0.81 and 95% CI 13:26-16:04 vs. 14:32-21:00) compared to the pre-treatment condition (Supplementary table 3). The differences between both pre-agomelatine and pre-placebo were assessed with paired samples t-tests and no significant differences were found (all *p*>0.05) in of the parameters analyzed. No carry-over effects and/or possible order effects of the treatment on circadian parameters were detected (all *p*>0.05) (Supplementary tables 1 and 2).

Sleep temporal series analysis

Figure 3 summarizes the 24-hour registry of the sleep circadian rhythm analyzed for both agomelatine and placebo treatments. Only agomelatine resulted in significant differences, showing higher sleep rhythm amplitude values during the night time; at

1:00 am (0.87 ± 0.19 vs. 0.70 ± 0.27; *p*=0.011; *d*=0.73 and 95% CI 0.76-0.98 vs. 0.53-0.87) and 2:00 am (0.84 ± 0.23 vs. 0.65 ± 0.26 *p*=0.015; *d*=0.77 and 95% CI 0.70-0.97 vs. 0.49-0.82) amplitudes were significantly higher when compared with pre-treatment. Furthermore, sleep values with agomelatine were increased across the whole night compared to pre-treatment conditions, which reflects an increase in the amplitude of the sleep rhythm following agomelatine treatment, but not after placebo.

DISCUSSION

This study evaluated the effectiveness and safety of agomelatine, an agonist of melatonin MT1 and MT2 receptors and an antagonist of 5HT2C receptor, for treating sleep problems in adults with ASD and co-morbid ID. The results of the RCT indicated that for these individuals, agomelatine was both effective in increasing the TST and safe. As well as increasing TST, agomelatine, but not placebo, resulted in improved rhythm stability, a phase delay in the skin temperature rhythm, reduction in the variability of the sleep-wake rhythm, and an advance in the motor activity rhythm. Additionally, the amplitude of the sleep rhythm increased. These findings support that agomelatine treatment also results in improvements in the circadian sleep-wake rhythm. Results indicated a phase delay after treatment as the phase of the 5 hours with maximal temperature values (measured by ACM) moved backwards. In addition, agomelatine corrected the phase of the night peripheral temperature increment, and corrected the phase and major motor activity values in the morning, indicating an increase in the daily functionality of individuals following agomelatine treatment.

The increases in TST and circadian sleep-wake parameters found with agomelatine are consistent with previous outcomes published for melatonin in children with ASD

(Wasdell et al., 2008; Wirojanan et al., 2009b). Melatonin has been the main pharmacological treatment for sleep problems in autism, and has been shown as effective in reducing insomnia symptoms including reducing SoL (Cortesi et al., 2012; Gringras et al., 2017; Malow et al., 2012; Wright et al., 2011), increasing sleep efficiency (Cortesi et al., 2012), reducing number of awakenings (Garstang and Wallis, 2006), increasing TST (Gringras et al., 2017; Wirojanan et al., 2009a; Wright et al., 2011) and also improving CRSWD (Wasdell et al., 2008; Wirojanan et al., 2009a). Only one study reporting that melatonin was effective at treating insomnia included young adults (Ishizaki et al., 1999). Melatonin is reported as safe and effective on its own (Maras et al., 2018) or in combination with other psychotropic treatments (Andersen et al., 2008) in children with autism. Apart from melatonin, donezepil has been studied in autism during early childhood in an open label study using polysomnography, (Buckley et al., 2011), and an increase in TST and normalization of Rapid Eye Movement (REM) sleep values was shown. Additionally, the use of other pharmacological treatments (e.g., risperidone) for autistic symptoms has indirectly improved participants' sleep performance (Kent et al., 2013; Gvozdjáková et al., 2014).

Sleep changes shown here are also consistent with agomelatine studies in depression (Poluéktov and Levin, 2013; Pribytkov et al., 2013). The increase in TST resulting from agomelatine treatment, according to ACM recordings, has been described in adults with major depressive disorder using polysomnography (Poluéktov and Levin, 2013; Pribytkov et al., 2013). TST was also increased following agomelatine treatment in a larger study (Quera-Salva et al., 2011) that compared agomelatine against escitalopram using polysomnography. In the results from an open label study (Salva et al., 2007)

similar increase in TST, and also improved sleep efficiency and an increase in non-rapid eye movement (NREM) Stage 3 were found with the same dose as used in our study. Other studies have found significant differences in other sleep parameters (e.g., day time sleepiness, shorter sleep onset latency) using questionnaires (Urade et al., 2015), but as our adults with ASD and ID are nonverbal, questionnaires are not validated for our population. Studies in participants with mood disorders or anxiety that used subjective sleep measures also have reported an increase in TST following agomelatine treatment (Stein et al., 2008; Hale et al., 2010) and a reduction in insomnia symptoms has been observed upon agomelatine treatment (Lemoine et al., 2007). However, some authors have described no effects on sleep after agomelatine treatment (Englisch et al., 2016; Calandre et al., 2014).

The increased rhythm stability, a phase delay of the peripheral temperature rhythm and a higher average value during the night found following agomelatine treatment indicate an improved circadian rhythm status, specifically a phase advancement, indicating an improvement of CRSWD (Laux and Group, 2012); peripheral temperature is a marker of circadian activity (Sarabia et al., 2008). It is well known that when targeting CRSWD with melatonin agonists the timing of administration as well as the pharmacokinetic parameters are important. To produce a phase advancement in individuals with a phase delay administration must occur several hours prior bedtime. On the other hand, in individuals with phase advancement some guidelines have recommended a low dose of melatonin (Auger et al., 2015), while several authors point to the need for further research into the effectiveness of melatonin or its agonists in treating CRSWD (Auger et al., 2015; Williams III et al., 2016; Mundey et al., 2005). An open label study described a

phase advancement of two hours in the temperature rhythm in older men using a larger dose of agomelatine than used here (Leproult et al., 2005). This is still consistent with our findings and may be due to the dual effect of agomelatine upon melatonin and serotonin receptors. Moreover, another study showed phase advance of the mid-range temperature decline with both 5 mg and 100 mg of agomelatine (Krauchi et al., 1997). Higher values of sleep at night in the amplitude of the wave of the 24 h rhythm, together with the greater stability of the sleep circadian rhythm in the agomelatine condition is consistent with what has been described by other authors using a sleep screening questionnaire Circscreen (Pjrek et al., 2007) to assess CRSWD.

Strengths and Limitations

In autism, most pharmacological interventions for sleep problems have focused on children and adolescents (from 2 to 18 years old) and use melatonin (Cortesi et al., 2012; Gringras et al., 2017; Malow et al., 2012). There is a lack of treatment studies for poor sleep in adults with ASD, both with and without comorbid ID. The current study is the first clinical trial in adults with ASD and ID that uses a robust design (a randomized, placebo-controlled, crossover clinical trial) to examine a new pharmacological treatment, agomelatine, for their presenting sleep problems. The study objectively examined both the effectiveness and safety of this treatment for sleep problems using ACM. Given the high variability in sleep reported among adults with ASD, this design also represents a strength compared to parallel designs because every participant acts as their own control. There are also few studies evaluating sleep problems in adults with ASD and co-morbid ID (Matson et al., 2008; Øyane and Bjorvatn, 2005; Hare et al., 2006b) and the ACM used here not only provides an objective measurement of sleep for

this population, but also allows associated circadian parameters to be evaluated. Accordingly, study findings would not necessarily be generalizable to autistic adults with no intellectual disability or those who are not in institutional care.

Despite our sample already being medicated with a median of five other drugs, the tolerability of agomelatine in our study was good, with only one withdrawal (4%) and one adverse event (4%), and the effect of drugs for depression (all serotonin reuptake inhibitors) on participants' sleep parameters was the same for both placebo and agomelatine treatment. This is in line with the safety and adverse events rates described in previous studies with agomelatine prescribed alone (Stahl, 2010), or in combination with other drugs including results described for agomelatine taken in combination with at least four other drugs (Soldatkin, 2013).

This study also had some limitations; although our sample size and agomelatine dose is in line with open agomelatine studies published in bipolar depression (Calabrese et al., 2007) and mild-major depressive episode (Pjrek et al., 2007), or sleep studies published in adults with autism and ID (Hare et al., 2006a), the number of participants is still low. Also, confirmation of ID was obtained from clinical registries, thus IQ scores were not available to allow examination of any IQ effects on outcomes. It would be interesting to perform these analyses in future research as there is no clear conclusion about the relation of sleep difficulties and IQ in adults on the autistic spectrum (van de Wouw et al., 2012). While the same number of participants (2) had normal SoL, night waking and TST following placebo and agomelatine treatment, this represented only 8% and agomelatine treatment resulted in a significant increase in total sleep duration. Adults

with ASD are at increased risk for cardiovascular conditions and diabetes (Croen et al., 2015; Jones et al., 2016), which are also known side effects associated with anti-psychotic medications these adults are frequently prescribed (Scahill et al., 2016; Scigliano and Ronchetti, 2013). Thus, as insufficient sleep duration is also associated with increased, cardiovascular risk and diabetes (McHill and Wright, 2017; Vgontzas et al., 2013), the increase in total sleep duration found with agomelatine is highly relevant to autistic adults. While we acknowledge that sleep efficiency is a more useful measure of poor sleep quality, when TIB is standardized, but still each subject remained under the same living conditions and schedules as they all lived in a residential facility. Also, in this study, TIB is lengthy, and that could be influencing participants' sleep. However, we were not able to change residential wake-up time or bedtimes and there were not significant differences across residential institutions.

We do not know if our results can be extended to adults with ASD and no ID, or to children with ASD and insomnia symptoms, or whether agomelatine may also improve the daytime sleepiness, shorter sleep onset latency, or increased night waking also found in individuals with ASD. While our participants had problems with sleep initiation, with an advanced sleep rhythm this is primarily due to the fixed schedules in their residential facilities, where they may be put in bed too early, and their fixed daily routines. While concomitant pharmacological treatments could affect the results, with only 8% of the participants free of other medications, all concomitant treatments remained constant across the study in both the treatment and placebo conditions; furthermore, all prescriptions were stable, with a prescription period greater than 6 months in all cases.

Conclusions

Sleep problems in autism persist into adulthood, and include insomnia and CRSWD (Baker and Richdale, 2015; 2017; Hare et al., 2006a; Matson et al., 2008), and when both poor night-time sleep and ID coexist in individuals with ASD, increased problematic daytime behaviours appear (Cohen et al., 2018). As happens in the general population (Crowley, 2011), sleep problems and problematic behaviour in individuals with autism lead to both families and residential services seeking treatment, including pharmacological treatment (Newcomb and Hagopian, 2018). Improving sleep patterns in individuals on the autism spectrum can have a beneficial impact both on the individuals and those who care for them (Devnani and Hegde, 2015).

This study is the first to evaluate, using objective sleep measurements, the effectiveness of a pharmacological sleep treatment for adults on the autism spectrum with ID. Our results indicate that agomelatine is effective in increasing night time TST, and also improving the CRSWD, as demonstrated through improvements in peripheral temperature, motor activity and sleep circadian rhythm amplitude. Furthermore, given that in individuals with autism, other than melatonin, most treatments are prescribed with no, or limited demonstrated effectiveness for treating poor sleep, agomelatine should be studied further and may be recommended for sleep problems in adults on the autism spectrum. The low number of adverse events, despite the high rate of polypharmacy, demonstrates a good, short-term safety profile for agomelatine. Further research is needed in order to assess the appropriateness of this and other treatments to improve their sleep quality in this vulnerable population.

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Table 1. Comparison of sleep parameters between pre-treatment vs. agomelatine or placebo obtained from ACM recordings.

Sleep parameters	Agomelatine (n=23)					Placebo (n=23)				
	Pre-treatment	**Post-treatment**	**95% CI**	**p-value**	**Cohen's *d***	**Pre-treatment**	**Post-treatment**	**95% CI**	**p-value**	**Cohen's *d***
Total Sleep Time (min)	449±177	532±121	**455-609**	**0.016**	0.55	542±106	574±67	531-616	0.148	0.36
Time in bed (min)	704±152	711±116	637-785	0.270	0.06	687±72	733±76	684-781	0.234	0.62
Sleep Onset Latency (min)	55±23	54±22	40-67	0.399	0.04	51±24	53±23	38-67	0.264	0.08
Number of awakenings	3.7±1.9	4.3±2.3	3-6	0.336	0.28	3.1±1.7	3.6±1.6	3-5	0.207	0.30
Wake After Sleep Onset (min)	134±112	116±63	76-156	0.278	0.20	91±35	90±64	49-131	0.469	0.02
Sleep Efficiency (%)	64	75	68-81	0.062	0.18	78	79	73-84	0.399	0.56

Table 2. Circadian sleep-wake rhythm indexes comparison for wrist temperature and sleep circadian rhythm in agomelatine and placebo treatments.

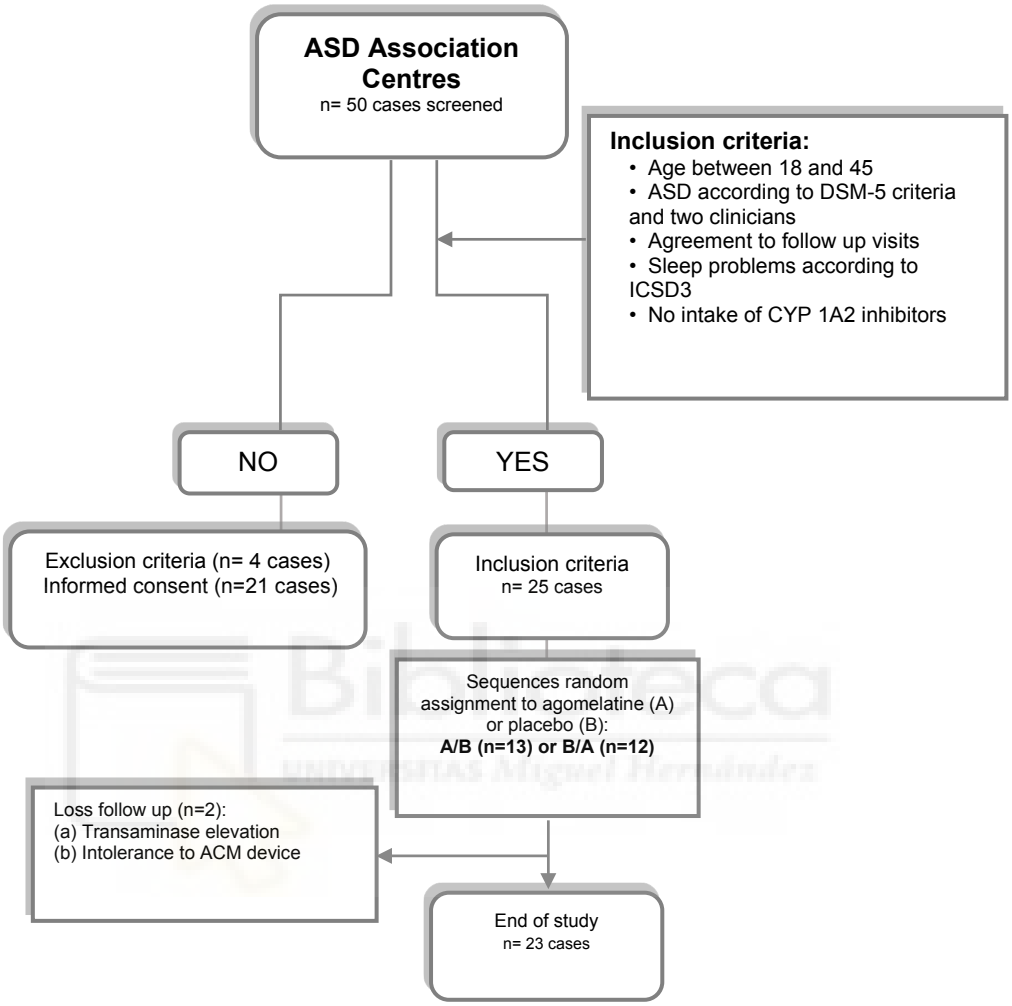
	Wrist Temperature								Sleep							
	Agomelatine				Placebo				Agomelatine				Placebo			
	Pre-treatment	Post-treatment	p-value	Cohen's d	Pre-treatment	Post-treatment	p-value	Cohen's d	Pre-treatment	Post-treatment	p-value	Cohen's d	Pre-treatment	Post-treatment	p-value	Cohen's d
IS	0.43±0.29	0.52±0.18	0.007	0.37	0.43±0.23	0.38±0.20	0.319	0.23	0.55±0.27	0.67±0.09	0.037	0.6	0.63±0.19	0.68±0.16	0.385	0.28
IV	0.14±0.07	0.15±0.05	0.313	0.16	0.11±0.06	0.12±0.10	0.396	0.12	0.38±0.17	0.40±0.13	0.180	0.13	0.30±0.13	0.34±0.12	0.053	0.32
RA	0.28±0.17	0.30±0.09	0.116	0.15	0.30±0.15	0.31±0.18	0.369	0.06	0.79±0.24	0.87±0.12	0.371	0.42	0.82±0.27	0.84±0.13	0.311	0.09
M5	1:45±2:28	3:15±2:20	0.037	0.62	00:45±3:18	2:24±2:09	0.111	0.59	00:32±3:11	1:38±1:16	0.082	0.51	2:11±1:26	2:18±1:46	0.134	0.07

VM5	34.58±1.	35.09±0.	0.02	0.47	34.62	34.59±0.	0.50	0.03	0.72±0.2	0.84±0.1	0.40	0.53	0.91±0.0	0.90±0.0	0.27	0.13
	31	82	7		±0.82	95	0		9	4	6		6	9	8	
L10	14:12±1:	14:00±2:	0.50	0.09	13:57±2:	13:42±3:	0.29	0.08	13:34±1:	14:09±1:	0.31	0.37	14:16±1:	14:36±2:	0.32	0.19
	11	41	0		09	47	6		32	38	2		10	08	3	
VL1	32.74±0.	33.07±0.	0.24	0.38	32.64±0.	32.49±0.	0.28	0.18	0.11±0.1	0.06±0.0	0.50	0.39	0.11±0.2	0.08±0.0	0.31	0.2
0	76	96	8		67	94	5		7	6	0		0	7	3	
CFI	0.50±0.1	0.50±0.1	0.36	0	0.55±0.1	0.51±0.1	0.24	0.27	0.72±0.1	0.78±0.0	0.11	0.76	0.77±0.1	0.79±0.0	0.46	0.17
	5	2	7		2	7	8		0	5	6		4	8	1	

IS inter-daily stability; IV intraday variability; RA relative amplitude; M5 and M10, L5 and L10 phase markers; CFI circadian function index.

Figure 1. (a) ASD recruitment and selection criteria flow chart and (b) study design. ACM: Ambulatory Circadian Monitoring, A agomelatine, B placebo.

a)



b)

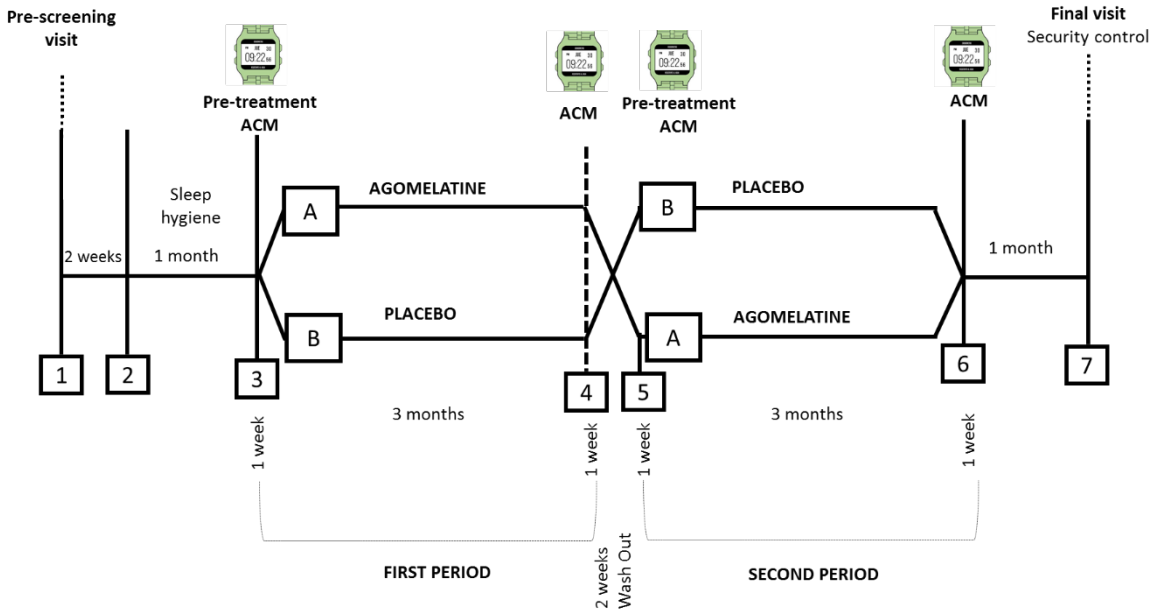


Figure 2. Consumption of medications with potential effects on sleep by the ASD participants during the study, drugs for psychosis (a), drugs for relapse prevention (b), drugs for depression (c) and drugs for anxiety (d). Results are expressed in %.

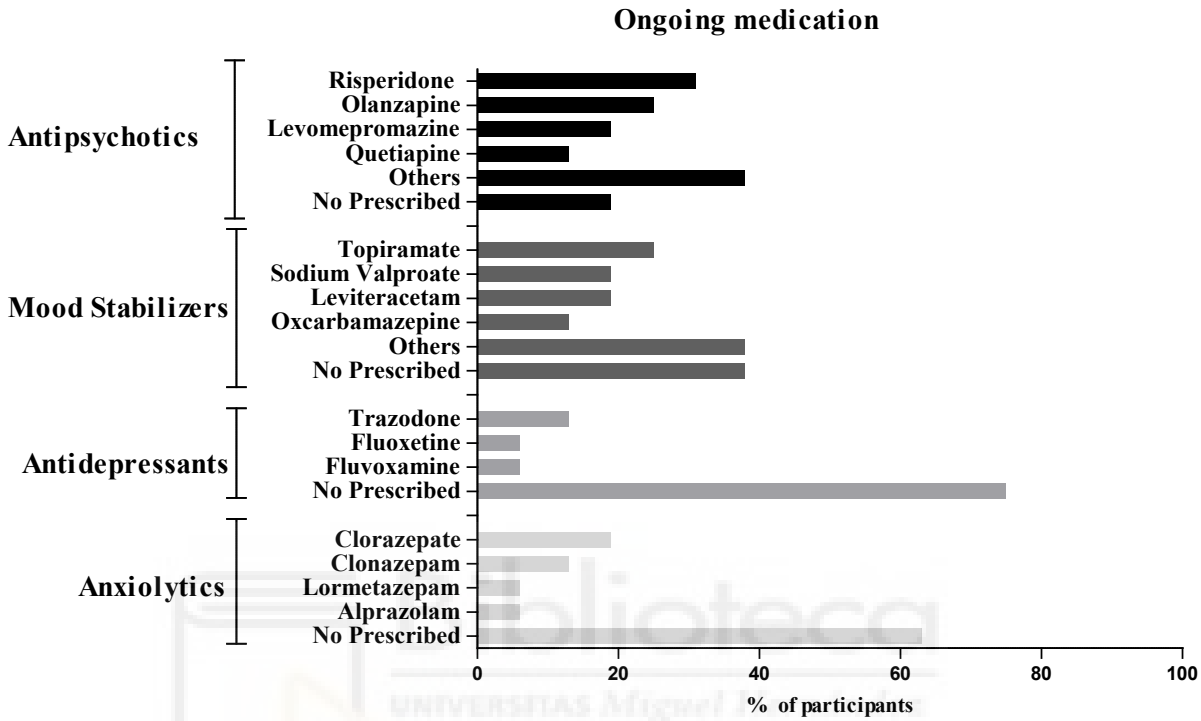
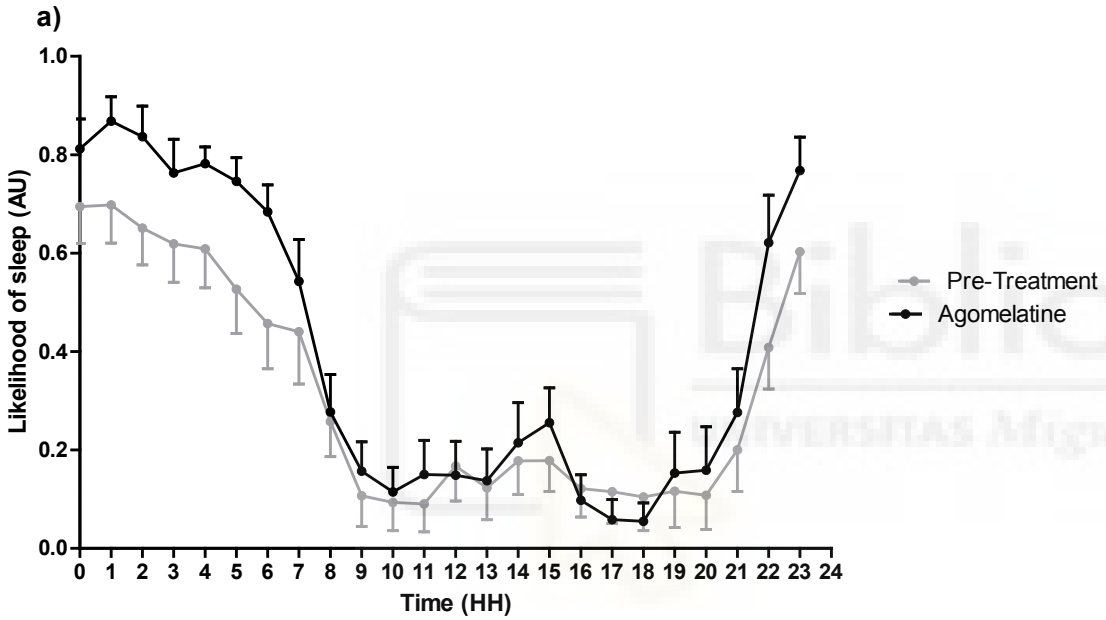
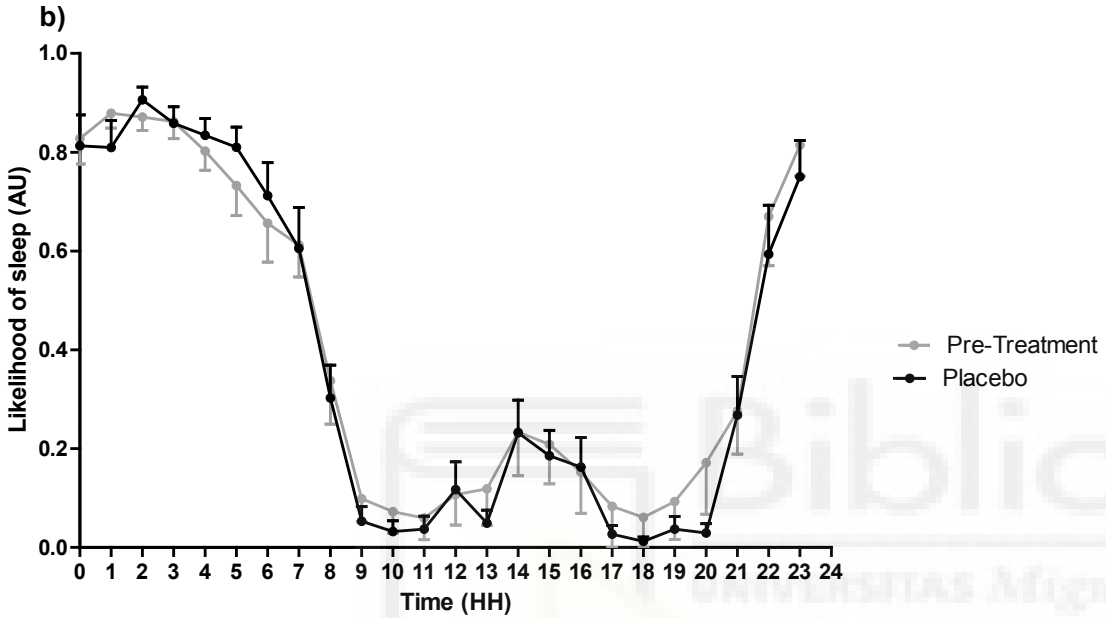


Figure 3. Sleep circadian rhythms for agomelatine (a) or placebo (b) period treatment in adults with ASD and ID. Sleep calculated by ACM does not have standardized measure that is why is expressed as AU: arbitrary units.





Supplementary

Supplementary Table 1. Comparison of sleep parameters in the pre-treatment attending to order sequence A/B vs. B/A for agomelatine or placebo obtained from ACM recordings.

Sleep parameters	Agomelatine – Pre-treatment (n=23)				Placebo – Pre-treatment (n=23)			
	A/B	B/A	p-value	Cohen's *d*	A/B	B/A	p-value	Cohen's *d*
Total Sleep Time (min)	459±202	420±84	0.473	0.25	551±76	535±136	0.628	0.15
Time in bed (min)	734±164	616±64	0.955	0.94	704±54	675±90	0.475	0.39
Sleep Onset Latency (min)	54±22	60±30	0.853	0.23	43±14	57±30	0.629	0.60
Number of awakenings	4±2	3±1	0.183	0.63	4±1	3±2	0.368	0.63
Wake After Sleep Onset (min)	148±129	94±13	0.824	0.59	91±8	90±50	1	0.03
Sleep Efficiency (%)	62	68	0.128	0.32	78	79	1	0.13

A: Agomelatine; B: Placebo. Data is expressed as mean ± standard deviation or as percentage. P values correspond to the comparison among individuals in sequence A/B vs B/A.

Supplementary Table 2. Comparison of sleep parameters in the post-treatment attending to order sequence A/B vs. B/A for agomelatine or placebo obtained from ACM recordings.

Sleep parameters	Agomelatine – Post-treatment (n=23)				Placebo – Post-treatment (n=23)			
	A/B	B/A	p-value	Cohen's *d*	A/B	B/A	p-value	Cohen's *d*
Total Sleep Time (min)	512±135	561±106	1	0.4	611±60	521±61	0.087	1.49
Time in bed (min)	696±140	732±84	0.639	0.31	761±66	694±79	0.639	0.92
Sleep Onset Latency (min)	61±17	43±25	0.104	0.84	51±22	55±27	0.806	0.16
Number of awakenings	4±2	5±3	0.804	0.39	4±1	3±2	0.452	0.63
Wake After Sleep Onset (min)	106±56	130±75	0.569	0.36	84±44	98±91	0.935	0.2
Sleep Efficiency (%)	73	77	0.626	0.35	80	76	0.512	0.41

A: Agomelatine; B: Placebo. Data is expressed as mean ± standard deviation or as percentage. P values correspond to the comparison among individuals in sequence A/B vs B/A.

Supplementary Table 3. Circadian sleep-wake rhythm indexes comparison for the motor activity rhythm in agomelatine and placebo treatments

	Agomelatine				Placebo			
	Pre-treatment	Post-treatment	p-value	Cohen's *d*	Pre-treatment	Post-treatment	p-value	Cohen's *d*
IS	0.27±0.11	0.33±0.11	0.188	0.54	0.34±0.11	0.36±0.23	0.285	0.11
IV	1.05±0.22	1.09±0.12	0.500	0.23	0.94±0.34	0.98±0.11	0.285	0.16
RA	0.71±0.17	0.67±0.20	0.461	0.22	0.79±0.15	0.81±0.13	0.284	0.14
M10H	18:16±5:53	14:45±1:57	0.024	0.80	14:43±1:36	15:26±2:08	0.180	0.38
VM10	27.54±16.43	31.44±11.28	0.216	0.28	29.21±14.98	26.78±11.11	0.285	0.18
L5H	2:21±1:50	1:19±2:01	0.161	0.54	2:40±1:50	2:10±1:48	0.065	0.28
VL5	6.23±7.14	6.41±4.91	0.461	0.03	3.86±3.14	2.41±1.42	**0.037**	0.60
CFI	0.48±0.07	0.49±0.09	0.188	0.12	0.55±0.13	0.56±0.11	0.500	0.08

IS inter-daily stability; IV intraday variability; RA relative amplitude; M5 and M10, L5 and L10 phase markers; CFI circadian function index.

Supplementary Table 4. Circadian sleep-wake rhythm indexes comparison for the body position rhythm in agomelatine and placebo.

	Agomelatine				Placebo			
	Pre-treatment	Post-treatment	p-value	Cohen's *d*	Pre-treatment	Post-treatment	p-value	Cohen's *d*
IS	0.36±0.17	0.48±0.15	0.213	0.75	0.42±0.20	0.57±0.21	0.039	0.73
IV	0.38±0.24	0.44±0.14	0.285	0.31	0.63±0.56	0.34±0.12	0.008	0.72
RA	0.42±0.18	0.44±0.17	0.252	0.11	0.52±0.08	0.58±0.13	0.078	0.55
M10H	15:21±2:30	14:50±1:08	0.422	0.27	15:01±1:01	15:37±1:32	0.337	0.46
VM10	42.43±14.36	41.58±7.71	0.064	0.07	46.29±6.39	44.97±6.45	0.422	0.21
L5H	23:26±4:38	1:57±2:13	0.074	0.69	2:54±2:18	2:21±2:31	0.200	0.23
VL5	19.02±13.00	16.54±6.79	0.180	0.24	15.05±4.39	12.17±4.74	0.156	0.63
CFI	0.53±0.11	0.57±0.11	0.180	0.36	0.59±0.08	0.66±0.09	0.031	0.822

IS inter-daily stability; IV intraday variability; RA relative amplitude; M5 and M10, L5 and L10 phase markers; CFI circadian function index.

Supplementary Table 5. Circadian sleep-wake rhythm indexes comparison for TAP rhythm in agomelatine and placebo treatments.

	Agomelatine				Placebo			
	Pre-treatment	Post-treatment	p-value	Cohen's *d*	Pre-treatment	Post-treatment	p-value	Cohen's *d*
IS	0.49±0.24	0.55±0.13	0.406	0.32	0.55±0.1010	0.62±0.20	0.371	0.44
IV	0.48±0.32	0.47±0.11	0.289	0.04	0.52±0.26	0.39±0.19	0.231	0.57
RA	0.47±0.17	0.52±0.13	0.156	0.33	0.59±0.06	0.65±0.11	0.05	0.67
M10H	15:46±2:17	14:51±1:26	0.242	0.31	15:11±1:14	15:06±2:03	0.10	0.05
VM10	0.60±0.18	0.56±0.06	0.125	0.30	0.56±0.09	0.56±0.09	0.473	0
L5H	23:36±7:39	1:33±1:45	0.469	0.35	2:25±1:12	2:10±1:57	0.472	0.15
VL5	0.21±0.13	0.18±0.07	**0.039**	0.29	0.15±0.04	0.12±0.05	0.074	0.66
CFI	0.57±0.14	0.61±0.09	0.074	0.34	0.63±0.08	0.69±0.10	0.273	0.67

IS inter-daily stability; IV intraday variability; RA relative amplitude; M5 and M10, L5 and L10 phase markers; CFI circadian function index.

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Title: Interplay of circadian and melatonin gene variants in adults with autism, intellectual disability and sleep problems

Short Running Title: Clock and melatonin genes in autistic adults

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CONFLICT OF INTEREST

The authors of this manuscript declare no conflict of interest.

ABSTRACT

People with Autism Spectrum Disorder and intellectual disability usually experience sleep problems, where circadian clock and melatonin pathway genes may play a role. To analyze the influence of circadian clock and melatonin pathway genetic variants in sleep-wake rhythms. Cases (n=83) and controls (n=25) were genotyped for PER1, ASMT, NPAS2, and MTNR1A by Sequenom MassARRAY. Sleep-wake rhythms were evaluated with ambulatory circadian monitoring. In cases (age 18-41-year-old), PER1 rs6416892-GG and ASMT rs5989681-GG genotypes, had a better sleep pattern according to sleep onset latency and awakenings, and worse sleep and/or temperature rhythms. Furthermore, NPAS2 rs1811399-CC affected temperature diurnal values. Sleep-wake rhythms and problems present could be related with circadian clock (PER1) and melatonin pathway (ASMT) gene variants.

Keywords: Autism spectrum disorder, sleep problems, circadian clock genes, melatonin pathway genes, ambulatory circadian monitoring.

One of the most common clinical symptoms in Autism Spectrum disorder (ASD) and ASD with intellectual disability (ID) is sleep problems, which have a significantly higher prevalence than that reported in the general population (Baker & Richdale, 2015; Ballester et al., 2018; Cohen, Conduit, Lockley, Rajaratnam, & Cornish, 2014; Richdale & Schreck, 2009). The most frequently reported sleep problems are insomnia symptoms (prolonged sleep latency, decreased sleep efficiency, reduced total sleep time, increased wake after sleep onset, bedtime resistance, and daytime sleepiness) and circadian rhythm sleep-wake disorders (CRSWD), primarily advanced or delayed sleep phase disorders (Baker & Richdale, 2017; Ballester et al., 2018; Kotagal & Broomall, 2012; Richdale & Schreck, 2009).

This high prevalence of poor sleep can be explained partially by the abnormalities in melatonin synthesis described in ASD including increases in its precursors (serotonin and N-acetylserotonin) (Gabriele, Sacco, & Persico, 2014) and decreased melatonin levels (Melke et al., 2008a; Tordjman et al., 2012). These alterations could influence insomnia symptoms by affecting the transition between sleep and wakefulness, as well as sleep maintenance, stages, structure and circadian timing (Baker, Richdale, Hazi, & Prendergast, 2017; Bourgeron, 2007; Malow et al., 2012). Additionally, some authors have pointed to the importance of melatonin receptor expression (MTNR1A) when sleep initiation is delayed (Simonneaux & Ribelayga, 2003), as gene variants in this receptor can alter its surface preventing any melatonin binding (Pauline Chaste et al., 2010). Another candidate gene that may affect melatonin levels is the enzyme that synthesizes melatonin from N-acetyl-serotonin (acetyl serotonin methyl transferase, ASMT), which has been evaluated as a genetic biomarker for autism, and also its implication in sleep problems in autistic individuals (Ackermann & Stehle, 2006; Jonsson et al., 2010;

Maronde et al., 2011; C Pagan et al., 2014; CV©cile Pagan et al., 2017; Toma et al., 2007; Veatch et al., 2015).

Furthermore, the circadian expression of circadian clock genes, is partly regulated by melatonin (Charrier et al., 2017), and the circadian clock genes, Period family (PER1) and Neuronal PAS domain-containing protein 2 (NPAS2), could also influence ASD sleep patterns. PER family (PER1, PER2 and PER3) are involved in the negative feedback loop of genes governing the central circadian clock (Crane & Young, 2014; Lowrey & Takahashi, 2011). PER family inactivation proved to decrease total sleep time in an animal model (Cirelli, 2009) and in combination with the NPAS2 gene could regulate the length of the rest-activity cycle (Ciarleglio, Resuehr, & McMahon, 2011; Vitaterna et al., 1994). The PER family is also a transcriptional regulator of non-rapid eye movement (NREM) sleep in mice (Franken et al., 2006). Furthermore, PER1 and melatonin pathway genes interact, as melatonin can modify the expression of the PER1 gene, worsening sleep disturbances (Agez, Laurent, Pevet, Masson-PV©vet, & Gauer, 2007).

Currently there are no published genetic association studies evaluating to which extent circadian clock or melatonin genes might account for phenotypic sleep problems commonly experienced by autistic adults with ID. In this study we analyzed the genetic influence of PER1, NPAS2, ASMT and MTNR1A on sleep in individuals with autism and ID.

Method

Participants

All participants were residents in Spanish autism institutions and were recruited there or in clinics specializing in ASD, after researchers met with parents and carers. Over 200 participants were pre-screened and informed consent was obtained from 123 adults with ASD.

Study inclusion criteria were: aged 18- to 50-years-old; a previous diagnosis of ASD meeting 5th Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria (American Psychiatric Association, 2013), confirmed by a clinician from the individual's residential facility and by a psychiatrist from our research team; and no currently prescribed sleep medication or melatonin. No participant was receiving cognitive behavioral therapy, so sleep was not influenced by this factor. Participants' intelligence quotient (IQ score <70) was obtained from medical records.

Procedure

Agreement to attend a clinical visit and a routine blood test was obtained from autistic participants and their carers. Control blood samples were chosen according to ethnic and geographical area conditions matching with the ASD cases. Complying with ethical and legal regulations, control samples were obtained from the Genotyping National Centre (CeGen, Santiago de Compostela, Spain) integrated within the Resources Network Platform (PRB2) sponsored by Carlos III Health Institute (Madrid, Spain, CEGEN-PRB2-ISCIII).

After screening was successfully completed, the autistic adults began the study during which they continued to take their regular medication. Participants could withdraw from the study at any time.

During their clinical visit, demographic information (age, sex, body mass index) and regular prescribed medications were recorded. Participants' sleep was assessed by ACM as described previously (Ballester et al., 2018). Blood samples for genotyping were taken during a routine blood test within 6-months of registration for the sleep study. All participants were asked to follow their regular routines, and sleep-wake diaries were completed by caregivers. Data from sleep diaries were used as backup for ACM records.

Genotyping

Following the routine blood test, mononuclear cells were isolated, DNA was extracted using the QIAamp¬Æ DNA Blood kit and concentrations were measured with a Nanodrop 2000 spectrophotometer. DNA for the control population was obtained from the National Spanish Gene Bank. Genotyping of PER1 (rs885747 G>C, rs6416892 T>G), ASMT (rs4446909 G>A, rs5989681 G>C), NPAS2 (rs1811399 T>G), and MTNR1A (rs28383652 NA, rs7654853 NA) was performed at CEGEN-PRB2-ISCIII.

In order to detect single nucleic acid changes, target regions were initially amplified using multiplex PCR and subsequently hybridized to custom-designed primers, and after that, subjected to a single base extension reaction using single mass-modified nucleotides. Spotting of the products onto a matrix chip and subsequent ionization enables real-time detection of nucleotides by Sequenom MassARRAY mass spectrometry. Genotypic and allelic frequencies were calculated and the results were compared for both populations to test if the presence of any of the SNPs studied was linked to having an ASD diagnosis (Gibbs et al., 2003).

Ambulatory Circadian Monitoring (ACM)

The sleep/wake circadian rhythm was obtained by ACM, a watch-like device placed on the non-dominant arm for a week. The device was removed during showering or any

other activity where it might get wet and data were filtered in order to eliminate erroneous measurements produced by temporary removal. The ACM device has three different sensors which calculate a single integrated variable, TAP, from the integration of wrist temperature (T, inverted), motor activity (A) and body position (P) where maximum values should occur at the same time of day and indicate a high level of activation (values near 1), or complete rest and sleep (values near 0). Sleep variables were inferred from TAP and converted into a binary code, with 1 corresponding to a resting period (mainly at night) and 0 to an active period (mainly during the day) (Ballester et al., 2018; Lopez, Jaussent, & Dauvilliers, 2014; Elisabet Ortiz-Tudela et al., 2014).

Sleep parameters

The sleep parameters analyzed were: (a) total sleep time (TST), defined as the number of minutes registered as sleep between night sleep onset and sleep offset; (b) time in bed (TIB), total minutes in bed at night until sleep offset; (c) sleep onset latency (SoL) the time until night sleep onset; (d) number of awakenings during the TST interval (num. awake); (e) waking (min) after sleep onset (WASO) during the TST interval and; (f) sleep efficiency (SE) percentage ([TST/TIB] x 100).

Circadian sleep-wake rhythm indexes

Circadian sleep-wake rhythm indexes were calculated from ACM recordings as previously described (Ballester et al., 2018). These indexes serve to characterize the status of the circadian clock and also the rest-activity cycle (Van Someren & Riemersma-Van Der Lek, 2007; Weitzman et al., 1981). Relative amplitude (RA, score range 0-1; where higher values indicate better circadian rhythmicity that is a greater difference between wake and sleep status values), inter-daily stability (IS, score range 0-1; higher

values indicate stronger circadian rhythm because values are similar across days) and intraday variability (IV, score range of 0-2; higher values indicate weaker circadian rhythm due to high variability within each day). M (5, 10) and L (5, 10) indicate consecutive 5- and 10-hour periods of maximum (M-onset) and least activity (L-onset); these phase markers are presented as a mean value in a hour basis and accompanied by a value (V). The diurnal phase marker should be between 12:00 and 2:00 pm, and the nocturnal marker between 3:00 and 5:00 am. These four phase makers were calculated for each rhythm. Finally, the circadian function index, which assesses circadian rhythmicity status was obtained from [(IS+ (2-IV) +RA)/3] (CFI, score range 0-1; higher values indicate more robust rhythmicity).

Study design and Ethics

This was a prospective observational study conducted from February 2012 to October 2016, excluding Summer months (June-August) because extremely hot weather could affect the accuracy of the sleep sensors used here (E. Ortiz-Tudela, Martinez-Nicolas, Campos, Rol, & Madrid, 2010). Following Hospital Ethics Committee approval, all participants, or their legal guardians received information about the design and purpose of the study and participants' or legal guardians' informed consent was obtained. The study was performed in accordance with the principles of the Helsinki Declaration.

Statistical Analyses

The Shapiro-Wilks test was used to assess normality in order to select parametric or non-parametric statistical tests. Continuous variables are presented as mean ¬± standard deviation (SD) or median and interquartile range (IQR), while categorical variables are expressed as percentages. Sleep parameters were compared to normal values (E. Ortiz-Tudela et al., 2010) and categorized in relation to the normal range [in range (‚úì), higher

( ), lower ( )] to indicate how participants' sleep varied from expected values. Frequencies were compared using the Chi-Square test, with Yate's continuity correction as appropriate, and X2 (df, n) are reported.

The distributions of observed allelic and genotypic frequencies were tested for Hardy-Weinberg equilibrium. Odds Ratio was calculated when studying the risk of having a certain SNP and a co-occurring ASD diagnosis. T-tests or Wilcoxon signed rank tests were used to assess group differences. The influence of genotype on the circadian rhythm indexes and sleep parameters was analyzed using regression analyses for co-dominant, dominant or recessive models and comparing results for the different genotypes; Bonferroni correction for multiple comparisons was performed.

Alpha-values <0.05 were considered to indicate significance for all analyses. Effect sizes were calculated using Cohen's d (range small (0.20), medium (0.50) or large (0.80)); or R squared ([negative or positive]: no linear correlation (0), weak (0.10), average (0.50), considerable (0.75), very strong (0.95) or perfect (1)). Also, 95% CIs are reported. All statistical analyses were performed with R 3.2.4 and GraphPad Prism 5.0 software.

Results

Participant demographics

While informed consent was obtained from 123 participants, blood samples were only obtained from 92 participants and of these, only 83 participants tolerated the ACM device. Screening and recruitment processes are described in Figure 1, while participants' demographical data are summarized in Table 1.

Cases (n=83, age range from 29- to 40-years, 78% males, body mass index 26.6 ¬± 6.4 Kg/m2) presented a median of three comorbidities per person (IQR25-75: 1-4, e.g.,

epilepsy, aggressive behaviour, or mood disorders) and three prescribed medications (IQR25-75: 2-5). Drugs most often prescribed were antipsychotics (86%), mood stabilizers (61%), anxiolytics (43%) and antidepressants (40%).

Healthy control samples (n=25) were provided from National Spanish Gene Bank and only geographical data were available and were matched with participants' geographical location.

Genotyping analysis

Genotypic and allelic frequencies are shown in Table 4. All polymorphisms analyzed were in Hardy-Weinberg equilibrium, except for MTNR1A (rs28383652 NA, rs7654853 NA) polymorphisms which were monomorphic in all our ASD individuals, and thus, excluded from the analysis. Hardy-Weinberg equilibrium implies that frequencies (allelic and genotypic) will be constant over generations when evolutionary influences are not present.All frequencies found in individuals with ASD and ID were similar to the healthy control population, except those for the PER1 gene. The autistic participants presented with a significantly higher prevalence of PER1 rs885747-CG and rs6416892-TT genotypes; the allele G and T frequencies presented for both SNPs in these individuals was 60%.

Genotyping influence on sleep problems

Sleep problems were present in all participants according to the normality percentage values of sleep parameters and circadian rhythm indexes (Table 2).

Insomnia

All participants presented with insomnia symptoms either reflected in a SoL duration that exceeded the normal value (46 ¬± 30 min, 62% outside normal values); higher values for TIB (667 ¬± 107 min, 81% outside normal values), or difficulty maintaining

sleep at night (WASO) reflected in both the number (3 ¬± 2, 80% outside normal values) and duration (122 ¬± 87 min, 79% outside normal values) of night awakenings. While the mean and SD values for TST were within the normal range, only 49% of the ASD subjects actually had a TST within the normal range. These sleep alterations were reflected in lower SE in autistic individuals compared with the general population (66% of autistic participants had SE outside normal values).

Genetic influence

Figure 2 shows that genotype significantly influenced sleep parameters in the autistic participants. PER1 rs6416892-GG cases (17%) needed less time to accomplish the transition from full wakefulness to sleep as evidenced by a higher percentage of SoL values inside the normal range (OR 0.2, 95% CI 0.06 to 0.70, p= 0.012, Figure 2). These individuals needed approximately 10 min less to fall asleep (TT and GT 47.5¬±30 vs. GG 36 ¬± 26.5 min, d=0.4, p=0.033). However, a significantly higher number of night awakenings were associated with the PER1 rs6416892-TT (2¬±1 GG, 3¬±2 GT, 4¬±3 TT, R2 =0.04, p=0.047) and the ASMT rs5989681-CC/GG (3¬±2 CC, 2¬±2 CG, 3¬±2 GG, R2 =0.09, p=0.049) genotypes, but both correlations were weak, see Figure 3. No other significant differences were found for the remaining sleep parameters (TST, TIB, WASO and SE) in relation to the polymorphisms analyzed.

Circadian sleep-wake rhythm indexes

Non-parametric circadian index values for the adults with autism and ID are presented in Table 3. A total of 79% of participants presented with a phase advanced sleep rhythm (based on consecutive 5- hour periods of maximum activity, M5 values) and 75% of the participants had abnormal circadian rhythmicity, with low stability (61% outside normal

range), higher fragmentation (100% outside normal range) or advanced sleep rhythm according to M5 and L10 values. In summary, non-parametric circadian index values indicate poorer quality circadian rhythms and suggest a CRSWD, mainly a phase advanced sleep rhythm.

Genetic influences

Figures 4 and 5 show significant genotype influences on autistic individuals' sleep circadian rhythms. While the primary focus of the study is sleep, the ACM device also measures wrist (skin) temperature. Night sleep should occur on the falling phase of the core body temperature rhythm and this is facilitated by a rise in skin temperature, especially at the extremities, thus the sleep-wake and temperature rhythms are expected to be in phase (Sarabia, Rol, Mendiola, & Madrid, 2008). Thus, any significant results related to the skin temperature rhythm were also examined.

When comparing the 24h wave of the sleep circadian rhythm by genotype, a sleep phase advancement was observed for the PER1 rs6416892-GG genotype with increased sleep values at 22:00 (0.88¬±0.2 vs. 0.81¬±0.19; d=0.36, p=0.032) and 1:00h (0.84¬±0.21 vs. 0.51¬±0.30; d=0.70, p=0.0001), for the major nocturnal (M5 index normal value: 3:00-5:00 h) sleep values. The same PER1 rs6416892-GG genotype showed also a temperature phase advancement across the day: for the major nocturnal (M5, 00:15¬±3:49GG, 2:07¬±2:40GT/ TT; respectively, d=0.59, p=0.045) values and diurnal least activity (L10, 12:49¬±2:21GG; 14:17¬±2:06GT/TT respectively, d=0.64, p=0.023) values.

A tendency to phase delay was found for the ASMT rs5989681-CC genotype, which moved the major nocturnal sleep circadian rhythm index backwards (M5, 2:24¬±1:25CC vs. 1:30¬±3:05CG/GG d=0.42, p=0.044). The ASMT rs5989681-GG genotype was

associated with a higher fragmentation of the wrist temperature rhythm (IV, 0.14¬±0.08GG, 0.11¬±0.05GC/CC d=0.57 p=0.033) with higher least activity values during the day (VL10, 32.46¬±1.67GG, 32.26¬±1.07GC/CC d=0.11, p=0.049), which were closer to a normal pattern. NPAS2 rs1811399-CC individuals showed higher temperature circadian rhythm values during the day (VL10, 32.46¬±0.01CC, 32.27¬±1.43AC/AA d=0.69, p=0.017) There were no further influences of these polymorphisms on circadian sleep-wake or temperature indexes.

Discussion

Our results show that PER1 rs6416892 and ASMT rs5989681 polymorphisms affect both sleep parameters and circadian rhythms as indicated by sleep and wrist temperature phase marker indexes, and thus may play a significant role in CRSWDs seen in ASD. Also, an isolated, significant effect was found for the gene NPAS2 rs181139 on diurnal wrist temperature values.

We found that specific polymorphisms on the PER1 gene (rs885747 G>C with a frequency of the 60% of allele G; rs6416892 T>G with a frequency of the 60% of allele T) appeared at a higher frequency in autistic individuals, than in control samples. This result is supported by a previous study in a sample of trios, formed by autistic progeny and parents, where they found the same allelic distribution which we had for rs6416892 and rs885747 and linked those frequencies with ASD (Nicholas et al., 2007). The frequencies of polymorphisms described for autistic individuals in this study and previously (Nicholas et al., 2007) could be linked to an abnormal expression of PER1 gene in brain areas that affect sleep-wake transition, e.g., in the suprachiasmatic nuclei,

a brain structure deeply involved in regulating the sleep process (Charrier, Olliac, Roubertoux, & Tordjman, 2017; Sun et al., 1997). The suprachiasmatic nuclei contains the principal circadian pacemaker. It controls circadian rhythms in rest and activity, and several other circadian rhythms including core body temperature and neuroendocrine functions (i.e., hormone release) (Sun et al., 1997). Some authors have also related the different allelic distribution of circadian clock genes found in autistic participants with a circadian rhythm dysregulation that could affect critical brain developmental periods and therefore be a causitive factor in the condition (Bourgeron, 2007; Geoffray, Nicolas, Speranza, & Georgieff, 2016; Wimpory, Nicholas, & Nash, 2002).

In our sample, those participants with a PER1 rs6416892-GG genotype needed less time to accomplish the transition from wakefulness to sleep and had fewer night awakenings. Little is known about how PER1 rs6416892 affects sleep in autism, but studies in animal models have described that when there is a lack of this gene there is a reduction in total sleep time (Cirelli, 2009), and autistic individuals often present with reduced total sleep (Baker & Richdale, 2017).

The ASMT rs5989681-GC genotype is also related to fewer night awakenings. Some authors have described that the rs5989681-G allele is associated with a dramatic decrease in activity of acetylserotonin O-methyltransferase (ASMT), the last enzyme in melatonin production and associated this with the lower melatonin levels described in autism (Melke et al., 2008b; CV©cile Pagan et al., 2017; Toma et al., 2007). As the analyses in our study were made by genotype, the C-allele could be protecting G-allele carriers and therefore positively influencing the number of awakenings per night, as the presence of both alleles is associated with a reduction in the mean number of nocturnal awakening.

PER1 helps to reset the biological clock (Crane & Young, 2014; Jonsson et al., 2010; Lowrey & Takahashi, 2011). Only one study with a small sample size has previously shown this circadian relevant variation to be more frequent in autistic individuals and related it with sleep problems (Zhiliang Yang et al., 2016). Related to circadian rhythmicity, this variation evidenced sleep and temperature phase advancement in the general population, and these findings are consistent with early morning awakening found in ASD (Utge et al., 2010). Furthermore, some authors have described that when silencing PER1 genetic expression, there is an alteration in circadian rhythms (Cirelli, 2009; Nagel, Clijsters, & Agami, 2009). In addition, disruptions in the PER1 complex with CRY1 and CRY2 are related to an early morning preference and an inability to reset the clock in response to environmental cues such as daylight (David M Berson, Felice A Dunn, & Motoharu Takao, 2002; Katzenberg et al., 1998).

According to our results, adults on the autism spectrum with a CC genotype in ASMT rs5989681 had a phase advancement in their sleep circadian rhythm. The C allele is associated with normal ASMT activity (Melke et al., 2008b; Cv©cile Pagan et al., 2017; Toma et al., 2007). This may result in normal levels of melatonin, however, the phase advancement could be related to an earlier time of melatonin release, as has been suggested previously (Lewy et al., 1998). Furthermore, alterations in melatonin release may be related with the role that PER1 plays in sustaining the oscillatory function of the clock, which could be influential in melatonin release and in maintaining the melatonin rhythm (Oster, van der Horst, & Albrecht, 2003).

Autistic adults with the ASMT rs5989681-GG genotype and NPAS2 rs1811399-CC presented with major temperature values during the day. Many previous studies in humans indicate that sleep is strongly linked to thermoregulation. Core body

temperature, which also cycles together with the sleep-wake rhythm, decreases during the nocturnal sleep phase and increases during the wake phase across the 24-hour circadian rhythm (Kryger & Sheldon, 2005). This phase relationship with temperature is important for maintaining sleep. In this study, PER1 rs6416892-GG was related to a phase advancement in the wrist temperature rhythm, and it has been described that they form a complex with CRY genes that influences sleep timing (Ciarleglio et al., 2011). However, the temperature rhythm results should be interpreted with caution as p values did not survive the Bonferroni correction. Nevertheless, this gene variant may be an area for future exploration with studies using bigger sample sizes.

Although individuals taking sleep medications were excluded, the influence of potential drug side effects on insomnia, circadian rhythm sleep-wake disorders and circadian clock gene brain expression cannot be excluded. There is some evidence that selective serotonin reuptake inhibitors (e.g., fluoxetine) alter the expression of the PER1 gene in brain areas related to sleep (Ammon, Mayer, Riechert, Tischmeyer, & HѴðllt, 2003; Uz et al., 2005).

While this study is the first to examine clock genes and their relationship with sleep phenotypes in autistic adults with ID using ACM, an objective measure, it also has some limitations. The circadian oscillation of clock-controlled gene expression is mainly regulated at the transcriptional level. Related to the phase advance described, PER1 forms a complex with CRY1 and CRY2, and these genes are involved in matching environmental time with biological time by resetting the clock in response to environmental cues such as daylight, thus, the phase advancement described for the variant of PER1 could be related to this complex (D. M. Berson, F. A. Dunn, & M. Takao, 2002). To further elucidate the mechanism of how positive and negative components of

the clock interplay, we will need to characterize these interactions and also those with BMAL1 in future studies. Also, in our population, the SNPs located in the melatonin receptor were monomorphic, so no analysis or comparison with previous studies could be performed (P. Chaste et al., 2010).

Though our sample could be considered small in terms of genetic analyses, it is a similar sample size to published studies analysing sleep problems and circadian genes in autistic populations. We acknowledge that the sample size of the control group is very small, thus the results must be confirmed by a larger study. It would have been interesting to know further details about the control sample used, however, knowing that they were healthy and from the same geographical area as our autistic participants is sufficient to test outcomes (Z. Yang et al., 2016). Although individuals taking sleep medications were excluded, the influence of potential drug side effects on sleep parameters or clock gene brain expression cannot be excluded (Ammon et al., 2003; Uz et al., 2005).

People with ASD commonly experience other comorbidities besides poor sleep. Our study showed that all our autistic participants had evidence of a sleep problem, either insomnia and/or a CRSWD with the PER1 rs6416892-GG genotype influencing this. Understanding the causes of poor sleep in ASD is necessary to improve treatment, which is a clinical priority. Clearly there is a need for clinically randomized trials to determine impact of pharmacogenetically-guided treatments for sleep problems or disorders in autistic adults with ID, in a variety of clinical settings, thus leading to improved diagnosis and therapy.

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Table 1. Demographic data for all subjects with Autism Spectrum Disorder (ASD) included, genotyped and analyzed by ambulatory circadian monitoring (ACM).

	Included (n=123)	Genotyped (n=92)	Genotyped+ACM (n=83)
Sex, Men/Women (n)	98/25	74/18	65/18
Age, mean ± SD (years)	29 ± 11	30 ± 10	29 ± 11
BMI, mean ± SD (Kg/m^2)	26.5 ± 6.4	26.2 ± 6.2	26.6 ± 6.4

Note: ACM stands for Ambulatory circadian Monitoring and Body Max Index (BMI) is measured in Kg/m^2.

Table 2. Sleep parameters obtained by ambulatory circadian monitoring (ACM) recordings in Autism Spectrum Disorder (ASD) participants.

Sleep parameters mean ± SD	ASD (n=83)	Normal values	In normal range %(n)	Tendency
Total Sleep Time (TST, min)	521±121	420-540	39 (49)	⇑
Time in bed (TIB, min)	667±107	420-569	15 (19)	⇑
Sleep Onset Latency (SoL, min)	46±30	30	30(38)	⇑
Number of awakenings (median, IRQ)	3±2	1	16(20)	⇑
Wake After Sleep Onset (WASO, min)	122±87	20	9(11)	⇑
Sleep Efficiency (SE, %)	77	>85	27(34)	⇓

Normal values came from Ortiz-Tudela et al. (27): ✓ (in range), ⇑ (higher) or ⇓ (lower); min =minutes.

Table 3. Non-parametric circadian indexes values for wrist temperature, motor activity, body position, TAP and sleep circadian rhythms.

	Sleep	Normal values	In normal range	Significance
IS	0.59±0.26	>0.70	39% (32)	⇓
IV	0.34±0.18	<1	0% (0)	⇑
RA	0.77±0.17	>0.80	63% (52)	⇓
M5	1:57±2:35	3:00-5:00	26% (22)	⇓
VM5	0.84±0.18	>0.90	48% (40)	⇓
L10	14:17±2:18	14:00-17:00	32% (27)	✓
VL10	0.12±0.20	<0.25	84% (70)	✓
CFI	0.73±0.17	>0.85	25% (21)	⇓

Note: IS : inter-daily stability; IV: intraday variability; RA: relative amplitude; M5 and M10, L5 and L10 indicate consecutive 10- and 5- hour period of maximum and minimum values respectively; and CFI: circadian function index,

Table 4. Genotypic and allelic distribution for *NPAS2* (rs1811399), *Per1* (rs885747 and rs6416892) and *ASMT* (rs4446909 and rs5989681) genes. Allelic and genotypic frequencies were calculated and expressed as percentages.

Gene	ASD	Control	P value
	%	%	X2 (df,n) or OR (IC 95%)
NPAS2 (rs1811399, G>T)			
GG	2	0	0.362
GT	42	50	2.03 (2,117)
TT	56	50	
Minor allele G	23	25	0.869
Major allele T	77	75	OR 0.896 (0.47 to 1.72)
Per1 (rs885747, G>C)			
CC	16	23	0.0003
CG	47	65	16.14 (2, 117)
GG	37	12	
Minor Allele C	40	55	0.047
Major Allele G	60	45	0.546 (0.311 to 0.957)
Per1 (rs6416892, T>G)			
GG	17	21	<0.0001
GT	46	69	20.53 (2, 117)
TT	37	10	
Minor Allele G	40	58	0.0175

Major Allele T	60	44	0.506 (0.289 to 0.886)
ASMT (rs4446909, G>A)			
AA	11	12	0.7773
AG	34	38	0.504 (2, 117)
GG	55	50	
Minor Allele A	28	31	0.757
Major Allele G	72	69	0.866 (0.471 to 1.591)
ASMT (rs5989681, G>C)			
CC	17	19	0.6388
CG	38	42	0.897 (2, 117)
GG	45	38	
Minor Allele C	36	40	0.564
Major Allele G	64	60	0.844 (0.476 to 1.495)

Figure 1. Flow chart of Autism Spectrum Disorder (ASD) subjects included, genotyped and analyzed by ambulatory circadian monitoring (ACM).

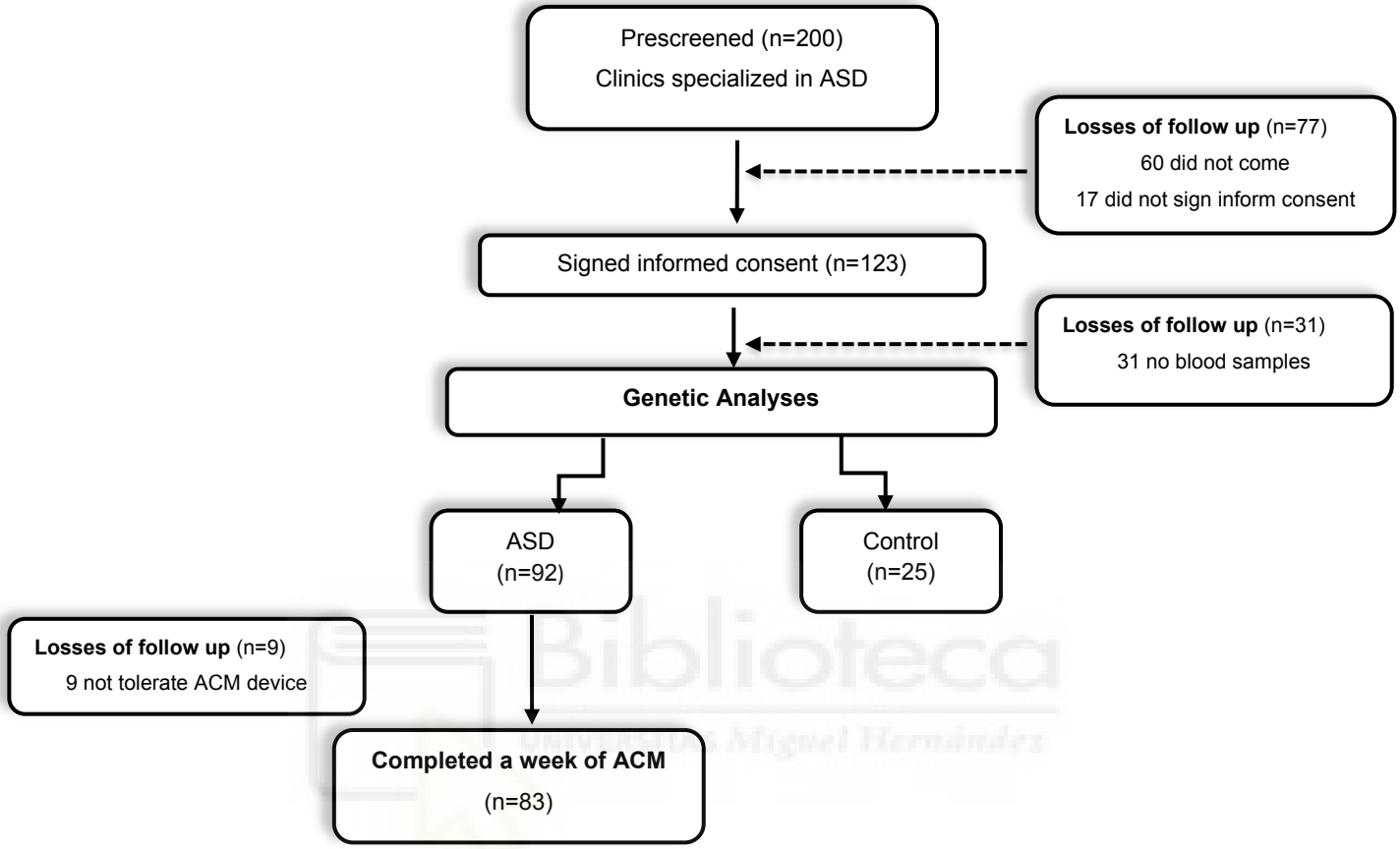


Figure 2. *Per1* rs6416892 *and ASMT* rs5989681 *g*enotype influence on sleep parameters.

Data is expressed as % of normal values.

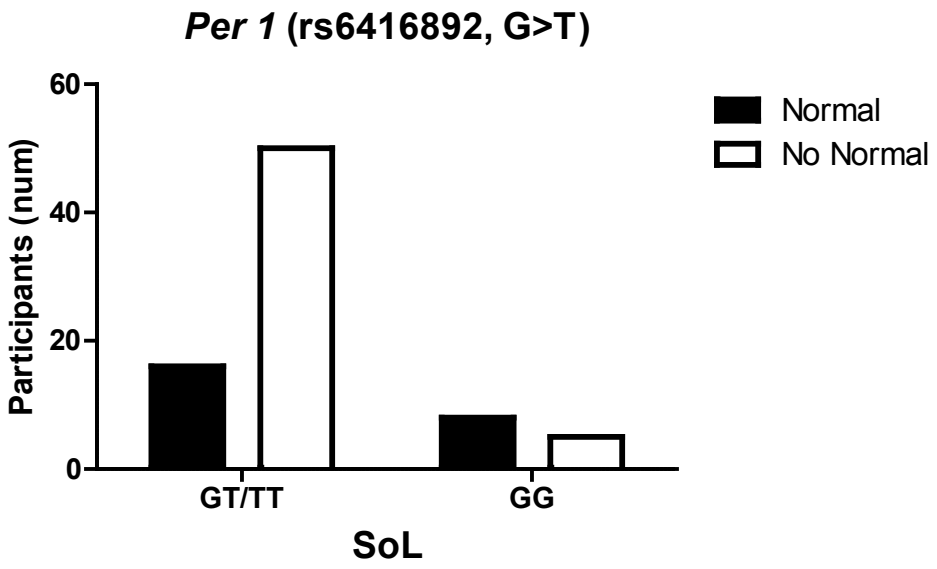


Figure 3. Genetic influence on Comparison of genotypes using a codominant model of the number of awakenings for *Per1* and *ASMT* genotypes.

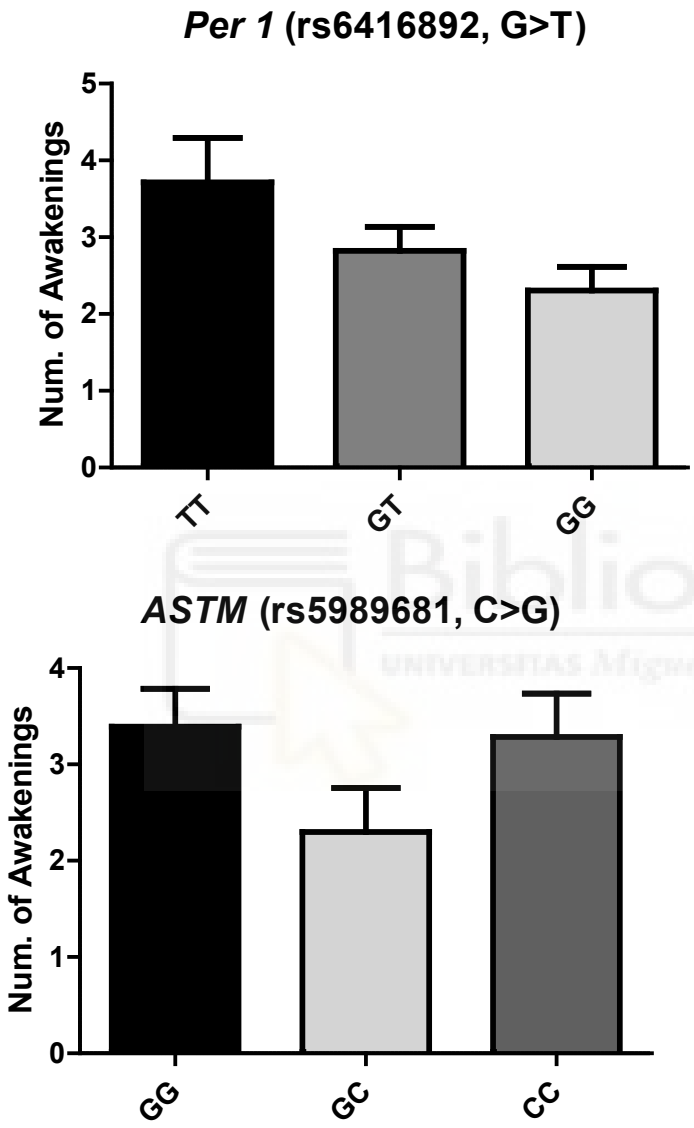
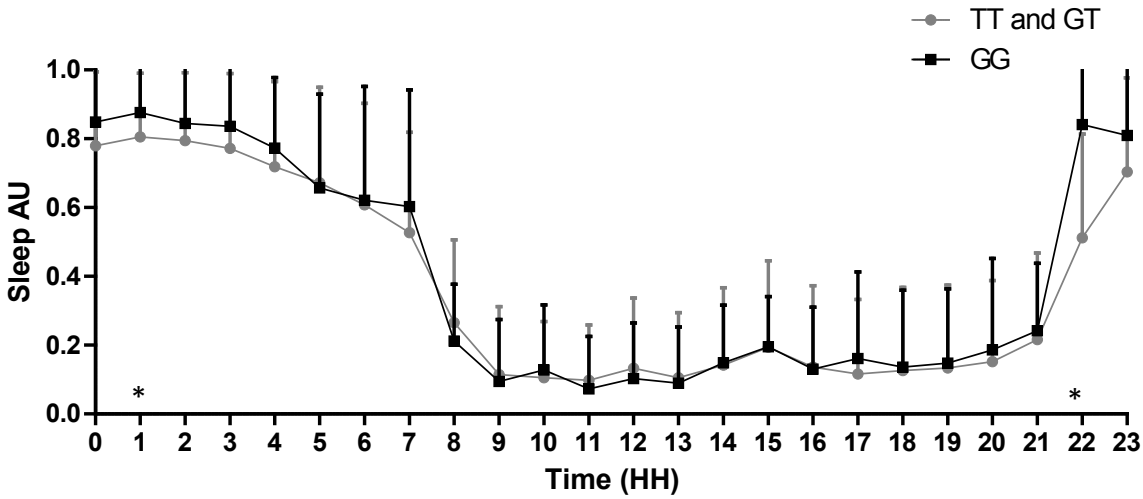
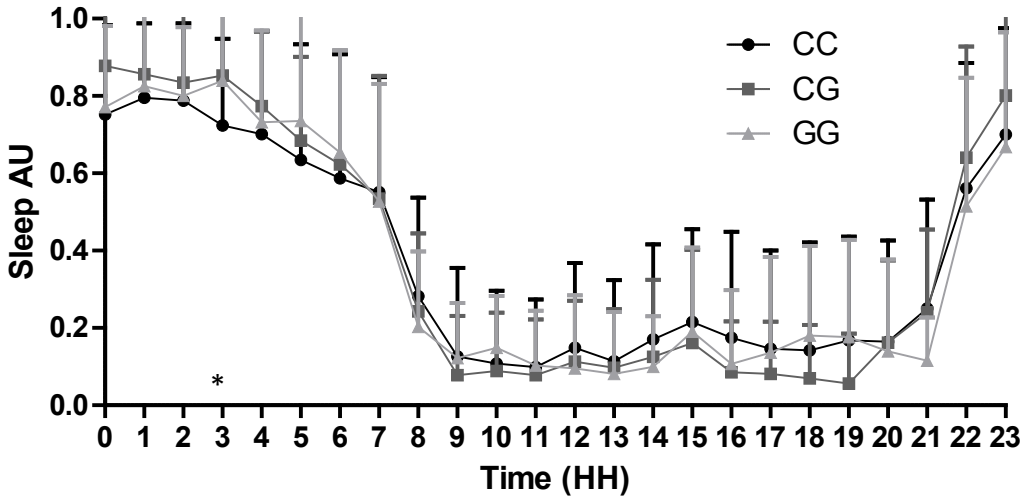


Figure 4. *Per1* rs6416892 genotype influence on sleep circadian phase in adults with ASD and ID.



Note: AU: Arbitrary Units, HH: hours, e.g., 1:00h

Figure 5. *ASMT* rs5989681 genotype influence on sleep circadian phase in adults with ASD and ID3**.**



Note: AU: Arbitrary Units, HH: hours, e.g., 1:00h

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