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Genetic risk factors of chronic insomnia disorder

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Résumé

Problématique: Le trouble d'insomnie chronique (TIC) affecte 3,5 millions de Canadiens. Malgré sa prévalence élevée, les mécanismes sous-jacents du TIC demeurent méconnus. Une meilleure compréhension de sa base biologique est possible grâce aux études génétiques. Deux études d'associations pangénomiques (GWAS) ont suggéré que le gène *MEIS1*, antérieurement associé au syndrome des jambes sans repos (SJSR), est indépendamment associé à l'insomnie. Cependant, la méthode de phénotypage des GWAS s'avère limitée et les résultats n'ont pas été répliqué.

Objectif: Évaluer l'association entre *MEIS1* et le TIC. Si le gène *MEIS1* est pléiotrope au TIC et au SJSR, la fréquence des variantes génétiques du gène sera équivalente chez les groupes TIC et TIC+SJSR.

Méthodologie: Au total, 646 patients insomniaques ont participé à l'étude. Trois variantes du gène *MEIS1* ont été génotypé. Nous avons comparé les distributions d'allèles et des génotypes de la cohorte TIC aux groupes contrôle et SJSR de la cohorte canadienne française.

Résultats: Des spécialistes en sommeil ont émis les diagnostics TIC+SJSR à 26% de la cohorte. Nos résultats suggèrent des différences significatives dans les distributions alléliques et génotypiques entre les groupes TIC et TIC+SJSR. De plus, les distributions d'allèles et de génotypes des trois variantes génétiques étaient similaires entre les groupes TIC et contrôle, et les groupes TIC+SJSR et SJSR ($p > 0.05$).

Conclusion: Nos données confirment l'association entre *MEIS1* et le SJSR mais elles ne sont pas en faveur de l'effet pléiotropique avec le TIC. Nous soulignons l'importance du phénotypage et le fait de distinguer le TIC du SJSR.

Mots-clés : Troubles du sommeil, génétique de l'insomnie, insomnie chronique, SJSR, GWAS, phénotypage, MEIS1.

Abstract

Background: Chronic insomnia disorder (CID) affects 3.5 million Canadians. Despite its high prevalence, we do not fully understand the underlying mechanisms of CID. Genetic studies of insomnia contribute to better understanding its biological basis. The latest two genome-wide association studies (GWAS) suggest that *MEIS1* gene, previously associated with restless legs syndrome (RLS), is independently associated to insomnia. However, the GWAS phenotyping method was limited and the finding was not yet replicated.

Objective: Evaluate the association between *MEIS1* and CID. If *MEIS1* is pleiotropic to CID and RLS, the minor allele frequency of *MEIS1* variants will be equivalent in CID patients with and without RLS.

Methods: Overall, 646 CID patients participated in the study. We genotyped three *MEIS1* variants. To confirm our results, we compared the allelic and genotypic distributions of the CID cohort to ethnically matched controls and RLS cases from the French Canadian cohort.

Results: Patients were diagnosed by sleep specialists and 26% of the sample were diagnosed with CID+RLS. We find significant differences in allele and genotype distributions between CID-only and CID+RLS groups. Allele and genotype distributions of the three *MEIS1* SNPs were similar in between CID-only and control groups and in between CID+RLS and RLS-only groups (all $p>0.05$).

Conclusion: Our data confirms the association between *MEIS1* and RLS but it does not support the pleiotropic effect of *MEIS1* in CID. Further, our study highlights the critical importance of phenotyping and the need to carefully isolate CID from other disorders that can cause sleep difficulties, particularly RLS.

Keywords : Sleep disorders, sleep genetics, insomnia genetics, chronic insomnia disorder, restless legs syndrome, genome wide association study, phenotyping, MEIS1.

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AIS*:* Athens Insomnia Scale

BHLHE41: basic helix-loop-helix family member e41 gene (also known as *DEC2*)

CACNA1A : calcium voltage-gated channel subunit alpha1 A gene

CACNA1C: Calcium Voltage-Gated Channel Subunit Alpha1 C gene

CBT-I: cognitive behavioral therapy for insomnia

CID: chronic insomnia disorder

DZ: dizygotic

EEG: electroencephalogram

FDA: Food & Drug Administration

GABA: γ -aminobutyric acid

GABRB3: GABA A receptor beta 3 subunit gene

GABRA3: A receptor gene beta 3 subunit gene

GABRA6: GABA A receptor gene 6 subunit gene

GWAS: genome wide association study

Hcrt: hypocretin gene

ICSD: International classification of sleep disorders

MAF: minor allele frequency

MDD: major depressive disorder

MSLT: multiple sleep latency test

MEIS1: Myeloid Ecotropic Viral Insertion Site 1 gene

Meis1: Myeloid Ecotropic Viral Insertion Site 1 gene in mice

MZ: monozygotic

NREM: non-rapid eye movement

OXA: orexin-A

OXB: orexin-B

PER: PERIOD gene

PLCB1: Phospholipase C Beta 1

PSQI: Pittsburgh Sleep Quality Index

RBFOX3: RNA-binding protein fox-1 homolog 3 gene

RLS: restless legs syndrome

ROR1: Receptor Tyrosine Kinase Like Orphan Receptor 1 gene

SDB: sleep disordered breathing

SNP: single nucleotide polymorphism

SSRI: serotonin selective reuptake inhibitors

SCI: Sleep Condition Indicator

5-HT: serotonin

5-HTT: serotonin transporter

5-HTTLPR: serotonin transporter linked polymorphic region

Liste des abréviations

NA

To my biological family and to CÉAMS family...

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It is on Febuary 28th 2016 that I received the offer of admission to the master's program. I was surrounded by my friends, celebrating my 24th birthday. I couldn't have wished for a better way to start the new year, feeling prosperous, enthusiastic and loved. Since that date a new journey started and made who I am today. During that journey I grew up on the personal and career levels. I met new people, whom I am blessed and grateful to have in my life. Others have left, they were just meant to share a limited period of this journey with me. Finally, my beloved ones who have always been there can't be thanked enough for who they are.

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Introduction

The field of sleep research was born almost fifty years ago but is still in its infancy to investigate how and why we sleep (Montplaisir, 2015). Sleep is "one of the least understood phenomena in biology" (Sehgal & Mignot, 2011) and advances in sleep genetics contribute to a better understanding of the underlying mechanisms of sleep (Gehrman, Keenan, Byrne, & Pack, 2015; Sehgal & Mignot, 2011). There is considerable evidence suggesting that genetic factors influence the amount and the organization of sleep (Dauvilliers, Maret, & Tafti, 2005; Sehgal & Mignot, 2011). A decade ago, it was shown that electroencephalogram (EEG) patterns are highly heritable (Ambrosius et al., 2008). For instance, twin studies demonstrate strong concordance between monozygotic (MZ) twins in slow wave sleep, sleep onset and sleep disruption, suggesting a heritability rate of 50% (Dauvilliers et al., 2005; Harvey, Gehrman, & Espie, 2014; Tafti, Maret, & Dauvilliers, 2005). Also, power spectral analysis of EEG frequencies during NREM and wakefulness of MZ twins were significantly higher than dizygotic (DZ) twins; which highlights genetic contributions (Ambrosius et al., 2008). These findings support the hypothesis that there are common inherited neuronal mechanisms that generate EEG oscillations in humans (Ambrosius et al., 2008).

There is also considerable evidence supporting the heritability of sleep disorders (Barclay & Gregory, 2013; Dauvilliers & Tafti, 2008; Gehrman, Keenan, Byrne, & Pack, 2015; Tafti, 2009; Tafti, Maret, & Dauvilliers, 2005). On average, the heritability rate of sleep disorders such as narcolepsy, restless legs syndrome (RLS) and sleep disordered breathing (SDB) ranges from 40 to 50% (Gehrman et al., 2015; Schormair et al., 2017; Tanizawa & Chin, 2018). Current knowledge on the sleep genetics of RLS, narcolepsy and SDB is more advanced than in other disorders such as insomnia disorder (Gehrman et al., 2015; Lind & Gehrman, 2016; Parish, 2013). This is partly due to clearer phenotyping strategies and large sample sizes used in RLS, narcolepsy and SDB genetic studies.

In the case of insomnia disorder, heterogeneous phenotyping of the disorder limits the advancement of the field and the replication of findings (Lind & Gehrman, 2016). Poor insomnia phenotyping results from the use of a wide diversity of questionnaires that are not validated to assess insomnia. The majority of previous genetic studies identified insomnia cases using broad

sleep related questionnaires (Barclay et al., 2011; Brower, Wojnar, Sliwerska, Armitage, & Burmeister, 2012; Polito et al., 2015), sleep queries in psychiatric questionnaires (Feusner et al., 2001; Serretti et al., 2003) or in population based surveys (Gass et al., 2010; Hammerschlag et al., 2017; Lane et al., 2017; Rétey et al., 2005). A few studies used validated insomnia questionnaires in community cohorts (Huang et al., 2014; Li, Huang, Lan, & Wang, 2015) or assessed insomnia in psychiatric samples (Perlis et al., 2003; Serretti et al., 2010; Utge et al., 2010) and a very few studies used clinically diagnosed insomnia samples (Buhr et al., 2002; Deuschle et al., 2010). However, the sample sizes of these latter studies were very small, ranging from a minimum of one clinical case (Buhr et al., 2002) to a maximum of 167 insomnia patients (Deuschle et al., 2010). To address this limitation, genome wide association studies are using very large samples (4×10^5 cases and 9×10^5 controls) but cases are very poorly phenotyped (Oexle, 2018). This project will illustrate how the use of large sample sizes do not compensate poor phenotyping. To do so, we will provide the clinical and research background on insomnia disorder, going from the current diagnostic criteria, economic impact, treatments, etiological models of insomnia to the current knowledge of the genetics of insomnia.

1.Insomnia disorder: diagnostic criteria, economic burden and treatment

1.1 Diagnostic classification of insomnia disorder

1.1.1 Current clinical diagnostic criteria of insomnia disorder

Insomnia disorder is the most common sleep disorder affecting 3.5 million Canadians (Morin et al., 2011). While 30% of the general population report insomnia symptoms, 10% meet the full diagnostic criteria of insomnia disorder (American Academy of Sleep Medicine, 2014; Morin et al., 2011). Females report higher prevalence of insomnia compared to males (Zhang & Wing, 2006) and insomnia prevalence increases with age (Riemann et al., 2017).

Insomnia disorder is characterized by a subjective complaint of poor sleep quality or quantity that is associated to difficulty initiating, maintaining or undesired early morning awakenings from sleep (American Academy of Sleep Medicine, 2014). The main criterion that distinguishes insomnia disorder from non-clinical insomnia symptoms is the impact of the sleep disturbance on the daytime functioning. For insomnia to be considered as a disorder, sleep difficulties or their consequences should cause significant clinical distress or occupational or social impairment (American Psychiatric Association, 2013; Bastien et al., 2014). When sleep disturbance and associated daytime symptoms have been present for at least three times a week for at least three months, it is called chronic insomnia disorder (CID) (American Academy of Sleep Medicine, 2014). When sleep disturbance and associated daytime symptoms have been present for less than three months, it is called short-term insomnia disorder (American Academy of Sleep Medicine, 2014).

The diagnostic criteria of insomnia disorder have greatly evolved with time (Vgontzas & Fernandez-Mendoza, 2013). While the previous diagnostic guidelines of insomnia disorder distinguished different subtypes of insomnia, current diagnostic criteria aggregate insomnia disorder into a single category due to the lack of empirical evidence to support the sub-categorizations (American Academy of Sleep Medicine, 2014; American Psychiatric

Association, 2013; Riemann et al., 2015). In 1997, the revised first edition of the International classification of sleep disorders (ICSD-R) included twenty-one insomnia subtypes (Vgontzas & Fernandez-Mendoza, 2013). The second edition of the ICSD listed twelve insomnia subtypes (Vgontzas & Fernandez-Mendoza, 2013). Finally, the third edition of the ICSD only distinguishes the periodicity of insomnia disorder: less (short-term insomnia disorder) or more than three months (CID) (American Academy of Sleep Medicine, 2014; Vgontzas & Fernandez-Mendoza, 2013). On a parallel note, our study included patients who had insomnia for more than three months so we focused on CID.

With the current diagnostic criteria, insomnia disorder is considered independent of other psychiatric disorders. In case of comorbidity, it is recommended to treat insomnia disorder and other psychiatric disorders simultaneously (Riemann et al., 2017). It is also important to note that insomnia is often comorbid to other sleep disorders and psychiatric disorders (Riemann et al., 2015). Insomnia can precede a comorbid condition, persist even after successfully treating the comorbid condition or aggravate symptoms of the comorbid condition (Riemann et al., 2015). In fact, more than 75% of RLS patients complain of insomnia symptoms resulting from their leg discomfort (Allen et al., 2014; Montplaisir et al., 1997; Ulfberg et al., 2007).

In psychiatric disorders, an illustrative example is the bidirectional relationship between insomnia disorder and major depressive disorder (MDD). Insomnia disorder and MDD are two of the most prevalent psychiatric disorders and they are frequently comorbid (American Psychiatric Association, 2013). Insomnia disorder is believed to be a predictor to MDD and vice versa (Staner, 2010). In a European study, insomnia preceded depression in 41% of mood disorder cases, 29% of the time both disorders were comorbid and in another 29% insomnia appeared after the onset of MDD (Sutton, 2014). Additionally, insomnia is one of MDD diagnostic criteria (American Psychiatric Association, 2013). When sleep disturbances are severe in MDD patients, response to antidepressant treatment and remission rates are lower than in those without sleep problems (Krystal, 2012).

Despite these clinical guidelines, it is still strongly believed in the research domain that there are at least two types of insomnia that merit attention: psychophysiological insomnia and paradoxical insomnia (Bastien et al., 2014; Edinger et al., 2004).

1.1.2 Current research evidence for the subcategorization of insomnia

In psychophysiological insomnia, the subjective complaints of the patient can be objectively observed using polysomnography (Bastien et al., 2014). Patients with psychophysiological insomnia with short sleep duration (less than six hours) are believed to have the most severe phenotype of the disorder (Vgontzas, Fernandez-Mendoza, Liao, & Bixler, 2013). Indeed, insomnia with objective short sleep duration is associated with physiological hyperarousal and higher risk of developing diseases such as hypertension, diabetes, neurocognitive impairment and mortality (Vgontzas & Fernandez-Mendoza, 2013). This subgroup of insomnia is also more likely to have a persistent course compared to insomnia with normal sleep duration (equal or more than six hours).

Paradoxical insomnia is characterized by normal sleep duration, but there is a discrepancy between subjective complaints and objective measures of sleep time. Despite the lack of objective sleep loss, there are subtle sleep microstructural differences that exist between good sleepers and patients with paradoxical insomnia (Bastien et al., 2014). Paradoxical insomnia is also associated with cognitive, emotional and cortical arousal (Bastien et al., 2014).

It is also important to note that these two types are not mutually exclusive. One can display objective sleep onset or sleep maintenance difficulties on polysomnographic records and express extreme subjective complaints of insomnia that are not objectively measured (Bastien et al., 2014). The distinction between these types of insomnia is crucial when considering treatment options. It is proposed that insomnia with short sleep duration may better respond to pharmacological treatments whereas insomnia with normal sleep duration may respond primarily to psychological therapy (Bathgate, Edinger, & Krystal, 2017; Vgontzas & Fernandez-Mendoza, 2013).

1.2 Economic burden of insomnia symptoms and chronic insomnia disorder

The economic burden of insomnia is excessively high on society. In 2009, the total direct and indirect annual cost of insomnia in the province of Quebec was estimated at 6.6 billion Canadian dollars (Daley, Morin, LeBlanc, Grégoire, & Savard, 2009). Direct costs include

medical consultations, transport to consultations, pharmacological prescriptions, over the counter products and alcohol used as a sleep aid (Figure 1) (Daley et al., 2009). Indirect costs include work absenteeism and productivity losses, which comprises 76% of economic burden (Figure 1) (Daley et al., 2009). The average annual direct and indirect cost per patient with insomnia disorder was five times higher than the annual cost of an individual with insomnia symptoms, $5,010 versus $1,431 respectively (Daley et al., 2009). Compared to good sleepers, the cost of those with insomnia disorder was eleven times higher, $5,010 versus $421 respectively (Daley et al., 2009). Moreover, untreated insomnia expenses are much higher than those of treated insomnia (Daley et al., 2009). The same research group has shown that in Canada, 74% of patients with insomnia disorder show persistent symptoms over the course of a year and 46% report insomnia persisting over three years (Morin et al., 2009). This finding indicates that insomnia disorder is often a persistent condition. Hence, there is a need to understand its biological basis. Such knowledge will lead to precision medicine and to methods of preventing insomnia.

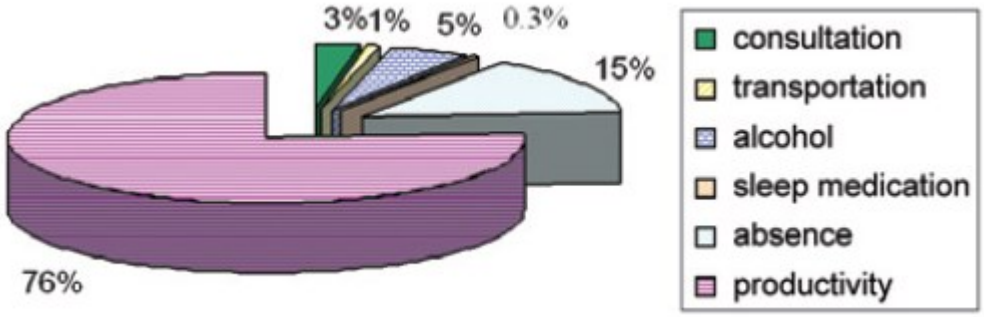

Figure 1. Direct and indirect economic burden of insomnia in the province of Quebec (Canada). Note that 76% of the economic burden of insomnia are attributed to reduced productivity (Daley et al., 2009).

1.3 Insomnia Treatment

1.3.1 Primary treatment for insomnia: Cognitive behavioral therapy for insomnia (CBT-I)

Cognitive behavioral therapy for insomnia (CBT-I) is the primary recommended treatment for insomnia (Morin et al., 2006). CBT-I also has positive therapeutic effects on

comorbid anxiety and depressive symptoms to insomnia (Riemann et al., 2017). CBT-I is composed of four main elements: psychoeducation about sleep–wake behaviour (sleep hygiene), behavioral strategies (sleep restriction and stimulus control), relaxation and cognitive techniques. The psychoeducation component of CBT-I instructs health practices (ie: clockwatching, exercise and substance use) and environmental factors (ie: light, noise and temperature) that promote or disrupt sleep (Riemann et al., 2017). Behavioral strategies are composed of sleep restriction method and stimulus control therapy. Sleep restriction method aims at reducing time in bed to the actual amount of sleep. Hence, a patient is recommended an individualized sleep window by the therapist, which is adjusted weekly over the course of treatment by the therapist. When sleep efficiency, ratio of the total time spent asleep to the total time spent in bed, reaches 85% or more the recommended time in bed increases by 15-30 minutes. However, if the sleep efficiency did not improve or is decreased (<80%), the sleep window is kept stable or decreased by 15-30 minutes until the optimal sleep duration is reached (Riemann et al., 2017; Spielman, Caruso, & Glovinsky, 1987).

Stimulus control therapy is a series of behavioural instructions that target the re-association of the sleep environment (bed/bedroom) with sleep and the reestablishment of a consistent sleep-wake schedule (Bootzin, 1972; Riemann et al., 2017). The set of behavioral instructions are as follows: going to bed only when sleepy, getting out of bed if unable to sleep after twenty minutes, using the bed/bedroom only for sleep and sex, waking up at the same time every morning and no napping during the day (Riemann et al., 2017).

Relaxation and cognitive techniques aim at reducing somatic tension (muscle relaxation and autogenic training) and intrusive thoughts at bedtime (ie: imagery training and meditation) (Riemann et al., 2017). Cognitive techniques identify and change misconceptions and beliefs about sleep and daytime consequences of insomnia (Riemann et al., 2017). Meta-analyses have shown that for long-term treatment, CBT-I is more prominent that pharmacological treatment (Smith et al., 2002). Nevertheless, for acute treatment pharmacological treatments and CBT-I have equal efficiency (Smith et al., 2002).

1.3.2 Pharmacological treatment of insomnia

Pharmacological treatment of insomnia includes benzodiazepines, benzodiazepines agonists, antidepressants, antipsychotics, antihistamines, phytotherapeutic substances and melatonin (Riemann et al., 2017). In 2014, Suvorexant, a reversible dual orexin receptor agonist, was approved by the U.S. Food & Drug Administration (FDA) and added as a possible pharmacological treatment for insomnia (Traynor, 2014).

Benzodiazepines and benzodiazepines agonists are the most prescribed classes of medication for insomnia (Riemann et al., 2015). These drugs bind to benzodiazepine receptor binding sites of the γ -aminobutyric acid (GABA) A receptor, which increases GABA inhibition in brain regions that promote arousal (ie: brain stem and hypothalamus) (Riemann et al., 2015). These classes of drugs are safe and effective for the short term treatment of acute insomnia (≤ 4 weeks) but their long term use is associated with great risk of tolerance and dependence (Riemann et al., 2017, 2015). In fact, millions of people worldwide are dependent on these drugs and suffer from long term side effects and increased morbidity and mortality (Ashton, 2005; Riemann et al., 2015). Moreover, a recent meta-analysis reported that more than 60% of the effectiveness of benzodiazepines and benzodiazepine agonists are due to placebo effect (Winkler & Rief, 2015). This placebo effect was proven using both subjective and polysomnography measures of sleep (Riemann et al., 2017; Winkler & Rief, 2015). Consequently, the use of these drugs can only be used in short term if CBT-I is ineffective or unavailable but its long term use is not recommended (Riemann et al., 2017, 2015).

Alternatively, antidepressants can be prescribed to for short term treatment of insomnia (Riemann et al., 2017, 2015). Dosages for antidepressants to treat insomnia are less than the recommended doses for depression (Riemann et al., 2017). However, the magnitude of antidepressants efficiency remains controversial. While two meta-analyses reported that antidepressants efficiency is less than benzodiazepines (Buscemi et al., 2007; Riemann et al., 2017; Winkler, Auer, Doering, & Rief, 2014), others report positive effects (McCleery, Cohen, & Sharpley, 2014; Yeung, Chung, Yung, & Ng, 2015). Even though antihistamines and antipsychotics are being prescribed to treat insomnia, there is very low quality evidence about their efficacy. Also, they have major side effects such as remission of insomnia after withdrawal, liver dysfunction, and heart rhythm disturbances (Riemann et al., 2015); therefore, they are not

recommended treatments of insomnia. Similarly, the low quality evidence of the use of melatonin and phytotherapy to treat insomnia limits their recommendation (Riemann et al., 2017).

To overcome these limitations and negative side effects, Suvorexant, a reversible dual orexin receptor agonist, was introduced to the U.S. market since 2014 as a potential insomnia treatment. Orexin is a neuropeptide secreted from the lateral hypothalamus neurons, known for the role it plays in regulating the sleep-wake cycle, particularly in maintaining the wake state (Krystal, Benca, & Kilduff, 2013). There are two types of orexin neuropeptides, orexin-A (OXA) and orexin-B (OXB). Both types act with different affinities through binding to two G-protein coupled receptors, OX1R and OX2R. Suvorexant binds reversibly to these two receptors and inhibits the activation of the arousal system, which facilitates sleep onset and sleep maintenance (Kishi, Matsunaga, & Iwata, 2015). A meta-analysis conducted on four clinical trials concluded that Suvorexant is effective in treating insomnia and it is better tolerated compared to the other pharmacological treatments in the market (Kishi et al., 2015). However, Suvorexant has major side effects such as next-morning somnolence and safety as seen in driving tests, with possible signs of muscle weakness, weird dreams, sleep walking, other night time behaviors and suicidal ideation (Jacobson, Callander, & Hoyer, 2014).

1.3.3 Limits of the current insomnia treatments

Meta-analyses have shown that for acute treatment of insomnia, CBT-I has equal efficiency to pharmacological treatment and for long-term treatment CBT-I is more effective (Smith et al., 2002). Despite this well documented efficacy of CBT-I, some questions remain unanswered. In fact, there is not enough evidence on the effect of CBT-I on long-term health outcomes (Morin, 2015). In other words, it remains unclear if treating insomnia disorder with CBT-I also decreases the risk for hypertension, depression and occupational disability (Morin, 2015). Moreover, 40% of CID patients treated with both CBT-I and medication do not sustain remission past six months (Morin et al., 2009).

In addition, CBT-I does not seem to be equally efficient for all types of insomnia (Vgontzas et al., 2013). Insomnia patients with the most severe phenotype of the disorder, with short sleep duration, are less responsive to CBT-I than those with normal sleep duration

(Bathgate et al., 2017). It is proposed that insomnia patients with short sleep duration might respond better to biologically-based treatments (Vgontzas & Fernandez-Mendoza, 2013). However, current pharmacological treatments are not convenient to all patients and have major adverse effects such as medication dependency (Ashton, 2005). Insomnia patients with comorbid depression and who experienced the onset of any of these disorders in childhood are less responsive to antidepressant medication compared to those with adulthood onset (Edinger et al., 2016). Hence, genetic studies can lead to better understanding the biological differences in this heterogeneous group of insomnia patients, which will optimize future treatment choices with less severe side effects. Genetic studies will also advance the field towards precision medicine, which will allow the identification of optimal treatments for patients based on their genetic screening.

2. Theoretical models of insomnia etiology

2.1 The "3P" model

The 3P model, proposed by Arthur Spielman (1987), is one of the most influential models of the etiology of insomnia (Spielman et al., 1987). The 3P model explains the factors that contribute to the development and the maintenance of insomnia. The 3P model suggests that insomnia results from the interaction between predisposing, precipitating and perpetuating factors (Figure 2). Predisposing factors are biological vulnerabilities that confer risk for insomnia (ie: genetic susceptibility); therefore, they are present before the manifestation of insomnia. When these predisposing factors interact with precipitating factors (ie: stressful life events), it is hypothesized that the risk of insomnia increases in vulnerable individuals. Finally, perpetuating factors are maladaptive behaviors ie daytime napping and prolonged stay in bed (Riemann et al., 2010) and beliefs that contribute to the persistence of insomnia over time. Perpetuating factors were further studied by the hyperarousal model hypothesizing that cognitive aspects such as hyperarousal (Riemann et al., 2010) and attention biases (Woods, Marchetti, Biello, & Espie, 2009) lead to the maintenance of insomnia over time.

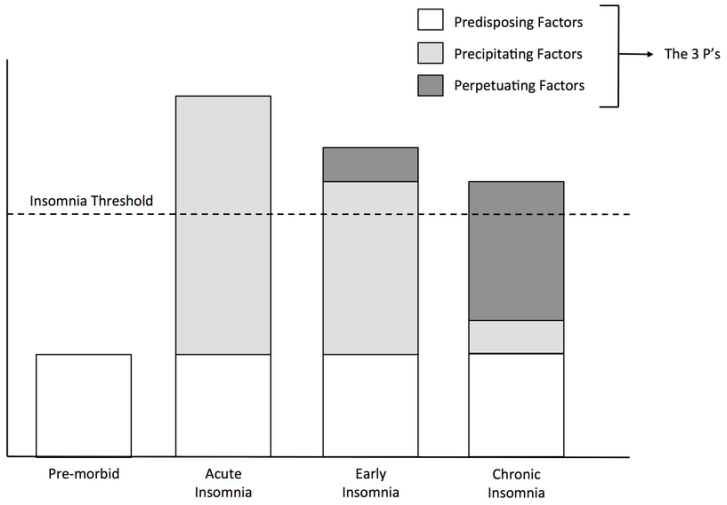

Figure 2. Representation of the "3P" Model of insomnia (Spielman et al., 1987)

2.2 The hyperarousal model of insomnia

2.2.1 What is the hyperarousal model of insomnia?

The hyperarousal model is inspired by Spielman behavioral paradigm explained above. The hyperarousal model expands the behavioral perspective by suggesting that conditioned arousal (somatic, cognitive and cortical activation that interfere with one's ability to disengage from the environment) may also act as a perpetuating factor (Levenson, Kay, & Buysse, 2015; Riemann et al., 2010). Conditioned arousal refers to classical conditioning by which the sleep environment (ie: bed and bedroom) and sleep circumstances become stimuli of arousal instead of de-arousal (Riemann et al., 2010). In fact, enhanced sensory processing at sleep onset and during sleep inhibit insomnia patients from disengaging from the environment, which leads to difficulties initiating or maintaining sleep. This constant alertness can also explain the discrepancy between objective polysomnography sleep measures and subjective reports of wake observed in paradoxical insomnia (Perlis, Giles, Mendelson, Bootzin, & Wyatt, 1997; Riemann et al., 2010). Physiologic hyperarousal can be in both central (cortical) (Perlis et al., 1997; Riemann et al., 2010) and peripheral (autonomic) nervous systems (Bonnet & Arand, 2010; Levenson et al., 2015). Arousal can be measured via cortisol level, heart rate variability, EEG and/or self-reports (Levenson et al., 2015).

Espie and colleagues (2006) also added a new perspective to the hyperarousal model called the AIE (attention-intention-effort) (Espie, Broomfield, MacMahon, Macphee, & Taylor, 2006). The AIE perspective suggests that patients' focused attention on sleep and their explicit intention to fall asleep lead to the development and maintenance of insomnia over time. In fact, selective direct attention to sleep counters the natural automaticity and involuntary process of sleep, which leads to maladaptive sleep preventing behavior.

2.2.2 The hyperarousal model explains the link between insomnia and other sleep and psychiatric disorders

The hyperarousal model also explains the link between insomnia disorder and other sleep and psychiatric disorders. As shown in neuroimaging studies of insomnia hyperarousal, patients with RLS also display arousal sleep disturbance related to the balance between glutamate and

GABA (Allen, Barker, Horská, & Earley, 2013; Spiegelhalder et al., 2016). The hyperarousal model also suggests that through classical conditioning, insomnia patients develop depression via learned helplessness (Riemann et al., 2010). Finally, the model demonstrates that insomnia patients are also at risk to suffer from anxiety as a consequence of their sleep related anxiety (Riemann et al., 2010). Consequently, the mechanism of stress is used by research studies to identify subjects who are likely to suffer from insomnia (Drake, Pillai, & Roth, 2014) and insomnia patients with low resilience (Palagini et al., 2018).

2.3 Stress diathesis model

The stress-diathesis model is the most robust etiological model of many psychiatric disorders such as in depression (Hammen, 2005). This model explains how a disorder results from the interaction between genetic predisposition and environmental stressors. Even though this model is incorporated in the 3P model (Figure 1), few studies examined how stressful life events trigger the onset of insomnia.

Studies on the stress-diathesis model of insomnia used sleep reactivity (sleep disturbance in response to a sleep challenge) to assess insomnia pre-morbidity (state that precedes the onset of a disorder) (Drake et al., 2014). The choice of using sleep reactivity was based on previous sleep research conducted on healthy participants. These studies indicated that sleep reactivity is subject to individual differences. Indeed, healthy participants exhibited different stress levels in response to four types of events that caused sleep disruption: first night effect, caffeine administration and advanced sleep phase (by 3 and 6 hours) (Bonnet & Arand, 2003; Drake, Jefferson, Roehrs, & Roth, 2006). In these studies, stress level was assessed using Ford Insomnia in Response to Stress Test (FIRST; questionnaire composed of nine items which assess the likelihood of experiencing sleep disturbance in response to common environmental stressors), nocturnal polysomnographic recordings, multiple sleep latency test (MSLT), performance testing, metabolic and heart-rate observations (Bonnet & Arand, 2003; Drake, Jefferson, Roehrs, & Roth, 2006).

Results of these studies show that the subgroup of healthy participants who displayed the highest level of stress in response to several stressful conditions were likely to experience sleep disruption (Bonnet & Arand, 2003; Drake et al., 2006). A subsequent study confirmed that individuals displaying the highest sleep reactivity have two times higher risk of

developing insomnia over a period of twelve months compared to good sleepers (Drake et al., 2014). Also, sleep reactivity seems to be a precipitant of depression, as mediated by insomnia (Drake et al., 2014).

Additionally, sleep reactivity plays a role in the maintenance of insomnia. In a group of patients diagnosed with insomnia disorder, sleep reactivity correlated to low resilience (psychobiological determinant of one's capacity to adapt successfully to stressful events) (Palagini et al., 2018). Overall, the theoretical models of insomnia suggest that there is a strong role for stress-response and predisposing factors, such as genetics, in the etiology of insomnia. In the following section we will review additional evidence of insomnia heritability that stems from family and twin studies. Particular focus will be presented regarding the link between genetic predisposition and age of onset, specific family members and stress induced insomnia.

3. Evidence of insomnia heritability

3.1 Family studies of insomnia

Familial aggregation is the clustering of disease within families (Matthews, Finkelstein, & Betensky, 2008). Familial aggregation studies determine one's risk to have a disease, given its presence in one or more family members, relative to others without a family history. If a disease is influenced by genetic factors, family members of an affected individual will be more likely to be affected compared to relatives of non-affected individuals (Lind & Gehrman, 2016). As the degree of relatedness is closer, it is expected that the genetic relationship increases, and family members will be more alike (Lind & Gehrman, 2016).

A positive family history of insomnia is common among insomnia patients. In the province of Québec, 35% of patients consulting for insomnia in a sleep clinic report positive family history of sleep disturbances (Bastien & Morin, 2000; Beaulieu-Bonneau, LeBlanc, Mérette, Dauvilliers, & Morin, 2007). The highest sleep disturbance among relatives was insomnia (76%) and 7% of the other reported sleep disturbances included apnea, RLS and daytime sleepiness (Bastien & Morin, 2000). Another study listed the prevalence of the other sleep disturbances among first degree relatives of insomnia patients as follows: sleep apnea (5%), RLS (3%) and excessive daytime sleepiness (2.4%) (Beaulieu-Bonneau et al., 2007). The same research group evaluated the risk factors of insomnia in a population-based sample. They observed that participants with positive family history of insomnia are three times more likely to experience new onset of insomnia over a period of twelve months compared to those without family history of insomnia (LeBlanc et al., 2009). This robust familial aggregation was stable even after adjustment of shared environment and socioeconomic factors (Zhang et al., 2009), which underscores the important role of genetic factors. Incidence of familial cases of insomnia is reported to be similar between males and females (Jarrin et al., 2017). Furthermore, positive family history of insomnia seems to be more frequent in individuals with childhood onset insomnia compared to adulthood onset.

3.1.1 Higher reports of positive family history before mid-adulthood

Three studies reported that positive family history of insomnia is more frequent in patients with childhood onset insomnia compared to adulthood onset insomnia (Bastien & Morin, 2000; Hauri & Olmstead, 1980). In fact, one study reported that patients with insomnia onset before the age of 18 have higher family history of insomnia compared to those with adulthood onset, 55% versus 39% respectively (Hauri & Olmstead, 1980). Similarly, another study reported higher familial incidence in patients with insomnia onset in childhood, adolescence and early adulthood compared to middle age or late life onset, 33 to 30% versus 13 to 25% respectively (Bastien & Morin, 2000). Finally, Dauvillers and colleagues reported that cases of familial insomnia are more likely to exist in those with early onset compared to sporadic insomnia (Dauvilliers et al., 2005).

3.1.2 The mother: most affected family member

Studies using different ethnic groups and different medical cases (InsomniaDisorder-only vs InsomniaDisorder+PsychiatricDisorder) report that the most frequently affected family member by insomnia is the mother (Bastien & Morin, 2000). Similar to a French Canadian cohort (Bastien & Morin, 2000), a French cohort (France) showed that 42% of patients with InsomniaDisorder-only and 45.5% of those with InsomniaDisorder+PsychiatricDisorder report that the family relative the most affected is the mother (Dauvilliers et al., 2005). In the latter study, the ratio of maternally versus paternally transmitted cases is at 1.85 in both InsomniaDisorder-only and InsomniaDisorder+PsychiatricDisorder (Dauvilliers et al., 2005). Two Chinese cohorts also suggested that there is stronger maternal than paternal association of familial insomnia (Wing et al., 2012; Zhang et al., 2009). Despite this evidence, the mode of inheritance of insomnia does not seem to be linked to the X (female sex) chromosome.

3.1.3 Increased risk of stress-induced insomnia and pre-sleep somatic arousal in offspring of parents suffering from stress-induced insomnia

The vulnerability to develop insomnia is present in both family members of insomnia patients and in the offspring of individuals with stress induced insomnia. A nuclear family study (estimating heritability rate based on the two parents and one offspring) has shown that 29% of stress-induced insomnia is heritable. Further, offspring of one or two parents with stress-induced

insomnia had threefold to sevenfold risk of having similar sleep reactivity as their parent(s) following stressful events (Fernandez-Mendoza et al., 2014). Mothers with such stress-induced insomnia were likely to have an offspring with increased anxiety levels. Fathers with stress-induced insomnia were likely to have offspring with high pre-sleep cognitive arousability (Fernandez-Mendoza et al., 2014). There is also evidence supporting that sleep-related stress is heritable.

The heritability rate of sleep related stress in non-insomnia participants is 37% (Drake, Scofield, & Roth, 2008). Hence, vulnerability to sleep disturbances caused by stressful events is common in the general population. Since participants in this study were not complaining of insomnia, this data presumes that there is an interaction between environmental and genetic factors that predisposes to insomnia disorder.

Overall, familial aggregation studies provide significant evidence of the heritability of insomnia disorder. Nevertheless, a major limitation of the family study approach is that familial aggregation studies do not discriminate between genetic or shared environmental factors (Lind & Gehrman, 2016). Consequently, twin studies are needed to disentangle these two factors (Lind & Gehrman, 2016).

3.2 Twin studies of insomnia

Twin studies examine the degree of genetic and environmental influences on the disorder of interest by comparing identical MZ, theoretically sharing 100% of the genome, to non-identical dizygotic twins DZ, sharing on average 50% of their genes (Lind & Gehrman, 2016). Increased concordance among MZ compared to DZ twins is suggestive of genetic contribution (Zondervan & Cardon, 2007). Typically, the MZ correlation is twice the correlation between DZ twins for traits that have strong genetic basis (Lind & Gehrman, 2016).

Twin studies report that the heritability rate of insomnia is moderate; reported estimates of the additive genetic variance of insomnia ranged from 0.22 (Lind, Aggen, Kirkpatrick, Kendler, & Amstadter, 2015) to 0.57 (Watson, Goldberg, Arguelles, & Buchwald, 2006). The difference in the heritability rate between studies is explained by methodological differences. While Lind et al. (2015), which reported the lowest heritability rate, used the Symptom

Checklist-90 questionnaire evaluating insomnia symptoms in adults in the past month, Watson et al. (2006), reporting the highest heritability rate, estimated the heritability of childhood onset insomnia and assessed insomnia using the following question: "How often do you have trouble falling asleep or staying asleep?".

Despite the methodological differences between studies, they have consistently shown that the MZ correlation is higher than DZ (Barclay, Gehrman, Gregory, Eaves, & Silberg, 2015; Drake, Friedman, Wright, & Roth, 2011; Heath, Kendler, Eaves, & Martin, 1990; Hublin, Partinen, Koskenvuo, & Kaprio, 2011; Lind et al., 2015; McCarren, Goldberg, Ramakrishnan, & Fabsitz, 1994; Watson et al., 2006). Some studies have even shown that the MZ correlation is twice or more the DZ twins correlation (Drake et al., 2011; Heath et al., 1990; Hublin et al., 2011; McCarren et al., 1994; Watson et al., 2006), which highlights the important role of genetic variance in the predisposition of insomnia.

3.2.1 Stability of insomnia heritability rate over the life span and between sexes

Evidence of the stability of insomnia heritability across time comes from children and adult twin studies. A twin study examined the stability of insomnia in children longitudinally at four ages: 8, 10, 14 and 15 years (Barclay et al., 2015). These results suggest that the genetic influence at the age of 8 impacts the subsequent timeframes. Interestingly, new genetic variance came into play at the age of 10, contributing to the presence of insomnia in adolescence. Hence, there are stable genetic influences that exist since the age of 8 and new ones interfere at the age of 10 (Barclay et al., 2015). Similarly, adulthood longitudinal twin studies have shown that the heritability of insomnia is consistent across time (Hublin et al., 2011; Lind et al., 2015). Further, twin studies conducted in youth (Gehrman et al., 2011; Gregory, Rijsdijk, Dahl, McGuffin, & Eley, 2006), young and senior adults (Gregory et al., 2016; Lind et al., 2017) also suggest that the shared genetic variants between insomnia disorder and MDD (genetic correlation ~0.6) are stable across the life-span.

Genetic stability over time is equivalent for both, females and males (Hublin et al., 2011; Lind et al., 2015). Even though there are no sex-based genetic differences, some studies reported that the heritability rate of insomnia in women is higher than in men (Drake et al., 2011; Lind et al., 2015). Drake et al. (2011) argued that this sex difference is mediated by gender differences

in sleep reactivity, showing that women experience more sleep disruption due to stressful events than men (Drake et al., 2011).

Taken together, familial aggregation studies and twin studies provide evidence of the heritability of insomnia disorder and its stability across time (Barclay et al., 2015; Hublin et al., 2011; Lind et al., 2015). Positive family history of insomnia is more common in first degree relatives of insomnia patients, especially in individuals with childhood onset insomnia (Riemann et al., 2015). Twin studies disentangled the genetic and environmental factors arguing that the heritability rate of insomnia is around 50%, which is equivalent to the heritability rate other sleep disorders (refer to introduction) (Riemann et al., 2015). Even though there is no statistically significant difference in the heritability rate of insomnia between sexes, reported heritability rate of insomnia is sometimes higher in women. Also, the mother is the most affected family member across ethnic groups and across medical conditions (Bastien & Morin, 2000; Dauvilliers et al., 2005; Wing et al., 2012; Zhang et al., 2009). Hence, investigation of sex differences need to be further studied in the future studies. Finally, we have seen that insomnia shares some genetic factors with other sleep and psychiatric disorders (ie: MDD). Positive family history of other sleep disorders (ie: sleep apnea and RLS) exists in insomnia patients (Bastien & Morin, 2000; Beaulieu-Bonneau et al., 2007).

4. Genetic Studies of insomnia

Candidate gene studies and genome wide association studies (GWAS) have contributed greatly to our understanding of biology and mechanisms of disease in humans (Lind & Gehrman, 2016). A comparison between the two basic types of genetic studies is presented in Table 1. Briefly, in candidate gene studies, genes of interest are identified a priori based on existing knowledge of biology and potential mechanisms (Lind & Gehrman, 2016). For instance, genes of interest can be identified for the role they play in a neurotransmitter system or based on animal studies or GWAS findings. Usually, the sample size of these studies includes hundreds of participants. Candidate gene studies compare the frequency of a genetic variation between affected and unaffected individuals or between different levels of symptoms (Lind & Gehrman, 2016). Genetic variations are base pair changes at a specific position in the gene, called single nucleotide polymorphism (SNP). SNP frequency differs between ethnic groups and between disease status (affected vs unaffected) (Lind & Gehrman, 2016). In a case-control design, we compare the minor allele frequency (MAF) to assess if the frequency of either of the SNP alleles (or genotypes) are altered in cases versus controls (Lind & Gehrman, 2016). Despite the popularity of candidate gene studies, the prior choice of gene does not lead to the discovery of new pathways, which limits what is believed to be "biologically plausible". In addition, the majority of candidate genes have failed to replicate, which can be due to phenotypic heterogeneity and false positives (Lind & Gehrman, 2016).

In contrast, GWAS are conducted with no prior hypotheses which allows to identify novel genes that one would not have hypothesized to be related to the phenotype of interest (Zondervan & Cardon, 2007). This methodology is deemed to be unbiased and results in the assessment of thousands to millions of SNPs (Lind & Gehrman, 2016). Usually, GWAS sample sizes include thousands of individuals. GWAS association analyses are obtained either from a chip of a specific set of SNPs or from whole genome-sequencing (Lind & Gehrman, 2016). However, this method is not without limitations. GWAS results can be false positives as a consequence to the large number of statistical tests that are run at once or due to population stratification (Lind & Gehrman, 2016). Consequently, independent replication of GWAS findings in smaller, but more precisely phenotyped cohorts is mandatory (Gehrman et al., 2013).

	Candidate Gene Studies	Genome Wide Association Studies
Hypothesis based	Yes	No
Sample Size	Hundreds	Thousands
Tested SNPs	A few	Thousands/ millions
Advantage(s)	• Quality of phenotyping is easier due to smaller sample sizes • Higher statistical power for each SNP	• Identify novel genes • Unbiased by prior knowledge, since all of the genes are being tested
Limitation(s)	• Prior gene choice • Failure to replicate findings (false positives or phenotypic heterogeneity)	False positives due to: • Large number of statistical tests run at once • Population stratification • Large sample sizes are required, reducing the possibility for high quality phenotyping

Tableau I.Comparison between candidate gene studies and genome wide association studies (GWAS). Abbreviations: SNP=single nucleotide polymorphism.

4.1 Candidate gene studies of insomnia

Studies on the predisposing factors of insomnia are scarce and have smaller sample sizes compared to genetic studies of psychiatric disorders (Gehrman et al., 2013; Lind & Gehrman, 2016). Candidate gene studies of insomnia investigated genes involved in the regulation of circadian rhythms and others related to the neurotransmitter systems involved in sleep-wake regulation such as serotonin and GABA pathways (Gehrman et al., 2013; Lind & Gehrman, 2016). The overview of these studies will provide an example of how candidate gene studies of insomnia can be hard to replicate due to the heterogeneity in phenotyping between studies.

4.1.1 Chronic insomnia disorder and circadian genes

Circadian genes (*CLOCK*, *TIMELESS* and *PERIOD*) were used as a starting point to assess the genetics of insomnia due to the interplay between circadian and sleep mechanisms (Gehrman et al., 2013). Studies that tested the role of circadian genes in insomnia did not assess insomnia directly. The majority of these studies evaluated sleep of patients with psychiatric disorders and results were mixed (Serretti et al., 2003, 2010; Utge et al., 2010). In fact, Serretti et al. (2003) found higher recurrence of insomnia in MDD patients with the homozygotes C variant of 3111T/*C CLOCK* (Serretti et al., 2003). However, the same research group was not able to replicate this finding in another sample of MDD patients with sleep disturbances (Serretti et al., 2010). Genetics of MDD patients with sleep problems was also studied in a large cohort from Finland (Utge et al., 2010). This study examined 113 SNPs across 18 clock genes and found an association between *TIMELESS* gene and early morning awakenings in male MDD patients only (Utge et al., 2010). Nonetheless, this finding was not replicated by other studies.

PERIOD (*PER*) genes -circadian rhythm related genes that contribute to individual differences in sleep timing- were also associated to insomnia symptomatology (Brower et al., 2012; Gehrman et al., 2013; Li et al., 2015; Lind & Gehrman, 2016; Viola et al., 2007). Viola and colleagues (2007) showed that healthy participants with the short allele of *PER3* gene (*PER3* 4/4) had longer sleep latency and less slow wave sleep compared to those with the long allele (*PER3* 5/5) (Viola et al., 2007). This finding was concordant to what was found in patients with alcohol dependence. Patients with *PER3* 4/4 genotype had the most severe insomnia symptoms compared to *PER3* 4/5 and *PER3* 5/5 carriers (Brower et al., 2012). Eventhough this finding

was never tested in a cohort of patients with insomnia disorder, another *PER* gene was associated to insomnia in a Chinese cohort.

PER2 gene is suggested to be mediating the interaction between work stress and the risk of insomnia. A Chinese study showed that Chinese workers with the C allele of *PER2* gene have five times higher risk of having insomnia than controls (Li et al., 2015). This allelic effect increased when combined to high work stress. In fact, those with high work stress and AC genotype were fifteen times more likely to have insomnia compared to those with AA genotype and low work stress (Li et al., 2015). These findings were never replicated by other studies and insomnia was assessed using Athens insomnia scale. Moreover, generalizability of these results are limited because the association between insomnia and *PER2* gene was not assessed in other ethnic groups than Chinese. Consequently, future genetic studies need to replicate the finding in patients with insomnia disorder and in other ethnic groups. Based on these studies, Barclay and colleagues (2011) tested the link between *PER3* and *CLOCK* genes and sleep quality (using the Pittsburgh Sleep Quality Index) but failed to replicate previous findings (Barclay et al., 2011). They argued that the effects of these genes on sleep quality are small and mixed findings between studies may be related to population composition (Barclay et al., 2011). In fact, we have seen that previous studies were focused on patients with MDD, alcohol dependence or they were questionnaire based. In addition to the circadian genes, other candidate genes studies of insomnia investigated serotonin and GABA pathways. The choice of these pathways was based on the role they play in sleep-wake regulation and because they are targeted by the most common insomnia pharmaceutical drugs.

4.1.2 Chronic insomnia disorder and serotonin (5-HT) pathway

The link between CID and the serotonin pathway is interesting for three main reasons. First, serotonin (5-HT) plays a role in sleep. In fact, 5-HT is a monoamine, group of neurotransmitters that are wake-promoting that receive excitatory input from the hypothalamic hypocretin/orexin system (Schwartz & Kilduff, 2015). Second, the third insomnia GWAS by Amin et al. (2016) suggested the implication of monoamines in insomnia disorder (Amin et al., 2016). Third, serotonin transporter (5-HTT) expression modulates serotonin selective reuptake inhibitors (SSRI), which is the primary pharmaceutical treatment for MDD and often prescribed

to treat insomnia disorder (Nautiyal & Hen, 2017; Riemann et al., 2017). Consequently, the serotonin transporter linked polymorphic region (5-HTTLPR) of the serotonin transporter gene -encoding 5-HTT protein and influencing synaptic serotonin levels- is an interesting genetic target for understanding the common biological basis between both disorders, CID and MDD (Harvey et al., 2014).

In a pharmacogenetic study of MDD, patients with the short (S) allele of 5-HTTLPR had greater risk of developing new or worsening insomnia and showed greater agitation with fluoxetine treatment compared to the long allele carriers (Perlis et al., 2003). This finding was replicated in a German cohort of CID patients (Deuschle et al., 2010). Nevertheless, the statistically significant difference between insomnia cases and controls at the genotype analysis (SS vs SL vs LL) was borderline, chi-square p-values of 0.052 (Deuschle et al., 2010). Furthermore, possible link between 5-HTTLPR, job-related stress, and the risk for insomnia was explored in a group of Chinese workers (Huang et al., 2014). This study reported that those with the 5-HTTLPR short allele had six times higher risk of developing insomnia when exposed to high work stress level compared to those with the long allele. Finally, a study conducted on community-dwelling individuals has shown that the 5-HTTLPR short allele is associated with sleep onset disturbance (Polito et al., 2015). Moreover, 5-HTTLPR genotype mediated the association between sleep onset latency and depressive symptoms (Polito et al., 2015). Taken together, only one study investigated the link between CID and 5HTTLPR. However, this study was underpowered (n=157) and genotyping results were borderline (Deuschle et al., 2010). Thus, assessment of the role of 5-HTTLPR in a larger CID cohort is needed.

4.1.3 Chronic insomnia disorder and γ -aminobutyric acid (GABA) pathway

The GABA system is another relevant neurotransmitter for the role it plays in sleep. In fact, GABA is the main inhibitory neurotransmitter in the brain and sleep-promoting nuclei are GABAergic in nature (Schwartz & Kilduff, 2015). These nuclei are found in the preoptic area, brainstem and lateral hypothalamus. A decrease in GABA function could lead to excitation of wake-promoting neurons instead, which leads to sleep disorders such as CID (Schwartz & Kilduff, 2015). To treat these dysfunctions, sleep medications such as benzodiazepines target

GABA A receptors (Bateson, 2004), which makes GABA A receptor genes relevant genetic candidates to understand the etiology of insomnia. Further, Amin et al. (2016) GWAS finding also suggested the implication of GABA in insomnia disorder (Amin et al., 2016). Despite its relevance, few studies have investigated the link between GABA A receptor genes and CID.

The four genetic studies that suggest a link between GABA receptor genes and insomnia did not assess insomnia directly (Agosto et al., 2008; Buhr et al., 2002; Feusner et al., 2001; Uhart, McCaul, Oswald, Choi, & Wand, 2004). The first study used a population of patients with post-traumatic stress disorder and phenotyped insomnia using the General Health Questionnaire (Feusner et al., 2001). Results suggest that heterozygosity of the GABA A receptor beta 3 subunit gene (*GABRB3*) major allele (G1) is associated to anxiety and insomnia symptoms (Feusner et al., 2001). Another study identified a missense mutation in GABA A receptor gene beta 3 subunit gene (*GABRA3*) in a patient diagnosed with CID with positive family history of insomnia (Buhr et al., 2002). Functional analysis showed a slower rate of the fast phase of desensitization of the beta3 subunit compared to the other GABA A receptor subunits. Moreover, current deactivation of the receptor revealed a slower rate of the fast phase of desensitization; which suggests that decreased GABAergic inhibition is potentially contributing to insomnia (Buhr et al., 2002). Finally, the T allele of GABA A receptor gene 6 subunit (*GABRA6*) was associated to increased blood pressure and activation of the HPA axis in response to psychological stress in healthy white Caucasians (Uhart et al., 2004). The association between GABA A receptor and sleep difficulty was also found in the animal model. Indeed, Drosophila with the mutant GABAA receptor gene, RdlA302S, displayed decreased sleep latency (Agosto et al., 2008).

To sum up, poor phenotying has been a major limitation in all of the above-mentioned studies. Another challenge in conducting candidate gene studies is that one must know which genes to examine while little is known about the underlying mechanisms of insomnia and sleep/wake regulation. To overcome this limitation, a great effort has been made since 2010 to conduct insomnia related GWAS.

4.2 Genome wide association studies of insomnia

To date, five GWAS examined potential gene involvement in insomnia related symptomatology (Amin et al., 2016; Ban, Kim, Seo, Kang, & Choi, 2011; Byrne et al., 2013, 2012; Hammerschlag et al., 2017; Lane et al., 2017). A summary of these GWAS is provided in Table 2. Briefly, the first insomnia related GWAS was conducted on a Korean sample (n=10,038) (Ban et al., 2011). The two lead SNPs reported by this GWAS are rs11208305 in Receptor Tyrosine Kinase Like Orphan Receptor 1 gene (*ROR1*) and rs718712 in Phospholipase C Beta 1 gene (*PLCB1*). These two genes were previously associated to bipolar disorder and schizophrenia respectively (Lind & Gehrman, 2016). However, results did not reach GWAS statistical significance.

The second insomnia related GWAS was conducted in a sample from the Australian twins registry (Byrne et al., 2013). This GWAS assessed the genetics of insomnia factor score and other sleep phenotypes including sleep time, sleep latency, sleep quality, sleep depth and sleep duration. The lead SNP of this GWAS rs7316184 in Calcium Voltage-Gated Channel Subunit Alpha1 C gene (*CACNA1C*), encoding a subunit of voltage-dependent calcium channels, was associated to sleep latency. This gene was previously associated with bipolar disorder (Sklar et al., 2008), schizophrenia (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) and narcolepsy (Shimada et al., 2010). Even though the result of this insomnia GWAS did not reach GWAS statistical significance (Byrne et al., 2013), the replication of the GWAS finding in candidate gene study conducted in a British cohort was successful (Parsons et al., 2013). Moreover, another calcium channel gene, calcium voltage-gated channel subunit alpha1 A gene (*CACNA1A)*, was among the first insomnia related GWAS (Ban et al., 2011). These evidences suggest that *CACNA1C* is involved in mechanisms regulating sleep function but there is not any support of its implication in insomnia disorder. Hence, this is a plausible candidate gene that needs to be further investigated in future genetic studies.

The third GWAS was conducted on seven European cohorts (Amin et al., 2016). This GWAS report significant association between sleep latency (assessed using the Munich Chronotype Questionnaire) and RNA-binding protein fox-1 homolog 3 gene (*RBFOX3*). This

finding was successfully replicated in twelve independent populations including 30,377 individuals, which confirms the robustness of the result. Functional analyses showed that *RBFOX3* gene is expressed in the brain and in the central nervous system. Further, these analyses suggest the involvement of this gene in GABA and monoamine signaling, two crucial neurotransmitters in triggering sleep onset (Amin et al., 2016; Lind & Gehrman, 2016).

Finally, the latest two insomnia GWAS (Hammerschlag et al., 2017; Lane et al., 2017) conducted on the UK Biobank sample associated a variant in Myeloid Ecotropic Viral Insertion Site 1 *(MEIS1*) gene to insomnia complaints. *MEIS1* gene is known for playing a role in development (Toresson, Parmar, & Campbell, 2000) and was previously associated with RLS (Schormair et al., 2017; Winkelmann et al., 2011, 2007, 2016; Xiong et al., 2009; Yang et al., 2011). The GWASs identified lead SNP - rs113851554 - was associated to RLS by Xiong and colleagues (2009) (Xiong et al., 2009) and is within the same linkage disequilibrium block as rs12469063 and rs2300478 (Xiong et al., 2009), two common genetic risk factors for RLS (Winkelmann et al., 2011, 2007; Xiong et al., 2009; Yang et al., 2011). Hammerschlag et al. (2017) conducted conditional phenotypic analysis and estimated that the UK Biobank insomnia trait was confounded by RLS in 12% of cases and 6% of controls, which partially contributed to the significant association they observed (Hammerschlag et al., 2017). However, they argue that there remained a significant effect of *MEIS1* on insomnia that could not be accounted for by this confounding. Therefore, they concluded that *MEIS1* is likely to have pleiotropic effects (independent association) on both RLS and insomnia.

To sum up, insomnia GWAS studies suggest potential contributions of *MEIS1*, *CACNA1A*, monoamine and GABA pathways. However, all of these GWAS phenotyped insomnia poorly (Table 2). Three of these GWAS assessed insomnia using one question, one relied on three questions and the fifth was based on five questions. Moreover, the majority of these findings have not yet been confirmed by replication studies. Hence, validation of GWAS findings is needed in well-phenotyped cohorts of patients with insomnia disorder.

Author (Year)	**Main Finding**	**Sample**	**Insomnia Phenotype(s)**	**Phenotype Assessment**
(Ban et al., 2011)	No GWAS significant results. Top SNPs: ➢ rs11208305 (*PCLB1*: previously associated to schizophrenia) ➢ rs718712 (*ROR1* prior association with bipolar disorder)	Korean epidemiologic study N = 8719 (1439 cases and 7280 controls)	Based on answers to questions as to overall insomnia (difficulty in falling asleep, difficulty in maintaining sleep or returning to sleep after awakening in the middle of the night and early awakening in the morning).	Self report (questionnaire)
(Byrne et al., 2013)	NoGWAS significant results. ➢ rs7316184 *CACNA1C* (previously associated to bipolar disorder and schizophrenia)	Australian twin registry N = 2323	Insomnia Factor score and the following questions: - Sleep time: "On WEEKDAYS after you go to bed, what time do you usually try to get to sleep?" - Sleep latency: "On WEEKDAYS, how long in minutes do you think it usually takes you to fall asleep from when you first try to go to sleep?" (Sleep Latency)	Self report (questionnaire)

			- Sleep Quality: "How would you describe the quality of your usual sleep over the last few months?" (Likert scale: 1(Very good) to 5 (Very poor)) - Sleep depth: "In particular, how would you describe the depth of your sleep?" (Likert scale: 1(Hard to wake) to 3 (easy to wake)) - Sleep duration: "On WEEKDAYS, how long would you usually sleep for?"	
(Amin et al., 2016)	Top SNPS associated to *RBFOX3*9 (involved in GABA and monoaminase): ➢ rs9900428 ➢ rs9907432 ➢ rs7211029	7 European Cohorts N=4 242	Munich Chronotype Questionnaire (used by the 7 cohorts) Sleep latency: "How long [does it] take [you] to fall asleep on free and workdays?"	Self Reported (Questionnaire)
(Lane et al., 2017)	Top SNPS: ➢ rs113851554 *MEIS1* ➢ rs145258459 *TMEM132E*	UK Biobank N=112 586 (European Ancestry)	Insomnia complaint "[Do you have] trouble falling asleep at night or wake up in the middle of the night?"	Self Reported (Questionnaire)

	➢ rs5922858 *CYCL1*			
(Hammerschlag et al., 2017)	Top SNP: ➢ rs113851554 *MEIS1*	UK Biobank N = 113,006 (European Ancestry)	Insomnia complaint "[Do you have] trouble falling asleep at night or wake up in the middle of the night?"	Self Reported (Questionnaire)

Tableau II.Brief description of the five insomnia Genome wide association studies

5. Objective and hypothesis

Considering the limitations of insomnia GWAS and the need to validate GWAS findings using well-phenotyped cohorts of patients with insomnia disorder, we aimed at conducting a candidate gene study to replicate the latest two insomnia GWAS findings (Hammerschlag et al., 2017; Lane et al., 2017). The three major limitations of these two GWASs are: use of a single question (asking participants if they have trouble falling asleep at night or wake up in the middle of the night) to classify subjects as cases or controls for insomnia symptoms, no RLS screening, and no replication of the findings in a cohort diagnosed with insomnia disorder.

Thus, we aimed to evaluate the association between CID and *MEIS1* gene in a large CID cohort. We hypothesized that if there is an independent association of *MEIS1* with insomnia, we expect to see similar minor allele frequencies between *MEIS1* genetic variants in the two groups, CID-only and CID+RLS.

6. Article

Reassessing GWAS findings for the shared genetic basis of insomnia and restless legs syndrome

by

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Co-author1 realized the following tasks: retrospective analysis of 761 clinical files, recruitment of a 150 insomnia patients, data entry, statistical analyses, literature review, presentation of the data, DNA extraction, genotyping and writing the manuscript.
Co-author2 extracted DNA, did the genotyping and helped writing the genotyping section.
Co-author3 provided the genotyping data of the French Canadian cohort and participated in writing the manuscript.
Co-author4 entered and helped analyzing the data of two RLS questionnaires.
Co-author5 helped in managing some samples and in retrieving DNA samples.
Co-author6 contributed in storing DNA samples.
Co-author7 diagnosed patients and participated in writing the manuscript.
Co-author8 diagnosed patients and participated in writing the manuscript.
Co-author9 wrote the research proposal, managed the project, validated statistical analyses and participated in writing the manuscript.

Abstract

Two genome-wide association studies (GWAS) suggest that insomnia and restless legs syndrome (RLS) share a common genetic basis. While the identified genetic variation in the *MEIS1* gene was previously associated with RLS, the two GWAS suggest a novel and independent association with insomnia symptoms.

To test the potential pleiotropic effect of *MEIS1*, we genotyped three *MEIS1* variants in 646 chronic insomnia disorder (CID) patients with and without RLS. To confirm our results, we compared the allelic and genotypic distributions of the CID cohort with ethnically matched controls and RLS cases in the French Canadian cohort.

The CID cohort was diagnosed by sleep medicine specialists and 26% of the sample received the combined diagnosis of CID+RLS. We find significant differences in allele and genotype distributions between CID-only and CID+RLS groups, suggesting that *MEIS1* is only associated with RLS. Genotype distributions and minor allele frequencies of the three *MEIS1* SNPs of the CID-only and control groups were similar (rs113851554: 5.3% vs 5.6%; rs2300478: 25.3% vs 26.5%; rs12469063: 23.6% vs 24.4%; all $p > 0.05$). Likewise, there were no differences between CID+RLS and RLS-only groups (all $p > 0.05$).

In conclusion, our data confirms that *MEIS1* is a genetic risk factor for the development of RLS but it does not support the pleiotropic effect of *MEIS1* in CID. While a lack of power precluded us from refuting small pleiotropic effects, our findings emphasize the critical importance of isolating CID from other disorders that can cause sleep difficulties, particularly RLS, for future genetic studies.

Keywords

Sleep disorders, sleep genetics, insomnia, RLS, GWAS, phenotyping, MEIS1

Statement of Significance

Genetic studies of insomnia are scarce and phenotypic definitions are heterogeneous. In fact, the majority of insomnia genetic studies focus on insomnia symptoms or measure sleep quality rather than the actual disorder. Moreover, few studies reassessed insomnia related GWAS findings despite the importance of the independent replication. Hence, our study plays a crucial role in revaluating the latest insomnia related GWAS findings while using a large and well phenotyped cohort of CID patients. Our results are not consistent with an independent association between insomnia and *MEIS1* gene, which highlights the importance of using well phenotyped cohorts and the necessity of isolating CID from confounding disorders such as RLS in future insomnia genetic studies.

Introduction

Chronic insomnia disorder (CID) and restless leg syndrome (RLS) are two common sleep disorders in the general population, with a prevalence of 10% (American Academy of Sleep Medicine, 2014; American Psychiatric Association, 2013; Morin, LeBlanc, Daley, Gregoire, & Mérette, 2006; Morin et al., 2011; Simon & VonKorff, 1997) and 2-4% (Allen, Stillman, & Myers, 2010) respectively. CID is characterized by a subjective complaint of poor sleep quality or quantity that is associated to difficulty initiating or maintaining sleep. The sleep disturbance significantly impacts daytime functioning and occurs at least 3 times a week for at least 3 months (American Academy of Sleep Medicine, 2014). CID is often comorbid with other sleep disorders. In fact, sleep initiation difficulty resulting from the leg discomfort observed in about 75% of RLS patients makes RLS a confounding factor of insomnia disorder (Allen et al., 2014; Montplaisir et al., 1997; Ulfberg et al., 2007). RLS is sensorimotor disorder defined by an urge to move legs that primarily occurs during the evening and/or at night. Symptoms intensify during rest or inactivity states and are relieved by movement (American Academy of Sleep Medicine, 2014).

Two genome wide association studies (GWAS) (Hammerschlag et al., 2017; Lane et al., 2017) recently reported that a specific genetic variant in Myeloid Ecotropic Viral Insertion Site 1 *(MEIS1*) gene*,* playing a role in development (Toresson et al., 2000) and previously associated with restless leg syndrome (RLS) (Schormair et al., 2017; Winkelmann et al., 2011, 2007, 2016; Xiong et al., 2009; Yang et al., 2011), is also independently linked to insomnia complaints. The lead single nucleotide polymorphism (SNP) identified - rs113851554 - was associated to RLS by Xiong et al. (Xiong et al., 2009) and is within the same linkage disequilibrium block as rs12469063 and rs2300478 (Xiong et al., 2009), two common genetic risk factors for RLS (Winkelmann et al., 2011, 2007; Xiong et al., 2009; Yang et al., 2011). The key finding of these GWAS (Hammerschlag et al., 2017; Lane et al., 2017) argues that insomnia and RLS share a common genetic basis.

To unravel the genetic relationship between these two intertwined disorders, Lane et al. (Lane et al., 2017) emphasized the necessity of conducting further analyses to determine if shared genetic associations are due to causality, partial mediation or pleiotropy. Hammerschlag et al.

(Hammerschlag et al., 2017) present several lines of compelling evidence to support pleiotropy, among them conditional analyses showing that the previous RLS GWAS leading SNPs (Winkelmann et al., 2007) – rs6710341, rs12469063 and rs2300478 – are not found by the insomnia GWAS. They also conducted a phenotypic analysis to determine the possibility that their findings were influenced by the presence of RLS in the participants. They indeed concluded that RLS contributes to the significant association between *MEIS1* and insomnia; however, this confounding effect is not sufficient to completely account for the association and rs113851554 has an independent effect on insomnia symptoms.

A shared genetic basis for RLS and insomnia would be intriguing for several reasons. A cardinal and common feature between these two disorders is the hyperarousal state that precedes sleep onset. In RLS, leg discomfort occurs (or is maximal) during the evening, at the restful wake state prior to falling asleep (Allen et al., 2014). In insomnia, patients engage in somatic, cognitive and cortical activation that prolongs sleep onset (Riemann et al., 2010). Similar hyperactive phenotype was observed in the animal RLS-model of heterozygous Meis1 knockout mice (Salminen et al., 2017; Spieler et al., 2014). Further, Meis1 knockout mice had slightly less delta power during sleep compared to the wild-type mice (Salminen et al., 2017), which is concordant with some previously reported observations in insomnia patients (Riemann et al., 2010). Hence, a plausible etiologic hypothesis is that one of the contributing genetic factors of these disorders could be *MEIS1* haploinsufficiency.

However, both GWAS have a major limitation. Despite the methodological differences between the two GWASs (Hammerschlag et al., 2017; Lane et al., 2017), both studies used of a single question asking participants if they have trouble falling asleep at night or wake up in the middle of the night to classify subjects as cases or controls for insomnia symptoms. Although a single question may be adequate to identify individuals with general sleep difficulties, it may not be sufficient to isolate insomnia from other self-reported conditions related to sleep quality, such as RLS. Additionally, this question does not clearly differentiate acute insomnia symptoms from the diagnostic criteria of CID (American Academy of Sleep Medicine, 2014), such as the duration of sleep difficulties and whether symptoms are accompanied by distress and/or daytime impairment.

Hence, the primary objective of this study is to evaluate the association between CID and *MEIS1* in a clinical setting. If there is an independent association of *MEIS1* with insomnia, we expect to see similar minor allele frequencies between *MEIS1* genetic variants in CID patients with and without RLS.

Methods

Participants

A total of 705 patients from the province of Québec with a diagnosis of primary CID recruited at the sleep clinic at the Centre d'Études Avancées en Médecine du Sommeil were considered for this study. All patients were interviewed by a sleep specialist prior to Cognitive Behavioral Therapy for insomnia and met the diagnostic criteria of primary CID (American Academy of Sleep Medicine, 2014) as per the normal practice for clinical care for insomnia. RLS diagnoses were made during the medical consultation based on the International RLS Study Group (IRLSSG) diagnostic criteria (Allen et al., 2014). Clinical patients that consented for research were retrospectively assigned to CID-only or CID+RLS (primary insomnia concomitant to RLS) groups by two clinicians specialized in sleep disorders (AD, JM).

Polysomnography (PSG) was used to quantify periodic leg movements in sleep (PLMS), which supports the diagnosis of RLS, and to screen for apnea-hypopnea. Subjects with uncertain RLS diagnosis and those with apnea-hypopnea index (AHI) greater than 15 were excluded. Based on these criteria, 59 subjects were excluded, leaving 646 patients in the CID cohort. Patients were free of any other neurological disease and provided written informed consent.

Polysomnography

Out of the 646 included subjects who met the inclusion criteria, 591 underwent a nocturnal PSG using a standard montage. Periodic leg movements during sleep (PLMS) as well as apnea and hypopnea were scored and analyzed according to standard criteria[23]. Electromyography (EMG) on both tibialis anterior was used to calculate the periodic leg movement index (PLMI). Overlapping movements between the two legs within 0.5 s are counted as one movement. PLM were defined as movements that lasted 0.5 to 10 seconds, were separated by intervals of 5 to 90 seconds and occurred in a series of at least four consecutive movements. Leg movements were detected with an increase in EMG ≥ 8 μV above the resting baseline for movement onset and a decrease in EMG < 2 μV above the resting level for movement offset.

AHI is the sum of the number of apneas and hypopneas per hour of sleep and was measured from oronasal flow and thoracoabdominal movements. Apnea was defined by the absence (≥90%) of airflow for more than 10 seconds and hypnopnea as an airflow reduction (≥30%) that lasted more than 10 seconds and resulted in either arousal (while SaO_2<3%) or oxygen desaturation (SaO_2≥4%).

Genotyping

In the CID cohort, genotyping was performed using a standard Taqman assay. Genomic Deoxyribonucleic acid (gDNA) was isolated from the patient buffy coat using FlexiGene DNA Kit (258) (Qiagen, Canada) and following manufacturer's standard protocol. Three SNPs from *MEIS1* gene located on chromosome 2 were examined, rs113851554 (assay ID:C_154329142_10; context sequence: GTATATGTGGAATTTATATGTTTCA[G/T]TTAGGTTGTTCTTATG), rs12469063 (assay ID: C__31123351_10 and context sequence: CAGCCTGCTTCCAGCTGTGGCAGGC[A/G]TGATGCAGTGAATTGCTTTTGAATG) and rs2300478 (assay ID: C__15754717_10 and context sequence: TAAGCCAGTCTTCTTGTTTTCAGTG[G/T]GTCTGTAAGTATCTGGTCAGAGAA) (Viia7 real time PCR, Thermo Fisher, Scientific, Canada). PCR reactions used 5ng of gDNA with the following cycling conditions (Step 1: 95°C for 10 min; Step 2: 95°C for 15 sec and 60°C for 60sec for 50 cycles).

For both groups, CID-only and CID+RLS, genotype distributions were within Hardy-Weinberg equilibrium for the 3 SNPS (rs113851554: $p = 0.37$ and $p = 1$, rs2300478: $p = 0.18$ and $p = 1$, rs12469063: $p = 0.70$ and $p = 0.49$ respectively). For the CID cohort, genotyping failed in 2 subjects for rs113851554 and 2 subjects for rs12469063 (ie the genotyping success rate was (99-100%). Reproducibility of the genotyping (100%) was confirmed by re-genotyping multiple samples within and across assay plates.

Allelic and genotypic distributions of the three SNPs were compared between the CID cohort and French Canadians Cohort (FCC) (486 FCC-controls and 385 FCC-RLS patients) from the province of Québec. The FCC was recruited and diagnosed at the same sleep clinic as the CID-cohort (Winkelmann et al., 2011, 2007; Xiong et al., 2009). RLS cases were diagnosed

according to standard criteria (Allen et al., 2003). Genotyping of the FCC was performed and described previously using the TaqMan SNP assay (Winkelmann et al., 2011, 2007; Xiong et al., 2009) on Applied Biosystems 7900 Fast Real-Time PCR System. Genotyping success rate in the FCC was >98%; genotyping failed in 16 subjects for rs113851554, 17 subjects for rs2300478, and 15 subjects for rs12469063.

Statistical Analysis

Statistical analyses were performed using R software. Independent t-tests were conducted to examine the differences between CID-only and CID+RLS groups in age, AHI, body mass index (BMI) and psychometric scores (Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI) and Insomnia Severity Index (ISI)) (Table 1). Non parametric test (Man-Whitney-Wilcoxon) was used to compare PLMI between the two groups. Chi-square analyses tested sex, genotype and allele distributions differences between groups. Finally, unadjusted and adjusted logistic regression models were used to predict the presence of RLS while using genotype, age and sex as predictors. Associations were presented as odds ratios (OR) and their 95% confidence intervals (CIs). Power calculations were computed using Quanto (http://biostats.usc.edu/Quanto.html).

Results

Twenty-six percent of the CID cohort was diagnosed with CID+RLS (Table 1). This group was significantly older by an average of 4 years and as expected, had higher periodic leg movement index (PLMI) than the CID-only group (27 versus 13 events/hour respectively). Considering the CID-only group mean age (of 49 years), the average PLMI is equivalent to what was previously reported of healthy subjects of equivalent age group (Montplaisir, Michaud, Denesle, & Gosselin, 2000; Pennestri et al., 2006; Youngstedt, Kripke, Klauber, Sepulveda, & Mason, 1998). On average, subjects with CID+RLS reported more anxiety and depressive symptoms than the CID-only group but BAI and BDI scores remained within the mild range for both groups (Beck, Epstein, Brown, & Steer, 1988; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). There were no between-group differences in sex distribution, BMI, insomnia severity index scores or AHI.

In the CID cohort, rs113851554, rs2300478 and rs12469063 minor allele frequencies (MAF) are significantly higher in the presence of RLS compared to its absence (Table 2). Importantly, MAF of the three *MEIS1* SNPs we obtained for the CID-only group are comparable to the MAF found in Canadian (FCC) and European controls (1000 Genomes Project Consortium et al., 2015). In fact, the risk allele frequency of the three SNPs in the two RLS-free groups [CID-only (CID cohort), FCC-controls (FCC)] and population-based European controls(1000 Genomes Project Consortium et al., 2015) (1000 Genomes project) are almost equivalent rs113851554: 5.3% vs 5.6% vs 5.3%; rs2300478: 25.3% vs 26.5% vs 24.8%; rs12469063: 23.6% vs 24.4% vs 23.9% respectively; all p-value > 0.05) (Table 3). In other words, the differences in MAF of rs113851554 between CID-only group (5.3%) and FCC-controls (5.6%) were very small and in a different direction from the effect on RLS. This concordance is also observed at the genotype distribution level (all p-value > 0.05) (Table 4). These results were confirmed with the logistic regression (Table 5); adjusted dominant model showed that the presence of at least one minor allele of rs113851554 increases the risk of RLS by 2.72 times (95% CI = 1.83–4.03, p<0.001). Similarly, adjusted dominant models of the two other SNPs were significantly linked to the presence of RLS (Table 5, Top panel). Unadjusted models had similar findings for the three SNPs. When comparing CID-only to the unaffected FCC controls for the three *MEIS1* SNPs, the MAF of the CID-only was lower than the MAF of unaffected controls, producing non-

significant OR scores less than one. However, our study was not sufficiently powered to find small effect sizes (Table 5, Bottom panel).

Discussion

This is the first study to examine the role of *MEIS1* in patients diagnosed with CID. Our data demonstrate that rs113851554, previously associated with RLS (Schormair et al., 2017; Winkelmann et al., 2011, 2007, 2016; Xiong et al., 2009; Yang et al., 2011) and recently associated with insomnia symptoms in the general population (Hammerschlag et al., 2017; Lane et al., 2017), is not associated with CID in the absence of RLS in our insomnia patients. Further, we did not find an association with two other RLS associated *MEIS1* SNPs (rs12469063 and rs2300478) (Winkelmann et al., 2011) in CID. This lack of association between *MEIS1* and CID is consistent with a recent finding reported by Salminen et al. (2017) which also did not find evidence to support a role for Meis1 deficiency in causing sleep disturbances such as sleep initiation or sleep maintenance difficulties in mice (Salminen et al., 2017). Since our data is not consistent with a pleiotropic effect of *MEIS1* as suggested by the recent insomnia-related GWASs (Hammerschlag et al., 2017; Lane et al., 2017), it is possible that these studies were confounded with the presence of RLS. The possibility of such confounding was directly addressed by Hammerschlag et al. (2017) using a variety of methods such as a conditional phenotypic analysis (Hammerschlag et al., 2017). They estimated that the UK Biobank insomnia trait was confounded by RLS in 12% of cases and 6% of controls which partially contributed to the significant association they observed. However there remained a significant effect of *MEIS1* on insomnia that could not be accounted for by this confounding, and they therefore concluded that *MEIS1* is likely to have pleiotropic effects on both RLS and insomnia. We attempted to verify this finding, but our data is not consistent with this conclusion, as we cannot find evidence for an association between *MEIS1* and CID independent of RLS. The consistency of the results between the two studies (Hammerschlag et al., 2017; Lane et al., 2017) is likely explained by

the fact that both studies used subjects drawn from the same general population cohort (UK Biobank). Therefore, our study further illustrates that the heterogeneity in the phenotyping definitions of insomnia is a major concern in the methodology of the previous genetic studies of insomnia (Gehrman et al., 2013; Lind & Gehrman, 2016).

Limitations also need to be considered while interpreting our results. First, our study is a retrospective study, and the collection of phenotypic data is not completely uniform between all subjects; for example, 9% of our cohort did not have a PSG and not all patients answered all questionnaires. Second, although we had sufficient statistical power to show an association between rs113851554 and RLS (Table 5, top panel), we did not have sufficient statistical power (Table 5, bottom panel) to replicate the effect sizes reported previously from the general population cohort for insomnia symptoms (OR =1.19) (Hammerschlag et al., 2017) or 1.26 (Lane et al., 2017)). We hypothesized that if there exists an association between rs113851554 and insomnia, the effect size should be stronger in a cohort of well-characterized patients with CID. However, the effect size we observed was smaller and in the opposite direction (OR = 0.932) relative to the previous findings. While it still may be statistically possible that there is an association between rs113851554 and insomnia, our data argues that the effect size is likely smaller and less biologically meaningful than previously reported.

It is important to emphasize that GWASs have made significant contributions in identifying genes and pathways involved in sleep and sleep disorders. An important part of this progress however, is the independent replication of GWAS findings in smaller, but more precisely phenotyped cohorts (Gehrman et al., 2013); the independent replication should be considered an integral part of the GWAS methodology. While our well phenotyped data does not conform

to the GWASs finding, it emphasizes the critical importance of isolating CID from other disorders that can cause sleep difficulties, particularly RLS, for future genetic studies.

In summary, we did not find an association between *MEIS1* and CID in our clinical cohort. However, we did replicate a clear association between *MEIS1* SNPs and RLS, even in patients with comorbid CID. Our findings also suggest that population-based cohorts should include psychometric tools with higher sensitivity and specificity in order to better characterize insomnia phenotypes, such as the insomnia severity index (ISI) (Morin, Belleville, Bélanger, & Ivers, 2011) and the Cambridge–Hopkins diagnostic questionnaire for RLS (Allen, Burchell, MacDonald, Hening, & Earley, 2009; Allen et al., 2014).

Abbreviations List

MEIS1, Myeloid Ecotropic Viral Insertion site 1

CID, chronic insomnia disorder

RLS, restless leg syndrome

GWAS, genome-wide association studies

SNP, single nucleotide polymorphism

PSG, polysomnography

PLMS, periodic leg movements in sleep

AHI, apnea-hypopnea index

PLMI, periodic leg movement index

MAF, minor allele frequency

BMI, body mass index

BAI, Beck Anxiety Inventory

BDI, Beck Depression Inventory

ISI, Insomnia Severity Index

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Table 1-Descriptive statistics of CID cohort.

	CID-only				CID+RLS				
	Avg.	**SD**	**Range**	**N**	**Avg.**	**SD**	**Range**	**N**	**p-value**
Age	49	14	14 - 83	476	53	12	14 - 83	170	**0.0008**
Sex, % female	64	NA	NA	476	68	NA	NA	170	0.3299
BMI	26	5	18 -50	421	27	5	18 -51	163	0.1314
BAI	10	8	0 - 47	428	13	10	0 - 52	151	**0.002**
BDI	13	10	0 - 60	427	15	10	0 - 51	153	**0.023**
ISI	17	5	5 - 28	170	18	5	8 - 28	34	0.5167
PLMI	13[a]	19	0-130	424	27	31	0 - 182	167	**1.664e-07**
AHI	1	2	0 - 14	405	1	2	0 - 12	157	0.6718

Abbreviations: BMI = body mass index; BAI = Beck Anxiety Inventory; BDI = Beck Depression Inventory; ISI = Insomnia Severity Index; PLMI = periodic leg movement index; AHI = apnea-hypopnea index; Avg. = average; SD = standard deviation; N = sample size.

[a] The average PLMI in the CID-only is equal to the PLMI of healthy subjects within the same age group(Pennestri et al., 2006)

Table 2-*MEIS1* SNPs genotypic and allelic frequencies.

***MEIS1* SNPs**	**Genotypes/Alleles**	**CID-only n (%)**	**CID+RLS n (%)**	**Chi-square p-value[a]**
rs113851554[T]	GG	428 (90)	128 (76.2)	**4.84e-05**
	GT	46 (9.6)	38 (22.6)	
	TT	2 (0.4)	2 (1.2)	
	G	902 (94.7)	294 (87.5)	**1.62e-05**
	T	50 (5.3)	50 (12.5)	
rs2300478[G]	TT	271 (56.9)	76 (44.7)	**0.021772**
	GT	169 (35.5)	76 (44.7)	
	GG	36 (7.6)	18 (10.6)	
	T	711 (74.7)	228 (67.1)	**0.008343**
	G	241 (25.3)	112 (32.9)	
rs12469063[G]	AA	279 (58.7)	73 (43.2)	**0.001956**
	AG	168 (35.4)	80 (47.3)	
	GG	28 (5.9)	16 (9.5)	
	A	726 (76.4)	226 (66.9)	**0.000767**
	G	224 (23.6)	112 (33.1)	

[a]Including Yate's correction

Table 3-Comparison of the minor allele frequencies (%) of *MEIS1* SNPs between CID cohort (CID-only; CID+RLS), French Canadian Cohort (FCC-controls and FCC-RLS cases) (Winkelmann et al., 2011, 2007; Xiong et al., 2009) and 1000 Genomes Project (1000 Genomes Project Consortium et al., 2015) European population.

	RLS Status	**Allele**	**CID Cohort**		**French Canadian Cohort**		**1000 Genomes Project**		**Chi-square p values**
			N	**%**	**N**	**%**	**N**	**%**	
rs113851554	Negative RLS	G	902	94.7	918	94.4	953	94.7	0.9456
		T	50	5.3	54	5.6	53	5.3	
	Positive RLS	G	294	87.5	612	82.9	NA	NA	0.292104
		T	50	12.5	126	17.1			
rs2300478	Negative RLS	T	711	74.7	703	73.5	757	75.2	0.6764
		G	241	25.3	253	26.5	249	24.8	
	Positive RLS	T	228	67.1	481	63.9	NA	NA	0.320825
		G	112	32.9	271	36.1			
rs12469063	Negative RLS	A	726	76.4	729	75.6	766	76.1	0.917039
		G	224	23.6	235	24.4	240	23.9	
	Positive RLS	A	226	66.9	485	64.8	NA	NA	0.515941
		G	112	33.1	263	35.2			

Table 4-Comparison of MEIS1 SNPs genotype distributions (n(%)) between CID cohort (number of CID-only and CID+RLS patients of each SNP is as follows: rs113851554: 476 and 168; rs2300478:476 and 170; rs1249063: 475 and 169 respectively) and French Canadian Cohort(Winkelmann et al., 2011, 2007; Xiong et al., 2009) (number of FCC-controls and FCC-RLS cases of each SNP is as follows: rs113851554:486 and 369; rs2300478: 478 and 376; rs12469063: 482 and 374 respectively).

	RLS Status	**Genotypes**	**CID Cohort**		**French Canadian Cohort**		**Chi-square p-value**
rs113851554[T]	Negative RLS	GG	428	(90.0)	433	(89.0)	0.7313
		GT	46	(9.6)	52	(11.0)	
		TT	2	(0.4)	1	(0.0)	
	Positive RLS	GG	128	(76.2)	253	(66.0)	0.1537
		GT	38	(22.6)	106	(28.0)	
		TT	2	(1.2)	10	(3.0)	
rs2300478[G]	Negative RLS	TT	271	(56.9)	252	(53.0)	0.1098
		GT	169	(35.5)	199	(41.5)	
		GG	36	(7.6)	27	(5.5)	
	Positive RLS	TT	76	(44.7)	155	(41.2)	0.5942
		GT	76	(44.7)	171	(45.5)	
		GG	18	(10.6)	50	(13.3)	
rs12469063[G]	Negative RLS	AA	279	(58.7)	266	(55.2)	0.1173
		AG	168	(35.4)	197	(40.9)	
		GG	28	(5.9)	19	(3.9)	
	Positive RLS	AA	73	(43.2)	157	(42)	0.6300
		AG	80	(47.3)	171	(45.7)	
		GG	16	(9.5)	46	(12.3)	

Table 5-Logistic regression predicting the risk for RLS or Chronic Insomnia Disorder (CID-only vs CID+RLS, top; CID-only vs FCC controls, bottom) using dominant genetic models for *MEIS1* SNPs. Sex and age are used as predictors in the adjusted model. The 80% Power is the OR at which this study has 80% power to detect a statistical difference based on the sample size. Abbreviations: SNP = single nucleotide polymorphism; OR= odds ratio; CI= confidence interval.

Chronic Insomnia Disorder, without vs with RLS (CID-only vs CID+RLS)

	Unadjusted model		**Adjusted model**		
MEIS1 SNPs	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**80% Power**
rs113851554	2.79 (1.88 – 4.11)	**1.48e-05**	2.72 (1.83 – 4.03)	**2.78e-05**	1.9
rs2300478	1.64 (1.22 – 2.20)	**0.00628**	1.58 (1.17 – 2.13)	**0.01212**	1.6
rs12469063	1.87 (1.39 – 2.53)	**0.000536**	1.81 (1.34 – 2.45)	**0.00118**	1.6

Chronic Insomnia Disorder vs unaffected controls (CID-only vs FCC)

	Unadjusted Models		**Adjusted Model**		
MEIS1 SNPs	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**80% Power**
rs113851554	0.92 (0.65 –1.29)	0.68	0.93 (0.65 – 1.33)	0.75	1.6
rs2300478	0.84 (0.680 – 1.04)	0.19	0.81 (0.65 - 1.00)	0.11	1.4
rs12469063	0.87 (0.697 – 1.07)	0.27	0.83 (0.66 - 1.03)	0.16	1.4

7. Discussion

The association between CID and a gene that plays a role in the embryonic development (Toresson et al., 2000), such as *MEIS1,* would have dramatically changed the way we conceptualize the etiology of insomnia. Such finding would suggest that insomnia has developmental origins. However, our data does not confirm this association (El Gewely et al., 2018). Our finding is concordant to a recent animal study reported by Salminen et al. (2017), which also did not find evidence to support a role for *Meis1* deficiency in causing sleep disturbances (sleep initiation or maintenance) in mice (Salminen et al., 2017).

Since our data is not consistent with a pleiotropic effect of *MEIS1* as suggested by the recent insomnia-related GWASs (Hammerschlag et al., 2017; Lane et al., 2017), it is possible that these studies were confounded with the presence of RLS. The possibility of such confounding was directly addressed by Hammerschlag et al. (2017) using a variety of methods such as a conditional phenotypic analysis (Hammerschlag et al., 2017). They estimated that the UK Biobank insomnia trait was confounded by RLS in 12% of cases and 6% of controls which partially contributed to the significant association they observed. However there remained a significant effect of *MEIS1* on insomnia that could not be accounted for by this confounding, and they therefore concluded that *MEIS1* is likely to have pleiotropic effects on both RLS and insomnia. We attempted to verify this finding, but our data is not consistent with this conclusion, as we cannot find evidence for an association between *MEIS1* and CID independent of RLS (El Gewely et al., 2018). The consistency of the results between the two studies (Hammerschlag et al., 2017; Lane et al., 2017) is likely explained by the fact that both studies used subjects drawn from the same general population cohort (UK Biobank). Therefore, our study further illustrates that the heterogeneity in the phenotyping definitions of insomnia is a major concern in the methodology of the previous genetic studies of insomnia (Gehrman, Pfeiffenberger, & Byrne, 2013; Lind & Gehrman, 2016).

7.1 Insomnia phenotying: a major concern in the methodology of previous genetic studies of insomnia

The first step in designing candidate gene and GWA studies is to have an accurate and specific definition of the phenotype of interest (Zondervan & Cardon, 2007). This is important for two reasons. First, non-specific definitions can decrease the power of detection of an effect (Zondervan & Cardon, 2007). Second, it can limit the ability to replicate the finding while replication is a crucial step in the validation of research findings (Zondervan & Cardon, 2007). In case of insomnia disorder studies, non-specific case definitions have been very common despite the availability of recommendations (Gehrman et al., 2013).

Recommendations of standardized assessment of insomnia using clinical history and/or questionnaires to create uniformity between studies has been proposed since early 2000s (Buysse, Ancoli-Israel, Edinger, Lichstein, & Morin, 2006; Edinger et al., 2004). Hence, structured clinical interviews are mandatory for diagnosing insomnia disorder but in practice, such interviews are time consuming and clinical expertise in insomnia disorder is scarce (Buysse et al., 2006). Also, sleep diaries do not capture the needed information to assess insomnia disorder as listed in diagnostic manuals (American Academy of Sleep Medicine, 2014; American Psychiatric Association, 2013; Bastien, Vallières, & Morin, 2001). To address these limitations, the use of questionnaires have been recommended in clinical and research practices (Buysse et al., 2006; Morin, 2003; Schutte-Rodin, Broch, Buysse, Dorsey, & Sateia, 2008). Despite the existence of these recommendations, heterogeneity in the definition of insomnia in research studies remains (Gehrman et al., 2013). Poor insomnia phenotyping is partly due to the use of non validated questionnaires to screen or measure the outcome of insomnia (Sateia et al., 2000).

Studies such as the latest two insomnia GWAS assessed insomnia by a single query: "Do you have trouble falling asleep at night or wake up in the middle of the night?" (Hammerschlag et al., 2017; Lane et al., 2017). Other than not conforming to the full diagnostic criteria of CID (American Academy of Sleep Medicine, 2014), this question omits the subsequent consequences of insomnia symptoms (ie: fatigue, attention impairment, mood disturbance, or impaired performance) while this is one of the main criterion in phenotyping insomnia in

research and clinical domains (check section 1.1.1). In fact, the impact of insomnia on daily functioning predicts the severity and the persistence of insomnia over time, which is associated to negative health outcomes and reduced quality of life (Kyle, Morgan, & Espie, 2010). Hence, it is crucial that future GWAS consider the recommendations of standardized assessment of insomnia using validated questionnaires. In the following section, we discuss the most common questionnaires that are used in research: Pittsburgh Sleep Quality Index (PSQI), Insomnia severity index (ISI), Athens Insomnia Scale (AIS) and Sleep condition indicator (SCI) and we justify our recommendation for the use of the ISI in future genetic studies (El Gewely et al., 2018).

7.1.1 Pittsburgh Sleep Quality Index (PSQI)

The PSQI was developed by Buysse and colleagues in 1989 and it has been extensively used in research for evaluating sleep disturbances in adults over a one-month time interval (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The PSQI is a reliable and valid instrument to discriminate between poor and good sleepers (Carpenter & Andrykowski, 1998) and a cutoff score of five (out of twenty-one) achieved maximum sensitivity and specificity for insomnia (Buysse et al., 2006). However, this instrument was not specifically designed for the assessment of insomnia disorder (Bastien et al., 2001; Carpenter & Andrykowski, 1998). Indeed, the PSQI does not assess daytime consequences of insomnia (impairment and social distress). To address this problem, Bastien and colleagues validated the use of the ISI in 2001 to facilitate the assessment of insomnia cases in clinical practices and in research (Bastien et al., 2001).

7.1.2 Insomnia Severity Index (ISI

The ISI has been used by many research teams in the past 20 years as a metric of treatment response in clinical research (Morin et al., 2011). The ISI corresponds in part to the diagnostic criteria of insomnia disorder as listed in the DSM-IV (American Psychiatric Association, 1994) and in the ICSD (American Sleep Disorders Association, 1990). The ISI is composed of seven questions, the first four questions are sleep related items and the other three are wake related. Precisely, the ISI assesses subjective symptoms of the three possible manifestations of insomnia (sleep onset difficulties, maintenance of sleep during the night, early morning awakenings), satisfaction with current sleep, degree of noticeable impairment

attributed to the sleep problem, distress or concerns caused by insomnia and interference with daily functioning. There are two distinct versions of the ISI that exist, one assesses insomnia severity in the last 2 weeks (Bastien et al., 2001) and the other evaluates insomnia in the last month (Morin et al., 2011). Despite this difference, the majority of research studies were conducted using the two weeks interval (Buysse et al., 2006). The ISI can be administered by patients, clinicians and significant others.

The ISI has been validated in samples composed of adolescents (Chung, Kan, & Yeung, 2011), young and older adults (Bastien et al., 2001). Also, it has been validated in cohorts of chronic insomnia disorder (Bastien et al., 2001), comorbid insomnia (Bastien et al., 2001; Savard, Savard, Simard, & Ivers, 2005), individuals from the community (Morin et al., 2011) and primary care cohorts (Gagnon, Bélanger, Ivers, & Morin, 2013). The ISI has also been translated and validated in many languages including French (Savard et al., 2005), English (Bastien et al., 2001), Spanish (Fernandez-Mendoza et al., 2012), Chinese (Chung et al., 2011; Wong et al., 2017), Korean (Cho, Song, & Morin, 2014), Italian (Castronovo et al., 2016) and German (Gerber et al., 2016). Finally, the psychometric property of the ISI in the evaluation of insomnia disorder as listed in the DSM-5 and in the ICSD-3 has recently been validated by Wong and colleagues (Wong et al., 2017). In the following section, we will review the details of the validation of the ISI and we will compare it to two common questionnaires: AIS and SCI.

Validation of the ISI

The validation of the ISI was done using the following measures: internal consistency, concurrent validity, test-retest reliability, sensitivity to change, predictive validity and content validity (Morin, 2003).

ISI internal consistency

The internal consistency is based on the correlation between different items on the same test and by the item-total correlations (Bastien et al., 2001; Morin, 2003). Internal consistency is estimated using Cronbach alpha coefficient (ranging from 0 to 1) (Morin, 2003). Validation studies of the ISI reported that the internal consistency of the ISI ranges from moderate (Cronbach alpha=0.74) (Bastien et al., 2001) to excellent (Cronbach alpha=0.90) (Savard et al., 2005). Differences between studies are due to the composition of the samples. While some studies used heterogeneous insomnia cohorts with multiple comorbid disorders and diverse age

groups (Bastien et al., 2001), others used homogenous ones (ie: community-based sample with no sleep disorders, adolescents only and insomnia in cancer patients only) (Chung et al., 2011; Savard et al., 2005). Also, differences can result from the of distinct ethnic groups (Chung et al., 2011). Several studies have shown that the weakest internal consistencies of the ISI are in the assessment of difficulties initiating sleep (r=0.36) and early morning awakenings (r=0.52) (Bastien et al., 2001; Morin, 2003) and the highest is in the assessment of the impact of sleep problems (interference) on daytime functioning (r=0.67) (Bastien et al., 2001). These findings suggest that ratings of sleep onset insomnia and early morning awakening may not contribute significantly to the overall insomnia severity (Morin et al., 2011).

Finally, studies have shown that the internal consistency of the ISI is very stable in pre and post treatment conditions (Bastien et al., 2001). The test-retest reliability was also assessed by Chung et al. (2011) over a two week interval (r=0.79) (Chung et al., 2011).

ISI concurrent validity

Concurrent validity is extent to which the results of a particular test or measurement correspond to those of a previously established measurement for the same construct. The concurrent validity of the ISI was estimated by two means. First, the severity ratings for the difficulties initiating and maintaining insomnia (nocturnal awakenings and early morning awakenings) obtained from the ISI (patient version and clinician version) were correlated with corresponding quantitative estimates of sleep onset latency, wake after sleep onset and early morning awakenings obtained from the sleep diary and polysomnography (Bastien et al., 2001). Second, the total ISI score was correlated with the sleep efficiency variable of the sleep diary as it is believed to be the best composite measure of overall sleep disturbances (Bastien et al., 2001). Results have shown that the correlation between ISI items and sleep diary variables are higher (correlations ranging from 0.32 to 0.55) than the correlations between ISI items and polysomnography variables (correlations ranging from 0.07 to 0.45) (Bastien et al., 2001). Moreover, the concurrent validity seems to be stronger post-treatment compared to pre-treatment conditions (Bastien et al., 2001). This difference is believed to be caused by the efficacy of treatment, especially CBT-I, rendering patients more aware and sensitive to their sleep (Bastien et al., 2001).

ISI content validity

The content validity is the relation of the items to the concept and the extent to which its components correspond to the diagnostic criteria of a disorder. The content validity of the ISI is supported by a component analysis (Bastien et al., 2001). Results of the analysis showed that three components of the ISI capture the diagnostic criteria of insomnia disorder: daytime consequences of insomnia (impact), severity of the symptoms and subjective sleep satisfaction (Bastien et al., 2001). Also, the content validity of the ISI was measured by correlating the total ISI score to the diagnostic criteria of insomnia disorder. The correlation between the ISI and the DSM-IV-TR insomnia disorder diagnostic criteria was satisfactory (Chung et al., 2011). Similar findings were recently published about the concordance between the ISI and the most recent diagnostic criteria of insomnia disorder as listed in the DSM-5 and the ICSD-3 (Wong et al., 2017).

ISI sensitivity to treatment changes

The ISI is sensitive to symptom changes with treatment. To estimate the ISI sensitivity of detecting treatment changes, correlations were assessed between changes in ISI scores from baseline to post-treatment and from post-treatment to 3-months follow-up (Bastien et al., 2001). On one hand, correlations were calculated based on dependent variable of the ISI and their corresponding items on the sleep diary and polysomnography. The ISI items significantly correlated to the four variables (sleep onset latency, wake after sleep onset, early morning awakenings and sleep efficiency) on sleep diary at pre-treatment, post-treatment and at 3-months follow up (Bastien et al., 2001). However, the correlations with the polysomnographic data were small. Only wake after sleep onset and sleep efficiency at pre-post treatment phase were significant. On the other hand, the sensitivity of the ISI for measuring treatment outcomes was also supported by its convergent changes over time as observed in the ISI clinician version (Bastien et al., 2001). Hence, the ISI is sensitive at detecting changes in the patient's perception of treatment outcome.

ISI predictive validity

The predictive validity of the ISI, ability to predict outcome in the future, was also studied. It was shown that at pre-treatment, the physician rating predicted the patient's ISI total score at baseline and post-treatment (Bastien et al., 2001). Also, sleep diary data seemed to be a reliable predictor of the patient's ISI score post-treatment (Bastien et al., 2001).

ISI clinical cut-off

The optimal cut-off point of the ISI is determined by the receiver operating characteristic (ROC) analysis. The area under the curve (AUC) of the ROC analysis measures the accuracy of the test based on how well it separates the group being tested into those with and without the disease. ROC curves of the ISI slightly differed between studies because of the sample composition. In Chinese adolescent students the ROC curve was 0.85 (95% CI: 0.77–0.92) (Chung et al., 2011); the optimal cut-off point for ISI was a total score of 9, with a sensitivity of 0.87 and specificity of 0.75 (Chung et al., 2011). Another study reported that a cutoff score of 10 (86.1% sensitivity and 87.7% specificity) is optimal for detecting insomnia cases in community samples (Morin et al., 2011). The same study reported that for the clinical samples, it is suggested to use a cut point of 11 (97.2% sensitivity and a perfect 100% specificity) (Gagnon et al., 2013; Morin et al., 2011). Recent data based on DSM-5 and the ICSD-3 showed that the ISI area under the curve is 0.85 and that ISI cutoff of 8 reached the best balance between sensitivity and specificity in detecting clinical insomnia cases from those without insomnia in a Chinese cohort (Wong et al., 2017).

Discussion on the ISI results in our study

The ISI averages of the CID cohort were 17 out of 28 in the CID-only group and 18 out of 28 in the CID+RLS group (El Gewely et al., 2018), which is an indication of severe chronic insomnia considering the cut-off of 11 in clinical settings as suggested by Morin and colleagues (2011). These high ISI averages were obtained despite the important number of missing data in 442 participants caused by the different times of recruitment (from 2001 to 2018); which further highlights the severity of the insomnia cases. As shown in Table 1 of the article, ISI scores are heterogeneous ranging from 5 to 28 yet they were normally distributed around the mean (not skewed). ISI scores below the clinical cut-off of 11 out of 28 can be explained by the use of medications that influence sleep, which is a common condition among clinical insomnia

patients. Hence, it is important that future studies using the ISI in clinical insomnia cohorts consider the effect of the use of medication influencing sleep on patients' response.

Another noteworthy comment is the similarity in the ISI averages between the two groups (CID-only and CID+RLS), 17 and 18 out of 28 respectively. This non-statistically significant difference (p-value=0.5167) suggests that the ISI does not discriminate between the causes of insomnia. Our results are concordant to a Korean study comparing the characteristics of CID-only patients to CID+RLS patients (Song et al., 2015). The Korean study noted that these two groups are indistinguishable from one another based on depression and quality of life (Song et al., 2015). Hence, we emphasize that the use of the ISI (cut-off of 10) in future community based genetic studies is not sufficient to identify RLS cases. Future studies will need to use the ISI in addition to the Cambridge–Hopkins diagnostic questionnaire for RLS to discriminate between the two disorders.

In brief, the short form of the Cambridge–Hopkins diagnostic questionnaire for RLS is easy to implement in research as it is self-administered, and it is quick to complete. The short form is composed of thirteen questions that assess the four diagnostic criteria of RLS and common RLS mimics such as positional discomfort and leg cramps. This questionnaire provides reasonable sensitivity and specificity (87% and 94%) that allow the assessment of RLS in population based studies (Allen, Burchell, MacDonald, Hening, & Earley, 2009). This questionnaire is also available in several languages (Allen et al., 2009).

Taken together, future studies will also need to evaluate the ability of the ISI to discriminate between CID and insomnia comorbid to other psychiatric and sleep disorders, as it was suggested by Morin and colleagues (2011). There are two other limitations of the ISI that need to be considered. First, the ISI does not address the minimum frequency criterion (three times a week) of the manifestation of insomnia symptoms as mentioned in the diagnostic manuals. Second, the ISI does not include many items to assess daytime impairment. These limitations have been addressed by the AIS and the SCI respectively; that is why it is important to compare the ISI to these questionnaires.

7.2 Insomnia severity index versus Athens Insomnia Scale and Sleep Condition Indicator

7.2.1 Athens Insomnia Scale (AIS)

In parallel to the creation of the ISI, the AIS was developed by Saldatos and colleagues in early 2000 (Soldatos, Dikeos, & Paparrigopoulos, 2000). The AIS assesses the severity following the diagnostic criteria of insomnia as listed in the International Statistical Classification of Diseases and Related Health Problems (ICD-10), which are similar to the diagnostic criteria listed in the DSM and the ICSD (Soldatos et al., 2000). The AIS is a self-reported questionnaire, composed of eight questions assessing sleep difficulties and their interference in the last month. The first five questions assess sleep difficulties (sleep induction, awakening during the night, early morning awakening, total sleep time and overall quality of sleep) and the last three questions evaluate the sense of well-being, overall functioning and sleepiness during the day. Each item of the AIS can be rated on a likert scale ranging from 0 (no problem at all) to 3 (very serious problem). The initial validation study of the AID reported that the Cranach's alpha of the questionnaire is high, ranging from 0.75 to 0.90 (Soldatos et al., 2000). The test–retest reliability over one week was 0.90 for the total score and ranging from 0.70 to 0.86 for individual items (Soldatos et al., 2000). The correlation of AIS with the Sleep Problems Scale, an external validator, was very high (Pearson's r = 0.90) (Soldatos et al., 2000). Thus, the AIS is well validated to assess insomnia disorder.

Insomnia severity index versus Athens Insomnia Scale

According to a comparative study, ROC curve of the ISI (0.85(95% CI: 0.77–0.92)) is slightly higher than the one obtained for the AIS (AUC:0.80 (95% CI: 0.72–0.89)) (Chung et al., 2011). Further, the AIS had the worst discriminatory capacity against DSM-IV-TR diagnosis of insomnia compared to the ISI and another questionnaire called Sleep Quality Index (Chung et al., 2011). Consequently, in our study we favored recommending the ISI for future genetic studies. Considering that daytime dysfunction is one of the most important criteria that distinguishes probable insomnia cases from those without, it is also important to compare the

ISI to the Sleep Condition Indicator, a psychometric instrument that has more items assessing daytime functioning (Espie et al., 2014; Wong et al., 2017).

7.2.2 Sleep Condition Indicator (SCI)

The SCI was developed by Espie and colleagues in 2014 to measure insomnia symptoms based on the DSM-5 criteria (Espie et al., 2014). The SCI is a self-reported measure composed of eight questions assessing nocturnal sleep disturbances and daytime functions (ex: productivity/ability to concentrate and energy/mood). The SCI score ranges from 0 to 32; a higher SCI score indicates that sleep is of better quality, and a lower score indicates greater symptom severity. The SCI has been validated (Espie et al., 2014; Palagini et al., 2015; Wong et al., 2017). The initial validation study of the SCI has shown that this questionnaire has robust internal consistency (Cronbach's alpha = 0.86) (Espie et al., 2014). Also, the SCI convergent validity was validated with its high correlation with the PSQI (r= -0.73) and the ISI (r= -0.79), suggesting that its measurement properties are consistent to these questionnaires (Espie et al., 2014). Hence, the SCI is an effective questionnaire to discriminate insomnia cases from healthy controls (Espie et al., 2014; Palagini et al., 2015). It was recently reported that a score of 21 in the SCI is an optimal cut off with the best sensitivity and specificity balance in detecting both DSM-5 insomnia disorder and ICSD-3 chronic insomnia from those without insomnia (Wong et al., 2017).

Insomnia severity index versus Sleep Condition Indicator

As mentioned previously, the SCI has more items assessing daytime functioning compared to the ISI. In fact, the SCI has the strongest correlations with all measures of daytime functions compared to the short form of the SCI and the ISI (Wong et al., 2017). Additionally, there are differences in the correlation between the SCI and ISI with sleep measures. While the SCI has stronger correlation with the sleep diary total sleep time, the ISI has stronger correlation with the sleep efficiency as assessed in the sleep diary.

In conclusion, the SCI seemed to be a convenient instrument to discriminate CID patients from healthy subjects. Nevertheless, its weak correlation with sleep efficiency needs to be further understood. Also, more studies need to evaluate the discriminate validity of the SCI in other populations that would be more representative of the clinical insomnia population (Wong

et al., 2017). Consequently, we preferred to recommend the ISI until further evidence of the SCI is available.

7.3 Subcategorization of insomnia in future genetic studies

To date, previous genetic studies assessed insomnia based on dichotic approach (presence versus absence of insomnia disorder) (Buhr et al., 2002; Deuschle et al., 2010; El Gewely et al., 2018; Serretti et al., 2010) or based on the presence of a single (ie: sleep onset latency only (Polito et al., 2015), early morning awakenings only (Gass et al., 2010; Utge et al., 2010)) or multiple insomnia symptoms (ie: sleep onset latency and nocturnal awakenings (Rétey et al., 2007). While the consideration of isolated insomnia symptoms confers limited morbidity and questions clinical significance of the results (Buysse et al., 2006), the dichotic approach seems more plausible but it remains a preliminary way to phenotype insomnia disorder. Future genetic studies should consider assessing insomnia based on the current research evidence of insomnia subcategorization, namely psychophysiological and paradoxical insomnia. If these subcategorizations of insomnia lie within a continuum ranging from paradoxical insomnia, less severe phenotype, to psychophysiological insomnia, the most severe phenotype; we would expect to observe differences in MAF between these subgroups.

It is suggested that within the psychophysiological subtype, additional subcategorization exists. It seems that insomnia with short sleep duration (less than six hours) has a stronger biological marker of genetic predisposition for chronic insomnia (Vgontzas & Fernandez-Mendoza, 2013). This suggestion was validated in a CBT-I study showing that individuals with short sleep duration are less responsive to CBT-I compared to those with normal sleep duration (equal or more than six hours) (Bathgate et al., 2017). Another recent study showed that insomnia patients with short sleep duration have higher cardiovascular autonomic dysfunction compared to those with normal sleep duration (Jarrin et al., 2018). To our knowledge differences between insomnia patients with short and long sleep duration has never been tested in insomnia genetic studies but it was shown in other samples.

In 2009, a study has shown that a missense mutation in the "basic helix-loop-helix family member e41" gene (*BHLHE41* also known as *DEC2*), transcription factor regulating the

circadian clock, was associated to short sleep duration in two healthy relatives (He et al., 2009). While generalizability of this finding is limited considering the small sample size, this study provides evidence that there are specific genes involved in sleep duration. Accordingly, it will be interesting to investigate if other variations of *DEC2* discriminates between insomnia patients with short sleep duration compared to those with normal sleep duration. Further, this gene is a potential candidate that can discriminate between psychophysiological and paradoxical insomnia.

Recently, the same research group has shown that the mouse model expressing the mutant human *DEC2* gene has an increased expression of the *hypocretin* (*Hcrt*) gene (involved in arousal maintenance) (Hirano et al., 2018). Considering that paradoxical insomnia is associated with cognitive, emotional and cortical arousal (Bastien et al., 2014), it will be interesting to assess the implication of *DEC2* gene in paradoxical insomnia as well. As it is hard to identify cases with paradoxical insomnia without the use of objective sleep measures, polysomnography data can be useful for future genetic studies.

7.3.1 The use of polysomnography for the subcategorization of insomnia in future genetic studies

Although polysomnography is not mandatory for screening insomnia patients (American Psychiatric Association, 2013), it can be used by future genetic studies to identify different insomnia subtypes. The literature shows that insomnia patients have increased high-frequency beta and gamma (> 30-Hz) non-rapid eye movement (NREM) EEG spectral power compared to good sleepers (Bastien et al., 2014; Krystal & Edinger, 2010). Further, a study has shown that patients with psychophysiological insomnia display significantly greater relative power in sigma activity compared to good sleepers whereas those with paradoxical insomnia had lower delta and greater alpha, beta and sigma activities compared to good sleepers (Krystal, Edinger, Wohlgemuth, & Marsh, 2002).

A decrease in the amount of light sleep in insomnia patients is possible via sleep restriction technique used in CBT-I. Sleep restriction increases the homeostatic sleep drive which results in an increase in delta power (Schwartz & Kilduff, 2015). As a consequence to this biological mechanism, patients with sleep onset insomnia solely are more responsive to

sleep restriction CBT-I technique compared to those with sleep maintenance insomnia or mixed insomnia (Harvey, 2002; Krystal & Edinger, 2010).

Considering the initial predisposition to high-frequency EEG spectral power in insomnia patients and its potential decrease using sleep restriction, genetic studies can help unravel other biological mechanisms involved in this process. Indeed, adenosine plays an important role in regulating sleep and defining sleep intensity (Basheer, Strecker, Thakkar, & McCarley, 2004; Gass et al., 2010); therefore, adenosine related genes seem to be plausible candidates to assess predisposed dysregulation in sleep intensity in insomnia patients. If adenosine related genetic results are plausible, findings will foster genetic screening in insomnia and lead to individualized treatment.

7.4 Clinical Implications

7.4.1 Phenotyping of insomnia disorder in clinical practices

Problems in phenotyping insomnia are not limited to research; insomnia disorder is often unrecognized and untreated in clinical practices (Araújo, Jarrin, Leanza, Vallières, & Morin, 2017; Morin, 2015). Part of the underdiagnosis of insomnia is explained by the way it was conceptualized in the past. Prior to the current diagnostic guidelines, insomnia was perceived as a secondary problem that would dissipate after treating the primary condition (Vgontzas & Fernandez-Mendoza, 2013). Another difficulty in phenotyping insomnia in clinical settings is the differential diagnosis, the process of differentiating between two or more conditions that share similar complaints or symptoms. For instance, difficulties in initiating and/or maintaining sleep are common complaints among RLS and sleep apnea patients, which makes them possible confounding factors of insomnia disorder (Allen et al., 2014; Montplaisir et al., 1997; Ulfberg et al., 2007). Thus, our study is an additional reminder to insomnia research and clinical practices to carefully phenotype insomnia as it has been recommended in the past twenty years (Buysse et al., 2006).

7.4.2 From insomnia genetic studies towards precision medicine

The current treatment approach for insomnia disorder is based on trials and errors; akin other psychiatric disorders (Jha & Trivedi, 2018). There are no standards to which treatment to be used. As mentioned in the *pharmacological treatment of insomnia* section, many pharmacological options are available going from benzodiazepines to antihistamines (Riemann et al., 2017). Consequently, patients undergo multiple medication trials for weeks to months before finding an effective treatment. This highlights that the field is suffering from the discrepancy in the perspectives between insomnia patients and clinicians (Araújo et al., 2017). It will be possible to further consider patient's experience of insomnia by developing new clinical measures and reaching targeted treatments (Araújo et al., 2017); which can be achieved using genetic studies.

Medication selection can be optimized with the identification of genomic and proteomic biomarkers involved in insomnia disorder. Genetic studies of sleep disorders, such as RLS, led to the identification of genes associated to the disorder (Keijzer et al., 2017). Our study replicated the association between RLS and *MEIS1* gene (El Gewely et al., 2018), which highlights that genotyping is a promising way to objectify a patient's sleep phenotype. Nevertheless, future genetic studies on the diagnostic and therapeutic benefits from analyzing sleep related genes are needed.

In insomnia disorder, the current status of genetic studies remains in a preliminary phase compared to RLS. Indeed, more studies are warranted to identify the genes involved in insomnia disorder (Lind & Gehrman, 2016). Our study played a role in guiding the genetic field towards the forthcoming steps. Accuracy in insomnia phenotyping requires the collaboration of medical practitioners, for both clinical and research purposes. Once information about genes involved in well phenotyped insomnia cohorts is available, individualized treatments of insomnia will be possible, which will optimize treatment choices for the different insomnia subtypes (Vgontzas et al., 2013). Well characterized CID samples, such as CEAMS Sleep biobank insomnia cohort, is the first step leading to precision medicine. As mentioned in a commentary about our article published in SLEEP November 2018 issue, " they introduce their well-characterized CID sample to the research community. This sample should be useful for evaluating large scale samples collected by simplified questionnaire phenotyping" (Oexle, 2018).

Conclusion

We examined the role of *MEIS1* gene in patients diagnosed with CID by genotyping three SNPs. We first genotyped rs113851554 SNP, previously associated with RLS (Schormair et al., 2017; Winkelmann et al., 2011, 2007, 2016; Xiong et al., 2009; Yang et al., 2011) and recently associated with insomnia symptoms in individuals from the general population (Hammerschlag et al., 2017; Lane et al., 2017). Our genotyping results of rs113851554 SNP demonstrate that *MEIS1* is not associated with CID in the absence of RLS in our insomnia cohort. Subsequently, we genotyped two other RLS associated *MEIS1* SNPs (rs12469063 and rs2300478) (Winkelmann et al., 2011) to observe if we obtain the same results as with rs113851554. Similarly to rs11381554 SNP, genotyping results of the two latter *MEIS1* SNPs did not show any association between *MEIS1* and CID in the absence of RLS.

Our study recognizes the importance and the scientific contributions that GWAS offer to the field of sleep genetics. As mentioned in the introduction, insomnia GWAS started a decade ago and they will help advancing our understanding on the mechanism of insomnia. Part of GWAS progress is the validation of the findings via replication studies. Our study underscores the critical importance of accurate phenotyping in the future insomnia GWAS. Since uniformity in the assessment of insomnia is needed for the success of this process, we suggest the use of validated psychometric tools that can be deployed in large population-based studies to better characterize insomnia and to isolate it from other sleep disorders such as RLS.

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