Fall 2014

Sleep-related phenotypes: adolescence and PAX6 haploinsufficiency

Alyson Elizabeth Hanish
University of Iowa

SLEEP-RELATED PHENOTYPES:

ADOLESCENCE AND *PAX6* HAPLOINSUFFICIENCY

by

Alyson Elizabeth Hanish

A thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Nursing
in the Graduate College of
The University of Iowa

December 2014

Thesis Supervisor: Professor Janet K. Williams

Graduate College
The University of Iowa
Iowa City, Iowa

CERTIFICATE OF APPROVAL

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PH.D. THESIS

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This is to certify that the Ph.D. thesis of

Alyson Elizabeth Hanish

has been approved by the Examining Committee
for the thesis requirement for the Doctor of Philosophy
degree in Nursing at the December 2014 graduation.

Thesis Committee: _____
Janet K. Williams, Thesis Supervisor

_____
M. Kathleen Clark

_____
Sandra E. Daack-Hirsch

_____
Anne Ersig

_____
Deborah Lin-Dyken

To Michael and Ellie for their love, support, and smiles along the way.

Sleep is the best meditation.

Dalai Lama

ACKNOWLEDGMENTS

I have been truly fortunate to have many people to thank for helping me complete this doctoral thesis. I would like to thank my dissertation committee members for their time, support, guidance, and mentorship. I am deeply grateful to Janet Williams for her intellectual guidance, editorial advice, and active participation in every step of this process. I feel incredibly privileged to have her as my supervisor as she has positively shaped my future career in nursing research. I would also like to thank Kathy Clark, Sandy Daack-Hirsch, Deborah Lin-Dyken, and Anne Ersig for their time and expertise in reading dissertation drafts and providing valuable input. I sincerely thank Joan Han for giving me the opportunity to conduct research in her lab at NIH, as well as for her time, training, mentorship, and patience during obstacles encountered.

To the Section on Growth and Obesity, NICHD, NIH, in particular Jack Yanovski and my fellow lab members: Sheila Brady, Gladys Palaguachi, Dezmond Taylor-Douglas, Daniela Reyes-Capo, Melanie Hicks, Louise Hannallah, Yael Caplan, and Jenny Strong for their personal and scholarly interactions. From them I have learned invaluable life lessons including the importance of coffee breaks and the enormous value in team-based research training and conducting transdisciplinary research.

I gratefully acknowledge the National Institute of Nursing Research for funding my doctoral fellowship. I would also like to acknowledge the NINR intramural program including Mary Engler and Ann Cashion for their training support and career advice. To all NIH GPP fellows, I have been truly privileged to experience this journey alongside some incredibly gifted and motivating individuals, and who, without a doubt, are the next

generation of worldwide scientific leaders. I would also like to thank my parents for reading to me every night and for investing in my education.

Above all, I would like to thank my husband, Michael Hanish, and my daughter, Elliot Hanish. I thank Michael for his patience and for his utmost faith in my ability to complete my dissertation, and I thank Ellie for the happiness she has brought our family.

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ABSTRACT

Sleep health and sufficient sleep are particularly important during adolescence when important physical, cognitive, emotional, and social changes occur. Given the potential role of PAX6 in pineal development and circadian regulation, adolescents with *PAX6* haploinsufficiency may be more likely to experience sleep-related problems compared to adolescents without these deletions or mutations. Haploinsufficiency of *PAX6* can result from WAGR syndrome, a contiguous gene deletion syndrome in which multiple genes are involved, or point mutations and microdeletions affecting only *PAX6*, which result in isolated aniridia. The purpose of this dissertation is to examine pineal volume, melatonin concentrations, and sleep disturbance in individuals with *PAX6* haploinsufficiency, as well describe validity of self-report measures of sleep problems in adolescents. Results are presented in three papers.

Although *PAX6* haploinsufficiency is rare and minimal research has focused on the role of *PAX6* in circadian regulation, irregular patterns of sleep-wake rhythm have been studied in children and adolescents with neurodevelopmental disorders (e.g. autism spectrum disorders), another population with possible abnormalities in melatonin physiology. The first paper presents an integrative review to synthesize the literature regarding the sleep-related measures currently being used to assess sleep disturbance in adolescents with a neurodevelopmental disorder. The second paper reports significantly reduced pineal volume, reduced melatonin secretion, and greater parent-report of sleep disturbance in individuals with *PAX6* haploinsufficiency versus controls. Paper 3 further characterizes the sleep-related-phenotype associated with an abnormality in the *PAX6* gene using self-report questionnaires and actigraphy in adolescents with *PAX6*

haploinsufficiency, as well as performs preliminary validation studies on age-appropriate self-report tools to measure sleep in adolescents. Results demonstrate similar self-reported daytime sleepiness, sleep disturbance, and sleep-related impairment in adolescents with *PAX6* haploinsufficiency compared to the healthy comparison group; however, actigraphy data documented increased time from lights off to sleep in the *PAX6* haploinsufficiency group. Self-reported sleep questionnaire scores and objective actigraphy variables (e.g. total sleep time) were significantly correlated in the healthy comparison group only; however, a lack of correlation among sleep-related measures in adolescents with *PAX6* haploinsufficiency suggests potential limitations in using self-reported sleep measures in this population. This study used a combination of physiological and patient-reported health measures, and although WAGR syndrome and isolated aniridia due to PAX6 insufficiency are rare disorders, describing the sleep-related phenotypes in this population contributes to knowledge of assessment and treatment of sleep disorders in general, facilitating research in additional adolescent populations.

PUBLIC ABSTRACT

Sleep health and sufficient sleep are particularly important during adolescence when important physical, cognitive, emotional, and social changes occur. Given the potential role of the *PAX6* gene in pineal development and circadian regulation, adolescents with an abnormality in the *PAX6* gene may be more likely to experience sleep-related problems compared to adolescents without these deletions or mutations. Describing the sleep-related phenotypes is necessary as sleep problems may go unrecognized and undertreated, which may compound existing conditions. However, measures used to describe sleep-related phenotypes are not well developed for adolescents, limiting the amount and type of data that can be gathered on sleep phenotypes and sleep problems in adolescent populations. The purpose of this dissertation is to examine pineal volume, melatonin concentrations, and sleep disturbance in individuals with an abnormality in the *PAX6* gene, as well as to describe validity of self-report measures of sleep problems in adolescents. We found significantly reduced pineal volume, reduced melatonin secretion, and greater parent-report of sleep disturbance in individuals with an abnormality in the *PAX6* gene versus controls. In addition, preliminary validation studies on age-appropriate tools to measure sleep in adolescents provided insight into the potential usefulness of self-reported sleep disturbance and sleep-related impairment measures. This study used a population with a rare genetic condition as a model to expand our knowledge of sleep-related phenotypes with the goal of more broadly improving sleep function and managing or eliminating sleep-related symptoms in adolescents.

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CHAPTER 1: INTRODUCTION

Introduction

An estimated 25-30% of the general adult population, and comparable children and adolescent percentages, are affected by decrements in sleep health that contribute to disability, morbidity, and mortality ("National Institutes of Health Sleep Disorders Research Plan," 2011). Sleep is increasingly recognized as important to public health, and insufficient sleep is a public health epidemic. An Institute of Medicine (IOM) report on sleep disorders and sleep deprivation estimated that 50-70 million Americans suffer from a chronic disorder of sleep and wakefulness, hindering daily function, and adversely affecting health ("Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem," 2006). The cumulative effects of sleep disorders and sleep deprivation have been associated with deleterious health consequences such as increased risk of diabetes, obesity, depression, hypertension, heart attack, and stroke. In addition, individuals with chronic sleep deprivation have an increased risk of injury and increased utilization of health care services. At a minimum, the total direct costs of sleep disorders and sleep deprivation in the United States are in the hundreds of billions of dollars (e.g. health care provider visits, hospital services, prescriptions, and over the counter medications) ("Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem," 2006).

Sleep and circadian disorders have been recognized by Congress and the Department of Health and Human Services as high priority targets for basic and clinical scientific investigation ("National Institutes of Health Sleep Disorders Research Plan," 2011). Sleep health has been added to the agenda for Healthy People 2020 with one of the objectives being to increase the proportion of adolescents in grades 9 through 12 who

get a sufficient amount of sleep. The term sleep health is not defined; however, Healthy People 2020 states the overall goal of sleep health is to increase public knowledge of how adequate sleep and treatment of sleep disorders can improve health, wellness, quality of life, productivity, and safety ("Sleep Health," 2014). Sleep health and sufficient sleep are particularly important during adolescence when important physical, cognitive, emotional, and social changes occur. Additionally, circadian rhythm changes make it more difficult for adolescents to fall asleep and also harder to wake up in the morning (Wolfson & Carskadon, 1998). There is no standard age range for defining adolescents; however, this study will use the age range provided by the World Health Organization, which defines adolescence as young people between the age of 10 and 19 ("World Health Organization, Adolescent Health ", 2014). Sleep health is especially critical for adolescents whose sleep-related phenotypes may reveal sleep problems (George & Davis, 2013). Sleep-related phenotypes utilize objective and subjective sleep characteristics to describe sleep health. Characterization of the sleep phenotype can indicate problems such as sleep disorders meeting clinical diagnostic criteria, as well as sleep problems and sleep deprivation. In the literature, inadequate sleep health is often described using the terms ‘sleep disorders’, ‘sleep problems’, and ‘sleep deprivation,’ and these terms are often used interchangeably without a definition.

To use sleep-related phenotypes to identify sleep problems, accurate description and identification of sleep characteristics are critical. One way to examine sleep-related phenotypes is through the use of sleep questionnaires. In 2010, extensive lists of published and unpublished instruments used to investigate sleep issues in children and adolescents were collected, and their psychometric properties were evaluated using

eleven methodological criteria outlined by the authors (Spruyt & Gozal, 2011). Two parent-report measures, the Sleep Disturbance Scale for Children (ages 6-15) and the Sleep Disorders Inventory for Students-Children and Adolescents (ages 11-18), met the eleven methodological criteria. In addition, two self-report measures, the Dream Content Questionnaire for Children (ages 9-14) and the Cleveland Adolescent Sleepiness Questionnaire (ages 11-17) nearly met the eleven methodological criteria. However, no self-report measures for adolescents 10-19 years of age fully met the 11 psychometric criteria (Spruyt & Gozal, 2011). Overall, there is a dearth of psychometrically sound adolescent self-report sleep questionnaires and lack of self-report measures may contribute to the underrepresentation of adolescents in studies of sleep phenotypes.

Sleep disorders present in children and adolescents include sleep-disordered breathing, narcolepsy, restless leg syndrome, period limb movement disorder, and insomnia (Owens, 2011). Some sleep disorders may be present from birth, including alterations in physiological processes that may contribute to adverse sleep-related phenotypes. One of these populations is persons with paired box gene 6 (*PAX6)* haploinsufficiency, who appear to be a particularly vulnerable population to adverse sleep-related phenotypes. Haploinsufficiency of *PAX6* can result from (1) WAGR (**W**ilms tumor, **a**niridia, **g**enitourinary anomalies, and cognitive impai**r**ment), a contiguous gene deletion syndrome in which multiple genes are involved, or (2) point mutations and microdeletions affecting only *PAX6*, which result in isolated aniridia. In both WAGR syndrome and isolated aniridia an abnormality in the *PAX6* gene results in aniridia. Although aniridia is medically defined as the absence of the iris, the term also includes a cluster of problems related to eye development (Fischbach, Trout, Lewis, Luis,

& Sika, 2005; Ivanov, Shuper, Shohat, Snir, & Weitz, 1995a; Lee, Khan, & O'Keefe, 2008a). In addition to the role of *PAX6* in eye development, *PAX6* also appears to play a role in development of the pineal, a gland in the brain that produces melatonin, a hormone involved in circadian regulation (Abouzeid et al., 2009; Estivill-Torrus, Vitalis, Fernandez-Llebrez, & Price, 2001; Hanish, 2012; Mitchell et al., 2003; Rath et al., 2009; Walther & Gruss, 1991). Given the potential role of PAX6 (PAX6 not italicized referring to protein) in pineal development and circadian regulation, individuals with deletions or mutations leading to *PAX6* haploinsufficiency (*PAX6* italicized referring to gene) may be more likely to experience sleep-related problems compared to individuals without these deletions or mutations. Parents of children and adolescents with *PAX6* haploinsufficiency have voiced concerns regarding sleep issues in their children. For example, one parent wrote in response to a recruitment letter, "I think she [her child] is a perfect candidate for your research, since she seems to have evidence of the sleep issues you are studying."

Sleep-related phenotypes, including the presence of sleep problems and types of sleep problems, have not been described in persons with *PAX6* haploinsuffciency, despite their vulnerability to sleep problems. Describing sleep-related phenotypes in adolescents with WAGR and isolated aniridia is necessary as sleep problems may go unrecognized and undertreated, which may compound existing conditions (e.g. behavioral problems). However, measures used to describe sleep-related phenotypes are not well developed for adolescents, limiting the amount and type of data that can be gathered on sleep phenotypes and sleep problems in adolescent populations.

Objective and subjective adolescent sleep health was assessed through measurement of sleep patterns, sleep disturbance, and sleep-related impairment. These

data were used to describe sleep-related phenotypes. Sleep patterns provide objective data regarding sleep-wake state and were measured using sleep logs and actigraphy. The variables of sleep onset latency and sleep efficiency were used as a means to describe sleep patterns. Sleep onset latency is defined as the time it takes to transition from wakefulness to sleep, while sleep efficiency is the percentage of time one spends in bed asleep (Meltzer, Montgomery-Downs, Insana, & Walsh, 2012) (Table 1.1). In addition, sleep questionnaires were used to examine the variables of sleep disturbance and sleep-related impairment, subjective assessments completed by the adolescents. Although these terms are not defined in the literature, this study used the definitions developed by the authors of the Patient Reported Outcome Information System (PROMIS) sleep questionnaires. Sleep disturbance was defined as self-reported perceptions of sleep quality, depth, and restoration, while sleep-related impairment was defined as self-reported alertness, sleepiness, tiredness, and functional impairments associated with sleep problems during waking hours (Table 1.2) (Cella et al., 2010).

The Biobehavioral Model of Altered Dysregulation in Circadian Systems (Figure 1.1) was used as an overarching model in which to study sleep in the context of several physiologic systems in humans. This model focuses on the interrelationships between the endocrine system, autonomic nervous system, and sleep system. According to the model, dysregulation in circadian systems can be measured by melatonin, cortisol, sympathetic nervous system activity, catecholamines, sleep duration, and sleep quality. The outcomes of dysregulation include: poor quality of life, poor treatment adherence, fatigue, disease progression, and poorer survival (Carlson, Campbell, Garland, & Grossman, 2007; Otte & Carpenter, 2009). This model provided an overall direction for research design and

assessment tools. Although the authors did not define sleep duration and sleep quality within this model, the study variables of sleep onset latency and sleep efficiency will capture aspects regarding sleep duration and quality. In addition, the study variables of sleep disturbance and sleep-related impairment provided information on the model outcomes of dysregulation such as quality of life, as by definition, sleep disturbance is a measure of sleep quality.

In addition, the sleep cycle provided a model for studying the physiology of sleep. Sleep architecture typically follows a pattern of alternating REM (rapid eye movement) and NREM (non-rapid eye movement) sleep throughout the night, with the timing and repetitions varying throughout the lifespan (George & Davis, 2013). The sleep cycle provided a physiological model in which to study sleep patterns, specifically, the study variables of sleep onset latency and sleep efficiency. Using the model, sleep onset latency was measured prior to the initiation of the first stage of sleep, while sleep efficiency was a percentage of time one spends in bed asleep, which was calculated throughout all of the stages of sleep.

As part of WAGR/Aniridia Protocol 08-CH-0213, an active phenotype-genotype protocol at the National Institutes of Health (NIH), participants have undergone extensive neuroimaging, neuropsychological testing, and metabolic/endocrine phenotyping. We reported on pineal gland volume, melatonin production, and parent-reported sleep disturbance in patients with *PAX6* haploinsufficiency (Hanish, 2012). In addition, our research describes both objective and subjective measurements of sleep patterns, sleep disturbance, and sleep-related impairment in adolescents with *PAX6* haploinsufficiency. Although WAGR syndrome and isolated aniridia are rare disorders, describing the sleep-

related phenotypes in this population will advance the knowledge of assessment and treatment of sleep disorders in general, facilitating research in adolescents without these conditions.

Purpose and Aims

The purpose of this dissertation is to examine pineal volume, melatonin concentrations, and sleep disturbance in individuals with *PAX6* haploinsufficiency, as well describe validity of self-report measures of sleep problems in adolescents. Given the potential role of PAX6 in pineal development and circadian regulation, individuals with *PAX6* haploinsufficiency may be more likely to experience sleep-related problems compared to individuals without these deletions or mutations. This study will elucidate sleep patterns, sleep disturbance, and sleep-related impairments in a population with plausible sleep problems, adolescents with *PAX6* haploinsufficiency, in comparison to healthy adolescents. Adolescents was defined as persons 10 to 19 years of age ("World Health Organization, Adolescent Health ", 2014). In addition, this study seeks to preliminarily validate the use of sleep disturbance and sleep-related impairment measures in a general adolescent population. This study used a combination of physiological and patient-reported health measures. The specific aims of are to:

1. Examine pineal volume, melatonin concentrations, and parent-reported sleep in individuals with an abnormality in the *PAX6* gene.
2. Further characterize the sleep-related phenotype associated with an abnormality in the *PAX6* gene in adolescents age 10-19 years.
3. Perform preliminary validation studies on age-appropriate self-report tools to measure sleep in healthy adolescents 10-19 years.

Significance

The public health consequences of sleep problems and sleep-related disorders are visibly seen in errors in judgment contributing to disastrous events such as the explosion of the *Challenger* space shuttle, as well as less visible consequences that are more prevalent and take a toll on mortality, morbidity, performance, accidents and injuries, functioning, quality of life, family well-being, and health care utilization ("Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem," 2006). Persons with *PAX6* haploinsufficiency appear to be a particularly vulnerable population to adverse sleep-related phenotypes. Pineal hypoplasia or absence has been previously reported in persons with known *PAX6* mutations (Abouzeid et al., 2009; Mitchell et al., 2003). Despite the known role of the pineal gland as the primary source of melatonin, little is known about the production of melatonin and sleep-related phenotypes in persons with genetic mutations that may result in pineal hypoplasia. Our research has been the first to report that both pineal volume and melatonin production are significantly lower in humans with *PAX6* haploinsufficiency (Hanish, 2012). In addition, parent-report of children (2-12 years) with *PAX6* haploinsufficiency suggests greater sleep problems in comparison to healthy controls (Hanish, 2012).

The sleep-related phenotypes, including the presence of sleep problems and types of sleep problems, need to be further explored in persons with *PAX6* haploinsuffciency. Describing the sleep-related phenotypes is necessary as sleep problems may go unrecognized and undertreated, which may compound existing conditions (e.g. behavioral problems). For example, sleep patterns, within the home setting, have not been assessed in persons with *PAX6* haploinsufficiency. In addition, despite parent-report data

suggesting greater sleep problems in children with *PAX6* haploinsufficiency (Hanish, 2012), self-report measures of sleep disturbance and sleep-related impairment have not been reported in adolescents with these mutations.

This gap in the science is particularly important, as adolescence is a critically important period for sleep. Circadian rhythm changes make it more difficult to fall asleep and harder to wake up in the morning. Adolescents require about nine to ten hours of sleep each night, and over the past two decades, researchers, teachers, parents, and adolescents have consistently reported inadequate sleep in adolescents (Carskadon, Acebo, Richardson, Tate, & Seifer, 1997; "National Sleep Foundation," 2011b; Wolfson & Carskadon, 1998). Studies in adolescents have found that inadequate sleep is associated with higher levels of depressed mood, anxiety, behavior symptoms, alcohol use, and attempted suicide (Liu, 2004). Additionally, studies indicate that there is an association between lower academic performance and shorter sleep duration (Wolfson & Carskadon, 2003). In 2010, extensive lists of published and unpublished instruments used to investigate sleep issues in children and adolescents (0-18 years) were collected and psychometric properties were evaluated using eleven methodological criteria outlined by the authors (purpose, research question, response format, generation of items, pilot, item-analysis and non-response analysis, structure, reliability, validity, confirmatory analysis, and standardization and norms development) (Spruyt & Gozal, 2011). In an adolescent population, none of the measures fully met the 11 psychometric criteria determined by the authors. Measures used to describe sleep-related phenotypes are not well developed for adolescent populations, and adolescent sleep research is underrepresented in the literature. Findings from this study will advance the knowledge of assessment and

treatment of sleep disorders in adolescents with *PAX6* haploinsufficiency, which may have relevance in other adolescent populations.

Patient Reported Outcomes Measurement Information System (PROMIS) measures are selected to provide valid and reliable, non-disease specific, standardized questionnaires allowing for comparability across conditions and across the lifespan. PROMIS questionnaires were established using literature searches of well-established existing measures as well as expert content review, qualitative research with patients, and pilot testing. There are currently minimal published studies using the adult PROMIS sleep disturbance and sleep-related impairment measures (Bajaj et al., 2011; Cook, Bamer, Amtmann, Molton, & Jensen, 2012); however, ongoing studies are underway. Within PROMIS, many measures are available for both pediatric and adult populations; however, currently no pediatric measures are available in the sleep disturbance or sleep-related impairment domains (Cella et al., 2010; "PROMIS," 2014). To our knowledge, for the first time, preliminary studies of the concurrent and construct validity of the PROMIS sleep disturbance and sleep-related impairment questionnaires were completed in an adolescent population (10-19 years).

Sleep is a readily accepted clinical construct, however it is a complex phenomenon that is difficult to define and measure. Currently there are over one hundred clinical trials focused on melatonin administration and thousands of clinical trials focused on sleep. Participants range from premature infants through the elderly ("Clinical Trials," 2014). The abundance of clinical trials highlights the need for standardized and validated sleep outcome measures that can be used across studies and across the lifespan. In this study, questionnaires were used to subjectively examine sleep disturbance and sleep-

related impairment, and actigraphy was used for a more objective measurement of sleep patterns. Actigraphy is an instrument that estimates sleep and wake times based on the correlation between sleep-wake state and motor activity. To our knowledge, this is the first study to use the self-report PROMIS sleep disturbance and sleep-related impairment measures in conjunction with actigraphy. Although WAGR syndrome and isolated aniridia are rare disorders, these diagnoses serve as models in which to study sleep-related phenotypes. For example, results from the questionnaires and actigraphy may be informative to other populations with possible abnormalities in melatonin physiology such as children with neurodevelopmental disorders (e.g. autism spectrum disorders) (Tordjman et al., 2013). This study used a population with a rare genetic condition as a model to expand our knowledge of sleep-related phenotypes with the goal of more broadly improving sleep function and managing or eliminating sleep-related symptoms.

Background

Sleep Architecture

Sleep architecture follows a pattern of alternating REM (rapid eye movement) and NREM (non-rapid eye movement) sleep throughout a typical night, with the cycle repeating itself about every 90 minutes. Humans spend approximately 75% of the night in NREM sleep, which is composed of the first three stages of sleep. During stage 1, persons are mostly between being awake and falling asleep. During stage 2, individuals become disengaged from their surroundings, breathing and heart rate are regular, and body temperature begins to drop. Stage 3 is the deepest and most restorative stage of sleep. During this stage, blood pressure drops, breathing becomes slower, muscles are relaxed with increased blood supply, tissue growth and repair occurs, and energy is

restored. The final stage of sleep is REM, which lasts 25% of the night. During REM, one's eyes dart back and forth and the body becomes immobilized. The brain is active, and this is the stage in which dreams occur ("National Sleep Foundation," 2011a). Overall, sleep architecture and the amount of time spent sleeping can vary from person to person and changes throughout the life cycles with adults typically needing 7-8 hours of sleep and newborns needing 16-18 hours of sleep (2011; "National Sleep Foundation," 2011a).

Sleep in Adolescence

Adolescence is a time of important physical, cognitive, emotional, and social change. Although sleep is a primary aspect of adolescent development, insufficient sleep in adolescence is common, growing progressively worse over the course of adolescence. Among the pediatric population (0-18 years), adolescents carry the strongest sleep debt. Adolescents physiologically need about 9 to 10 hours of sleep, while sleep averages around 7 hours a night (Wolfson & Carskadon, 1998). Too little sleep can leave one feeling lethargic, sluggish, irritable, and moody, and can inhibit focus and concentration, slow response time, and decrease learning of cognitive tasks (Randazzo, Muehlbach, Schweitzer, & Walsh, 1998). Studies have found that inadequate sleep in adolescence is associated with higher levels of depressed mood, anxiety, behavior symptoms, and alcohol use. Findings also demonstrate an association between short sleep duration and nightmares and suicidal behavior (Liu, 2004; Wolfson & Carskadon, 1998). Additionally, studies indicate that there is an association between lower academic performance and sleep duration (Wolfson & Carskadon, 2003). Furthermore, inadequate sleep can also pose safety risks. For example, almost 20 percent of all serious car crash injuries within

the general population are associated with driver sleepiness (Connor et al., 2002). Adolescents and young adults are the mostly likely group to be involved in crashes caused by a driver falling asleep (Pack et al., 1995).

Sleep in Persons with Visual Impairments and Multiple Disabilities

Children and adolescents with visual impairment appear to be a particularly vulnerable population, and there is a gap in the literature on appropriate evidence-based therapeutic strategies to improve sleep. A systematic review on the management of sleep disorders in children with a visual impairment expressed the need to validate outcome measures in this population using subjective standardized quality of life and behavioral parameters, as well as objective measures, such as actigraphy (Khan et al., 2011). Although minimal data are available, the literature suggests that children and adolescents with visual impairments may suffer from sleep disorders due to the lack of light perception (Khan et al., 2011; Warman et al., 2011). Several studies in adults who are blind suggest that sleep-wake disorders may occur in up to 50% of patients; however the paucity of data on the prevalence of sleep disorders in the blind makes it difficult to put into context the extent of sleep problems. Despite the reports that sleep disorders are a problem in persons who are blind, the overall incidence of sleep disorders is unknown (Lockley, Arendt, & Skene, 2007; Warman et al., 2011).

One of the groups of patients with PAX6 haploinsufficiency, WAGR syndrome, has multiple disabilities including visual and cognitive impairment. Although the sleep-related phenotype has not been studied specifically in patients with WAGR syndrome, one study reports that more than two-thirds of children and adolescents with multiple

disabilities suffer from severe and sustained disturbances in sleep (Tietze et al., 2012). This study also noted that in the majority of children and adolescents with multiple disabilities, self-report is not possible, and therefore is interpreted by caregivers thus sleep disturbance is heavily dependent on the assessment instrument and the parent's perception of and tolerance for the observed symptoms. Currently, there is no defined standard for questionnaires used in assessment of quality of sleep in children and adolescents with multiple disabilities (Tietze et al., 2012).

Circadian Rhythm Sleep Disorders

Circadian rhythm sleep disorders (CRSD) are classified as persistent or recurrent patterns of sleep disturbance primarily due to alterations in the circadian system or a misalignment between endogenous circadian rhythm and exogenous factors that affect the timing or duration of sleep. Currently there are six distinct CRSDs recognized by the International Classification of Sleep Disorders: 1) delayed sleep phase type; 2) advanced sleep phase type; 3) irregular sleep-wake phase type; 4) free-running type; 5) jet lag type; and 6) shift work type (Sack et al., 2007a). Other recognized CRSDs include those secondary to medical conditions, drug or substance abuse, as well as those not otherwise specified. Although progress is being made on the understanding of the biology of circadian rhythms, classification remains primarily on criteria related to a constellation of symptoms, at times supplemented by questionnaires and laboratory tests (Sack et al., 2007a). Based on the evidence for the role of *PAX6* in pineal function, persons with *PAX6* haploinsufficiency may have a circadian rhythm disorder due to possible melatonin-related deficiency. Depending on the extent of ocular involvement and light perception, persons with *PAX6* haploinsufficiency may demonstrate free-running rhythm.

Free-running rhythms, a type of circadian related sleep disorder, are very rare in normally-sighted people; however, they are quite common in persons who have visual impairments such as blindness who have no access to the entraining (synchronizing) effects of the light/dark cycle. Patients with free-running rhythms have circadian cycles that mimic those of persons in time-free environments that are thought to reflect failure of entrainment. The occurrence of free-running rhythms in persons who are blind indicates that some light/dark is crucial for normal entrainment of humans (Sack et al., 2007b).

WAGR Syndrome

WAGR syndrome (**W**ilms tumor, **a**niridia, **g**enitourinary anomalies, and cognitive impai**r**ment) is a rare genetic disorder (1 case per 500,000 to 1,000,000) caused by heterozygous contiguous, variably-sized deletions in the 11p13 chromosomal region (Fischbach et al., 2005). Diploid organisms have two sets of chromosomes and one copy of each allele on each chromosome. When both alleles are the same, they are homozygous, and when the alleles are different, they are heterozygous (Lewis, 2010). Haploinsufficiency occurs when a diploid organism only has a single functional copy of a gene due to inactivation of the second copy through mutation. The single functional copy does not produce enough protein to bring about the wild-type condition (phenotype of a typical form of a species), thus leading to an abnormal state ("National Library of Medicine ", 2014). In WAGR syndrome, multiple genes are involved including the *WT1* and the *PAX6* gene. Haploinsufficiency of *WT1* accounts for Wilms tumor and genitourinary anomalies, while *PAX6* haploinsufficiency accounts for problems with eye development. The *WT1* gene and the *PAX6* gene are approximately 700 kb apart, with the *WT1* gene centromeric to *PAX6* (Chao, Huff, Strong, & Saunders, 2000).

Isolated Aniridia

Haploinsufficiency of *PAX6* can occur as part of contiguous gene deletion syndrome (WAGR) or as point mutations and microdeletions affecting only *PAX6*, which result in isolated aniridia (Fischbach et al., 2005; Ivanov et al., 1995a). It is likely that the single normal copy of *PAX6* is simply not enough to produce sufficient biologically active PAX6 protein. Different mutations may result in variable phenotypic presentation. In humans, *PAX6* is located on band p13 of chromosome 11 with mutations scattered throughout the gene. The majority of mutations reported are nonsense mutations, frameshift mutations, or splicing errors predicted to cause premature truncation of the PAX6 protein (Kokotas & Petersen, 2010). The prevalence of missense mutations in *PAX6* genes may be underrepresented due to ascertainment bias in that missense mutations may cause milder or clinically distinct eye phenotypes that are not always considered for analysis (Hanson et al., 1999).

Isolated aniridia occurs in 1/50,000 to 1/100,000 individuals, is mostly inherited in an autosomal dominant pattern, and mutations in *PAX6* have been shown to be the main cause of aniridia (Glaser, Walton, & Maas, 1992; Mannens et al., 1989). Approximately two-thirds of the children with aniridia have an affected parent (inherited in an autosomal dominant pattern) and one-third of the cases are sporadic with *de novo* mutations (Kokotas & Petersen, 2010). The term ‘aniridia’ is most often used to denote the entire spectrum of eye abnormalities caused by the *PAX6* defects (Clericuzio, 2005). In most cases, aniridia is characterized by the absence or hypoplasia of the iris, but may also involve the retina, optic nerve, lens, and cornea. Aniridia is associated with a range of other ocular anomalies such as cataract, glaucoma, nystagmus, strabismus, ptosis,

foveal hypoplasia, corneal pannus, anterior chamber angle, optic nerve malformations, and Peter's anomaly. Iris deficiency and the above mentioned ocular anomalies in aniridia are associated with decreased visual acuity, glare, and photophobia (Abouzeid et al., 2009; Fischbach et al., 2005; Ivanov et al., 1995a; Mannens et al., 1989).

PAX6: An Overview

In animal and human studies, the phenotypic spectrum associated with *PAX6* mutations is wide, and there is a correlation between the level of *PAX6* activity and the severity of the phenotype (Glaser et al., 1994; Glaser et al., 1992; Hanson et al., 1999). The *PAX6* gene is essential for eye development in all species in which it has been studied, and it has been termed the master regulatory gene of eye organogenesis (Gehring, 1996). Homozygous *PAX6* mutations in flies to humans completely lack eyes (Pichaud & Desplan, 2002). In addition to the importance of *PAX6* in eye development, *PAX6* plays a role in neural, pancreatic, and pituitary development (Glaser et al., 1992; Hanson et al., 1999; Simpson & Price, 2002; Solomon et al., 2009; Wen et al., 2009). In mammals, PAX6 is expressed in the eyes, nasal structures, brain, spinal cord, pancreas, and pituitary. PAX6 may also regulate cell proliferation, determination, and death in both normal development and oncogenesis by affecting gene expression or function of other transcription factors (Simpson & Price, 2002).

PAX6: Phenotypes in animals

The human *PAX6* gene is a homolog of the mouse *Pax6*. The small eye (Sey) mutant mouse and rat have been associated with mutations in *Pax6* and findings indicate that human aniridia and murine small eye phenotypes arise due to the defects in *PAX6* (Glaser et al., 1992; Hill et al., 1991; Walther & Gruss, 1991). Mice with homozygous

spontaneous point mutations in the *Pax6* gene die neonatally with anophthalmia as well as neurodevelopmental anomalies including absence of the corpus callosum, olfactory bulb, cerebellar vermis, and pancreas. Homozygous *Pax6-/-* mice that lack the PAX6 protein also fail to develop the pineal gland and subcommisural organ and have an abnormal posterior commissure (Estivill-Torrus et al., 2001). Heterozygous *Pax6+/-* mice have iris hypoplasia, cataracts, and anomalies of the cornea, olfactory bulb, and cerebrum. Heterozygous *Pax6+/-* mice exhibit a grossly normal posterior commissure and a present, but often smaller pineal gland (Estivill-Torrus et al., 2001). Similar abnormalities were described in humans with heterozygous *PAX6* mutations, but some features in humans appear to be more striking than in murine heterozygotes. For example, anterior commissure and pineal absence were noted in humans with heterozygous *PAX6* mutations, while neither was found in heterozygous murine *Pax6* mutants (Mitchell et al., 2003).

PAX6: Phenotypes in humans

In 2000, Chao et al. examined *PAX6* mutations in twenty human subjects. All mutations in *PAX6* resulted in similar phenotypic presentation (e.g. aniridia, cataracts, glaucoma, and, nystagmus), and their mutational data supports the notion that haploinsufficiency of *PAX6* causes aniridia in humans (Chao et al., 2000). In 2001, Sisodiya et al., assessed fourteen human subjects with aniridia due to *PAX6* mutations using MRI to look for alterations in brain structure and the Smell Identification Test to assess olfactory capacity. In ten of the fourteen subjects (71.4%), the anterior commissure was absent, two were hypoplastic (14.3%), and only two were normal size (14.3%). The anterior commissure is implicated in both olfactory and auditory processes, and the

authors noted that smell testing demonstrated reduced olfaction in thirteen of the fourteen subjects (Sisodiya et al., 2001). In 2003, Mitchell et al. recruited twenty-four human subjects with ocular anomalies with various *PAX6* mutations and one hundred neurologically normal subjects to undergo high resolution MRI. The pineal gland was found to be absent in thirteen and abnormal in seven of the twenty-four subjects with *PAX6* mutations, but normal in all healthy subjects. Prior to this study, no cases of human pineal absence had been previously reported (Mitchell et al., 2003).

In 2009, Abouzeid et al. examined ten human subjects from three families with aniridia. All participants underwent ophthalmologic and neurological examination as well as cerebral magnetic resonance imaging (MRI) of the index case from each family. Common ocular features included absence of iris tissue, corneal pannus, and foveal hypoplasia with severely reduced visual acuity. Neurologic exam was normal in all ten patients. Cerebral MRI showed absence of the pineal gland in all three index patients. Sleep studies were not performed, thus the functional consequences of the absence of the pineal gland could not be assessed (Abouzeid et al., 2009).

Compound heterozygosity (two heterozygous alleles with different mutations) for *PAX6* mutations has been described in two case reports. The first reported case was a female born by cesarean section at 43 weeks gestation who inherited a different *PAX6* mutation from each parent. The infant presented with severe and multiple craniofacial and CNS defects and died on the 8^{th} day of life (Glaser et al., 1994). The only living patient was described at 4 years of life and also inherited a different *PAX6* mutation from each parent. MRI showed agenesis of corpus calosum, hypoplastic pons and vermis, pituitary and hypothalamic hypoplasia, and globular basal ganglia. This family

demonstrated the wide phenotypic spectrum with mutations in *PAX6* with the father's missense mutation resulting in milder anomalies than the mother's nonsense mutation (Solomon et al., 2009).

Pineal

As outlined, *PAX6* appears to play a role in development of the brain including the pineal, a gland in the brain that produces melatonin, a hormone involved in circadian regulation. Descriptions of the pineal can be traced back to antiquity and identification of the pineal can be traced back to the third and fourth centuries BC. Around AD 130-200, Galen of Pergamon is said to have provided the first description of the pineal location within the human brain. The philosopher René Descartes (1596-1650) is coined as conceptualizing the belief of the pineal as the 'seat of the soul' or the organ that coordinates psychophysiological functions (Lopez-Munoz, Molina, Rubio, & Alamo, 2011). The pineal is located proximally on the posterior aspect of the diencephalon. The pineal is positioned 1-2mm from the midline (between the two hemispheres) making it a great reference point for the midsagital plane. The pineal is remarkable for its rich vascularization. In most mammals, the pineal is a circumventricular organ, lacking an endothelial blood-brain barrier and reacts to peripherally acting drugs. The pineal functions as an endocrine gland, a regulator of hormones, and a circadian oscillator and although other pineal products and substances (e.g. 5-methooxytryptophol) have been noted, melatonin remains the most widely researched (Macchi & Bruce, 2004).

Melatonin

In 1958, the bovine pineal was used to identify the indoleamine N-acetyl-5 methoxytryptamine that became known as melatonin due to its ability to affect frog skin

melanophores as well as the chemical similarity to serotonin (Macchi & Bruce, 2004). Biochemically, pinealocytes take up tryptophan from the blood and convert it to serotonin; serotonin is then converted to N-acetylserotonin, which is then methylated into melatonin (Axelrod, 1974). In the 1960s, development of fluorescent techniques have allowed for the measurement of pineal melatonin with identification of diurnal secretion of melatonin in rodents (Macchi & Bruce, 2004). Melatonin has been detected in blood, saliva, urine, breast milk, and cerebral spinal fluid. Studies have also confirmed the existence of melatonin synthesis in humans in the retina, the gut, the liver, and peripheral cells such as bone marrow cells, lymphocytes, and epithelial cells (Bubenik, 2002; Cagnacci, 1996; Conti et al., 2000; Tan et al., 1999). In 1999, sheep were studied to see whether the genetic differences in plasma melatonin concentration differ due to either pineal size or ability of the pineal to synthesize and/or secrete melatonin. Genetic variability in plasma melatonin in sheep was found to be due to pineal weight, not variations in the enzymatic activity of the pinealcytes (Coon et al., 1999b).

Pineal melatonin modulates circadian rhythm, exerts seasonal rhythmicity, and regulates sleep. In humans, the melatonin cycle is essential for circadian timing and impels the normal sleep/wake cycle which is important for optimal health (Reiter, Tan, Korkmaz, & Ma, 2011). Melatonin has been termed a chronobiotic, a substance that adjusts the timing of internal biological rhythms. Biological circadian rhythms are internally generated, and humans have a slightly longer than 24 hour period (Arendt & Skene, 2005). In healthy humans, melatonin concentration starts to rise to detectable thresholds in the evening. Melatonin reaches maximum levels in the middle of the night and then decreases before habitual wake up time (Arendt & Skene, 2005). Research

points to a web of temporal interactions between melatonin, body temperature, and sleep. Approximately two hours prior to habitual bedtime, melatonin rises and that peak coincides with the nocturnal trough in body temperature (Cagnacci, 1996; Lavie, 1997). Melatonin levels are normally higher in the night than in the daytime in all organisms studied thus far, and in nocturnal animals, melatonin is elevated during the active phase (Macchi & Bruce, 2004). Many circadian rhythms including temperature, cortisol, and melatonin depend on the interaction of retinally perceived light and the suprachiasmatic nucleus (SCN) (Kennaway, Goble, & Stamp, 1996). The circadian rhythm is endogenously driven as even organisms under constant darkness persist in rhythm. The SCN is the main endogenous pacemaker and synchronizes with the external day-night cycle, and the circadian rhythm of melatonin is abolished by lesions in the SCN (Klein & Moore, 1979). In humans, melatonin levels show no circadian variation until two to three months after birth, but circumstances related to intrauterine development or post-natal environmental exposure may modify the expression of the rhythms. Serum melatonin has been found to be the highest during childhood with a decrease starting near puberty. Serum melatonin levels tend to stabilize in adults with decreases in late adulthood (Attanasio, Borrelli, & Gupta, 1985; Kennaway et al., 1996; Waldhauser, Kovacs, & Reiter, 1998). In most vertebrates seasonal variations in the day length lead to opposing changes in the duration of nocturnal melatonin secretion; however due to the use of artificial light, humans are largely shielded from seasonal changes (Arendt & Skene, 2005).

In humans, administration of melatonin during the day or early evening, when physiological levels are low, generally increases sleep propensity, subjective sleepiness,

and fatigue (Dollins et al., 1993; Hughes & Badia, 1997; Pires et al., 2001; Reid, Van den Heuvel, & Dawson, 1996; Zhdanova et al., 1995). Low doses of melatonin have been shown to facilitate sleep onset without alterations in sleep architecture or changing alertness the next day (Leu et al., 2011; Pires et al., 2001; Zhdanova et al., 1995). Melatonin is metabolized primarily in the liver, but also in the kidney. Pathologies of the liver and kidney (e.g. cirrhosis or chronic renal failure) are known to alter clearance rate. After oral administration of 2 mg of melatonin, peak serum levels are reached in 52.0 ± 31.5 minutes with an elimination half-life of 60.8 ± 13.2 minutes. An investigational study demonstrated that only 15% of the ingested dose (2mg or 4mg) reached systemic circulation with oral melatonin undergoing significant first-pass metabolism (DeMuro, Nafziger, Blask, Menhinick, & Bertino, 2000). Certain medications have been found to decrease endogenous serum melatonin levels such as benzodiazepines, beta-blockers, and caffeine (Kabuto, Namura, & Saitoh, 1986; McIntyre, Burrows, & Norman, 1988; Shilo et al., 2002; Stoschitzky et al., 1999; K. P. Wright, Jr., Badia, Myers, Plenzler, & Hakel, 1997). In humans, the primary metabolite is melatonin sulfate (6-sulfatoxymelatonin), and in studies using labeled exogenous melatonin, the urinary concentration of melatonin sulfate reflects nearly 90% of administered melatonin (Arendt, 1995).

In addition to circadian and sleep regulation, melatonin may have reproductive, gastrointestinal, immunological, psychiatric, metabolic, and weight-related effects. In mice, melatonin variation is used as a seasonal clock and aids in reproduction. In seasonal breeders, melatonin transmits information about day length to aid in reproduction (Waldhauser et al., 1998). In humans, melatonin's role in reproduction is not clear, although it has been shown that there is a significant decline in serum melatonin

concentration at the onset of puberty (Attanasio et al., 1985; Waldhauser et al., 1984). Melatonin appears to have gastroprotective effects, and it has been considered for prevention and treatment of colorectal cancer, ulcerative colitis, gastric ulcers, irritable bowel syndrome, and childhood colic (Bubenik, 2002). Melatonin appears to have an immune-enhancing effect with suppression of endogenous melatonin in mice resulting in a decrease in spleen and thymus activity, which is reversed by melatonin administration (Maestroni, 1993). Another potential mechanism of action of melatonin is a role in free radical scavenging as melatonin has been shown to be a powerful antioxidant (Reiter et al., 1995). Melatonin has also been shown to demonstrate oncostatic properties by slowing tumor progression in animal models (Karasek & Pawlikowski, 1999). In humans, several studies have found lower melatonin levels in neurological and psychiatric disorders such as depression and schizophrenia (Macchi & Bruce, 2004). In 2011, Reiter et al. reviewed the potential role that the circadian system (including the sleep/wake cycle and melatonin rhythm) may play in determining the accumulation of fat, as well as the health consequences of circadian perturbations, melatonin suppression, and sleep deficiency. Melatonin may have anti-obesity effects, as rats provided with melatonin in their drinking water reduced body weight gain and amount of visceral fat compared to animals that drank only water. Melatonin also stimulates the growth and metabolic activity of brown adipose tissue (BAT), which has high metabolic activity and is involved in thermogenesis in rodents. Thus, melatonin has the possibility of weight inhibitory effects due to stimulatory actions on BAT (Reiter et al., 2011).

Pinealectomy and disorders with altered melatonin

Pinealectomy in rats attenuates nighttime levels of melatonin in the blood, while daytime levels are unaffected. There are minimal data in human's post-pinealectomy, but the limited studies suggest a possible increase in sleep problems. Case reports observed that melatonin replacement therapy subjectively improved sleep quality in patients after pinealectomy (Jan, Tai, Hahn, & Rothstein, 2001a; Lehmann, Cockerell, & Rudge, 1996). Besides iatrogenic melatonin deficiency due to post-surgical pinealectomy, other human conditions exist that have altered melatonin production which may shed light on the role of melatonin in humans. Smith-Magenis Syndrome (SMS) is a rare multisystem disorder caused by a heterozygous interstitial deletion of chromosome 17p11.2. The phenotype of SMS includes infantile hypotonia, generalized complacency and lethargy in infancy, minor skeletal and craniofacial features, ocular abnormalities, middle ear and laryngeal abnormalities, and psychomotor and growth retardation. Persons with SMS have a uniquely inverted circadian rhythm of melatonin secretion (high daytime levels and low nighttime levels), and sleep disturbance is a universal feature of SMS from infancy through adulthood. Findings from polysomnography, actigraphy, sleep diaries, and questionnaires have been used to describe features of sleep disturbance in SMS which support reduced sleep time and sleep disturbance in persons with SMS (Gropman, Duncan, & Smith, 2006).

Irregular patterns of sleep-wake rhythm, as well as abnormalities in melatonin physiology, have also been found in children and adolescents with neurodevelopmental disorders (e.g. autism spectrum disorders). For example, monitoring of serum melatonin levels revealed that individuals with autism spectrum disorders have lower nocturnal

serum melatonin levels as well as urinary melatonin sulfate, in comparison to controls (Kulman et al., 2000; Melke et al., 2008; Tordjman, Anderson, Pichard, Charbuy, & Touitou, 2005b). Melatonin supplementation appears to be a safe, well-tolerated treatment for sleep disorders in children with neurological impairments (Andersen, Kaczmarska, McGrew, & Malow, 2008a; Braam, Didden, Smits, & Curfs, 2008a; De Leersnyder, Zisapel, & Laudon, 2011; Giannotti, Cortesi, Cerquiglini, & Bernabei, 2006; Sanchez-Barcelo, Mediavilla, & Reiter, 2011). Melatonin has been found to help treat children with neurological impairments who experience chronic sleep disorders with benefits extending not only to the children but also the family (Carr et al., 2007; De Leersnyder et al., 2011; Giannotti et al., 2006; Wasdell et al., 2008b). In one study, forty-seven children (age 2.05-17.81 years) with neurodevelopmental disabilities completed a randomized double-blinded, placebo-controlled, crossover trial of controlled release melatonin (5 mg) followed by a 3 month open-label study. The crossover trial consisted of 10 days of treatment, followed by a placebo washout for 3-5 days, followed by 10 days of alternative treatment. Wrist actigraphy assessed sleep patterns and caregivers completed sleep logs. The Clinical Global Impression-Severity (CGI-S) and the Clinical Global Impression Improvement (CGI-I) rating scales were used by clinicians to evaluate effectiveness of treatment. The CGI-S provided a cross-sectional evaluation of the severity of sleep difficulty and the CGI-I provided a longitudinal evaluation of improvement from baseline (Wasdell et al., 2008b). To capture the caregiver's perspective on the severity of the children's impairment across several functional and health dimensions, caregivers completed the Parents' Global Assessment Scale. The Family Stress Scale was used to access family stress due to the sleep disorder. The study

found that total night-time sleep was significantly greater and sleep latency was significantly shorter in the melatonin group compared to placebo. Significant improvements were observed in all clinician and parent ratings scales in the melatonin group compared to placebo. During the subsequent open-label trial, significant continued improvements were observed in all clinician and parent ratings scales in the open-label melatonin group compared to the randomized trial of melatonin (Wasdell et al., 2008b).

Research Paradigm and Conceptual Framework

An overall biobehavioral research paradigm linking genetics, biology, and behavior guided the study. According to the 2011 *NINR Strategic Plan: Bringing Science to Life*, nurse scientists are well suited for patient-centered biobehavioral research with a primary focus on health promotion, symptom management, quality of care, and quality of life. To advance the science of health, one of the National Institute of Nursing Research (NINR) investments in research is to improve quality of life by managing symptoms of acute and chronic illness. NINR seeks to provide a better understanding of symptoms and symptom clusters that will improve clinical management of illness including the management of pervasive symptoms such as pain, fatigue, depression, and impaired sleep. These symptoms cross multiple conditions across the lifespan and impair quality of life. According to the NINR Strategic Plan, symptom management research improves our understanding of the genomic and biological mechanisms associated with symptoms while at the same time increasing our knowledge of the behavioral aspects of symptoms that reduce one's ability to live a typical life ("NINR Strategic Plan: Bringing Science to Life," 2012).

The Biobehavioral Model of Altered Dysregulation in Circadian Systems was used to provide direction for research design and assessment tools (Carlson et al., 2007) (Figure 1.1). In addition, the sleep cycle was used as a model for studying the physiology of sleep. We have reported on pineal gland volume, melatonin production, and parent-reported sleep disturbance in patients with *PAX6* haploinsufficiency (Hanish, 2012). An additional study will focus on objective and subjective measurements of sleep patterns, sleep disturbance, and sleep-related impairment in adolescents with *PAX6* haploinsufficiency.

Design

An observational study design was used to fulfill the aims of the study. The study recruited participants with haploinsufficiency of *PAX6* either through a contiguous gene deletion syndrome (WAGR) or point mutations and microdeletions affecting only *PAX6*, which result in isolated aniridia. The study is an amendment to an existing Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) protocol, entitled "WAGR Syndrome and Other 11p Contiguous Gene Deletions: Clinical Characterization and Correlation with Genotype" (08-CH-0213, Joan Han; Jack Yanovski, PIs).

Sleep logs and actigraphy were used to objectively examine sleep patterns in adolescents with *PAX6* haploinsufficiency. Preliminary studies of the concurrent and construct validity of the PROMIS sleep disturbance and sleep-related impairment questionnaires were completed in an adolescent population. Validity is the extent to which an instrument measures what it claims to measure. In establishing concurrent validity, scores on an instrument are correlated with scores on another measure of the

same construct or a highly related construct. This type of validity is often accomplished when comparing a measure to the gold standard measure to see if it reflects the same construct (Kimberlin & Winterstein, 2008). The Cleveland Adolescent Sleepiness Questionnaire (CASQ) was used as a comparison sleep measures, as it is an age-appropriate self-report questionnaire that has met the most psychometric criteria as outlined by the review of pediatric sleep questionnaires by Spruyt and Gozal (2011). Although in the sleep field, polysomnography is the gold standard for sleep assessment, it is not routinely indicated and requires equipment and facilities (T. I. Morgenthaler et al., 2007), thus actigraphy was used as the gold standard objective comparison. Establishing construct validity requires examining the relationship of the measure being evaluated with variables known to be related to the construct being measured by the instrument (Kimberlin & Winterstein, 2008). For this study, construct validity was assessed by comparing the CASQ and PROMIS scores in a population with plausible sleep problems, adolescents with *PAX6* haploinsufficiency, to healthy adolescents.

Summary

Haploinsufficiency of *PAX6* can result from (1) WAGR, a contiguous gene deletion syndrome in which multiple genes are involved, or (2) point mutations and microdeletions affecting only *PAX6*, which result in isolated aniridia. In addition to the role of *PAX6* in eye development, *PAX6* also appears to play a role in development of the pineal, a gland in the brain that produces melatonin, a hormone involved in circadian regulation. Although *PAX6* haploinsufficiency is rare, and minimal research has focused on the role of *PAX6* in circadian regulation, irregular patterns of sleep-wake rhythm have been studied in another population with possible abnormalities in melatonin physiology,

children and adolescents with a neurodevelopmental disorder. Chapter 2 presents an integrative review on sleep methodology used in adolescents with a neurodevelopment disorder. Our study (Chapter 3) is the first to report that both pineal volume and melatonin production are significantly lower in humans with *PAX6* haploinsufficiency. In addition, parent report of children (2-12 years) with *PAX6* haploinsufficiency suggests greater sleep problems in comparison to healthy controls (Hanish, 2012).

The purpose of the observational study in Paper 3 is to determine if adolescents who have an abnormality in the *PAX6* gene have sleep problems and to describe the sleep-related phenotype associated with PAX6 haploinsufficiency. For this study, objective and subjective adolescent sleep health was assessed through measurement of sleep patterns, sleep disturbance, and sleep-related impairment in adolescents with *PAX6* haploinsufficiency in comparison to healthy adolescents. Describing sleep-related phenotypes in adolescents with WAGR and isolated aniridia is necessary as sleep problems may go unrecognized and undertreated, which may compound existing conditions. However, measures used to describe sleep-related phenotypes are not well developed for adolescents, limiting the amount and type of data that can be gathered on sleep phenotypes and sleep problems in adolescent populations. In addition, this study seeks to preliminarily validate the use of sleep disturbance and sleep-related impairment measures in a general adolescent population. Although WAGR syndrome and isolated aniridia are rare disorders, describing the sleep-related phenotypes in this population will advance the assessment and treatment of sleep disorders in general, facilitating research in adolescents without these conditions.

Table 1.1. Objective Sleep Patterns

Variables to Measure	Conceptual Definition	Operational Definition
Sleep onset latency	How long it takes to transition from wakefulness to sleep	Time between bedtime and sleep onset as measured by self-report sleep diary and actigraphy
Bedtime	When one attempts to fall asleep	Clock time to fall asleep as measured by self-report sleep diary
Sleep Onset	When one falls asleep	Clock time for first of predetermined number of consecutive minutes of sleep following reported bedtime as measured by actigraphy
Sleep efficiency	Percentage of time one spends in bed asleep	TST/sleep opportunity * 100 as measured by self-report sleep diary and actigraphy
Total Sleep Time (TST)	How long one is actually sleeping	Duration of sleep in sleep period as measured by actigraphy
Sleep opportunity	How long one attempts to fall asleep until one intends to wake up	Time between bedtime and wake time as measured by self-report sleep diary

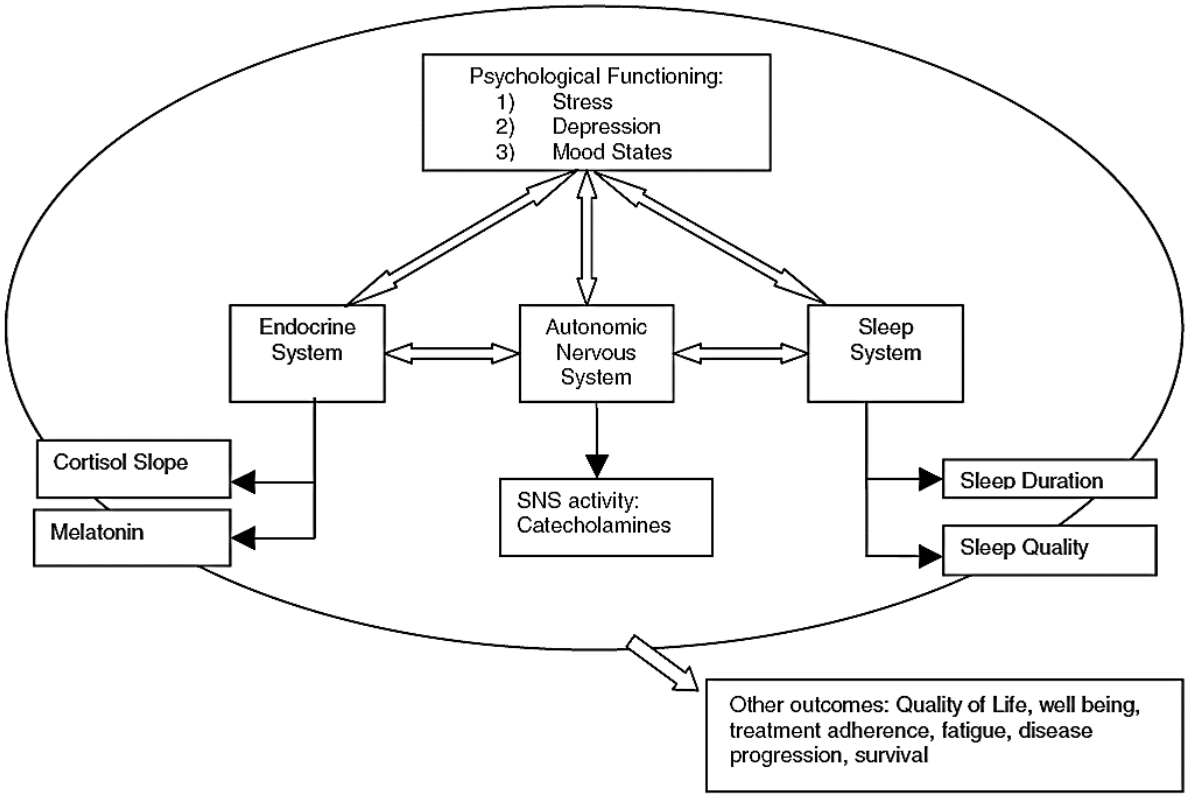
Adapted from (Meltzer et al., 2012)

Table 1.2. Subjective Report: Sleep Disturbance, Sleep Related Impairment, & Daytime Sleepiness

Variables	Conceptual Definition	Operational Definition	Measurement Tool
Sleep Disturbance	Self-reported perceptions of sleep quality, depth, and restoration	Total score as measured my PROMIS Sleep Disturbance	PROMIS Questionnaire
Sleep-related Impairment	Self-reported alertness, sleepiness, tiredness, and functional impairments associated with sleep problems during waking hours	Total score as measured my PROMIS Sleep-related impairment	PROMIS Questionnaire
Daytime Sleepiness	Inability to stay awake or alert during major waking episodes of the day resulting in unintended lapses into drowsiness or sleep (no clear definition given by authors)	Total score as measured by Cleveland Adolescents Sleepiness Questionnaire (CASQ)	CASQ

Adapted from (PROMIS, 2012)

Figure 1.1. Biobehavioral Model of Altered Dysregulation in Circadian Systems



(Carlson et al., 2007)

CHAPTER 2: SLEEP RESEARCH METHODOLOGY IN ADOLESCENTS WITH A NEURODEVELOPMENTAL DISORDER: INTEGRATIVE REVIEW

Abstract

Studies in the past decade suggest that adolescents with a neurodevelopment disorder report high rates of sleep disturbance. The purpose of this review is to synthesize the literature regarding the sleep measures used to assess sleep disturbance in adolescents with a neurodevelopmental disorder. Research from 21 countries (n=66 studies) provided an international perspective. This review identified that sleep-related measures included: parent/caregiver report questionnaires (n=37), self-report questionnaires (n=3), sleep logs (n=13), actigraphy (n=7), and polysomnography (n=20). Sixteen studies included multiple types of sleep-related measures. Fifteen different sleep questionnaires were reported, although the majority of studies used unspecified or author-made questionnaires. None of the studies reported used only an adolescent sample (10-19 years), however all samples contain adolescents, in addition to other age groups across the lifespan. A greater understanding of sleep measures specific to adolescents with a neurodevelopmental disorder was proposed as a possible avenue for future research.

Keywords: sleep, adolescent, neurodevelopmental disorder, autism, integrative review

Introduction

Sleep and circadian disorders are recognized by Congress and the Department of Health and Human Services as high priority targets for basic and clinical scientific investigation ("National Institutes of Health Sleep Disorders Research Plan," 2011). Sleep health has been added to the agenda for Healthy People 2020 with one of the

objectives being to increase the proportion of adolescents in grades 9 through 12 who get a sufficient amount of sleep ("Sleep Health," 2014). Sleep health and sufficient sleep are particularly important during adolescence, when important physical, cognitive, emotional, and social changes occur. In the literature, inadequate sleep health is often described using the terms ‘sleep disorders’, ‘sleep problems’, ‘sleep deprivation’, and ‘sleep disturbance,’ and these terms are often used interchangeably without clear definitions.

Although sleep is a primary aspect of adolescent development, insufficient sleep in adolescence is common, growing progressively worse over the course of adolescence. Among the pediatric population, adolescents carry the strongest sleep debt. Adolescents physiologically need about 9 to 10 hours of sleep; however, sleep averages around 7 hours a night with circadian rhythm changes making it more difficult for adolescents to fall asleep and also harder to wake up in the morning (Wolfson & Carskadon, 1998). Too little sleep can leave one feeling lethargic, sluggish, irritable, and moody, and can inhibit focus and concentration, slow response time, and decrease learning of cognitive tasks (Randazzo et al., 1998). Inadequate sleep in adolescence is associated with higher levels of depressed mood, anxiety, behavior symptoms, alcohol use, as well as suicidal behavior (Liu, 2004; Wolfson & Carskadon, 1998). There is also an association between lower academic performance and shorter sleep duration (Wolfson & Carskadon, 2003). In addition, inadequate sleep can pose safety risks. For example, almost 20 percent of all serious car crash injuries within the general population are associated with driver sleepiness (Connor et al., 2002) and adolescents and young adults are the mostly likely group to be involved in crashes caused by a driver falling asleep (Pack et al., 1995).

Children and adolescents with neurodevelopmental disorders appear to be at an increased risk of sleep-related disturbances with prevalence rates ranging from 50-80% (Couturier et al., 2005; Engelhardt, Mazurek, & Sohl, 2013; Krakowiak, Goodlin-Jones, Hertz-Picciotto, Croen, & Hansen, 2008). Sleep problems include bedtime resistance, as well as difficulty with sleep initiation and maintenance (Sivertsen, Posserud, Gillberg, Lundervold, & Hysing, 2012). Sleep-related disturbances are a clinically important issue in children and adolescents with a neurodevelopmental disorder as sleep problems can exacerbate and worsen repetitive and stereotypic behavior, inattention, and hyperactivity, as well as interfere with learning and cognition (Malow, McGrew, Harvey, Henderson, & Stone, 2006; Taylor, Schreck, & Mulick, 2012; Wiggs & Stores, 1996). In addition to the impact of sleep disturbance on the quality of life of the child, sleep problems in children and adolescents with a neurodevelopmental disorder can also adversely affect family functioning (Jan et al., 2008; Wasdell et al., 2008a). To date, the majority of sleep-related research in neurodevelopmental disorders has focused on autism spectrum disorders (ASD). Although it is known that sleep problems persist over time in children with ASD, less is known about sleep problems during adolescence (Baker, Richdale, Short, & Gradisar, 2013; Goldman, Richdale, Clemons, & Malow, 2012).

According to the National Institute of Nursing Research (NINR) strategic plan, symptom management research improves our understanding of the genomic and biological mechanisms associated with symptoms while at the same time increases our knowledge of the behavioral aspects of symptoms that reduce one's ability to live a typical life. The strategic plan notes that NINR seeks to provide a better understanding of symptoms and symptom clusters that will improve clinical management of illness

including the management of pervasive symptoms such as impaired sleep, which crosses multiple conditions across the lifespan and impairs quality of life ("NINR Strategic Plan: Bringing Science to Life," 2012). Sleep is a readily accepted clinical construct, however it is a complex phenomenon that is difficult to define and measure. Examination of impaired sleep in adolescents requires measures that are valid and reliable for this population. Measures used to assess sleep include questionnaires, sleep logs, actigraphy, and polysomnography.

Sleep questionnaires provide a subjective measurement of sleep and can be completed by a parent and/or caregiver, as well through self-report. In 2010, extensive lists of published and unpublished instruments used to investigate sleep issues in children and adolescents were collected, and their psychometric properties were evaluated using eleven methodological criteria outlined by the authors (purpose, research question, response format, generation of items, pilot, item-analysis and non-response analysis, structure, reliability, validity, confirmatory analysis, and standardization and norms development) (Spruyt & Gozal, 2011). A total of 57 questionnaires were evaluated and were included in the review. Using the methodological criteria outlined by Spruyt and Gozal, two parent-report measures, the Sleep Disturbance Scale for Children (ages 6-15) and the Sleep Disorders Inventory for Students-Children and Adolescents (ages 11-18), met the eleven established conditions. In addition, two child self-report measures, the Dream Content Questionnaire for Children (ages 9-14) and the Cleveland Adolescent Sleepiness Questionnaire (ages 11-17) nearly met the eleven methodological criteria (both missing standardization and norms development). However, no self-report measures for adolescents fully met the 11 psychometric criteria (Spruyt & Gozal, 2011).

Although sleep questionnaires may provide a valuable subjective measurement of sleep, limitations have been noted. The majority of sleep questionnaires used to investigate sleep issues in children and adolescents are completed by a parent or caregiver (Spruyt & Gozal, 2011), which is heavily dependent on the assessment instrument and the parent's perception of and tolerance for the observed symptoms (Tietze et al., 2012). Questionnaires tend to assess information from one point of time, either retrospective or concurrent, and researchers are often uncertain of the accuracy of the participant's reported behavior (e.g. bedtime) (Wolfson et al., 2003). Although many sleep questionnaires have been developed and the psychometrics have been evaluated (Spruyt & Gozal, 2011), there is no standard sleep-related questionnaire used to measure sleep in pediatric populations. In addition, it is unclear as to what questionnaires are being used to assess sleep in children and adolescents with a neurodevelopmental disorder, a population vulnerable to sleep disturbances.

Sleep logs are also completed by a parent and/or caregiver, as well through self-report. They contain reported data such as sleep and wake time, quality of sleep, and number of naps. Sleep logs have face validity and provide both qualitative and quantitative data in regards to sleep quality and sleep duration. Sleep logs are used in the assessment of patients with a suspected sleep disorder; however, there is no widely accepted standardized sleep log (Sack et al., 2007a). Sleep logs can be used to aid in data analysis when using actigraphy by providing information regarding approximate sleep and wake times. However, discrepancies between subjective report and objective measurement have been reported. For example, one study noted that parental report of their child's night wakings did not correspond with actigraphy measures of night wakings

thus demonstrating that parents are often unaware of when and for how long children are awake during the night (Holley, Hill, & Stevenson, 2010). In comparison to polysomnography, participants tend to underestimate sleep duration and number of night wakings and overestimate sleep latency (Chambers, 1994; Sadeh, Sharkey, & Carskadon, 1994; Wolfson et al., 2003).

Actigraphy, an objective measurement of sleep patterns, is based on the correlation between sleep-wake state and motor activity. An ambulatory device can be worn on the wrist to provide an estimate of sleep based on the wrist motion. The device can be worn for a prolonged period of time, in the home setting, and can provide a continuous estimate of the activity-rest cycle during the period of study. Actigraphy is a valid way to assist in determining sleep patterns in normal, healthy populations as well as patients with suspected sleep disorders (Acebo et al., 1999; Sadeh, 2011; Sadeh & Acebo, 2002; Sadeh et al., 1994). There is relatively good agreement in studies that actigraphy data correlate with parent and child-reported sleep logs and polysomnography (T. Morgenthaler et al., 2007).

Polysomnography is a sleep study that comprehensively records biophysiological changes that occur during sleep. A sleep study is typically conducted in a health care facility and monitors eye movement, brain waves, heart rate, electrical activity of muscles, body position, breathing effort, rate, and blood oxygen levels. Polysomnography is the gold standard for sleep assessment, however it is not routinely indicated and requires equipment and facilities (T. I. Morgenthaler et al., 2007).

Adolescence is a time of transition which can be challenging in typically developing children, and although sleep is a primary aspect of adolescent development,

insufficient sleep in adolescence is common and may be magnified and exacerbate underlying conditions in children with neurodevelopmental disorders. Examination of impaired sleep in adolescents requires measures that are valid and reliable for this population; however, it is unclear as to what measures are currently being used to assess sleep in adolescents with a neurodevelopmental disorder, and whether they meet psychometric criteria. The purpose of this review is to synthesize the literature regarding the sleep measures currently being used to assess sleep disturbance in adolescents with a neurodevelopmental disorder in order to develop recommendations on sleep-related measures to be used in future research.

Methods

An integrative review methodology (Whittemore & Knafl, 2005) was purposefully selected to summarize existing empirical evidence. This method allows for inclusion of research from diverse methodologies and for various purposes (e.g. methodological issues of phenomena of interest) and provides a rigorous and systematic approach. The integrative review process includes problem identification, literature search, data evaluation, data analysis, and presentation of the study findings (Whittemore & Knafl, 2005). The problem identified is it is unclear as to what sleep-related measures are currently being used to assess sleep in adolescents with a neurodevelopmental disorder.

Literature Search

Electronic searches of CINAHL, PsycINFO, and PubMed were conducted to identify all relevant studies. The search used predetermined inclusion and exclusion criteria (Table 2.1) and keywords of relevance to the aim of the integrative review.

Search terms included neurodevelopment disorder or disability and sleep and adolescent, as well as diagnosis specific disorders (e.g. autism) used in combination with the search terms sleep and adolescents (Table 2.2). We limited our study to papers that were published from July 2004 to July 2014 to capture literature that focuses on the current state of the sleep literature. In total, the initial search yielded 307 citations. After removal of duplicates and screening of titles and abstracts, 72 relevant studies were identified for evaluation. After full text read of the 72 articles, 6 were excluded due to the sample not including any adolescents (n=3) or not including sleep-related measures (n=3). Although the purpose of this review was to synthesize the literature regarding the sleep measures used to assess sleep disturbance in adolescents with a neurodevelopmental disorder, none of the articles included an adolescent-only (10-19 years) sample. One study does claim to include only adolescents in the sample; however the age range was not described (Baker et al., 2013). To be included in the review, the study sample contained at least one adolescent. The reference lists were reviewed, which did not lead to inclusion of any additional studies, but confirmed already selected references. Studies originated from 21 countries, the majority of which were conducted in the United States (Table 2.3). The final sample included 66 articles.

Data Evaluation

Each article was evaluated using generic criteria for evaluating the literature (McCarthy & O'Sullivan, 2008). Components of the criteria include key questions related to the abstract, literature review, aim/objectives, design, sample, ethical approval, data collection, data analysis, findings, and discussion. In addition to using the generic criteria, qualitative and quantitative research-specific criteria were utilized allowing for

data of studies using both research designs (McCarthy & O'Sullivan, 2008). The purpose of this review is to synthesize the literature regarding the sleep measures currently being used to assess sleep disturbance in adolescents with a neurodevelopmental disorder, not to directly assess quality of measures (e.g. psychometric criteria).

Data Analysis

The constant comparison method: data reduction, data display, data comparison, conclusion drawing, and verification were used for analysis (Whittemore & Knafl, 2005). Data were extracted from the studies including the authors, year of publication, sample (e.g. age, number of participants, and diagnosis), sleep measures, main findings, and recommendations for sleep-related research. The constant comparison method was used to analyze the final sample of 66 articles, as well as in a subset of articles including participants ≥ 7 years of age through ≤ 20 years of age that may more closely mirror an adolescent sample (n=8).

Results

Study Characteristics

The final sample for this integrative review included 66 articles published from July 2004 through July 2014 (Table 2.6). Research from 21 countries provided an international review (Table 2.3); however, it should be noted that authors did not always clearly state the country in which the sample was obtained thus author affiliations of country of origin were used as a proxy when not found within the sample characteristics of the manuscript. Sample size ranged from 4 to 1,849 participants. About one-quarter of the studies had less than 20 participants with a neurodevelopmental disorder (n=18/66), and over one-half of the studies had less than 40 participants with a neurodevelopmental

disorder (n=36/66). At least seventeen diagnoses were included in this review with an autism-related diagnosis (e.g. autism spectrum disorders, autism, Asperger syndrome) being the most commonly reported (n=20) (Table 2.4). In addition, 10 of the studies included participants with different neurodevelopmental disorders in one sample or participants were diagnosed with multiple disorders within the sample (e.g. participants with both autism and Fragile X syndrome).

Sleep Measures Reported

Reported sleep measures included: parent/caregiver or self-report questionnaires (n=40), sleep logs (n=13), actigraphy (n=7), and polysomnography (n=20) (Table 2.5). Sixteen studies included multiple types of sleep-related measures (e.g. questionnaire and polysomnography within one study).

Questionnaires

The most commonly reported sleep measure was a parent/caregiver reported sleep questionnaire (n=37) (Table 2.5). The majority of studies used unspecified or author-made questionnaires (n=14) (Table 2.5). Fifteen different sleep questionnaires were identified, with the Child Sleep Habits Questionnaire (CSHQ) was the most common measure used (n=11) (Table 2.5). The CSHQ is a reliable and valid questionnaire that examines sleep disturbance in children (Goodlin-Jones, Sitnick, Tang, Liu, & Anders, 2008; Owens, Spirito, & McGuinn, 2000). The psychometric properties have been reported including internal consistency in both a community sample (0.68) and clinical sample (0.78). Test-retest reliability ranged from 0.62 to 0.79 (Owens, Spirito, & McGuinn, 2000). The CSHQ contains 33 items that measure sleep disturbance and 3 items collecting information about bedtime, wake-up time, and sleep duration. Parents

rate the frequency of each item using a 3-point Likert scale with higher scores indicating more frequent sleep disturbances. The CSHQ includes 8 sub-scales reflecting the following sleep domains: 1) Bedtime Resistance; 2) Sleep Onset Delay; 3) Sleep Duration; 4) Sleep Anxiety; 5) Night Wakings; 6) Parasomnias; 7) Sleep-Disordered Breathing; and 8) Daytime Sleepiness (Owens, Spirito, & McGuinn, 2000).

Although parent-report may provide useful information, parent-report of sleep may underestimate children's sleep problems, and as children get older, parents may be unaware of increased sleep onset delay experienced by their children, as well as frequency and duration of any night wakings that may also affect total sleep time (Baker et al., 2013; Goldman, Richdale, et al., 2012; Owens, Spirito, McGuinn, & Nobile, 2000). Three studies included sleep questionnaires that were used as self-report measures completed by the child or adolescent (Baker et al., 2013; Goldman, Malow, Newman, Roof, & Dykens, 2009; Paavonen et al., 2008). In 2008, Paavonen et al. evaluated the prevalence of sleep disturbance in children with Asperger syndrome (n=52) compared to typically developing (TD) controls (n= 61). Children and adolescents (5 to 17 years of age) completed the Sleep Self Report (SSR) questionnaire (Paavonen et al., 2008). The SSR questionnaire is a 26-item, 1 week retrospective survey designed to be administered to or self-administered in children 7 to 12 years of age (Owens, Spirito, McGuinn, et al., 2000). To our knowledge, the reliability and validity of the SSR questionnaire has not been published. According to the authors, this is the first study to use self-report of sleep in children with ASD, and the child and adolescent report obtained from the SSR yielded additional data not obtained through parent-report measures including sleep-related fears and attitude toward sleeping (Paavonen et al., 2008). Parents were informed of how to

help their children if they had difficulties in filling out the SSR questionnaire, however, the authors note that despite parental instruction, it is possible that children with ASD may have had more problems understanding the questions than TD peers which may have affected their responses (Paavonen et al., 2008).

In 2009, Goldman et al. described sleep patterns of adolescents and adults (17-35 years of age) with Williams syndrome (n=23). Participants completed an interviewer-administered Epworth Sleepiness Scale (ESS), an 8-item validated adult questionnaire that measures excessive daytime sleepiness. A score of <10 out of 24 is indicative of increased daytime sleepiness (Johns, 1994). The psychometric properties have been reported including internal consistency (α=0.88) and test-retest reliability (r=0.82) (Johns, 1991, 1992). In this study, the authors note that in regards to the use of self-report questionnaires, individuals with Williams syndrome have a strong desire to please others and may give answers that they think others want to hear, which may lead to biased results; however actigraphy data supported the validity of self-report of sleep in Williams syndrome (Goldman et al., 2009).

In 2013, Baker et al. investigated sleep disturbance and sleep patterns in adolescents with high functioning autism spectrum disorder (HFASD) (n=27) compared to TD adolescents (n=27). Adolescents (mean age of 15.5 years) completed the Sleep Habits Survey (modified by the authors), Pediatric Daytime Sleepiness Scale, and Flinders Fatigue Scale (Baker et al., 2013). Although the authors note that they used an adolescent-only sample, no age range is reported. The Sleep Habits Survey (SHS) is an eight page, 63-item (with additional sub-questions) measure that has been used in adolescents 13-19 years of age (Wolfson & Carskadon, 1998). Evidence for the validity

of the SHS has been suggested in that strong correlations of survey reports with diary and actigraphy data have been reported (school-night values r=0.53 to 0.77) (Wolfson et al., 2003). The Pediatric Daytime Sleepiness Scale (PDSS) is an 8-item, retrospective self-report measure that has been used in adolescents 11-15 years of age to evaluate the experience of sleepiness, during given situations, over a two week period of time. Adolescents rate how sleepy they felt using a five-point Likert scale ranging from 'always' to 'never.' Scores range from 0 to 32, with higher scores indicating greater sleepiness (Drake et al., 2003). The internal consistency for the final 8-item scale has been reported as α=0.80 (Drake et al., 2003) and α=0.81 (Baker et al., 2013). The Flinders Fatigue Scale is a 7-item self-report measure to assess daytime fatigue over a two week period of time. Six items are presented in Likert format with responses ranging from 'not at all' to 'extremely'. Scores range from 0 to 31, with higher scores indicating greater fatigue. The internal consistency for the FFS has been reported as α=0.91 (Gradisar et al., 2007) and α=0.76 (Baker et al., 2013). According to the authors, the return rate of questionnaires and sleep diaries may be reduced in participants with HFASD due to the nature of the disorder and the possibility exists that only motivated individuals may participate (Baker et al., 2013).

Sleep Logs

Thirteen studies reported the use of a sleep log (Table 2.6). Four included a child or adolescent self-reported sleep log (Allik, Larsson, & Smedje, 2008; Baker et al., 2013; Goldman et al., 2009; Oyane & Bjorvatn, 2005), while the other nine studies used a parent or caregiver-reported sleep log. In three studies, a sleep log was the only sleep-related measure used (Garstang & Wallis, 2006; Memari et al., 2013; Takaesu, Komada,

& Inoue, 2012), while the other ten studies used a sleep log in combination with questionnaires, actigraphy, polysomnography, and/or measurement of melatonin concentration. Length of data collection ranged from three days (Memari et al., 2013) up to 9 months (Wirojanan et al., 2009) and included sleep variables such as start of bedtime routine, time asleep, night wakening, time awake, naps, and total sleep time. Only one study cited the sleep log as being previously validated in another population (Baker et al., 2013; Wolfson et al., 2003).

Actigraphy

Actigraphy was reported in seven of the studies, all of which also included a sleep log. Four different actigraph devices, from three companies, were used including: Micro-Mini Motionlogger (Ambulatory Monitoring Inc., Ardsley, New York) (Baker et al., 2013; Wasdell et al., 2008a), Actiwatch (Mini-Mitter, Bend, Oregon) (Goldman et al., 2009; Wirojanan et al., 2009), Actiwatch (Respironics Actiwatch 2, Bend, Oregon) (Gibbs, Wiltshire, & Elder, 2013), and Actiwatch (Cambridge Neurotechnology, Ltd., Cambridge, UK) (Allik et al., 2008; Oyane & Bjorvatn, 2005). The majority of studies included a seven-day period of data collection (Allik et al., 2008; Baker et al., 2013; Gibbs et al., 2013; Goldman et al., 2009) , while one study included two weeks of actigraphy data (Oyane & Bjorvatn, 2005). Two of the studies collected actigraphy data for an extended period of time (several weeks to months), as the aim of the studies was to determine the efficacy of melatonin administration (versus placebo) for sleep problems in children and adolescents with neurodevelopmental disorders (Wasdell et al., 2008a; Wirojanan et al., 2009).

Polysomnography

Polysomnography was reported in twenty of the studies (Table 2.6). All polysomnography studies were completed in an inpatient sleep laboratory setting (data typically collected on a single or two night stay); however, one study completed polysomnography both within a laboratory, as well as in the home environment (Bodizs, Gombos, & Kovacs, 2012). Half of the studies used polysomnography as the only sleep-related measure within the study, not including any additional questionnaire, sleep log, or actigraphy data. All studies reported polysomnography procedure, equipment, and analysis methods used, however a wide range was reported and description is beyond the scope of this review. Although child or adolescent-specific issues in regards to compliance with the polysomnography procedure were not found in any articles, other issues regarding the use of polysomnography were discussed. For example, one author notes that clinicians may consider investigations using polysomnography impractical due to limited access, geographic, and economic reasons (Fitzgerald, Paul, & Richmond, 2007).

Other Measures Reported

Ten studies have a component reported in the "other" category: four studies involve measurement of melatonin concentration, four involve patient chart review data and/or qualitative interview during patient assessment, and two studies used the Multiple Sleep Latency Test (all of which also used other measures e.g. polysomnography) (Table 2.6). The specificities of these studies will not be further discussed, as they are outside the scope of this review; however, they do add to additional measures being reported to assess sleep in adolescents with a neurodevelopmental disability.

Multiple Measures Used

Sixteen studies included multiple types of sleep-related measures, and it is difficult to draw conclusions regarding studies using multiple measures due to the multiple variables in specific populations and differing aspects of sleep being documented. Three of these studies included only a sleep log and actigraphy (Allik et al., 2008; Wasdell et al., 2008a; Wirojanan et al., 2009). As discussed previously, all studies that included actigraphy also included a sleep log, as sleep logs can be used to aid in data analysis when using actigraphy. Four studies included at least one sleep questionnaire in addition to a sleep log and actigraphy (Baker et al., 2013; Gibbs et al., 2013; Goldman et al., 2009; Oyane & Bjorvatn, 2005). In these studies, it appears as though sleep questionnaires were used to provide supplementary data rather than being used as a comparison to actigraphy variables. However, in their discussion Oyane and Bjorvatn did provide data comparing actigraphy variables to retrospective parent-report sleep questionnaires of adolescents with Asperger syndrome and autism. In this study, only two of the seven adolescents with low actigraphy-scored sleep efficiency had a parent report of a sleep problem using sleep questionnaires. Only one of the five individuals with high sleep onset latency had a parent-reported sleep problem and none of the individuals with low sleep efficiency and high sleep onset latency had a parent-reported sleep problem using sleep questionnaires. This suggests that despite objective sleep disturbances recorded using actigraphy, parents and caretakers did not always report sleep problems (Oyane & Bjorvatn, 2005). One possible explanation that has been suggested for this discrepancy is that parameters such as time to fall asleep and night wakings may be difficult for parents to estimate, as they may not directly be observed during the night.

For example, night wakings may be underestimated if the adolescent does not get out of bed to wake the parents (Oyane & Bjorvatn, 2005).

Seven studies included at least one sleep questionnaire and polysomnography (Bruni et al., 2007; Buckley et al., 2010; Hagebeuk, Bijlmer, Koelman, & Poll-The, 2012; Hagebeuk, van den Bossche, & de Weerd, 2013; Mason et al., 2011; Miano et al., 2007; Williams, Scheimann, Sutton, Hayslett, & Glaze, 2008). Although, it again appears that sleep questionnaires were used to provide supplementary data rather than used as a comparison to polysomnography variables, however a few studies did discuss congruency and discordance between the measures. For example, Hagebeuk et al. discussed that the Sleep Disturbance Scale for Children score showed significant nighttime respiratory complaints in three patients with Rett syndrome, in which polysomnography recordings confirmed obstructive sleep apnea syndrome in all three patients (2012). In another study, Mason et al. found significant correlations between parental reports of repetitive leg movements during sleep and periodic limb movements on polysomnography in children with Williams syndrome. On the other hand, the study also shows that polysomnography data reveals a high prevalence of sleep disorders even in participants with Williams syndrome who had not been considered by their parents to have sleep problems (Mason et al., 2011). In another study, parents of children with ASD reported a higher prevalence of disorders of initiating and maintaining sleep, enuresis, and repetitive behavior when falling asleep in comparison to controls, however the questionnaires were not completely confirmed by polysomnography data which showed a reduced total sleep time, but normal latency (Miano et al., 2007).

One study included a sleep diary and questionnaire (B. Wright et al., 2011), which also demonstrated similarities and differences between sleep diary and questionnaire results. In this study, the effects of melatonin administration were examined in children with autism. Sleep diary data corroborated the Sleep Difficulties Questionnaire, which showed significant improvements for questions related to sleep onset in participants taking melatonin, however the questionnaire data also showed significant improvements for questions related to night wakening, even though no differences were found in wakening rate in sleep diaries (B. Wright et al., 2011).

Only one study included both polysomnography and actigraphy and no differences were found between the polysomnography and actigraphy variables (Goldman, Bichell, Surdyka, & Malow, 2012), thus confirming that there is relatively good agreement in studies that actigraphy data correlate with polysomnography (T. Morgenthaler et al., 2007). In conclusion, it is difficult to synthesize studies using multiple, different types of sleep-related measures due to the multiple variables in specific populations and differing aspects of sleep being documented. Generally, studies use multiple sleep-related measures to collect supplementary patient data or use self or parent-report measures as a means to corroborate objective measures using actigraphy or polysomnography.

Sub-Sample Analysis

As none of the articles included an adolescent-only (10-19 years) sample, a subset of articles including participants ≥ 7 years of age through ≤ 20 years of age were also analyzed (Figure 2.1). This subsample was chosen to more closely mirror an adolescent specific sample due to known age-related changes in sleep patterns. The sample included

8 articles from 7 different countries (n=2 United States). Sample size ranged from 16 to 123 participants. Six of the studies contained a sample with an autism-related diagnosis (Allik et al., 2008; Bruni et al., 2007; Engelhardt et al., 2013; Memari et al., 2013; Sivertsen et al., 2012; Tordjman, Anderson, Pichard, Charbuy, & Touitou, 2005a), one study included participants with Down syndrome (Breslin, Edgin, Bootzin, Goodwin, & Nadel, 2011), and one study contained a sample of participants with an unspecified developmental disorder (Moss, Gordon, & O'Connell, 2014). This review identified that sleep research measures included: parent-report questionnaires (n=4) (Breslin et al., 2011; Engelhardt et al., 2013; Moss et al., 2014; Sivertsen et al., 2012), sleep diary (Memari et al., 2013), sleep diary and actigraphy (Allik et al., 2008), parent-report questionnaire and polysomnography (Bruni et al., 2007), and measurement of melatonin concentration (Tordjman et al., 2005a). Two studies included an author-made questionnaire (Engelhardt et al., 2013; Sivertsen et al., 2012), one study included both the Sleep Disturbance Scales for Children and the Pediatric Daytime Sleepiness Scale (Bruni et al., 2007), and two studies included the Child Sleep Habits Questionnaire (Breslin et al., 2011; Moss et al., 2014). This sub-sample contained a wide range of study purposes including the examination of the relationship between sleep and media use, as well as sleep and cognition, age-related sleep trajectories, sleep architecture, and melatonin alterations. No studies in the sub-sample used self-report measures.

Discussion

To our knowledge, this is the first review to synthesize the literature regarding the sleep measures used to assess sleep disturbance in adolescents with a neurodevelopmental disorder. There is a wide variation in sleep-related measures being

used in adolescents with neurodevelopmental disorders, and this variation may contribute to ambiguity in how sleep problems are defined, assessed, and reported. Understanding sleep disturbance and sleep patterns in adolescents with a neurodevelopmental disorder is important in assessing risk for sleep problems and for the prevision of appropriate prevention and interventional strategies.

A major limitation in the current literature is that older children and adolescents are combined with much younger children and infants in the same sample despite known age-related changes in sleep patterns. Adolescence is a time of transition which can be challenging in typically developing children, and these challenges may be magnified in children with neurodevelopmental disorders. Children and adolescents with neurodevelopmental disorders appear to be at an increased risk of sleep-related disturbances (Couturier et al., 2005; Engelhardt et al., 2013; Krakowiak et al., 2008), which can exacerbate and worsen repetitive and stereotypic behavior, inattention, hyperactivity, as well as interfere with learning and cognition (Malow et al., 2006; Taylor et al., 2012; Wiggs & Stores, 1996). Future studies using adolescent-only samples would be beneficial, allowing for the assessment of a more homogenous group, minimizing age-related differences in sleep patterns that are often seen when infants, children, and adolescents are combined in one sample.

The majority of studies in this review used parent-reported measures of sleep. Although parent report may provide useful information, these questionnaires did not always correspond to objective measurement of sleep using actigraphy and polysomnnography (Mason et al., 2011; Miano et al., 2007; Oyane & Bjorvatn, 2005). Parent report of children's sleep may underestimate sleep problems, and as children get

older, parents may be unaware of increased sleep onset delay experienced by their children, as well as frequency and duration of night wakings that may also affect total sleep time (Baker et al., 2013; Goldman et al., 2009; Owens, Spirito, McGuinn, et al., 2000). One limitation of our study is that the quality of the study measures was not directly assessed, and the search was limited to published articles in the past decade. Potential discrepancies in objective and subjective sleep variables suggests that need to further explore assessment of adolescent sleep using multiple modes of data collection allowing for studies of comparability both within and between sleep-related measures.

In this review, only three studies used self-report questionnaires in children and adolescents with a neurodevelopmental disorder, and these studies suggest the potential usefulness of self-report sleep measures, at the same time, the limited number of studies highlights the need for additional studies using self-report sleep questionnaires. Although not specific to sleep, recent research in other areas suggests that self-report measures are possible and useful in children with high functioning ASD. In one study, thirty children with ASD and twenty-one controls (10-16 years, IQ and reading ≥70) completed measures to assess symptoms of anxiety and depression using the Spence Children's Anxiety Scale (SCAS) and Children's Depression Inventory (CDI) (Ozsivadjian, Hibberd, & Hollocks, 2014). The SCAS is a 44-item questionnaire that is widely used to screen for anxiety in clinical and research settings and is available in both parent and child versions. Results have shown good internal consistency (α=0.60 to 0.92) and high test-retest reliability (α=0.45 to 0.62) in a community sample (n=2,052; age 8-12 years). The CDI is also available in both parent and child versions and is designed to screen for depression and high internal consistency has been reported α (=0.86). In this study,

intraclass correlations revealed good agreement between parent and child reports on both the anxiety and depression measures (Ozsivadjian et al., 2014), thus indicating the possibility and usefulness of self-report data in children and adolescents with ASD.

This review examined measures used to assess sleep in adolescents with a neurodevelopmental disorder. At least seventeen different clinical disorders were included in the studies reviewed. Although the intent of the review was not to compare sleep methods by diagnosis, it is unclear whether sleep measures should be diagnosis specific or if and where there is overlap between different neurodevelopmental disorders. Children and adolescents with neurodevelopmental disorders often have a large range in IQ and present with multiple disorders and co-morbidities. It has been reported that seventy-five percent of children with ASD have one co-morbid condition and over forty percent of children with ASD have two or more co-morbidities (e.g. ASD and ADHD) (Mannion et al, 2013). The large range of diagnosis, co-morbidities, and IQ in children and adolescents with neurodevelopmental disorders may likely contribute to variations in the type and choice of sleep-related measures (e.g. self-report data is not always possible) that would be appropriate, perhaps complicating comparison of study result. Future research would be beneficial to ascertain if classification and usefulness of sleep-related measures should be diagnoses specific, or rather instead rely on proxies of cognition such as IQ.

Conclusion

Sleep is a readily accepted clinical construct, however it is a complex phenomenon that is difficult to define and measure. Examination of impaired sleep in adolescents with neurodevelopmental disorders requires measures that are valid and

reliable for this population, and this review identified common measures used to assess sleep including questionnaires, sleep logs, actigraphy, and polysomnography. However, there is a wide variation in sleep-related measures being used in children and adolescents with neurodevelopmental disorders, and this variation in measurement may likely contribute to ambiguity in how sleep problems are being defined, assessed, and reported. Sleep problems persist over time in children with ASD (Baker et al., 2013; Goldman, Richdale, et al., 2012), and additional research is needed in adolescent-only samples. Nursing in particular seeks to provide a better understanding of symptoms and symptom clusters that will improve clinical management of illness including the management of pervasive symptoms such as impaired sleep, which crosses multiple conditions across the lifespan and impairs quality of life ("NINR Strategic Plan: Bringing Science to Life," 2012). Development, validation, and standardization of age-appropriate sleep-related measures that can be utilized in adolescents with neurodevelopmental disorders, a population vulnerable to sleep problems, is a necessary avenue for future research in order to assess risk for sleep problems and for the prevision of appropriate prevention and interventional strategies.

Table 2.1. Inclusion and Exclusion Criteria

Inclusion	Exclusion
• Empirical qualitative and quantitative research • Published between 2004-2014 • Peer-reviewed primary research reports • Sleep-related research measures including at least one adolescent (10-19 years) within sample	• Interventional pharmaceutical drug studies • Research primarily focused on diagnosis of ADHD, Tourette syndrome, epilepsy, and traumatic brain injury • Reporting of study in duplicate papers • Unpublished research • Sample containing no adolescents (10-19 years)

Table 2.2. Literature Search Terms

Search Terms Used (across all databases)	Article Count
Neurodevelopmental disorder and sleep and adolescent	25
Neurodevelopmental disability and sleep and adolescent	5
Autism and sleep and adolescent	125
Down syndrome and sleep and adolescent	50
Prader Willi and sleep and adolescent	30
Williams syndrome and sleep and adolescent	21
Smith Magenis and sleep and adolescent	14
Rett syndrome and sleep and adolescent	12
Angelman and sleep and adolescent	12
Fetal Alcohol syndrome and sleep and adolescent	5
Fragile X and sleep and adolescent	8
TOTAL	**307**

Table 2.3. Literature Sorted by Country

Country	Article Count
United States	23
Australia	8
Netherlands	5
Italy	4
England	3
Canada	3
China	2
France	2
Hungary	2
Norway	2
Scotland	2
Argentina	1
Czech Republic	1
Finland	1
Iran	1
Ireland	1
Japan	1
New Zealand	1
Qatar	1
Sweden	1
UK unspecified	1
TOTAL	**66**

Table 2.4. Literature Sorted by Diagnosis of Sample

Diagnosis of sample	Article Count
Autism-related diagnosis	20
Combination diagnosis*	10
Down syndrome	8
Prader Willi syndrome	5
Angelman syndrome	4
Unspecified intellectual disability	4
Williams syndrome	4
CDKL5 mutation	2
CHARGE syndrome	1
Cri du Chat syndrome	1
Duplication of the proximal short arm of chromosome 17	1
Fetal Alcohol Spectrum Disorder	1
Fragile X syndrome	1
Interstitial 15q duplication	1
MECP2 mutation	1
Rett syndrome	1
Smith Magenis syndrome	1
TOTAL	**66**

*Purpose of study was to look at two different diagnoses or needed to meet two diagnoses to be included in study.

Table 2.5. Literature Sorted by Sleep-related Measures Used

Sleep-related Measures Used	Article Count
Sleep Questionnaire (any used within article): [1,3,4*,6,8,9,10,11,12,13,14,15,17,22,23,25*,27,28,30,31,32,35,37,39*,40,41,46,47,48,49,51,54,55,56,58,61,63,64,65,66]	40
Author-made or Unspecified [1,6,8,13,23,28,30,31,32,35,49,56,61,66]	14
Children's Sleep Habits Questionnaire [3,11,12,14,40,48,51,58,63,64,65]	11
Sleep Disturbances Scale for Children [17,22,25,54,55]	5
Epworth Sleepiness Scale [30,39,40,41,58]	5
Sleep Questionnaire [46,47]	2
Pediatric Daytime Sleepiness Scale [4,22]	2
Family Inventory of Sleep Habits [3]	1
Sleep Habits Survey [4]	1
Flinders Fatigue Scale [4]	1
Infant Sleep Questionnaire [10]	1
Sleep Difficulties Questionnaire [15]	1
Atypical Behavior Patterns Questionnaire [27]	1
Insomnia Severity Index [58]	1
Sleep Self Report Questionnaire [25]	1
Behavioral Evaluation of Disorders of Sleep [9]	1
Periodic Limb Movement Questionnaire [37]	1
Multiple Questionnaires Used [3,4,22,25,30,40,58]	7
Sleep Logs [2,4*,15,16,18*,19,20,26,30*,39*,40,57,59]	13
Actigraphy [4,16,18,20,30,39,40]	7
Polysomnography [5,14,22,23,24,34,36,37,38,41,42,43,44,45,52,53,54,55,58,60]	20
Other (multiple sleep latency test, melatonin, interviews, notes/chart review) [7,19,21,29,33,41,44, 50, 59,62]	10

* Self-report by child or adolescent

Table 2.6 Characteristics and Main Findings of Studies

	Author Year Location	Sample	Purpose	Sleep-related Measure(s) Used	Studies with Multiple Measures	Major findings	Recommendations:
*1	(Engelhardt et al., 2013) United States	n=49 boys with Autism Spectrum Disorders (ASD), n=38 with Attention Deficit Hyperactivity Disorder (ADHD), and n=41 Typical Development (TD) 8-17 years	To examine the relationship between media use (television, computer, and video games) and sleep	Single question made by investigator (parent report)	No	In-room access to a television or a computer significantly related to a reduction in sleep in boys with ASD, but not in ADHD or TD and average day time exposure to video games associated with reduced sleep among boys with ASD	Screen based media time and bedroom media access should be routinely accessed, may be important intervention target for sleep problems in children with ASD
*2	(Memari et al., 2013) Iran	n=123 children with Autism Spectrum Disorders (ASD) 7-14 years	To investigate cognitive flexibility in children and adolescents with ASD in conjunction with sociodemographic correlates	Total sleep time (two weekdays and one weekend) (parent report)	No	Perseveration were inversely correlated with sleep time	Further studies are needed to determine if daily activities (e.g. total sleep time) are directly linked to cognitive flexibility
3	(Urraca et al., 2013) United States	n=14 confirmed interstitial 15q duplication 2-17 years	To conduct a phenotype/genotype analysis and determine relationship of 15q duplication in autism	Family Inventory of Sleep Habits (parent report) Children's Sleep Habits Questionnaire (parent report)	No	Child Sleep Habits Questionnaire suggested sleep problems with more severity in subjects with paternal origin of duplication	Further sleep studies are needed to determine how sleep is affected and if it depends on parental origin of duplication
4	(Baker et al., 2013) Australia	n=27 with high functioning ASD (HFASD) and n=27 TD Mean age=15.5 (no age range given)	To investigate sleep disturbance and sleep patterns in adolescence with HFASD	Sleep Habits Survey Pediatric Daytime Sleepiness Scale Flinders Fatigue Scale Sleep diary Actigraphy (all self-report)	Yes	Adolescents with HFASD had longer sleep onset latencies, less percent sleep efficiency, and less total sleep time than TD adolescents	Further research is recommended to confirm findings and for the development of treatments for adolescent sleep difficulties

Table 2.6 Continued

5	(Limoges, Bolduc, Berthiaume, Mottron, & Godbout, 2013) Canada	n=17 adults with autism and normal IQ and n=14 TD adults 16-27 years	Explore relationship between sleep and cognitive daytime performance	Polysomnography	No	Objective signs of poor sleep in persons with ASD are associated with some limitations in cognitive performance	Further research needed to investigate if improvement in sleep in ASD is always accompanied by better cognitive performance
6	(Maskey, Warnell, Parr, Le Couteur, & McConachie , 2013) England	n=863 children with Autism Spectrum Disorders (ASD) 2-18 years	To investigate the type and frequency of co-existing conditions in children with ASD	Single item made by investigator (parent report)	No	Parents reported sleeping problems in 45% of the children	No sleep recommendations were found
7	(Braam et al., 2013) Netherlands	n=15 patients with an intellectual disability and with chronic sleep onset insomnia showing disappearance of melatonin treatment effect after 4-8 weeks of treatment 4-32 years	To explore an association between a SNP in the CYPIA2 gene and slow melatonin metabolism in patients who showed a disappearance of treatment affect	Salivary melatonin measured by Radioimmunoassay (RIA)	No	A SNP was found in 8 of the 15 patients which may have caused the disappearing efficacy of exogenous melatonin due to slow melatonin metabolism	Further studies are needed to determine the role of melatonin metabolism and susceptibility to ASD
8	(Kheir et al., 2012) Qatar	n= 56 caregivers of a child diagnosed with Autism Spectrum Disorders (ASD) and n=42 caregivers of non-autistic child 3-17 years	To assess the level of concern and pessimism of primary caregiver of children with autism in Qatar	Single question made by investigator (parent report)	No	Children with autism on average sleep less hours a day than the non-autism group	No sleep related recommendations were made
9	(Taylor et al., 2012)	n=335 children diagnosed with autism or pervasive	To evaluate the effects of sleep problems on day-time cognitive and	Behavioral Evaluation of Disorders of Sleep (parent report)	No	IQ across all domains was related to sleep quality and quantity	Elaborate on these findings, specific diagnostic sleep

Table 2.6 Continued

	United States	developmental disorder 1-18 years	adaptive performance in an ASD population				disorders must be evaluated in conjunction with these types of specific cognitive and adaptive skills, further analysis of specific daily living skill deficits and relation to sleep disorders
10	(Claro, Cornish, & Gruber, 2011) United Kingdom	n=69 children and adolescents with Cri du Chat syndrome (CDC) and n=47 comparison children with moderate to severe intellectual disabilities 3-18 years	Examine whether the fatigue level of children diagnosed with CDC was associated with the expression of autistic symptoms	Infant Sleep Questionnaire (parent report)	No	Children with high levels of fatigue, regardless of diagnosis, are more likely to exhibit autistic symptoms	Objective measurement is needed (e.g. Multiple Sleep Latency Test); longitudinal studies needed to delineate the casual effects of fatigue
11	(Rzepecka, McKenzie, McClure, & Murphy, 2011) Scotland	n=167 parents or guardians of children with an intellectual disability (ID) and/or Autism Spectrum Disorders (ASD) 5-18 years	To examine the relationship between sleep problems, anxiety and challenging behaviors in the child and adolescent ID/ASD population	Children's Sleep Habits Questionnaire (parent report)	No	Significant positive correlations between sleep problems and anxiety, sleep problems and challenging behavior, and anxiety and challenging behavior in clinical sample of children with ID and/or ASD	Future research could further examine the relationship between sleep problems, anxiety and challenging behaviors in the child and adolescent ID/ASD population, as well as their relationship to specific demographic factors
12	(Goldman, Richdale, et al., 2012)	n=1859 children diagnosed with Autism Spectrum	To characterize the sleep habits of older children and	Children's Sleep Habits Questionnaire (parent report)	No	Sleep problems persist through the age span from early childhood to	Include self-reported questionnaires in adolescents and

Table 2.6 Continued

	United States	Disorders (ASD) participating in the Autism Treatment Network 3-18 years	adolescents with ASD compared to younger age groups, as well as identify areas defined by parental report to be problematic			adolescents in children with ASD, but types of problems change with age (e.g. young children experience bedtime resistance while adolescents have trouble falling asleep)	actigraph measurements of sleep patterns, validation study of the CSHQ in adolescents with ASD is needed
*13	(Sivertsen et al., 2012) Norway	n=28 with autism spectrum problems (ASP) 7-9 yrs and 11-13 years	To assess sleep problems in children with ASP when the children were 7-9 and 11-13 years old	Single question made by investigator (parent report)	No	Children with ASP have a high rate of sleep problems and developed more sleep problems over time	Future epidemiological studies in children with ASD are needed including use of a validated sleep questionnaire
14	(Buckley et al., 2010) United States	n=60 children with autism, n=15 with typical development (TD), and n=13 with developmental delay 2-13 years	To compare objective polysomnographic parameters between 3 cohorts: children with autism, typical development, and developmental delay without autism	Children's Sleep Habits Questionnaire (parent report) Polysomnography	Yes	No differences between the TD group and developmental delay groups; children with autism had shorter total sleep time, greater slow-wave sleep percentage, and smaller REM percentage compared to TD children and children with developmental delay	Future studies should focus on the linking the social and emotional processing deficits of autism and fundamental deficits in physiological regulation
15	(B. Wright et al., 2011) United Kingdom	n=17 children with Autism Spectrum Disorders (ASD) 3-16 years	To examine the effects (sleep latency, night waking, total sleep time) of melatonin versus placebo in children with ASD who had not responded to behavior management strategies	Sleep Difficulties Questionnaire (SDQ) (parent report) Sleep diary (parent report)	Yes	Melatonin significantly improved sleep latency and total sleep time compared to placebo, but not number of night wakening's	Additional studies on the synthesis and bioavailability of melatonin in children with autism are needed

Table 2.6 Continued

16	(Wirojanan et al., 2009) United States	n=12 participants diagnosed with Fragile X syndrome (FXS) and Autism Spectrum Disorders (ASD) 2-15 years	To determine the efficacy of melatonin for sleep problems in children with ASD and FXS	Sleep diaries (parent report) Actigraphy	Yes	Mean night sleep duration was longer, mean sleep-onset latency was shorter, and sleep-onset time was earlier on melatonin than placebo	Melatonin can be considered a safe and effective treatment in addition to behavioral therapies and sleep hygiene for the management of sleep problems in children with ASD and FXS
17	(Hartshorne et al., 2009) United States	n=87 children with CHARGE syndrome 6-18 years	To document the extent of sleep disturbance in CHARGE syndrome and explore relationship between sleep disturbance, challenging daytime behavior, and parental well-being	Sleep Disturbances Scale for Children (SDSC) (parent report)	No	Over half of the participant's received scores considered significant for sleep disturbance with correlations between challenging behavior, sleep disturbance, and parental well-being	Importance of screening for sleep disturbance in children with CHARGE syndrome as early identification and appropriate therapy may reduce negative consequences of poor sleep
*18	(Allik et al., 2008) Sweden	n=16 children with Asperger syndrome and high functioning autism (HFA) and n=15 typically developing children 8-13 years	Longitudinal follow-up study to determine if differences observed at baseline are also evident at follow-up and if sleep patterns from baseline to follow-up differ in children with Asperger syndrome or HFA and controls	Sleep diary (child and parent report) Actigraphy	Yes	No differences in total sleep time, but children with Asperger syndrome/HFA spent a longer time awake in bed before falling. Both Asperger syndrome/HFA and controls had similar age-related trajectories in their sleep development, mainly sleep delay and decreased sleep duration	Findings should be replicated in larger samples, as well as within different cultural contexts
19	(Andersen, Kaczmarska, McGrew, & Malow, 2008b)	n=107 children diagnosed with Autism Spectrum Disorders (ASD) who were prescribed	Describe the effects of using melatonin to treat insomnia in children with ASD	Follow-up clinic notes documenting parent concerns of sleep after doses of melatonin taken	No	Melatonin appears to be a safe and well tolerated treatment for insomnia in children with ASD	Future prospective randomized blinded placebo clinical trials of melatonin in ASD are warranted and

Table 2.6 Continued

	United States	melatonin 2-18 years		Sleep diary (parent report)			studies should consider the formulation of melatonin, dose-response, and effects of sleep hygiene counseling
20	(Wasdell et al., 2008a) Canada	n=47 children with multiple neurodevelopmental disabilities (NDD's) and chronic delayed sleep phase syndrome (DSPS) or impaired sleep maintenance (ISM) 2-18 years	To determine the efficacy of controlled release melatonin in the treatment of DSPS and ISM in children with NDD's including ASD	Somnologs (caregiver report) Actigraphy	Yes	Total sleep time and sleep latency showed significant improvements with the use of melatonin therapy	Future research should assess flexibility of sleep schedules, and/or longer periods of sleep measurements
21	(Ming, Brimacombe, Malek, Jani, & Wagner, 2008) United States	n=160 children diagnosed with Autism Spectrum Disorders (ASD) 2-18 years	To characterize the clinical co-occurrences and potential subgroups in 160 children with ASD	Retrospective chart review and 14-page clinical intake form (completed by caregivers)	No	Approximately half of the children with ASD exhibited a sleep disorder sometime during their life	No sleep related recommendations were found
*22	(Bruni et al., 2007) Italy	n=8 with Asperger syndrome, n=10 with autism, and n=12 healthy controls 7-15 years	To evaluate sleep architecture and NREM sleep microstructure	Sleep Disturbance Scales for Children (parent report) Pediatric Daytime Sleepiness Scale (parent report) Polysomnography	Yes	Children with Asperger syndrome had a higher prevalence of problems in sleep initiation and daytime sleepiness, as well as minor differences in sleep architecture parameters	No sleep related recommendations were found
23	(Miano et al., 2007) Italy	n=31 children with Autism Spectrum Disorders (ASD)	To evaluate sleep in children with ASD and to analyze their sleep	Sleep questionnaire (modified, name not given) (parent report)	Yes	Parents of children with ASD reported a high prevalence of disorders	Future studies needed to compare the CAP parameters

Table 2.6 Continued

		and n=893 age-matched controls 3-19 years	architecture and cyclic alternating pattern (CAP)	Polysomnography		of initiating and maintaining sleep, enuresis, repetitive behavior when falling asleep, and daytime sleepiness. CAP measures showed alterations of NREM sleep in children with ASD	of ASD patients to matched groups of cognitively impaired subject in order to characterize CAP in subjects who are cognitively impaired, but not autistic
24	(Potocki et al., 2007) United States	n=35 subjects with duplication of the proximal short arm of chromosome 17 25 months-14 years	To characterize the genotype and phenotype of Potocki-Lupski Syndrome	Polysomnography	No	Sleep disorders breathing characterized by obstructive sleep apnea, central sleep apnea, and significant oxygen desaturation was found	Abnormalities noted during sleep studies may have substantial consequences for daytime function and behavior and overnight sleep study should be considered as part of clinical evaluation
25	(Paavonen et al., 2008) Finland	n =52 children with Asperger syndrome and n= 61 healthy controls 5-17 years	To evaluate the prevalence of sleep disturbances in children with Asperger syndrome compared to controls using child and parent report	Sleep Disturbance Scale for Children (parent report) Sleep Self Report Questionnaires (child report)	No	Average sleep duration was longer in the control group and sleep onset difficulties were more common in Asperger syndrome group. Children with Asperger syndrome report short sleep duration and tiredness, a negative attitude toward sleep, and suffered sleep-related fears	The role of anxiety warrants further research as increased anxiety may cause sleep problems or vice versa
26	(Garstang & Wallis, 2006) United	n=7 children with Autism Spectrum Disorders (ASD)	To establish whether melatonin is an effective treatment for children with ASD	Sleep diary (parent report)	No	Melatonin improved sleep latency, waking's per night, and sleep duration	Larger sample size and multicenter studies are needed

Table 2.6 Continued

	Kingdom	4-16 years	and sleep problems				
27	(Dominick, Davis, Lainhart, Tager-Flusberg, & Folstein, 2007) United States	n=39 with a history of language impairment and n=67 with Autism Spectrum Disorders (ASD) 4-14 years	To investigate atypical behaviors in children with a history of language impairment (HLI) and children with ASD	Atypical Behavior Patterns Questionnaire (ABPQ) (parent interview)	No	Atypical behaviors in sleep patterns were more common in children with ASD than HLI. There was a relationship between sleep abnormalities and a lifetime history of major depression, which was diagnosed several years after the onset of sleep problems, in children and adolescents with ASD.	Replication studies needed
28	(Cotton & Richdale, 2006) Australia	n=98 children with an intellectual disability (ID) (n=37 with autism, n=15 with Down syndrome (DS), n=17 with Prader Willi syndrome (PWS),and n=29 with presumed familial ID) and n=55 typically developing adolescents 2-18 years	To examine parental description of sleep problems in children with an ID and how these sleep problems impacted the child and his/her family	Sleep questionnaire (unspecified parent report)	No	Across disorder groups over half of the participants had a sleep problem with the highest percentage found in autism group (73%), however no differences across groups in terms of severity of sleep problems. Differing types of sleep problems by group (e.g. autism group and co-sleeping; PWS group and daytime sleeping)	Further research with larger samples is necessary to delineate the scope of sleep disturbances across developmental disorders for prevention and treatment strategies
*29	(Tordjman et al., 2005a) France	n=50 children and adolescents with Autism Spectrum Disorders (ASD)	To better characterize the possible melatonin alteration in autism by studying nocturnal	Nocturnal urine 6-sulphatoxymelatonin (6SM)	No	Patients with autism had significantly lower 6-SM excretion	Further research is warranted in order to understand the mechanisms

Table 2.6 Continued

		and n=88 controls 7-16 years	melatonin secretion				underlying the lower melatonin production, to assess the impact of altered melatonin on the pathophysiology, and to determine the utility of melatonin administration in individuals with autism
30	(Oyane & Bjorvatn, 2005) Norway	n=15 adolescents with Asperger syndrome and autism 15-25 years	To investigate prevalence of sleep disturbances in young adults with autism and Asperger syndrome	Sleep questionnaire (unspecified) Epworth Sleepiness Scale (parent report) Sleep diary (adolescent, parent, or caregiver report) Actigraphy	Yes	Objective sleep disturbances are still present in adolescents with Asperger syndrome and autism, although they are less frequently reported by parents compared to childhood	Larger studies with actigraphy in adolescents with ASD, including intervention studies, could provide more accurate data on the persistence of sleep disturbance
31	(Gail Williams, Sears, & Allard, 2004) United States	n=210 children with autism 2-16 years	To better understand sleep problems in autism	Modified version of sleep survey used by Kosair Children's Hospital Center (parent report)	No	Most commonly reported sleep problems included: difficulty falling asleep, restless sleep, unwillingness to fall asleep in own bed, and frequent waking during the night	Objective data is needed to delineate sleep architecture in children with autism and efforts are needed to correlate objective and subjective data. Further research is needed to assess impact of sleep problems on academic performance
32	(Kronk et al., 2010) United	n=978 families who had one or more children with the	To report on current child sleep difficulties reported by parents of	Sleep questionnaire (author-made parent report)	No	Approximately one-third of the children with FXS currently experience	Additional research is needed to address why gender

Table 2.6 Continued

	States	full mutation for Fragile X syndrome (FXS) 5- 20 years	children with FXS, including prevalence, type, and treatment			sleep difficulties (84% had 2 or more current sleep difficulties (e.g. problem falling asleep or frequent nighttime awakenings)	differences were not as apparent as expected and why male sleep disturbance appears to improve over time
33	(Ipsiroglu, McKellin, Carey, & Loock, 2013) Canada	n=33 parents of children with fetal alcohol spectrum disorders (FASD), n= 7 health care professionals, and n=27 children with FASD 2-15 years	To determine the factors and processes that contribute to the lack of recognition and treatment of sleep problems in FASD	Qualitative interviews and Comprehensive Clinical Sleep Assessments	No	The most striking finding was the desperation of the families and the effect of sleep deprivation on their QOL	Need for a screening concept, which explores not only the child's day and night time sleep behaviors, but also quality of life and family well-being
34	(Miano et al., 2008) Italy	n=14 patients with Fragile X syndrome (FXS), n= 9 patients with Down syndrome (DS), and n=26 controls 7-26 years	To analyze CAP in patients with DS and FXS to assess alterations in sleep microstructure	Polysomnography	No	Patients with FXS had reduced time in bed compared to patients with DS. Patients with DS had lower sleep efficiency, higher percentage of wake after sleep onset, and reduced Stage 2 NREM compared to other groups. Patients with FXS showed most disrupted sleep microstructure	Replication needed in larger cohorts of children with DS and FXS, as well as other groups with intellectual disabilities
35	(Fehr et al., 2013) Australia, but database study	n=86 children with a CDKL5 mutation n=920 females with MECP2 mutations 6 months-22.4 years	To describe the clinical profile of the CDKL5 disorder and compare with Rett Syndrome	Sleep questionnaires (unspecified) (family and clinician report)	No	Sleep problems occurred in about ninety percent of the children with a CDKL5 mutation, which was higher than the sleep disturbances reported in children MECP2	No sleep related recommendations were found

Table 2.6 Continued

						mutations	
36	(Bodizs et al., 2012) Hungary	Study 1: n=9 patients with Williams syndrome (WS) and n= 9 controls 14-29 years Study 2: : n=20 patients with WS and n= 20 controls 6-29 years	Describe the sleep EEG fingerprint in WS	Polysomnography (lab and home)	No	Patients with WS have a disturbed sleep pattern with alterations in sleep architecture including: higher sleep latency, more wake time after sleep onset, lower sleep efficiency, higher NREM and slow wave sleep percent, and lower REM sleep percent. Spectral analysis showed alterations in band power values: increases in delta power and less global decreases in alpha, sigma, beta, and gamma power	No sleep related recommendations were found
37	(Mason et al., 2011) United States	n=35 patients with Williams syndrome (WS) and n= 35 controls 2-18 years	Describe sleep in children with WS and explore association between disturbed sleep and behavior	Modified Periodic Limb Movement questionnaire (parent report) Polysomnography	Yes	Patients with WS had decreased sleep efficiency, increased respiratory-related arousal, and increased slow wave sleep. They also had more difficulty falling asleep, greater restlessness, and more arousals	Further research is needed to determine whether children with WS might offer a distinct opportunity to assess genetic contributions to sleep disturbances in childhood and therapeutic interventions were suggested
38	(Gombos, Bodizs, & Kovacs, 2011)	n=9 patients with Williams syndrome (WS) and n= 9 controls	Determine whether alterations in sleep architecture and EEG spectra in adolescents	Polysomnography	No	Continuity of sleep problems from childhood through adolescence and adulthood with	Further studies unraveling the relationship between sleep alterations and

Table 2.6 Continued

	Hungary	14-29 years	and young adults with WS are similar to those found in children with WS			demonstrated decreased sleep time, sleep efficiency, and REM sleep percentage, as well as increased WASO, NREM percentage, SWS, and leg movements	daytime symptoms and learning disabilities are of special interest
39	(Goldman et al., 2009) United States	n=23 adolescents and adults with Williams syndrome (WS) 17-35 years	Describe the sleep patterns of adolescents and adults with WS	Epworth Sleepiness Scale (self-report) Sleep diary (self-report) Actigraphy	Yes	Reduced sleep efficiency, prolonged sleep latency, increased wake time after sleep onset, and elevated movement	Additional studies are needed using polysomnography and controls subjects, as well as relationship between sleep problems and behavioral correlates
40	(Gibbs et al., 2013) New Zealand	n=8 children with Prader Willi syndrome (PWS) and n=16 controls 4-15 years	To assess total nocturnal sleep duration and to explore the relationship with BMI and daytime sleepiness	CSHQ (parent report) Epworth Sleepiness Scale (parent report) Sleep diary (parent report) Actigraphy	Yes	PWS group had a shorted sleep latency, longer WASO, and higher daytime sleepiness score	Safety implications as children with PWS could be awake and unsupervised for long periods
41	(Williams et al., 2008) United States	n= 37 patients with Prader Willi syndrome (PWS) 15 months-24 years	To review polysomnograms and multiple sleep latency tests in a cohort of PWS patients to determine the relationship between BMI-Z, daytime sleepiness, growth hormone treatment, and sleep disordered breathing	Epworth Sleepiness Scale (parent report) Multiple Sleep Latency Test Polysomnography	Yes	All patients exhibited some form of sleep disordered breathing and there was a positive correlation between BMI-Z and apnea-hypopnea index (AHI)	Further larger, prospective, normalized studies could better define the incidence and severity of sleep disordered breathing, as well as benefits of clinical intervention
42	(Torrado et al., 2007) Argentina	n=91 children with Prader Willi syndrome (PWS)	To better understand the phenotype variability of PWS, a	Polysomnography	No	Individuals with and without a deletion were found to have OSAS and	No sleep related recommendations were found

Table 2.6 Continued

		(n=35 for polysomnography) 12 days- 17 years	phenotype-genotype study was performed			one or more central events (apnea and/or hypopneas), however the deleted groups had more desaturations greater than or equal to 10% associated to central events	
43	(O'Donoghue et al., 2005) Australia	n=13 patients with Prader Willi syndrome (PWS) 1-28 years	To determine the prevalence and type of sleep-disordered breathing in patients with PWS and its relationship to neurobehavioral abnormalities	Polysomnography	No	Obstructive sleep apnea is prevalent amount subjects with PWS and is associated with increased BMI, daytime sleepiness, and behavioral disturbances	No sleep related recommendations were found
44	(Nevsimalova et al., 2005) Czech Republic	n=4 (cases studies of patients with Prader Willi syndrome) Ages 18, 23, 6, 10	To evaluate sleep patterns to assess hypocretin CSF levels in PWS	Multiple Sleep Latency Test Polysomnography	No	Nocturnal PSG revealed fragmented sleep, low sleep efficiency, and hypopnea/apnea index in PWS cases as being increased from borderline up to very high values in proportion to patients' age. CSF hypocretin-1 deficiency was noted	No sleep related recommendations were found
45	(Capone, Aidikoff, Taylor, & Rykiel, 2013) United States	n=28 patients with Down syndrome (DS) with major depressive episode and n=9 controls 14-30 years	To determine the association between depression and sleep apnea.	Polysomnography	No	Almost ninety percent of patients with DS met criteria for obstructive sleep apnea syndrome. OSAS may be a common co-morbidity in adolescents and younger adults with DS and depression	Further studies in sleep, obstructive sleep apnea syndrome and its association with cognition, behavior, and mental health issues in persons with DS are warranted

Table 2.6 Continued

46	(Chen, Spano, & Edgin, 2013) United States	n=29 adolescents and young adults with Down syndrome (DS) 14-31 years	To examine the relationship among sleep disorders/behaviors and executive dysfunction	Sleep Questionnaire (caregiver report)	No	Individuals with high ratings of sleep disruption also show greater difficulties with executive function. First study to examine correlations between parent rating of sleep disruption and cognitive deficits in patients with DS	Further studies are needed to examine the relation between sleep problems and measures of brain structure and function in DS. Future research should examine the relationship between poor sleep, cognitive impairment, and decline in adults with DS
47	(Maas, Didden, Korzilius, & Curfs, 2012) Netherlands	n=100 individuals with an intellectual disability (n=25 Cri du Chat syndrome (CDC), n=25 Jacobsen syndrome (JS), n= Down syndrome, and n=25 none-specific intellectual disability) 3-31 years	To assess the prevalence and level of severity of sleep disturbances	Sleep Questionnaire (caregiver report)	No	In CDC, JS, and DS snoring was the most prevalent type of sleep disturbance, whereas in the NS group no type pf sleep disturbance was identified as most prevalent	Future studies should use samples consisting of either children and adolescents or adults with an intellectual disability to control for developmental effect on the prevalence of different types of sleep problems
*48	(Breslin et al., 2011) United States	n=35 children with Down syndrome (DS) 7-18 years	To examine sleep in DS	Children's Sleep Habits Questionnaires (parent report)	No	Eight-five percent of the sample had sleep disturbance scores in the clinic range with elevated scores on: bedtime resistance, sleep anxiety, night waking, parasomnias, sleep disordered breathing, and daytime sleepiness	Findings suggest that many sleep disturbances persist across development and given that sleep is important for memory, learning, and behavior, interventions for sleep problems are

Table 2.6 Continued

						subscales	clearly warranted across a wide range of ages in DS
49	(Rosen, Lombardo, Skotko, & Davidson, 2011) United States	n= 255 children with Down syndrome (DS) <18 years	As part of a quality improvement initiative, to better address needs of children with DS in the areas of sleep and sleep disordered breathing, parents were asked to complete sleep questionnaires	Sleep questionnaire (non-specified; parent report)	No	Difficulty initiating and maintaining sleep and excessive daytime sleepiness were frequently/almost always present in more than half of the children. Parents of children post adenotonsillectomy reported witnessed apnea (47.5%) and gasping/choking (28.9%) more than once a month	Findings indicate a need to educate families of children with DS about difficulty initiating and maintaining sleep, excessive daytime sleepiness, obstructive sleep apnea, and there consequences
50	(Yam et al., 2008) China	n= 407 with Down syndrome (DS) 0.06- 17.16 years	To examine the prevalence of medical problems in children and teenagers with Down syndrome in Hong Kong	Medical questionnaire including portion on sleep apnea or other sleep problems	No	Using a review of hospital records, nine percent of pediatricians reported sleep problems	Advocate for age-specific medical checklist including pamphlet on anticipatory health care in children with DS
51	(Carter, McCaughey, Annaz, & Hill, 2009) United Kingdom	n=58 children with Down syndrome (DS) 0.65-17.9 years	To determine the prevalence of sleep problems in children with DS	Child Sleep Habits Questionnaire (parent report)	No	Parents universally reported sleep problems in school aged children with DS that persist into teenage years	Parents report sleep problems in school aged children with DS and pediatricians should routinely assess sleep behavior
52	(Fitzgerald et al., 2007) Australia	n=33 children with Down syndrome (DS) who snore 0.2-19 years	To consider if polysomnography should be recommended as a routine test in children with DS who snore	Polysomnography	No	Ninety-seven percent of the children in the study had moderate to severe OSA	Findings support the use of polysomnography in children with DS as a routine investigation

Table 2.6 Continued

53	(Ng et al., 2006) Hong Kong	n= 22 children with Down syndrome (DS) Mean age= 10.82 n=22 snoring controls Mean age= 10.27	To compare the prevalence of obstructive sleep apnea (OSA) in children with DS (with or without snoring) to snoring children	Polysomnography	No	Prevalence of OSA in children with DS and snoring controls was similar with sixty-one percent of children with DS with OSA neither having habitual snoring nor observed apnea, highlighting the difficulty of screening OSA in DS children by clinical history	Suggest polysomnography be offered to all children with DS from ages four to six years
54	(Hagebeuk et al., 2013) Netherlands	n=4 female children with CDKL5 mutations 2-15 years	To examine the presence of breathing and sleep abnormalities in a small series of patients with CDKL5 mutations	Sleep Disturbance Scale for Children (parent report) Polysomnography	Yes	The SCSC indicated disorders of initiating and maintaining sleep, daytime somnolence, and sleep breathing disorders and polysomnography revealed central apnea when awake, low REM sleep, frequent awakening, and low sleep efficiency	Polysomnography seems warranted to evaluate breathing and sleep disturbances
55	(Hagebeuk et al., 2012) Netherlands	n= 12 patients with Rett syndrome Mean age = 8 years	To evaluate the presence of overnight respiratory disturbances in a cohort of patients with Rett syndrome	Sleep Disturbance Scale for Children (parent report) Polysomnography	Yes	Respiratory disturbances were present in all patients and were clinically significant in most	Recommend polysomnography in patients with Rett syndrome
56	(Young et al., 2007) Australia	n=237 patients with Rett syndrome 2-29 years	To investigate the type of frequency of sleep problems, relationships with age and MECP2 mutation type, and to evaluate changes over time	Author-made questionnaire (caregiver report)	No	High prevalence of sleep problems across age groups, but most frequent in younger age group. Most frequently reported problems: daytime napping, night-	Assessment of sleep patterns should be considered as part of the overall multidisciplinary management and future research is

Table 2.6 Continued

						time laughter, teeth grinding, night screaming, and seizure. Some differences by mutation time (e.g. large deletion greater sleep problems)	needed to see if sleep dysfunction is amenable to pharmacological or behavioral intervention
57	(Takaesu et al., 2012) Japan	n=15 patients with Angelman syndrome 6-27 years	To investigate circadian rhythm sleep disorder in patients with Angelman syndrome and the relationship between Angelman syndrome and serum melatonin levels	Sleep logs (caregiver report)	No	High prevalence of circadian rhythm sleep disorders in patients with Angelman syndrome, which may be related to lower overnight serum melatonin levels	Future research should measure serum melatonin at least hourly to obtain a detailed melatonin profile, including dim light melatonin onset and actigraphy and randomized clinical trials of melatonin are needed in this population
58	(Goldman, Bichell, et al., 2012) United States	n=16 children/adolescents with Angelman syndrome and the primary caregiver 2-16 years	To obtain objective and subject data on sleep in children/adolescents with Angelman syndrome and related these sleep patterns to the primary parental caregivers sleep, daytime sleepiness, and stress	Children's Sleep Habits Questionnaire (parent report) Insomnia Severity Index (caregiver completed) Epworth Sleepiness Scale (caregiver completed) Actigraphy Polysomnography	Yes	Children/adolescents and their parents exhibit over an hour of wake time after sleep onset and fragmented sleep. Variability in child total sleep time was associated with parental stress	Future reach is needed to identify underlying causes of poor sleep and the relationship to daytime function, behavior, and the family unit
59	(Braam, Didden, Smits, & Curfs, 2008b) Netherlands	n=8 children/adults with Angelman syndrome (n=4 placebo and n=4 melatonin group) 4-20 years	To conduct a randomized placebo-controlled trial in patients with Angelman syndrome	Sleep diary (parent report) Salivary melatonin	No	During melatonin treatment, sleep latency decreased, sleep onset advanced, and total sleep time increased	Further studies need to be performed on melatonin metabolism in Angelman syndrome to establish best dose

Table 2.6 Continued

60	(Miano et al., 2005) Italy	n=10 patients with Angelman syndrome 2-16 years Two age-matched groups: n=15 patients with mental retardation without epilepsy 2-10 years n=13 patients with mental retardation and epilepsy 3-9 years	To evaluate sleep breathing patterns and to detect periodic leg movements in patients with Angelman syndrome	Polysomnography	No	Sleep period time and total sleep time were not significantly different in the three groups. Patients with Angelman syndrome showed higher WASO% and sleep onset latency; percentage of REM sleep was reduced in Angelman syndrome and in patients with mental retardation with epilepsy All groups had, high frequency of sleep apneas and periodic limb movement	Need to further explore sleep disorders in children affected by mental retardation and set up correct treatment
61	(Barry, Leitner, Clarke, & Einfeld, 2005) Australia	n= 62 patients with Angelman syndrome, n=29 patients with presumed Angelman syndrome, and n=340 control subjects 1-40 years	To better define the "behavioral phenotype" in Angelman syndrome (e.g. prevalence and types of behavior characteristics)	Angelman syndrome questionnaire as part of normal case management procedure (twelve items from developmental behavior checklist were chosen to be included in the questionnaire) (caregiver report)	No	Sleep disturbance prevalence was 67.8% in Angelman syndrome and 26.4% in controls	Sleep disturbance should be included in the "behavioral phenotype" of Angelman syndrome
62	(Chik, Rollag, Duncan, & Smith, 2010) United States	n= 30 confirmed patients with Smith Magenis syndrome (SMS) and n=5 controls 16 mon ths-20 years	To determine the utility of daytime salivary melatonin as a diagnostic test in SMS	Salivary melatonin	No	Elevated daytime melatonin production was demonstrated in the majority of patients with SMS	The utility of midday salivary measurement appears insufficient to distinguish patients with SMS form other conditions. Multiple samples at additional time points may increase the sensitive

Table 2.6 Continued

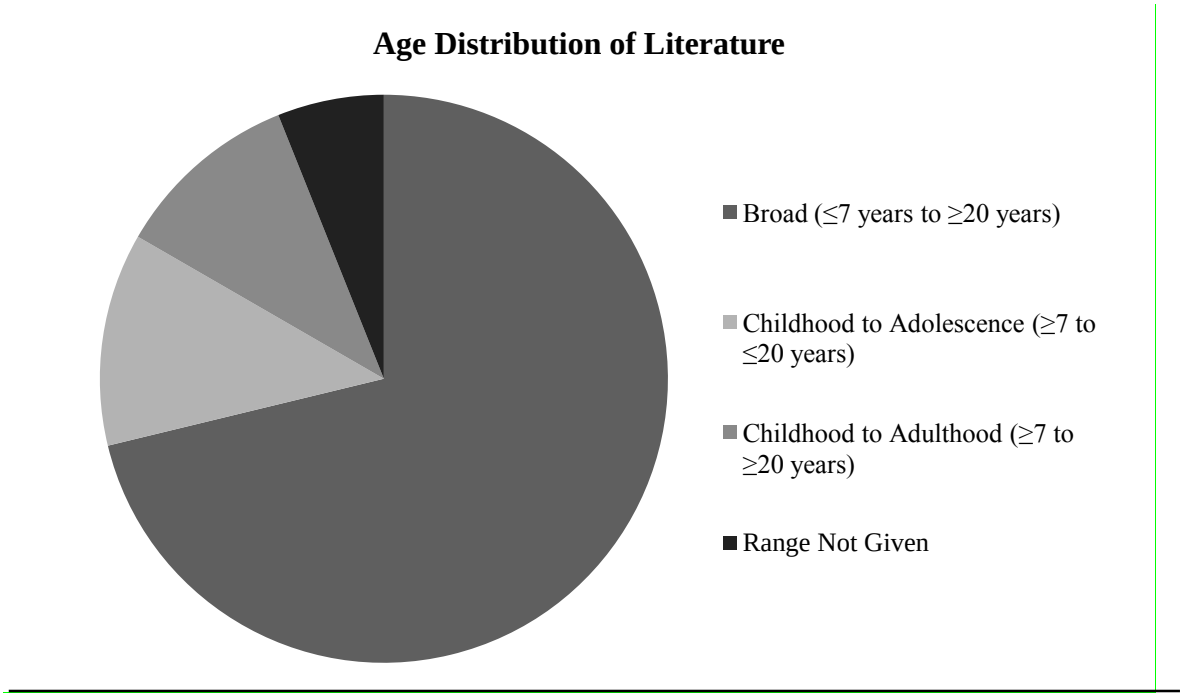
							if a SMS salivary melatonin test
63	(Hoffman, 2006) United States	n= 106 children with autism and n=169 controls from community sample 4-16 years	To compare parents' reports of sleep problems of their children with autism to parents' reports of typically developing children in a way that addresses methodological limitations of past studies	Children's Sleep Habits Questionnaire (parent report)	No	Significant differences in bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnia, and sleep disordered breathing were found for the autism versus community group	Research that addresses methodological shortcoming is needed (e.g. disentangle children's sleep problems, their autistic symptomology, and coexisting conditions), as well as examination of relationship between children's and parents' sleep problems and impact on parental stress and family process
64	(Mannion, 2013) Ireland	n= 89 children and adolescents with ASD 3-16 years	To investigate the prevalence of current comorbid psychological disorders and behaviors that are associated with these disorders, as well as investigate the prevalence of intellectual disability, epilepsy, gastrointestinal symptoms, and sleep problems	Children's Sleep Habits Questionnaire (parent report)	No	The prevalence of a comorbid disorder was 46.1% (excluding an intellectual disability) and 78.7 % (including an intellectual disability). The prevalence of sleep problems in this sample was over 80%	Future research could also examine the role of behavioral interventions in relation to comorbid disorders, including sleep problems

Table 2.6 Continued

*65	(Moss et al., 2014) Australia	n= 26 children and adolescents with a developmental disability 8-17 years	To examine the impact of the Sleepwise intervention on children's and teenager's sleep, in terms of overall level of sleep disturbance reported by parents, in comparison to a control group	Children's Sleep Habits Questionnaire (parent report)	No	Sleepwise approach was effective in reducing sleep disturbance and parent stress	Further areas of interest may include investigation of the physiological effects of improved sleep, as well as variables influencing parents' engagement with behavior change 3once involved in treatment program
66	(Vanhelst, Bui-Xuan, Fardy, & Mikulovic, 2013) France	n= 410 children with an intellectual disability Mean age= 15.1 years	To explore the relationship between sleep habits and overweight/obesity, physical activity, and sedentary behaviors in adolescents with an intellectual disability	Author made questionnaire	No	The highest percentage of overweight and obese adolescents was in the late to bed late to rise group and the lowest percentage of overweight and obese subjects was in the early to bed early to rise	Further investigations should include energy intake assessed by a dietician and an objective assessment of physical activity, such as an accelerometer

*= subsample study
ASD= Autism Spectrum Disorders
CDC= Cri du Chat syndrome
DS= Down syndrome
FASD= Fetal Alcohol Spectrum Disorders
FXS= Fragile X syndrome
HFASD= High Functioning Autism Spectrum Disorders
ID= Intellectual Disability
JS= Jacobsen syndrome
NDD= Neurodevelopmental Disorder
TD= Typical Development
WS= Williams syndrome

Figure 2.1. Literature Sorted by Sample Age Range



CHAPTER 3: PINEAL HYPOPLASIA, REDUCED MELATONIN, AND SLEEP DISTURBANCE IN PATIENTS WITH *PAX6* HAPLOINSUFFICIENCY

Authors: Alyson E. Hanish, MSN, RN, John A. Butman, MD, PhD, Francine Thomas, BS, Jianhua Yao, PhD, Joan C. Han, MD

Institutions: Section on Growth and Obesity, Program in Developmental Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health (NIH), Bethesda, MD 20892 USA; National Institute of Nursing Research, NIH, Bethesda, MD 20892 USA; University of Iowa, College of Nursing, Iowa City, IA 52242 USA; Radiology and Imaging Sciences, Clinical Center, NIH, Bethesda, MD 20892 USA; Department of Pediatrics, Le Bonheur Children's Hospital, University of Tennessee Health Science Center, Memphis, TN 38103 USA

Site of Study: National Institutes of Health (NIH), Bethesda, MD 20892 USA

Correspondence: Joan C. Han, Associate Professor, Department of Pediatrics, Le Bonheur Children's Hospital, 50 North Dunlap Street, Room 476R, Memphis, TN 38103 USA; Telephone: 901-287-6408; Fax: 901-287-4478; Email: jhan14@uthsc.edu

Financial Disclosures: The authors have no conflicts of interest.

Acknowledgments: This study was funded by the Intramural Research Programs of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (1ZIAHD008898-02) and the National Institute of Nursing Research of the National Institutes of Health (NIH), and by the NIH Bench-to-Bedside Program. We thank the members of International WAGR Syndrome Association, Aniridia Foundation

International, and Aniridia Network UK for assistance in identifying families who were willing to participate in this study. We thank Jack Yanovski and David Klein for helpful discussions. We thank the following individuals for their assistance: Sheila Brady, Kristen Danley, Shannon Fuhr, Amanda Huey, Melanie Hicks, Daniela Reyes-Capo, Lindsay Hunter, Matthew Tsang, Mark Lee, Tanvee Singh, Emily Yin, and Yael Caplan.

Abstract

Study Objectives: Paired box 6 (PAX6) appears to play an important role in the development of the pineal, the primary source of the circadian regulating hormone, melatonin. Pineal hypoplasia has been previously reported in patients with *PAX6* haploinsufficiency (+/-); however, pineal measurement, melatonin concentrations, and sleep quality have not been reported. This study examined pineal volume, melatonin secretion, and sleep disturbance in patients with *PAX6+/-*.

Design: Cross-sectional descriptive

Setting: Inpatient setting, National Institutes of Health, Bethesda, MD

Participants: Thirty-seven patients with *PAX6+/-* (age 15.3±9.9 years) and 17 healthy controls (16.0±7.2 years)

Interventions: None

Measurements: Pineal volume was evaluated by magnetic resonance imaging (MRI). Diurnal serum cortisol, serum melatonin, and urine 6-sulfatoxymelatonin (6SM) concentrations were measured by enzyme-linked immunosorbent assay. The Child Sleep Habits Questionnaire (CSHQ) was administered for patients <13y.

Results: Pineal volume was 5-fold lower in *PAX6+/-* vs. controls (mean±SD: 25±15 vs. 129±50 µL, $p<0.001$). Midnight serum cortisol was similar in *PAX6+/-* vs. controls

(p=0.14). Midnight serum melatonin was >2-fold lower in *PAX6*+/- vs. controls (median [25th-75th]: 28 [22-42] vs. 71 [46-88] pg/mL, p<0.001). First morning void urinary 6SM was 4-fold lower in *PAX6*+/- vs. controls (11 [6-26] vs. 45 [34-61] ng/mgCr, p=0.001). CSHQ score was higher in *PAX6*+/- vs. controls (48±6 vs. 41±5, p=0.03).

Conclusions: Our findings suggest that *PAX6*+/- is associated with smaller pineal size, lower melatonin secretion, and greater parental report of sleep disturbances in children. Further studies are needed to explore the potential use of melatonin replacement for improving sleep quality in patients with *PAX6*+/-.

Keywords: pineal, melatonin, sleep, MRI, CSHQ, ELISA, WAGR syndrome, 11p deletion, PAX6

Introduction

Heterozygous loss of function for the transcription factor, paired box 6 (*PAX6*, located on chromosome 11p13), is the most common cause of aniridia, a rare developmental eye disorder (prevalence 1/50,000-100,000) associated with absence or hypoplasia of the iris and other ocular defects (Glaser et al., 1992; Ivanov, Shuper, Shohat, Snir, & Weitz, 1995b). *PAX6* haploinsufficiency *(+/-)* can occur due to mutations involving only *PAX6* in patients with isolated anirida or as part of contiguous 11p13 gene deletions that involve *PAX6* and adjacent genes in patients with WAGR (Wilms tumor, Aniridia, Genitourinary anomalies, cognitive impaiRment) syndrome (Fischbach et al., 2005; Lee, Khan, & O'Keefe, 2008b).

In addition to its ocular role, *PAX6* also appears to be important for the development of the pineal, the primary source of the circadian regulating hormone, melatonin (Estivill-Torrus et al., 2001; Rath et al., 2009; Walther & Gruss, 1991). In

wild-type mice, *Pax6* is expressed in the pineal during early embryologic developmental stages. Homozygous *Pax6-/-* mice lack pineal-specific immunomarkers at all embryonic stages (Estivill-Torrus et al., 2001; Walther & Gruss, 1991). Heterozygous *Pax6+/-* mice exhibit a present, but hypoplastic pineal gland (Estivill-Torrus et al., 2001).

In 2003, Mitchell et al. described the first observation of human pineal absence in a study of 24 subjects with *PAX6* mutations, 7 of whom had pineal hypoplasia and 13 had pineal absence on brain MRI (Mitchell et al., 2003). A subsequent study described 3 patients with *PAX6* mutations who underwent brain MRI, and all were found to have pineal absence (Abouzeid et al., 2009). Neither of these studies described sleep assessments or melatonin concentrations, and thus the functional consequences of pineal absence were not assessed.

We assessed pineal volume, diurnal concentrations of serum melatonin and its urinary metabolite, 6-sulfatoxymelatonin (6SM), and sleep disturbance in 37 patients with *PAX6+/-* and 17 healthy control subjects matched for age, sex, race, and body mass index. We hypothesized that *PAX6+/-* would be associated with smaller pineal volume, lower melatonin, lower 6SM, and greater sleep disturbance.

Methods

Study Population

Subjects in the *PAX6+/-* group included patients with WAGR syndrome who had prior genetic testing showing 11p deletion (deletion boundaries, identified by oligonucleotide array comparative genomic hybridization as previously described, (Han et al., 2008) are shown in Supplemental Table 3.2), and patients with isolated aniridia who had documented mutations or intragenic deletions affecting only *PAX6* (shown in

Supplemental Table 3.2). Healthy control subjects included individuals who had no chronic medical conditions and were neurologically normal on physical examination. Subjects were excluded from analysis if they reported beta-blocker use (which could suppress melatonin secretion), renal insufficiency (which could affect urinary clearance of melatonin), or complete lack of light perception (which could lead to free-running circadian patterns). The study was approved by the institutional review board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD and registered at ClinicalTrials.gov (NCT00758108). Written informed consent was obtained from adults who were competent to provide consent and from the parents or legal guardians of children and adults with cognitive impairment. Assent was obtained from patients who were unable to give consent.

Brain Imaging

Non-contrast brain MRI was performed using an 8 channel 12 element head array coil on a 3.0 tesla system (Philips Achieva, Netherlands) on all patients who could comply with the procedure or who were undergoing sedation for clinically indicated eye examination under anesthesia. In addition to routine clinical MRI sequences (e.g., T1-weighted, T2-weighted, fluid attenuated inversion recovery [FLAIR]), we performed two different dedicated high resolution sequences to evaluate the pineal gland. The first was a T2 weighted sequence using a 2D fast spin echo technique (performed in 34 *PAX6+/-* patients and 16 control subjects). The second was a T2/T1 weighted 3D balanced fast field echo sequence (performed in 18 *PAX6+/-* patients and 14 control subjects). Both methods generate high signal in CSF and low signal in pineal tissue.

Contrast parameters for the 2D FSE sequence were: repetition time (TR) 2260 ms, effective echo time (TE) 180 ms, flip angle (FA) 90°, echo train length 32, bandwidth (BW) 218 Hz/pixel. Geometric parameters were: field of view 150 mm anterior-to-posterior (AP) × 150 mm right-to-left (RL) × 38 superior-to-inferior (SI), sequence resolution 0.39 mm AP × 0.52 mm RL × 1.8 mm SI. Two averages were performed with an imaging time of 3m00s.

Contrast parameters for the 3D BFFE sequence were: TR 5.5 ms, TE 2.2 ms, FA 30°, BW 389 Hz/pixel. Geometric parameters were: field of view 80 mm anterior-to-posterior (AP) × 80 mm right-to-left (RL) × 88 superior-to-inferior (SI), sequence resolution 0.4 mm AP × 0.4 mm RL × 0.8 mm SI. Two averages were performed with an imaging time of 8m26s.

Two-dimensional analyses of pineal images were used to estimate volume by multiplication of the AP dimension with the square of the RL dimension. For the 3D measurements, pineal borders were manually traced slice-by-slice on the dedicated B3D BFFE images, using the manual verification painting tool in RTHR CT-Slicer software, a custom-designed program developed at the NIH (software available upon request). The pixels inside the traced contour were summed to determine the total pineal volume.

Serum Cortisol and Melatonin

Venous blood was drawn from an indwelling intravenous catheter at 08:00 in the morning and at midnight (in a dark room while the subject was in bed). Serum cortisol concentrations were measured using a solid-phase, competitive chemiluminescent enzyme immunoassay on the Siemens Immulite 2500 Analyzer (NIH Clinical Center Chemistry Laboratory). Serum samples were stored at -80°C, and at the completion of the

study, melatonin concentrations were measured by a commercially available competitive enzyme-linked immunosorbent assay (ELISA) (Genway, San Diego, CA) according to the manufacturer's protocol, with the following modification: serum melatonin samples were diluted 2.5-fold prior to assay. The intra-assay coefficient of variation was 3.0-11.4% and the inter-assay coefficient of variation was 6.4-19.3%. The detection limit of the assay was 1.6 pg/mL. Serum albumin was measured on the Siemens Dimension Vista (NIH Clinical Center Chemistry Laboratory) so that concentrations of serum melatonin could be adjusted for albumin concentration since endogenous melatonin is weakly protein bound (Kennaway & Voultsios, 1998).

Urine 6-sulfatoxymelatonin (6SM)

Urine was collected from the first morning void and during the evening. Melatonin secretion was estimated by measuring the urinary concentrations of 6-sulfatoxymelatonin (6SM) using a competitive ELISA (Genway, San Diego, CA) according to the manufacturer's protocol. The intra-assay coefficient of variation was 5.2-12.2% and the inter-assay coefficient of variation was 5.1-14.9%. The detection limit of the assay was 1.0 ng/mL. Urine 6SM concentrations were expressed in ng per mg of creatinine (ng/mg Cr) to control for renal function and dilution of urine. Urinary creatinine was measured using the Siemens Dimension Xpand (NIH Clinical Center Chemistry Laboratory). The first morning void concentration of 6SM, normalized to urinary creatinine, has been extensively used as an estimate of overnight melatonin secretion (McMullan, Schernhammer, Rimm, Hu, & Forman, 2013).

Child Sleep Habits Questionnaire

The Child Sleep Habits Questionnaire (CSHQ) is a validated parent-report questionnaire that examines sleep disturbance in children (Goodlin-Jones et al., 2008; Owens, Spirito, & McGuinn, 2000). The CSHQ contains 33 items that measure sleep disturbance and 3 items collecting information about bedtime, wake-up time, and sleep duration. Parents rate the frequency of each item using a 3-point Likert scale: "usually" (5–7 times per week), "sometimes" (2–4 times per week) and "rarely" (0–1 time per week), with higher scores indicating more frequent sleep disturbances. The CSHQ was completed by parents of children <13 years of age.

Statistical Analyses

Statistical analyses were performed using SPSS version 19.0 (IBM Corp., Armnok, NY). Skewness, kurtosis, and Kolmogorov–Smirnov test for normality were assessed. BMI-Z scores were calculated from subject heights and weights using the modified LMS method (Cole, 1990) of the Centers for Disease Control and Prevention 2000 growth charts (Kuczmarski et al., 2000). Parametric tests were used for normally distributed data, while skewed data were assessed using nonparametric tests. Independent samples *t*-tests, Mann-Whitney U tests, and Fisher's exact tests were used to compare unadjusted differences in demographic and clinical characteristics between patients with *PAX6*+/- and healthy controls. Skewed data were also normalized by log-transformation so that parametric analyses of covariance could be used to assess differences in pineal volume, 6SM, and CSHQ (adjusting for age, sex, race, BMI-Z), and serum melatonin (additionally adjusting for albumin). Back-transformed adjusted means and 95% confidence intervals are reported in the results for variables that were log-transformed.

Diurnal variation in morning and evening cortisol concentrations within subjects were compared using the Wilcoxon signed rank test. Pearson and Spearman correlations were calculated to assess the relationships among pineal volume, melatonin secretion, and CSHQ scores. P-values <0.05 were considered nominally significant, while P-values <0.0083 were considered significant after Bonferroni correction for multiple comparisons.

Results

Patients included 37 individuals with *PAX6*+/- (age 2 - 40y) and 17 healthy controls (age 7 - 32y). Subject characteristics are shown in Table 3.1. Within the *PAX6*+/- group, there were no significant differences between patients with WAGR syndrome versus isolated aniridia for pineal volume, cortisol, melatonin, and sleep questionnaire score (all p's>0.1; data not shown). Due to these similarities, participants with WAGR/11p deletion syndrome and isolated aniridia were combined for all subsequent analyses.

PAX6 Haploinsufficiency is Associated with Reduced Pineal Volume

Pineal volume, estimated by 2D measurements, was approximately 5-fold lower in *PAX6*+/- versus controls (p<0.001, Table 3.1). A subset of subjects also had 3D volume measurements, yielding a similar 5-fold lower volume in *PAX6*+/- versus controls (p<0.001, Table 3.1). The 2D-calculated and 3D-measured volumes were well correlated (r=0.95, p<0.001). After controlling for age, sex, race, and BMI-Z, pineal volumes were still found to be significantly lower in patients with *PAX6*+/- versus controls (p's< 0.001, Figure 3.1).

PAX6 Haploinsufficiency is Associated with a Normal Diurnal Pattern of Cortisol

Midnight serum cortisol was similar in *PAX6*+/- versus controls (p=0.14, Table 3.1). The 8AM serum cortisol values were also similar in *PAX6*+/- versus controls (p=0.77, Table 3.1). After adjusting for age, sex, race, and BMI-Z, midnight serum cortisol was similar in *PAX6*+/- versus controls (adjusted mean [95%CI]: 2.2 [1.6 - 2.9] vs. 1.6 [1.1 - 2.3] µg/dL, p=0.19). The 8AM serum cortisol values were also similar in *PAX6*+/- versus controls (11.7 [9.9 - 13.9] vs. 9.9 [7.9 - 12.5] µg/dL, p=0.26). Subjects in the *PAX6*+/- and control groups both had normal diurnal patterns of cortisol with higher levels at 8AM compared to midnight (p's <0.001).

PAX6 Haploinsufficiency is Associated with Lower Serum Melatonin

Midnight serum melatonin was lower in *PAX6*+/- versus controls (p<0.001, Table 3.1). After controlling for age, sex, race, BMI-Z, and albumin, midnight serum melatonin remained lower in patients with *PAX6*+/- versus controls (p<0.001, Figure 3.2). Morning serum melatonin was also lower in *PAX6*+/- versus controls (p=0.008, Table 3.1). After controlling for age, sex, race, BMI-Z, and albumin, morning serum melatonin remained lower in patients with *PAX6*+/- versus controls (p=0.006, Figure 3.2).

PAX6 Haploinsufficiency is Associated with Lower Morning Urinary 6SM

Morning urinary 6SM (reflecting nighttime melatonin production) was lower in *PAX6*+/- versus controls (p=0.001, Table 3.1). After controlling for age, sex, race, and BMI-Z, morning urinary 6SM melatonin remained lower in patients with *PAX6*+/- versus controls (p=0.001, Figure 3.2). Evening urinary 6SM was not significantly different in *PAX6*+/- versus controls (p<0.11, Table 3.1). After controlling for age, sex, race, and BMI-Z, however, evening urinary 6SM melatonin was found to be nominally higher in

patients with *PAX6+/-* versus controls (p=0.04, Figure 3.2), but this difference did not remain significant after correction for multiple comparisons.

PAX6 Haploinsufficiency is Associated with Nominally Greater Reported Sleep Disturbances

Total CSHQ score was 15% higher (reflecting increased sleep disturbance) in children with *PAX6+/-* versus controls (p<0.03, Table 3.1), but this nominal difference was no longer significant after adjustment for multiple comparisons. After adjustment for age, sex, race, and BMI-Z, a trend toward higher CSHQ score was observed for the *PAX6+/-* group as compared to the control group (p=0.06).

Pineal Volume is Correlated with Serum Melatonin and Urine 6SM

Among all subjects combined, pineal volume was significantly correlated with midnight serum melatonin (rho = 0.57, p<0.001 for 2D-calculated; rho=0.69, p<0.001 for 3D-measured) and first morning void 6SM (rho=0.44, p=0.006 for 2D-calculated; rho=0.39, p=0.05 for 3D).

Discussion

In patients with *PAX6+/-* compared to controls, pineal volume, midnight serum melatonin, morning serum melatonin, and morning urinary 6SM were lower, while cortisol diurnal patterns were similar. Total CSHQ scores showed nominally greater parental report of sleep disturbances in children with *PAX6+/-*. Pineal volume was significantly correlated with midnight serum melatonin and first morning void 6SM. Although pineal hypoplasia and absence have been previously reported in patients with various *PAX6* mutations (Abouzeid et al., 2009; Mitchell et al., 2003), this current study is the first to describe pineal volume, melatonin secretion, and sleep disturbance in this

population. Our findings are consistent with animal studies that show correlation of pineal size with melatonin secretion. Melatonin in pinealectomized rats and humans is reduced and lacks circadian rhythmicity (Acuna-Castroviejo et al., 2014; Jan, Tai, Hahn, & Rothstein, 2001b; Kocher, Brun, Borson-Chazot, Gonnaud, & Claustrat, 2006; Lehmann et al., 1996; Murata, Sawamura, Ikeda, Hashimoto, & Honma, 1998; Ozaki & Lynch, 1976). Furthermore, the size of the pineal has been correlated with melatonin concentrations in sheep (Coon et al., 1999a).

Although we originally hypothesized that serum melatonin concentrations and urinary melatonin metabolite levels would be lower in the morning and evening in patients with *PAX6+/-* as compared to controls, we observed that evening urinary 6SM was similar after adjusting for multiple comparisons. This similarity may be due to release into circulation of small amounts of extrapineal sources of melatonin (e.g. gastrointestinal tract) (Acuna-Castroviejo et al., 2014).

One limitation of our study is that blood was drawn at midnight, which may have preceded the typical melatonin peak (median for healthy adults usually between 2- 4AM) (Arendt, 1995). We found midnight serum melatonin was approximately 2-fold lower in *PAX6+/-* vs. controls, whereas first morning void urinary 6SM was approximately 5-fold lower in *PAX6+/-* vs. controls. It is possible that midnight blood samples were collected prior to attaining peak serum melatonin levels, thus blunting the difference between groups. An additional limitation of our study is that total, rather than free, melatonin concentration was measured because we did not have salivary samples available. Because melatonin is weakly protein bound in peripheral circulation, (Kennaway & Voultsios, 1998) differences in albumin concentration could alter free melatonin concentrations. In

our cohort, serum albumin was comparable between groups and did not alter the results when used as a covariate for analyses. Another limitation was that sleep disturbance was only assessed in children using parent report and no objective sleep measures were analyzed between groups. Further studies are needed to assess self-reported sleep disturbance in patients with *PAX6+/-* vs. controls. In addition, objective measures such as actigraphy monitoring in a home-based environment would be useful to examine sleep patterns, as well as provide insight into clinical assessment and sleep-related interventions in this population.

This is the first study to explore the functional consequences of pineal hypoplasia in patients with *PAX6+/-*, and our findings support the hypothesis that *PAX6* plays an important role in human pineal development and sleep regulation. Further studies examining sleep disturbance and sleep patterns would be beneficial in elucidating the *PAX6+/-* sleep-related phenotype, which will aid in establishing effective sleep management in these patients.

Although this study examined these outcomes in a small group of indivuals with rare genetic conditions, there are implications for the general population. Furthermore, common genetic polymorphisms that affect the function of PAX6 may be associated with insomnia in the general population (Ban, Kim, Seo, Kang, & Choi, 2011). Additional studies of these genetic variants and their impact on pineal size, melatonin secretion, and sleep patterns could lead to genotype-specific treatment of insomnia using melatonin as a targeted therapy in PAX6-insufficient individuals.

In conclusion, the present study is the first to demonstrate significantly reduced pineal volume, reduced melatonin secretion, and greater sleep disturbance in patients

with *PAX6*+/- versus controls. Further research is needed to determine if melatonin replacement could be particularly beneficial in *PAX6* +/- patients, and potentially also in individuals in the general population with common genetic variants affecting PAX6 functioning.

Table 3.1. Characteristics of Patients with *PAX6*+/- and Healthy Controls

	***PAX6*+/-** **(n=37)**	**Healthy Controls** **(n=17)**	**P-Values**
Age (y)	15.3 ± 9.9 (2.4 – 40.5)	16.0 ± 7.2 (7.8 - 32.3)	0.80
Sex (% Female)	57	65	0.77
BMI (kg/m^2)	25.3 ± 7.4 (14.0 - 44.0)	26.6 ± 8.0 (15.2 - 39.2)	0.56
BMI-Z	1.4 ± 1.0 (-1.4 to +2.7)	1.2 ± 1.2 (-1.3 to +2.5)	0.64
Race/Ethnicity (%) Non-Hispanic Caucasian Other	 86 14	 65 35	0.08
Pineal Volume by 2D Calculation (µL)	31 ± 22	156 ± 76	<0.001
Pineal Volume by 3D Measurement (µL)	25 ± 15	129 ± 50	<0.001
Midnight Serum Melatonin (pg/mL)	28.6 [22.1 - 42.6]	71.6 [46.4 - 88.1]	<0.001
8AM Serum Melatonin (pg/mL)	25.4 [18.1 - 29.3]	36.8 [24.1 - 68.0]	0.008
Morning Urinary 6SM (ng/mL)	11.8 [6.6 - 26.1]	45.2 [34.0 - 61.4]	0.001
Evening Urinary 6SM (ng/mL)	8.6 [3.7 - 16.7]	4.7 [3.5 - 7.0]	0.11
Midnight Serum Cortisol (µg/dL)	1.7 [1.2 - 2.9]	1.1 [1.0 - 2.6]	0.14
8AM Serum Cortisol (µg/dL)	11.9 [7.6 - 15.8]	11.5 [7.6 - 16.1]	0.77
Albumin (g/dL)	3.9 [3.7 - 4.0]	4.0 [3.8 - 4.15]	0.15
CSHQ Total Score	48 ± 6	41 ± 5	0.03

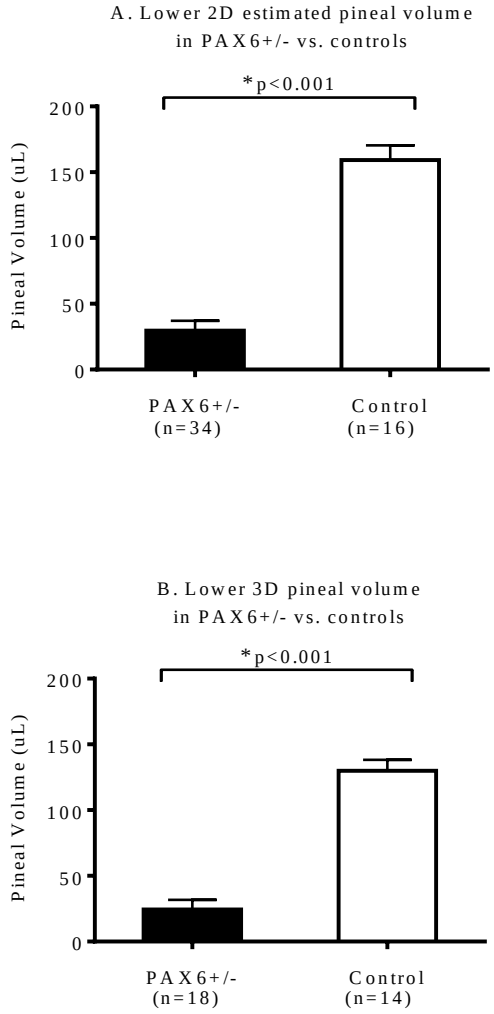
Table 3.2. Supplemental Data: Genetic Abnormalities in Patients with WAGR Syndrome (W) and Isolated Aniridia (A)

ID	Age (y)	Sex	Genetic Abnormality (NCBI Build, Hg 18)
W1	9.5	F	Chromosome 11 deletion (20,759,560-35,124,632)
W2	8.3	F	Chromosome 11 deletion (22,437,763-33,647,959)
W3	11.9	F	Chromosome 11 deletion (23,887,358-42,707,993)
W4	6.5	F	Chromosome 11 deletion (24,744,722-32,740,440)
W5	12.6	M	Chromosome 11 deletion (24,748,424-36,203,063)
W6	16.7	F	Chromosome 11 deletion (24,770,593-37,835,652)
W7	10.3	M	Chromosome 11 deletion (25,059,584-40,571,441)
W8	22.4	F	Chromosome 11 deletion (25,330,891-36,039,207)
W9	8.0	M	Chromosome 11 deletion (25,968,860-43,778,471)
W10	6.2	M	Chromosome 11 deletion (26,690,778-37,341,623)
W11	24.3	F	Chromosome 11 deletion (27,057,396-36,959,349)
W12	12.2	M	Chromosome 11 deletion (27,773,897-33,998,994)
W13	12.0	F	Chromosome 11 deletion (27,824,678-42,622,913)
W14	6.3	M	Chromosome 11 deletion (28,200,894-32,820,697)
W15	6.1	F	Chromosome 11 deletion (28,221,617-33,679,832)
W16	13.6	M	Chromosome 11 deletion (28,901,513-39,755,713)
W17	16.1	F	Chromosome 11 deletion (29,703,168-38,673,618)
W18	27.1	F	Chromosome 11 deletion (30,035,387-33,602,848)
W19	25.4	F	Chromosome 11 deletion (30,232,199-37,070,116)
W20	7.1	F	Chromosome 11 deletion (31,459,056-33,682,710)
W21	13.1	M	Chromosome 11 deletion (24,391,751-38,869,102)
W22	8.4	M	Chromosome 11 deletion (17,776,012-44,236,020)
W23	14.0	F	Chromosome 11 deletion (25,176,956-38,039,876)
W24	10.5	F	Chromosome 11 deletion (25,751,539-35,156,921)
W25	7.1	M	Chromosome 11 deletion (29,641,313-47,114,592)
W26	6.8	M	Chromosome 11 deletion (19,309,352-32,405,969)

Table 3.2. Continued

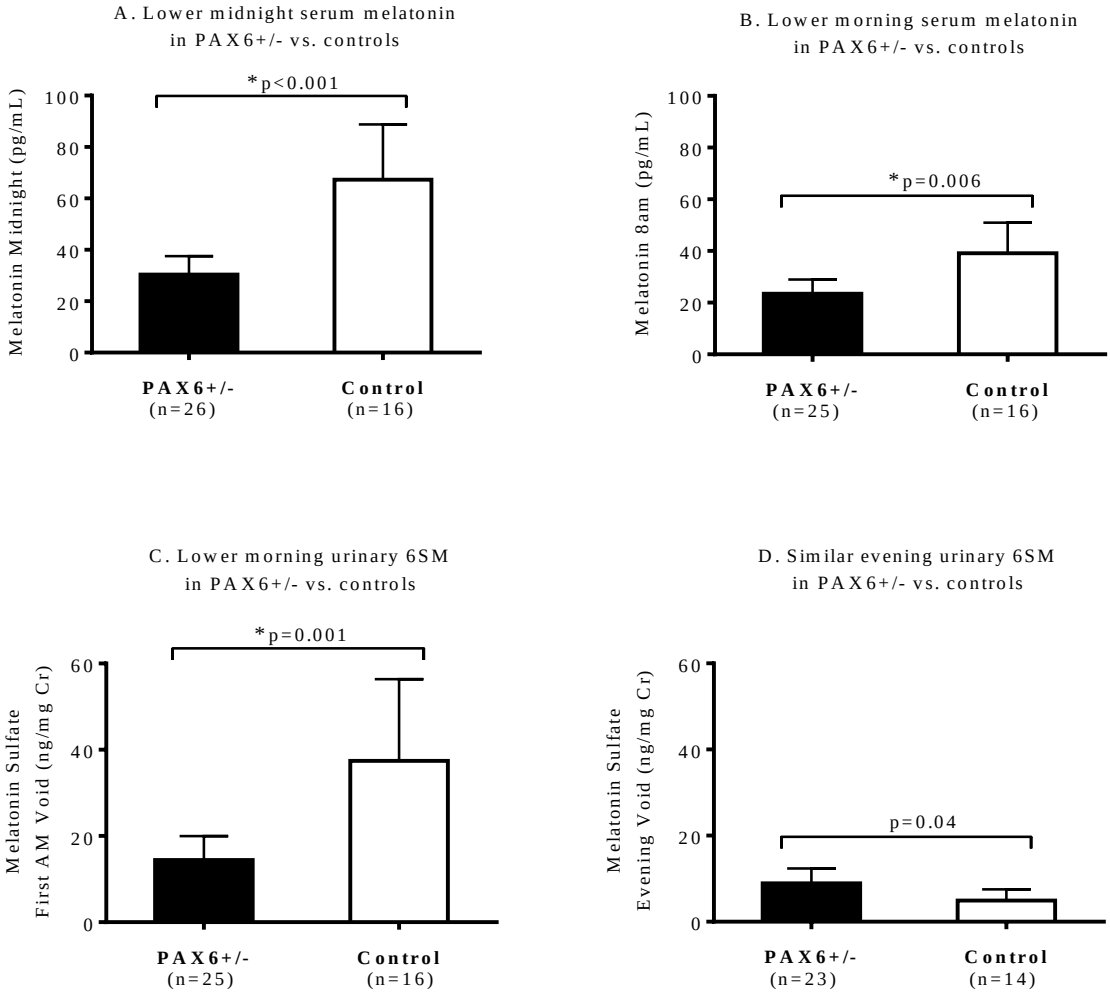
W27	2.4	M	Chromosome 11 deletion (17,932,124-35,841,004)
W28	13.1	F	Chromosome 11 deletion (23,952,397-37,862,266)
A1	7.3	F	heterozygous G161VfsX46 (missense, frame-shift, premature stop)
A2	8.3	M	heterozygous W266X (premature stop in exon 10)
A3	17.5	M	heterozygous T>A substitution in intron 2 (predicted to alter splicing)
A4	23.4	F	heterozygous Q2X (premature stop)
A5	28.3	F	heterozygous deletion of exons 1-4
A6	32.3	F	heterozygous deletion of exons 8-13
A7	33.0	M	heterozygous R240X (premature stop in exon 9, homeobox domain).
A8	39.3	M	heterozygous R38W (missense mutation in paired box domain)
A9	40.5	F	heterozygous R240X (premature stop in exon 9, homeobox domain)

Figure 3.1. Lower Pineal Volume in Patients with *PAX6* haploinsufficiency (*PAX6+/-)* as Compared to Controls



(A) Pineal volume was calculated by brain magnetic resonance imagining (MRI) using 2D analysis. (B) Pineal volume was calculated by brain MRI using 3D analysis. Covariates for (A) and (B) were age, sex, race, & BMI-Z. Adjusted means ± SEMs and nominal p-values, with asterisk (*) indicating significance after adjustment for multiple comparisons, are shown.

Figure 3.2. Melatonin in Patients with *PAX6* haploinsufficiency (*PAX6+/-)* as Compared to Controls



(A, B) Patients with *PAX6*+/- had lower adjusted midnight and morning serum melatonin as compared to controls. Melatonin was measured using a competitive enzyme-linked immunosorbent assay (ELISA). Covariates were age, sex, race, BMI-Z, and serum albumin. (C, D) Patients with *PAX6+/-* had lower adjusted first morning urinary 6-sulfatoxymelatonin (6SM) but no significant difference in evening urinary 6SM as compared to controls. 6SM was normalized to urinary creatinine (to control for differences in dilution) and adjusted for age, sex, race, and BMI-Z. For (A-D), skewed data were normalized by log transformation. Backtransformed adjusted means with ± 95% confidence intervals are shown. Nominal p-values, with asterisk (*) indicating significance after adjustment for multiple comparisons, are shown

CHAPTER 4: DAYTIME SLEEPINESS, SLEEP DISTURBANCE, SLEEP RELATED IMPAIRMENT, AND SLEEP PATTERNS IN ADOLESCENTS WITH *PAX6* HAPLOINSUFFICIENCY

Abstract

Pineal hypoplasia, reduced melatonin concentration, and parental report of sleep disturbance in children have been previously reported in patients with *PAX6* haploinsufficiency (+/-); however, self-report measures and sleep patterns have not been described. This study examined self-reported daytime sleepiness, sleep disturbance, sleep-related impairment, and sleep patterns in adolescents with *PAX6*+/-. The sample included 9 adolescents with *PAX6*+/- (age 15.5 ± 3.3 years) and 25 healthy adolescents (13.6 ± 2.3). The Cleveland Adolescent Sleepiness Questionnaire (CASQ) and PROMIS sleep questionnaires were administered to adolescents, and sleep patterns were assessed using actigraphy. Total scores on all questionnaires were similar in *PAX6*+/- vs. healthy comparison group; however, *PAX6*+/- was associated with significantly greater time from lights off to sleep onset after adjustment for age, sex, and race (adjusted mean ± 95% CI for *PAX6*+/- vs. healthy comparison group: 20.1 [8.1-49.7] vs. 6.2 [3.7-10.4] minutes, p=0.04). Total scores on the CASQ and PROMIS sleep measures were correlated (r's>0.7, p's<0.001) in the healthy comparison group only. Total sleep time was negatively correlated with CASQ and PROMIS sleep measures total scores (p's<0.02) in adolescents in the healthy comparison group. Our study suggests the potential research and clinical utility of adult versions of PROMIS sleep measures in an adolescent population. Keywords: sleep, WAGR syndrome, PAX6, CASQ, PROMIS

Introduction

PAX6 haploinsufficiency *(+/-)* can occur due to mutations involving only *PAX6* in patients with isolated anirida, a rare developmental eye disorder (prevalence 1/50,000-100,000) associated with absence or hypoplasia of the iris and other ocular defects (Glaser et al., 1992; Ivanov et al., 1995b) or as part of contiguous 11p13 gene deletions that involve *PAX6* and adjacent genes in patients with WAGR (Wilms tumor, Aniridia, Genitourinary anomalies, cognitive impaiRment) syndrome (Fischbach et al., 2005; Lee et al., 2008b). In addition to its ocular role, *PAX6* also appears to be important for the development of the pineal, the primary source of the circadian regulating hormone, melatonin (Abouzeid et al., 2009; Estivill-Torrus et al., 2001; Mitchell et al., 2003; Rath et al., 2009; Walther & Gruss, 1991). Given the potential role of *PAX6* in pineal development and circadian regulation, individuals with deletions or mutations leading to *PAX6+/-* may be more likely to experience sleep-related problems compared to individuals without these deletions or mutations.

Sleep health and sufficient sleep are particularly important during adolescence when important physical, cognitive, emotional, and social changes occur. Although sleep is a primary aspect of adolescent development, insufficient sleep in adolescence is common, growing progressively worse over the course of adolescence. Among the pediatric population, adolescents carry the greatest sleep debt, physiologically needing about nine hours of sleep, while sleep averages around seven hours a night (Wolfson & Carskadon, 1998). Too little sleep can leave one feeling lethargic, sluggish, irritable, and moody, and can inhibit focus and concentration, slow response time, and decrease learning of cognitive tasks (Randazzo et al., 1998). Inadequate sleep in adolescence is

associated with higher levels of depressed mood, anxiety, alcohol use, suicidal behavior, and lower academic performance (Liu, 2004; Wolfson & Carskadon, 1998; Wolfson et al., 2003).

We were the first to report significantly reduced pineal volume, reduced melatonin secretion, and greater parent-report of sleep disturbance in individuals with *PAX6+/-* versus healthy controls (Hanish, 2012). Although *PAX6+/-* is rare and minimal research has focused on the role of *PAX6* in circadian regulation, irregular patterns of sleep-wake rhythm, as well as abnormalities in melatonin physiology, have also been studied in children and adolescents with a neurodevelopmental disorder (e.g. autism spectrum disorders) (Tordjman et al., 2005a). Sleep-related disturbances are a clinically important issue in children and adolescents with a neurodevelopmental disorder as sleep problems can exacerbate and worsen repetitive and stereotypic behavior, inattention, hyperactivity, as well as interfere with learning and cognition (Malow et al., 2006; Taylor et al., 2012; Wiggs & Stores, 1996). In addition to the impact of sleep disturbance on the quality of life of the child, sleep problems in children and adolescents with a neurodevelopmental disorder can also adversely affect family functioning (Jan et al., 2008; Wasdell et al., 2008a). Describing sleep-related phenotypes in adolescents with WAGR and isolated aniridia is necessary as sleep problems may go unrecognized and undertreated, which, like other conditions with abnormal melatonin physiology, may exacerbate existing conditions.

We examined daytime sleepiness, sleep disturbance, sleep-related impairment, and sleep patterns in adolescents with *PAX6+/-*. We hypothesized that *PAX6+/-* would be associated with greater daytime sleepiness, sleep disturbance, and sleep-related

impairment, as well as decreased sleep efficiency and increased sleep onset latency compared to a healthy comparison group.

Methods

Study Population

Subjects in the *PAX6+/-* group included adolescents with WAGR syndrome who had prior genetic testing showing 11p deletion (including *PAX6* deletion) and adolescents with isolated aniridia who had documented mutations or intragenic deletions affecting only *PAX6*. To be included in the study, participants met inclusion/exclusion criteria for WAGR/Aniridia Protocol 08-CH-0213, an active phenotype-genotype protocol at the National Institutes of Health (NIH) (Appendix A). To be included in the sleep portion of the study, adolescents with *PAX6+/-* had the ability to perceive light (lack of light perception could lead to free-running circadian rhythm), and were currently not taking any sleep aids including melatonin. Twelve adolescents with *PAX6+/-* were eligible for the study; however, three subjects were unable to stop melatonin for the purposes of this study and were not included in the sample. One subject was on melatonin prior to the study, and stopped melatonin from seven days prior to study initiation through study completion. Because WAGR syndrome is associated with cognitive impairment, only subjects with IQ>70 or, if IQ test results were not available, parent-reported functional capacity to complete study questionnaires were included in the study (IQ unavailable for two participants). Healthy comparison subjects included individuals who had no chronic medical conditions and were not taking any chronic medications including sleep aids. The study was an amendment to WAGR/Aniridia Protocol 08-CH-0213 and was approved by the institutional review board of the *Eunice Kennedy Shriver* National

Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD. Written informed consent was obtained from adults who were competent to provide consent and from the parents or legal guardians of children and adults with cognitive impairment. Assent was obtained from patients who were unable to give consent. All participants were compensated $50 for completion of the 7-day actigraphy recording and $10 for completion of study questionnaires. Healthy subjects were compensated an additional $30-$40 for their time, based on an hourly rate, as they came to Clinical Center at the National Institutes of Health (Bethesda, MD) to complete a physical exam.

Cleveland Adolescent Sleepiness Questionnaire

The Cleveland Adolescent Sleepiness Questionnaire (CASQ) is a sixteen-item instrument used to measure daytime sleepiness in adolescents 11-17 years of age. Adolescents rate the frequency of each item using a 5-point Likert scale: "never" (0 times per month), "rarely" (less than 3 times per month), "sometimes" (1–2 times per week), "often" (3–4 times per week) and "almost every day" (5 or more times per week), with higher scores indicating increased daytime sleepiness (Spilsbury, Drotar, Rosen, & Redline, 2007). The normative sample consisted of 411 participants who were recruited from churches, schools, and a community-based cohort study. Participants in the cohort study did not have evidence of sleep disordered breathing by overnight polysomnography. In addition, a sample of 62 adolescents with diagnosed sleep-disordered breathing comprised the sleep-disordered breathing subsample. The CASQ demonstrates good internal consistency (α=0.89) and correlations (r=0.66 to 0.75) between the CASQ and two other measures of daytime sleepiness (Pediatric Daytime

Sleepiness Scale and Sleep Habits School Survey) demonstrate evidence of construct validity in community samples (Spilsbury et al., 2007). The CASQ was completed as a self-report measure of daytime sleepiness in our sample of adolescents.

PROMIS Sleep Disturbance and Sleep Related Impairment

Sleep disturbance and sleep-related impairment were measured using Patient Reported Outcomes Measurement Information System (PROMIS) 8-item short forms (v. 1.0; 8a and 8b). PROMIS is an NIH-funded initiative with the mission of creating a state-of-the art assessment system for self-reported health in both children and adults. PROMIS provides clinicians and researchers access to efficient, precise, valid, reliable, non-disease specific, and standardized questionnaires, allowing for comparability across conditions and across the lifespan. PROMIS measures were established using literature searches of well-established existing measures, as well as expert content review, qualitative research with patients, and pilot testing (Cella et al., 2010; "PROMIS," 2014). Within PROMIS, many self-report measures are available in both pediatric (8-17 years) and adult populations (18+); however, currently no pediatric measures are available in the sleep disturbance or sleep-related impairment domains (Cella et al., 2010; "PROMIS," 2014).

PROMIS sleep disturbance and sleep-related impairment item banks were developed to improve self-report regarding the sleep-wake function in adults. PROMIS defines sleep as a reversible recurrent state of reduced awareness of and interaction with the environment, while wakefulness is an active engagement with the environment (Buysse et al., 2010). In this study, the 8-item PROMIS Sleep Disturbance questionnaire was used to measure self-reported perceptions of sleep quality, depth, and restoration.

This includes perceived difficulties getting to sleep, staying asleep, as well as sleep satisfaction (Cella et al., 2010; "PROMIS," 2014). The 8-item PROMIS Sleep Related Impairment questionnaire measured self-reported alertness, sleepiness, tiredness, and functional impairments associated with sleep problems during waking hours. Both measures used a 5-point Likert scale and the raw score was converted to a standardized T-score using conversion tables published on the PROMIS website (nihpromis.org) with higher scores indicating greater sleep/wake disturbances. In reference to one of the adult legacy measures, the Pittsburgh Sleep Quality Index (PSQI), the sleep disturbance bank was correlated at r=0.85 and the sleep-related impairments was correlated at r=0.70. Within PROMIS, many self-report measures are available in both pediatric (8-17 years) and adult populations (18+); however, currently no pediatric measures are available in the sleep disturbance or sleep-related impairment domains (Cella et al., 2010; "PROMIS," 2014). PROMIS sleep disturbance and sleep-related impairment questionnaires were administered to our sample of adolescents and preliminary studies of concurrent and construct validity were completed.

Actigraphy

The Actiwatch Spectrum device (Philips Respironics, Bend, OR) was used to provide an objective measure of sleep-wake patterns. The device was worn on the non-dominant wrist, in the home setting, for seven consecutive days and nights to provide an estimate of sleep based on movement. The actigraph was set to record in 30 second epochs at a medium sensitivity level for scoring sleep and wake time. Data from actigraphs were downloaded to a computer and total bedtime, total wake time, total sleep time (TST), sleep efficiency, and sleep onset latency were calculated using an automated

algorithm (Respironics Actiware 6, Philips Respironics, Bend, OR). A custom interval was generated to calculate the time from lights off to sleep onset, as an additional measure of sleep latency. In addition to software generated data for the seven day record, sleep variables were split into school day (Sunday-Thursday) versus weekend (Friday and Saturday). All data were collected during a typical school week. The illness and unexpected event days were deemed by the investigator to have caused minimal disruption to the adolescent's sleep pattern and were therefore included in the analysis. Six adolescents had unscorable actigraphy data on one out of the seven nights due to off-wrist detection sensors; all were weekdays, and all participants were included in analysis. In addition, patients recorded approximate time of sleep and wake during the seven days of data collection using a sleep/wake log (Philips Respironics, Bend, OR) to aid in analysis of actigraphy data.

Statistical Analyses

Statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armnok, NY). Skewness, kurtosis, and Kolmogorov–Smirnov test for normality were assessed. Parametric tests were used for normally distributed data, including questionnaires, while skewed data were assessed using nonparametric tests. Nonparametric tests were performed for actigraphy variables due to the small sample size. Independent samples *t*-tests, Mann-Whitney U tests, and Fisher's exact tests were used to compare unadjusted difference in demographic and clinical characteristics between adolescents with *PAX6*+/- and the healthy comparison group. Pearson correlations were calculated to assess the relationships among CASQ, PROMIS Sleep Disturbance, and PROMIS Sleep Related Impairment total scores. Using an estimated

correlation coefficient of 0.7, a sample size of twenty in the healthy comparison group will have ≥90% power to detect a correlation between questionnaires at a level of significance <0.01. Skewed data were normalized by log-transformation so that parametric analyses of covariance could be used to assess differences in actigraphy variables (adjusting for age, sex, race). A sample size of seventeen participants with *PAX6*+/- and seventeen participants in the healthy comparison group would have ≥ 80% power to detect a difference in sleep onset latency at a level of significance of <0.01. Wilcoxon tests compared differences in weekday versus weekend bedtime and wake time. P-values <0.05 were considered nominally significant.

Results

Patients included 9 adolescents with *PAX6*+/- (age 10-19y) and 25 healthy adolescents (age 10 - 18y). Subject characteristics are shown in Table 4.1. Within the *PAX6*+/- group there were no significant differences between adolescents with WAGR syndrome versus isolated aniridia for total sleep questionnaire scores, sleep onset latency, and sleep efficiency (all p's>0.15; data not shown). Due to these similarities, participants with WAGR/11p deletion syndrome and isolated aniridia were combined for all subsequent analyses.

Questionnaires

Total CASQ, PROMIS Sleep Related Impairment, and PROMIS Sleep Disturbance scores were not statistically different in adolescents with *PAX6*+/- versus the healthy comparison group (p's>0.15, Table 4.1). Although no cut off score for daytime sleepiness has been established for the CASQ, a previous study reported normative sample CASQ scores (mean ± standard deviation: 31.2 ± 9.4), as well as CASQ scores

for primary snorers (35.0 ± 12.3) and sleep apnea (37.7 ± 11.5) (Spilsbury et al., 2007). Total CASQ scores were nominally greater, indicating greater daytime sleepiness, in adolescents with *PAX6+/-* versus the healthy comparison group (comparable to reported normative sample), but this difference did not reach statistical significance. Both groups of adolescents with *PAX6+/-* and the healthy comparison group had average PROMIS Sleep Related Impairment and Sleep Disturbance T-scores (lower scores indicating less sleep disturbance and sleep-related impairment) less than reported normative values for adults (mean ± standard deviation: 50 ± 10) (nihpromis.org). CASQ score was significantly correlated with PROMIS Sleep Disturbance ($p<0.001$, Figure 4.1) and Sleep Related Impairment ($p<0.001$, Figure 4.1) total scores in adolescents in the healthy comparison group only. PROMIS Sleep Disturbance and Sleep Related Impairment scores were also significantly correlated ($p<0.001$, Figure 4.1) in adolescents in the healthy comparison group only.

Actigraphy

Total bedtime (average of week), weekday bedtime, weekend bedtime, total wake time (average of week), weekday wake time, weekend wake time, total sleep time (average of week), sleep onset latency, and sleep efficiency were not statistically different in adolescents with *PAX6+/-* versus the healthy comparison group ($p's>0.08$, Table 4.1). Although it has been reported that adolescents physiologically need about 9 hours of sleep (Wolfson & Carskadon, 1998), both groups had an average total sleep time of less than eight hours per night (Table 4.1). To our knowledge, standardized norms for sleep onset latency and sleep efficiency have not been established in adolescents; however, typical values for healthy adult populations are approximately twelve minutes for sleep

onset latency and approximately ninety-two percent for sleep efficiency (Natale, Plazzi, & Martoni, 2009). Both adolescents with *PAX6+/-* and the healthy comparison group had average sleep onset latency below that of the adult norms; however, sleep efficiency was similar to that of the adult norms (Table 4.1). Time from lights off to sleep onset was over 2-fold higher in *PAX6+/-* versus the healthy comparison group (p=0.07, Table 4.1). After controlling for age, sex, and race, time (minutes) from lights off to sleep onset was found to be significantly higher in adolescents with *PAX6+/-* versus the healthy comparison group (adjusted mean [95% CI]: 20.1 [8.1 - 49.8] vs. 6.2 [3.7 - 10.4], p=0.04, Figure 4.2).

Total bedtime (average of week) was positively correlated with CASQ (r=0.68, p<0.001), PROMIS Sleep Disturbance (r=0.63, p=0.001) and Sleep Related Impairment (r=0.66, p<0.001) total scores in adolescents in the comparison group only. In addition, total sleep time was negatively correlated with CASQ, PROMIS Sleep Disturbance, and Sleep Related Impairment total scores (p's<0.02, Figure 4.3) in adolescents in comparisongroup only. Weekday versus weekend bedtime, as well as weekday versus weekend wake time were significantly different in the healthy comparison group (p=0.02; p<0.001); however no differences were found in patients with *PAX6+/-* (p=0.26; p=0.14).

Discussion

In adolescents with *PAX6+/-* compared to the healthy comparison group, self-reported daytime sleepiness, sleep disturbance, and sleep-related impairment scores were similar. Adolescents in our comparison group had average self-reported daytime sleepiness scores comparable to the average CASQ scores of the control group used to develop the questionnaire (Spilsbury et al., 2007); however, to our knowledge, PROMIS

Sleep Disturbance and Sleep Related Impairment measures have not been reported in an adolescent sample. CASQ, PROMIS Sleep Disturbance, and Sleep Related Impairment scores were significantly correlated in adolescents in the comparisongroup only

Bedtime, wake time, total sleep time, sleep onset latency, and sleep efficiency were similar in *PAX6+/-* compared to the healthy comparison group; however time from lights off to sleep onset was higher in adolescents with *PAX6+/-* versus the healthy comparison group. Differences in weekday versus weekend bedtime and wake time were found in the healthy comparison group, but not in adolescents with *PAX6+/-*. The significant differences in weekday versus weekend bedtime and wake times found in the comparisongroup are consistent with the literature as adolescent sleep patterns on weekends typically show a considerable delay and lengthening versus the weekdays, with sleep onset and offset occurring later (Wolfson & Carskadon, 1998).

Although reduced pineal volume, melatonin secretion, and sleep disturbance in children have been previously reported in patients with *PAX6+/-* (Hanish, 2012), this study found no differences in self-reported daytime sleepiness, sleep disturbance, or sleep-related impairment in adolescents with *PAX6+/-* compared to the healthy comparison group. One possible explanation is the overall inadequate sleep reported in general adolescent populations. Adolescents require about nine to ten hours of sleep each night and over the past two decades, researchers, teachers, parents, and adolescents have consistently reported inadequate sleep in adolescents (Carskadon et al., 1997; "National Sleep Foundation," 2011b; Wolfson & Carskadon, 1998). Chronic sleep deprivation in adolescents stems from social, employment, recreational, academic, as well as biological changes (Wolfson & Carskadon, 2003). In our sample, both groups on average slept less

than 8 hours per night, and it is possible that overall sleep deprivation in adolescents has masked any differences between adolescents with *PAX6*+/- compared to the healthy comparison group. Another possible explanation is that the sample size for the *PAX6*+/- group was insufficient to identify differences on sleep deprivation.

Although we originally hypothesized that *PAX6*+/- would be associated with decreased sleep efficiency and increased sleep onset latency in comparison to healthy subjects, no significant differences were found. The actigraphy device and software used in this study measured sleep onset latency by calculating the time between rest and sleep intervals, which is based on thresholds of activity count. Although no differences were found using the activity-based method to measure sleep onset latency, we did find differences in time from lights off to sleep onset by setting custom intervals within the software program. This finding is also potentially clinically significant as the adjusted mean time from lights off to sleep was six minutes for the healthy comparison group, compared to twenty minutes in adolescents with *PAX6*+/-. Increased time from lights off to sleep may cause decreased total sleep time, as well as difficulty in establishing bed time routines. For example, parents of children/adolescents with *PAX6*+/- have reported that bedtime generates a great deal of anxiety for their child, causing the child to leave his/her room to seek parent support. In addition, increased time from lights off to sleep may pose safety risk in children/adolescents who need further adult supervision. Another study found similar total sleep time, but longer time awake in bed before falling asleep in children (8-13 years) with Asperger syndrome (n=16) compared to typically developing controls (n=15) (Allik, 2008). These similarly reported sleep patterns may be due to reduced melatonin concentration reported in patients with *PAX6*+/- (Hanish, 2012), as

well as in patients with Asperger syndrome (Melke et al., 2008). In addition, adolescents with *PAX6+/-* had more stable sleep as demonstrated by similar weekday versus weekend bedtime. One possible explanation for greater sleep stability is adolescents with *PAX6+/-* may have more parental involvement in bedtime routines due their parent's perception of sleep difficulties. For example, one parent of an adolescent with *PAX6+/-* wrote: "I think she is a perfect candidate for your research, since she seems to have evidence of the sleep issues you're studying."

One limitation of our study is that polysomnography was not completed; however, actigraphy has several advantages as it is minimally invasive, less expensive, and enables data collection in an adolescent's natural sleep environment. One concern of the investigators was the potential for lost or broken actigraphs due to the high expense of the equipment. In this study, all actigraphy equipment was properly returned to the investigator, and adolescents found the watch to be aesthetically acceptable. Another limitation to this study is the small sample of adolescents with *PAX6+/-*, however both WAGR syndrome and isolated aniridia are rare disorders, and this is the first study to examine sleep in an adolescent-only sample. Three subjects were unable to stop melatonin for the purposes of this study and one parent described that her child had a "panic attack" when it came time to withhold melatonin and "sleep has been such an issue for her, we just can't go back to how it was before." The small sample could have led to insufficient power to detect differences in sleep questionnaires scores, as well as lack of correlations between sleep measures. However, it is also possible that self-report of sleep may be problematic in adolescents with *PAX6+/-* given the lack of correlations found between objective actigraphy variables and subjective self-report questionnaires,

which were seen in comparisonsubjects. Self-report measures including neurocognitive functioning have been used in this population (Han et al., 2013), and although patients with isolated aniridia are of normal intelligence, patients with WAGR syndrome have varying degrees of cognitive impairments. To be included in the study participants with WAGR syndrome had an IQ >70 or parent-reported functional capacity to complete study questionnaires in an attempt to mitigate any self-report problems due to cognitive impairment, however it is unknown if completion of self-report measure was more challenging for these participants. Future research using self-report questionnaires in larger adolescent samples, with varying IQ's, would shed light into potential cognitive-related challenges in completion of self-report sleep questionnaires in adolescents.

Our preliminary validation study found significant correlations among all three sleep questionnaires (PROMIS Sleep Disturbance, PROMIS Sleep Related Impairment, and CASQ), as well as objective actigraphy variables (e.g. total sleep time and bedtime) in the healthy comparison group only. PROMIS provides clinicians and researchers access to efficient, precise, valid, reliable, non-disease specific, and standardized questionnaires, allowing for comparability across conditions and across the lifespan. Within PROMIS, many self-report measures are available in both pediatric and adult populations; however, currently no pediatric measures are available in the sleep disturbance or sleep-related impairment domains (Cella et al., 2010; "PROMIS," 2014). Our study suggests the potential research and clinical utility of adult versions of PROMIS sleep measures in an adolescent population.

Although *PAX6+/-* is rare and recruitment in this population may prove difficult, common genetic polymorphisms that affect the function of PAX6 may be associated with

insomnia in the general population (Ban et al., 2011). Additional studies of these genetic variants and their impact on pineal size, melatonin secretion, self-reported sleep problems, as well as sleep patterns could lead to genotype-specific treatment of insomnia such as using melatonin as a targeted therapy in PAX6-insufficient individuals.

Conclusion

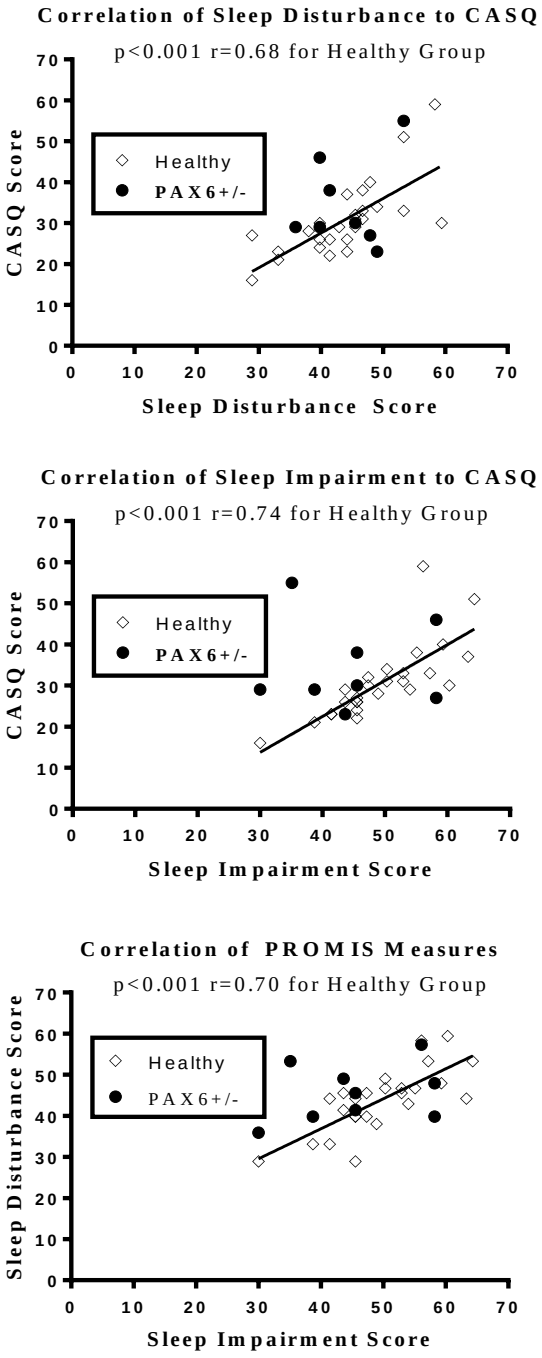
In conclusion, this is the first study to explore sleep disturbance, sleep-related impairment, and sleep patterns in adolescents with *PAX6+/-* versus the healthy comparison group. Our study found increased time from lights off to sleep, which may be a potentially useful outcome measure for sleep interventions (e.g. melatonin replacement) in PAX6-insufficient individuals, as well as other populations with reduced melatonin concentrations. In addition, future studies should explore additional psychometric properties of PROMIS sleep measures to provide age-appropriate, validated, and reliable measures of sleep in adolescents.

Table 4.1. Characteristics of Adolescents with *PAX6*+/- and Healthy Comparison Group

	***PAX6*+/-** **(n=9)**	**Healthy Comparison Group (n=25)**	**P-Values**
Age (y)	15.5 ± 3.3 (10–19)	13.6 ± 2.3 (10-18)	0.07
Sex (% Female)	66.7	44.0	0.44
Race/Ethnicity (%)			
Non-Hispanic Caucasian	88.9	80.0	1.00
Other	11.1	20.0	
CASQ Total Score	34.6 ± 10.9	31.3 ± 8.7	0.39
PROMIS Sleep Disturbance	45.5 ± 7.0	44.3 ± 7.2	0.67
PROMIS Sleep Related Impairment	45.7 ± 10.2	50.2 ± 7.1	0.15
Total Bedtime	22:19 [21:34 – 23:41]	22:46 [22:25 – 23:48]	0.11
Weekday Bedtime	22:08 [21:23 – 23:33]	22:18 [21:56 – 23:32]	0.25
Weekend Bedtime	22:40 [21:38 – 01:33]	23:26 [23:04 – 00:14]	0.14
Total Get up Time	7:09 [6:43 – 7:30]	7:19 [7:00 – 8:00]	0.11
Weekday Get up Time	6:36 [6:19 – 7:10]	7:05 [6:43- 7:51]	0.08
Weekend Get up Time	7:24 [6:44 – 10:01]	8:16 [7:51 – 8:43]	0.25
Total Sleep Time	7:42 [6:30 – 8:51]	7:55 [7:19 – 8:15]	0.86
Sleep Onset Latency (minutes)	1.8 [1.2 – 3.9]	2.4 [1.3 – 3.4]	0.77
Lights off to Sleep (minutes)	17.0 [6.1 – 74.4]	7.9 [2.5 – 13.5]	0.07
Sleep Efficiency (%)	91.2 [89.8 – 92.2]	91.1 [89.8 - 92.5]	0.74

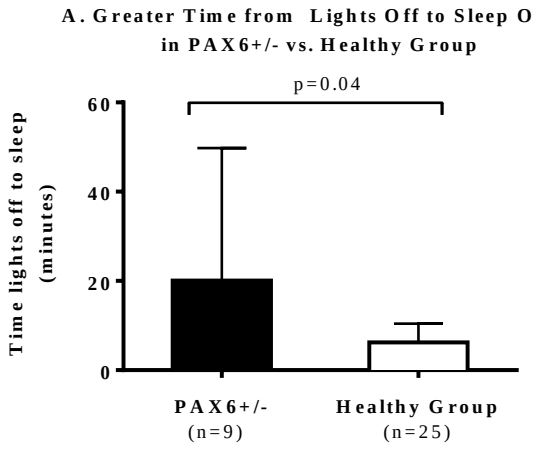
Normally distributed variables are shown as mean ± SD and were analyzed using independent samples t-test. Nonparametric data are shown as median [25^{th} - 75^{th} percentile] and were analyzed using Mann-Whitney U test. Percentages were compared using Fisher's exact test. Abbreviations: CASQ=Cleveland Adolescent Sleepiness Questionnaire; PROMIS: Patient Reported Outcome Measurement Information System.

Figure 4.1. Sleep Questionnaire Correlations in Adolescents with *PAX6* Haploinsufficiency (*PAX6*+/-) and Healthy Comparison Group



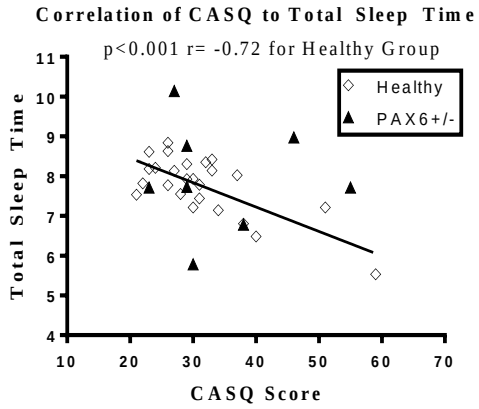
Pearson correlations were calculated to assess the relationships among CASQ, PROMIS Sleep Disturbance, and PROMIS Sleep Related Impairment total scores. Nominal p-values for healthy comparison group are shown.

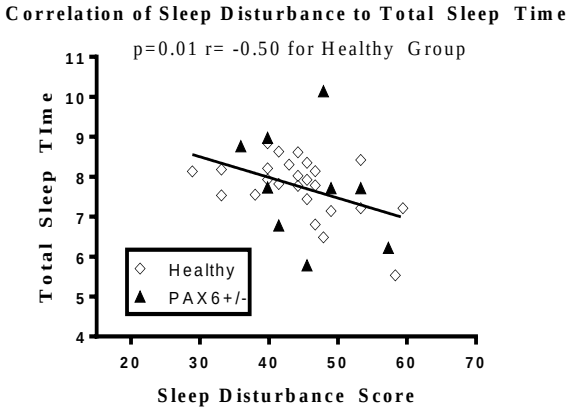
Figure 4.2. Greater Time from Lights Off to Sleep in Patients with *PAX6* Haploinsufficiency (*PAX6+/-)* as Compared to Healthy Comparison Group

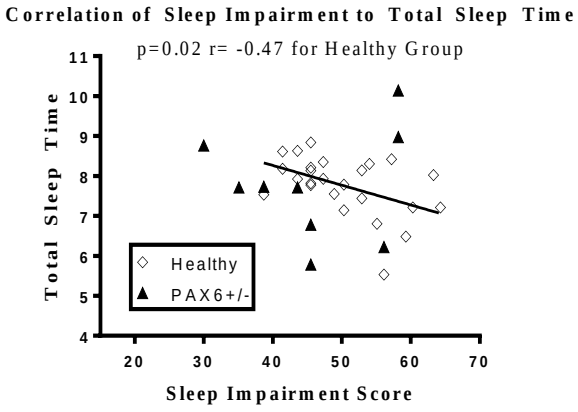


Greater time from lights off to sleep onset in patients with PAX6 haploinsufficiency (*PAX6+/-)* as compared to healthy comparison group. (A) Lights off to sleep onset was measured by actigraphy using Actiwatch software. Covariates were age, sex, and race. Skewed data were normalized by log transformation and backtransformed adjusted means with ± 95% confidence intervals are shown. Nominal p-values are shown.

Figure 4.3. Sleep Questionnaire and Total Sleep Time Correlations in Adolescents with *PAX6* Haploinsufficiency (*PAX6+/-*) and Healthy Comparison Group







Pearson correlations were calculated to assess the relationships among CASQ, PROMIS Sleep Disturbance, and PROMIS Sleep Related Impairment total scores and total sleep time (weekly average). Nominal p-values for healthy comparison group are shown.

CHAPTER 5: CONCLUSIONS

Introduction

Approximately one-third of the general adult population, and comparable children and adolescent percentages, are affected by decrements in sleep health that contribute to disability, morbidity, and mortality ("National Institutes of Health Sleep Disorders Research Plan," 2011). Given the potential role of PAX6 in pineal development and circadian regulation, individuals with *PAX6* haploinsufficiency may be more likely to experience sleep-related problems compared to individuals without these deletions or mutations. Haploinsufficiency of *PAX6* can result from WAGR syndrome, a contiguous gene deletion syndrome in which multiple genes are involved, or point mutations and microdeletions affecting only *PAX6*, which result in isolated aniridia. Although *PAX6* haploinsufficiency is rare, and minimal research has focused on the role of *PAX6* in circadian regulation, irregular patterns of sleep-wake rhythm have been studied in children and adolescents with neurodevelopmental disorders, another population with possible abnormalities in melatonin physiology. An integrative review was conducted to synthesize the literature regarding the sleep-related measures currently being used to assess sleep disturbance in adolescents with a neurodevelopmental disorder. In addition, our research was the first to report that both pineal volume and melatonin production are significantly lower in humans with *PAX6* haploinsufficiency, and parent-report of children (2-12 years) with *PAX6* haploinsufficiency suggests greater sleep problems in comparison to healthy subjects (Hanish, 2012). Our research also further characterizes the sleep-related-phenotype associated with an abnormality in the *PAX6* gene by using self-report questionnaires and actigraphy in adolescents with *PAX6* haploinsufficiency.

Preliminary validation studies of age-appropriate tools to measure sleep were completed in a healthy adolescent comparison group.

The concluding chapter includes a review of the major findings from each paper, a discussion of combined findings from the three papers, as well as the applicability of the study framework, limitations, and implications for clinical practice and research.

Paper 1

The purpose of the integrative review was to synthesize the literature regarding the sleep-related measures used to assess sleep disturbance in adolescents with a neurodevelopmental disorder. The integrative review included 66 articles published from July 2004 through July 2014, and research from 21 countries provided an international perspective. Parent-report questionnaires were the most common sleep-related measure utilized, which may be problematic as parent-report of children's sleep may underestimate sleep problems. As children get older, parents may be unaware of increased sleep onset delay experienced by their children, as well as frequency and duration of night wakings that may also affect total sleep time (Baker et al., 2013; Goldman et al., 2009; Owens, Spirito, McGuinn, et al., 2000). One of the major problems identified through this review was the abundance and lack of specificity of reported sleep measures. For example, fifteen different sleep questionnaires were reported in this review. In addition, the majority of studies used unspecified or author-made questionnaires, and the validity and reliability of these measures were not addressed. Although many pediatric sleep questionnaires have been developed and the psychometrics have been evaluated (Spruyt & Gozal, 2011), there is no standard sleep-related questionnaire used to measure sleep in adolescents, as well as in adolescents with

neurodevelopmental disorders. Lack of clarity and consistency in utilization of sleep-related measures may likely contribute to ambiguity in how sleep problems are being defined, assessed, and reported. In addition, none of the studies included in this review used an adolescent-only sample, and a major limitation in the current literature is that older children and adolescents are combined with much younger children and infants in the same sample, despite known age-related changes in sleep patterns.

Regardless of age, children and adolescents with neurodevelopmental disorders appear to be at an increased risk of sleep-related disturbances (Couturier et al., 2005; Engelhardt et al., 2013; Krakowiak et al., 2008), which can exacerbate and worsen underlying conditions (Malow et al., 2006; Taylor et al., 2012; Wiggs & Stores, 1996). Among the pediatric population, adolescents carry the strongest sleep debt (Wolfson & Carskadon, 1998), making them particularly vulnerable to sleep-related problems. Examination of sleep in adolescents with neurodevelopmental disorders requires measures that are age-appropriate, valid, and reliable for this population, which is important in order to assess risk for sleep problems and for the prevision of appropriate prevention and interventional strategies. Future research should focus on the development of subjective self-reported sleep measures that can be used in adolescents with a neurodevelopmental disorder, as well as used in combination with more objective measures of sleep such as actigraphy. Standardization of the sleep-related measures used in adolescent sleep research would allow for comparability across studies and across conditions shedding light into potential recommendations on the use of sleep-related measures in diagnosis specific, as well as healthy adolescent populations.

Paper 2

PAX6 appears to play an important role in the development of the pineal, the primary source of the circadian regulating hormone, melatonin. Although pineal hypoplasia and absence have been previously reported in patients with various *PAX6* mutations (Abouzeid et al., 2009; Mitchell et al., 2003), this study is the first to describe pineal volume, melatonin secretion, and sleep disturbance in this population. Thirty-seven patients with *PAX6+/-* (age 15.3 ± 9.9 years) and seventeen healthy controls (16.0 ± 7.2 years) were evaluated during an inpatient admission to the National Institutes of Health (Bethesda, Maryland). Pineal volume was evaluated by MRI, and diurnal serum cortisol, serum melatonin, and urine 6-sulfatoxymelatonin (6SM) concentrations were measured by enzyme-linked immunosorbent assay. The Child Sleep Habits Questionnaire (CSHQ) was administered for patients less than 13 years of age. In patients with *PAX6+/-* compared to controls, pineal volume, midnight serum melatonin, morning serum melatonin, and morning urinary 6SM were lower, while evening urinary 6SM and cortisol diurnal patterns remained similar. Total CSHQ scores showed greater parental report of sleep disturbances in children with *PAX6+/-*.

This is the first study to explore the functional consequences of pineal hypoplasia in patients with *PAX6+/-*, and our findings support the hypothesis that *PAX6* plays an important role in human pineal development and sleep regulation. In healthy humans, melatonin concentration starts to rise in the evening, reaching maximum levels in the middle of the night, and then decreasing before habitual wake up time (Arendt & Skene, 2005). Although we originally hypothesized that serum melatonin concentrations and urinary melatonin metabolite levels would be lower in the morning and evening in

patients with *PAX6+/-* as compared to controls, we observed that evening urinary 6SM (reflection of daytime melatonin concentration) was similar after adjusting for multiple comparisons. This similarity may be due to release into circulation of small amounts of extrapineal sources of melatonin (e.g. gastrointestinal tract) (Acuna-Castroviejo et al., 2014), and although the similarity was unexpected, this measurement was a reflection of daytime melatonin concentration when we already anticipate low levels.

Melatonin is important for circadian regulation including sleep initiation and maintenance (Arendt & Skene, 2005). Individuals with *PAX6+/-* appear vulnerable to adverse sleep-related phenotypes due to decreased pineal volume and subsequent melatonin deficiency. It is important to note that participants in this study were not currently taking exogenous melatonin, which is readily available as an over-the-counter supplement in the United States, and it is unclear as to the prevalence of melatonin usage and usefulness in this population. In this study, sleep disturbance was only assessed in children using parent-report and no objective sleep measures were analyzed between groups. Although this study found greater parental report of sleep disturbances in children with *PAX6+/-*, further studies are needed to assess self-reported sleep disturbance and sleep-related impairment. In addition, objective measures such as actigraphy monitoring in a home-based environment would be useful to examine sleep patterns including sleep onset latency and sleep efficiency, as well as provide insight into clinical assessment and sleep-related interventions in PAX6-insufficient individuals.

Paper 3

Our previous study was the first to report significantly reduced pineal volume, reduced melatonin secretion, and greater parent-report of sleep disturbance in individuals

with *PAX6+/-* versus healthy comparison subjects. However, adolescent self-report of sleep and sleep patterns have not been described. This study examined self-reported daytime sleepiness, sleep disturbance, sleep-related impairment, and sleep patterns in adolescents with *PAX6+/-*. The sample included 9 adolescents with *PAX6+/-* (age 15.5 ± 3.3 years) and 25 adolescents in the healthy comparison group (13.6 ± 2.3 years). The Cleveland Adolescent Sleepiness Questionnaire (CASQ) and PROMIS Sleep Disturbance and Sleep Related Impairment questionnaires were administered to adolescents, and sleep patterns were assessed using actigraphy. Our results showed that total scores on all sleep questionnaires were similar in adolescents with *PAX6+/-* vs. the healthy comparison group. Adolescents in our healthy comparison group had average self-reported daytime sleepiness scores comparable to the average CASQ scores of the control group used to develop the questionnaire (Spilsbury et al., 2007); however, to our knowledge, PROMIS sleep measures have not been reported in an adolescent sample, limiting our ability to compare our findings with those of a normative adolescent sample. Using actigraphy, total bedtime (average of week), weekday bedtime, weekend bedtime, total wake time (average of week), weekday wake time, weekend wake time, sleep onset latency, and sleep efficiency were also similar in adolescents with *PAX6+/-* versus the healthy comparison group. Although no differences were found using the activity-based method to measure sleep onset latency, we did find differences in time from lights off to sleep onset. This finding is potentially clinically significant as the adjusted mean time from lights off to sleep was approximately 6 minutes for the healthy comparison group, compared to 20 minutes in adolescents with *PAX6+/-*. Increased time from lights off to sleep may cause decreased total sleep time, as well as difficulty in establishing bed time

routines. For example, parents of children/adolescents with *PAX6+/-* have reported that bedtime caused a great deal of anxiety to their child, causing the child to leave his/her room to seek parent support. In addition, increased time from lights off to sleep may also pose safety risks in children/adolescents who need further adult supervision.

Although we originally hypothesized that *PAX6+/-* would be associated with greater daytime sleepiness, sleep disturbance, and sleep-related impairment, as well as decreased sleep efficiency and increased sleep onset latency in comparison to healthy subjects, no significant differences were found. One possible explanation is that the small sample of adolescents with *PAX6+/-* could have led to insufficient power to detect differences in sleep questionnaires scores, as well as lack of correlations between sleep measures. Another possible explanation is the overall sleep insufficiency reported in general adolescent populations. Adolescents require about nine to ten hours of sleep each night and over the past two decades, researchers, teachers, parents, and adolescents have consistently reported inadequate sleep in adolescents (Carskadon et al., 1997; "National Sleep Foundation," 2011b; Wolfson & Carskadon, 1998). In our sample, both groups, on average, slept less than 8 hours per night, and it is possible that overall sleep deprivation in adolescents has masked any differences between adolescents with *PAX6+/-* compared to the healthy comparison group; however, it is also possible that self-report of sleep may be problematic in adolescents with *PAX6+/-* given the lack of correlations found in objective actigraphy variables to subjective self-report questionnaires, which were seen in the healthy comparison group. Self-report measures, including neurocognitive functioning, have been used in this population (Han et al., 2013), and although patients with isolated aniridia are of normal intelligence, patients with WAGR syndrome have

varying degrees of cognitive impairments. To be included in this study participants with WAGR syndrome had an IQ >70 or parent-reported functional capacity to complete study questionnaires in an attempt to mitigate any self-report problems due to cognitive impairment. However, it is unknown if completion of self-report measure was more challenging for these participants, and future research using self-report questionnaires in larger adolescent samples, with varying IQ's, would shed light into potential cognitive-related challenges in completion of self-report sleep questionnaires in adolescents.

Although *PAX6+/-* is rare, common genetic polymorphisms that affect the function of PAX6 may be associated with insomnia in the general population (Ban et al., 2011). Additional studies of these genetic variants and their impact on pineal size, melatonin secretion, self-reported sleep problems, as well as sleep patterns could lead to genotype-specific treatment of insomnia such as using melatonin as a targeted therapy in PAX6-insufficient individuals. Our study found increased time from lights off to sleep, which may be a potentially useful outcome measure for sleep interventions (e.g. melatonin replacement) in PAX6-insufficient individuals, as well as other populations with reduced melatonin concentrations.

In addition to characterizing the sleep-related phenotype associated with an abnormality in the *PAX6* gene in adolescents, our study performed preliminary validation studies on PROMIS sleep measures in adolescents in the healthy comparison group. In the healthy comparison group, total scores on CASQ and both PROMIS measures were correlated ($r's>0.7$, $p's<0.001$). In addition, total bed time (average weekly bedtime) was positively correlated with CASQ, PROMIS Sleep Disturbance, and Sleep Related Impairment ($r's>0.63$, $p's<0.001$), and total sleep time was negatively correlated with

CASQ, PROMIS Sleep Disturbance, and Sleep Related Impairment (r's<-0.47, p's<0.02) in the healthy comparison group. Future studies should explore additional psychometric properties of PROMIS sleep measures to provide age-appropriate, validated, and reliable measures of sleep in adolescents.

Combined Findings

According to the 2011 *NINR Strategic Plan: Bringing Science to Life*, nurse scientists are well suited for patient-centered biobehavioral research with a primary focus on health promotion, symptom management, quality of care, and quality of life. A better understanding of symptoms and symptom clusters will improve clinical management of illness including the management of pervasive symptoms such impaired sleep, which crosses multiple conditions across the lifespan and impairs quality of life ("NINR Strategic Plan: Bringing Science to Life," 2012). All three studies have utilized a biobehavioral research paradigm, linking genetics, biology, and behavior.

Paper 2 focused on the genetic and biological portion of the biobehavioral research paradigm and provided new direct evidence for an important role of *PAX6* in human pineal development and melatonin secretion. In 2011, the National Human Genome Institute (NHGRI) published *Charting a Course for Genomic Medicine from Base Pairs to Bedside*, a vision for the future of genomics research. The vision for genomics is organized around five domains that span from basic research to clinical application. This vision reflects the view that the most effective way to improve human health is to understand normal biology as a basis for understanding disease biology, which then becomes the basis for improving health. Using genomics is essential to understanding both normal and disease-related function of the genetic contributors to

disease as well as the cellular and biological process in which they are involved to develop strategies for diagnosis, prevention, and therapeutic intervention (Green & Guyer, 2011). Given the role of the *PAX6* gene in pineal development and circadian regulation, individuals with *PAX6* haploinsufficiency may be more likely to experience sleep-related problems compared to individuals without these deletions or mutations. In addition, common genetic polymorphisms that affect the function of PAX6 may be associated with insomnia in the general population (Ban et al., 2011). Paper 2 discussed the need to further examine self-reported sleep disturbance and sleep patterns in adolescents with *PAX6* haploinsufficiency, with the goal of developing strategies for diagnosis, prevention, and therapeutic intervention for sleep problems in this population. Additionally, studies of these genetic variants of PAX6 in broader populations, and their impact on pineal size, melatonin secretion, self-reported sleep problems, as well as sleep patterns could lead to genotype-specific treatment of insomnia such as using melatonin as a targeted therapy in PAX6-insufficient individuals.

Papers 1 and 3 focused on the behavioral portion of the biobehavioral research paradigm. Sleep is a readily accepted clinical construct, however it is a complex phenomenon that is difficult to define and measure. Paper 1 synthesized the literature regarding the sleep-related measures used to assess sleep disturbance in adolescents with a neurodevelopmental disorder, thus providing a foundation and knowledge in sleep-related measures used in another population with possible alterations in melatonin physiology. This review highlighted the importance of further development of age-appropriate, standardized, valid, and reliable sleep measures that can be utilized in adolescents with neurodevelopmental disorders in order to assess risk for sleep problems

and for the prevision of appropriate prevention and interventional strategies. Currently, there are no standard sleep questionnaires utilized in adolescents, as well as in adolescents with neurodevelopmental disorders, and this variation may likely contribute to ambiguity in how sleep problems are being defined, assessed, and reported. The American Academy of Pediatrics has created an adolescent sleep health working group that has largely advocated for later school start times in middle schools and high schools allowing students the opportunity to achieve optimal levels of sleep ("School start times for adolescents," 2014). Although recommendations for sleep-related measures have not been published, validation and implementation of standardized sleep-related measures in adolescents would allow for comparability across school districts, strengthening evidence-based rationales regarding the potential benefits of delayed school start times.

Paper 3 described sleep behavior, using sleep questionnaires and actigraphy, in adolescents with *PAX6* haploinsufficiency and a healthy comparison group. Future studies should explore additional psychometric properties of PROMIS sleep measures to provide age-appropriate, valid, and reliable measures of sleep in adolescents. In addition, the study found significantly greater time from lights off to sleep in adolescents with *PAX6* haploinsufficiency versus the healthy comparison group which is a potentially useful outcome measure for sleep interventions (e.g. melatonin replacement) in PAX6-insufficient individuals.

In combination, all three studies include a portion of the biobehavioral research paradigm with the overall goal of linking genetics, biology, and behavior to improve sleep health in adolescents. Together, these studies address various elements of the current state-of-the science and future directions regarding sleep-related measurement in

adolescents. In particular, Paper 1 addressed the lack of validated self-report sleep measures in adolescents with neurodevelopmental disorders. There is an overall need for better self-report sleep measures in adolescents, as well as a need to understand if these measures are appropriate for adolescents with a neurodevelopmental disorder. If general adolescent sleep measures are not appropriate for patients with a neurodevelopmental disorder, potentially due to the spectrum of cognitive-related issues, research is needed to find an alternative way of assessing sleep in this population due to drawbacks of parental report of child sleep. Paper 2 outlines physiological vulnerabilities (e.g. reduced pineal volume and reduced melatonin concentration) to sleep problems in patients with *PAX6* haploinsufficiency, and Paper 3 further characterized the sleep-related phenotype in adolescents with *PAX6* haploinsufficiency. In addition, preliminary validation studies on PROMIS sleep measures in adolescents in the healthy comparison group demonstrated potential clinical and research utility of these measures; however, further research is needed to examine additional psychometric properties of PROMIS sleep measures, including applicability to adolescents with a neurodevelopmental disorder in larger sample sizes, to provide age-appropriate, validated, and reliable measures of sleep in adolescents.

Study Framework

The Biobehavioral Model of Altered Dysregulation in Circadian Systems provided an overarching model in which to study sleep in the context of several physiologic systems in humans. This model focuses on the interrelationships between the endocrine system, autonomic nervous system, and sleep system. According to the model, dysregulation in circadian systems can be measured by melatonin, cortisol, sympathetic

nervous system activity, catecholamines, sleep duration, and sleep quality. The outcomes of dysregulation include: poor quality of life, poor treatment adherence, fatigue, disease progression, and poorer survival (Carlson et al., 2007; Otte & Carpenter, 2009). Using this model, this study aimed to examine both dysregulation in circadian systems and outcomes of dysregulation in adolescents with *PAX6* haploinsufficiency. Paper 1 synthesized the sleep-related instruments currently reported in the literature to measure dysregulation in circadian systems in adolescents with a neurodevelopmental disorder. Paper 2 focused on the dysregulation in circadian systems by measuring melatonin, cortisol, as well as parent-report of sleep quality in patients with *PAX6* haploinsufficiency in comparison to controls. In addition, Paper 3 used sleep questionnaires and actigraphy to measure dysregulation in circadian systems in terms of sleep duration and sleep quality in adolescents with *PAX6* haploinsufficiency in comparison to the healthy adolescent group. According to the model, one outcome of circadian dysregulation is poor quality of life. Paper 3 examines quality of life using the sleep-related impairment questionnaire, which measures self-reported alertness, sleepiness, tiredness, and functional impairments associated with sleep problems during waking hours. The common goal of these three papers is to prevent adverse outcomes of dysregulation by developing strategies for diagnosis, prevention, and therapeutic intervention for sleep problems to improve overall health in adolescents.

Although this model was useful in terms of overall direction for research design and assessment tools, one major limitation of this model is that genetics is not included. Over the past decade, the genetic analysis of sleep has emerged as an important discipline (Crocker & Sehgal, 2010) and inclusion of genetics in this model would broaden the

models timeliness and applicability. In addition, this model would benefit from further development of sleep definitions (e.g. the model does not define sleep duration and sleep quality).

Limitations

This is the first study to describe sleep-related phenotypes, including sleep disturbance, sleep-related impairment, and sleep patterns in adolescents with *PAX6* haploinsufficiency. Describing sleep-related phenotypes in adolescents with *PAX6* haploinsufficiency is necessary as sleep problems may go unrecognized and undertreated, which, like other conditions with abnormal melatonin physiology, may exacerbate existing conditions. The foremost limitation to this study is the small sample size of adolescents with *PAX6* haploinsufficiency. Both WAGR syndrome (1 case per 500,000 to 1,000,000) and isolated aniridia (1 case per 100,000) are rare disorders, and although an adolescent-only sample can also be seen as a study strength, using an adolescent-only sample restricted an already rare population, and was an obstacle in our ability to recruit participants. The small sample of adolescents with *PAX6* haploinsufficiency could have led to insufficient power to detect differences in sleep questionnaires scores, as well as lack of correlations between sleep measures in adolescents with *PAX6* haploinsufficiency in comparison to the healthy adolescent group. One of the reasons for the small sample was issues regarding stopping the use of melatonin, which excluded three subjects from the study. For example, one parent described that her child had a “panic attack” when it came time to withhold melatonin and “sleep has been such an issue for he, we just can’t go back to how it was before.” Despite the small sample, we did find greater time from lights off to sleep in adolescents with *PAX6* haploinsufficiency in comparison to the

health adolescent group. All adolescents in this study completed self-report measures, adhered to study procedures, returned actigraphy equipment promptly, and in good condition, suggesting the potential feasibility of enrollment of adolescents in sleep-related studies.

The small sample also did not allow us to complete subgroup analyses to compare participants with WAGR syndrome to participants with isolated aniridia. This is important because WAGR syndrome is a contiguous gene deletion syndrome in which multiple genes are involved, while isolated aniridia involves point mutations and microdeletions affecting only *PAX6*. The majority of mutations reported in patients with isolated aniridia are nonsense mutations, frameshift mutations, or splicing errors predicted to cause premature truncation of the PAX6 protein (Kokotas & Petersen, 2010), and different mutations may result in variable phenotypic presentation. We were unable to determine if deletions vs. mutations in *PAX6* lead to any differences in sleep-related phenotypes. It has been reported that common genetic polymorphisms that affect the function of PAX6 may be associated with insomnia in the general population (Ban et al., 2011), and furthering the genotype to phenotype characterization would advance the knowledge of the role of PAX6 in sleep health.

Although our previous study was the first to report significantly reduced pineal volume, reduced melatonin secretion, and greater parent-report of sleep disturbance in individuals with *PAX6* haploinsufficiency versus controls, these measures were not collected at the time of the current study. Although a portion of our current sample also completed the previous pineal and melatonin study, these data were collected several years prior to participation in the current sleep questionnaire and actigraphy study. This

limited the type and scope of analyses that could be performed. For example, we were unable to determine if pineal volume and melatonin secretion were correlated with self-reported daytime sleepiness, sleep disturbance, or sleep-related impairment in adolescents. In addition, for this study, we did not collect parent-reported sleep questionnaires so we could not directly compare parent-report to self-report sleep measures completed by adolescents with *PAX6* haploinsufficiency and the healthy comparison group. If we would have collected parent-report data, it would have allowed us to examine parental perception of child sleep. This would have been potentially useful data as greater sleep stability was reported in adolescents with *PAX6* haploinsufficiency, and it was suggested that this could have been due to parent's perception of sleep problems leading to a more active role in bedtime routines.

To be included in the study, participants with *PAX6* haploinsufficiency were currently not taking any sleep aids including melatonin. Participants with *PAX6* haploinsufficiency were excluded from the study if unable to stop melatonin one week prior to and during the one week of actigraphy data collection. One participant was on melatonin prior to the study and was able to stop melatonin to meet study requirements; however, two subjects were unable to enroll in the study due to inability to stop melatonin. The remaining sample reported that they were not on any sleep aids for the past six months. It is not clear how many participants chose not to contact the investigator to participate due to inability to stop sleep aids. It is possible that the adolescents with *PAX6* haploinsufficiency who completed this study, and were able to stop melatonin, had less sleep-related problems than those who were unable to stop melatonin, possibly leading to sample bias. It remains unclear as to why some adolescents were on melatonin

and others were not; however, melatonin is available as an over-the-counter supplement in the United States, while melatonin does require a prescription in other countries. Only one participant in this study would have been required to get a melatonin prescription, and this participant was not currently taking melatonin prior to study enrollment.

Implications for Clinical Practice

Sleep health and sufficient sleep are particularly important during adolescence, when important physical, cognitive, emotional, and social changes occur. Although sleep is a primary aspect of adolescent development, insufficient sleep is common in adolescence and is associated with higher levels of depressed mood, anxiety, alcohol use, suicidal behavior, and lower academic performance (Liu, 2004; Wolfson & Carskadon, 1998; Wolfson et al., 2003). Despite its significance and frequency, sleep disturbance is an area that is largely unaddressed in the nursing literature (Vallido, Peters, O'Brien, & Jackson, 2009). A few studies have advocated for the need for formal nursing education regarding adolescent sleep issues (Vallido, Jackson, & O'Brien, 2010), as well as the need for assessment of sleep to become a routine part of adolescent health care including education to adolescents and their families regarding the importance of adequate sleep (George & Davis, 2013). In 2011, the first nursing textbook on sleep and sleep disorders was published presenting the latest scientific evidence on health promotion, prevention, and treatment of sleep and sleep disorders across the lifespan (Redecker, 2011). Nursing has a role to play in assisting adolescents and their families to recognize the importance of sleep to general health and well-being (Vallido et al., 2009), and nurses working with adolescents in the clinical or school-setting are uniquely poised to screen adolescents for sleep insufficiency.

Although this is the first study to report on the sleep-related phenotype in adolescents with *PAX6* haploinsufficiency, irregular patterns of sleep-wake rhythm have also been reported in children and adolescents with a neurodevelopmental disorder. Sleep-related disturbances are a clinically important issue in children and adolescents with a neurodevelopmental disorder as sleep problems can exacerbate and worsen repetitive and stereotypic behavior, inattention, hyperactivity, as well as interfere with learning and cognition (Malow et al., 2006; Taylor et al., 2012; Wiggs & Stores, 1996). In addition to the impact of sleep disturbance on the quality of life of the child, sleep problems in children and adolescents with a neurodevelopmental disorder can also adversely affect family functioning (Jan et al., 2008; Wasdell et al., 2008a). Describing sleep-related phenotypes in adolescents with WAGR and isolated aniridia is necessary as sleep problems may go unrecognized and undertreated, which, like other conditions with abnormal melatonin physiology, may exacerbate existing conditions. Our studies have found greater parental report of sleep disturbances in children with *PAX6+/-* and one parent even wrote, "I think she [her child] is a perfect candidate for your research, she seems to have evidence of the sleep issues you are studying".

In our study, total sleep time was measured using actigraphy, and insufficient sleep was found in adolescents with WAGR and isolated aniridia, as well as adolescents in our healthy comparison group. The median total sleep time in both groups was less than eight hours per night, which is below the recommended nine to ten hours per night. Although no differences were found using the activity-based method to measure sleep onset latency, lights off to sleep onset was found to be significantly higher in adolescents with *PAX6+/-* versus the healthy comparison group. This finding is potentially clinically

significant as the adjusted mean time from lights off to sleep was approximately 6 minutes for the healthy comparison group, compared to 20 minutes in adolescents with *PAX6*+/-, and additional evidence is needed to support a clinical guideline for sleep management in adolescents with *PAX6*+/-. Due to our genetic, biological, and behavioral research demonstrating potential circadian dysfunction in patients with WAGR or isolated aniridia, clinical assessment of sleep should be considered in this population, and referral to a sleep specialist may be necessary. In addition, further research is needed to determine if melatonin replacement would be clinically beneficial in patients with WAGR or isolated aniridia, and in particular if melatonin replacement decreases the time from lights off to sleep. Although WAGR syndrome and isolated aniridia are rare disorders, describing the sleep-related phenotypes in this population will advance the knowledge of assessment and treatment of sleep disorders in general, facilitating their use in other populations such as genetic variants of *PAX6* in the general population, as well as other populations with abnormal melatonin physiology such as adolescents with neurodevelopmental disorders.

Implications for Research

Sleep and circadian disorders have been recognized by Congress and the Department of Health and Human Services as high priority targets for basic and clinical scientific investigation ("National Institutes of Health Sleep Disorders Research Plan," 2011), and sleep health has been added to the agenda for Healthy People 2020 with one of the objectives being to increase the proportion of adolescents who get a sufficient amount of sleep. In addition, organizations such as the National Sleep Foundation have advocated for interdisciplinary research to further examine sleep's role in adolescent

development, health and behavior ("Adolescent Sleep Needs and Patterns: Research Report and Resource Guide," 2011).

In an integrative review of the sleep literature in adolescents with a neurodevelopmental disorder, none of the studies included in this review used an adolescent-only sample, and an abundance of sleep questionnaires were reported. The variation in sample age, as well as questionnaires may likely contribute to ambiguity in how sleep problems are being defined, assessed, and reported. Future research should focus on the development of subjective self-reported sleep measures that can be used in adolescents with a neurodevelopmental disorder, as well as used in combination with more objective measures of sleep such as actigraphy.

Although *PAX6+/-* is rare and recruitment in this population may prove difficult, common genetic polymorphisms that affect the function of PAX6 may be associated with insomnia in the general population (Ban et al., 2011). Additional studies of these genetic variants and their impact on pineal size, melatonin secretion, self-reported sleep problems, as well as sleep patterns could lead to genotype-specific treatment of insomnia such as using melatonin as a targeted therapy in PAX6-insufficient individuals. Our study identified that time from lights off to sleep may be a potentially useful outcome measure for sleep interventions (e.g. melatonin replacement) in PAX6-insufficient individuals, as well as other populations with reduced melatonin concentrations.

To our knowledge, for the first time, preliminary studies of the concurrent and construct validity of the PROMIS sleep disturbance and sleep-related impairment questionnaires were completed in an adolescent population, and our findings demonstrated the potential utility of PROMIS sleep measures in adolescents. Further

research is needed to establish additional psychometrics in adolescent-specific samples, which is important as PROMIS measures would allow for comparability across conditions and across the lifespan. Our findings suggest that PROMIS questionnaires could be a potential useful outcome measures in research aimed at improving adolescent sleep health.

Conclusion

Results from the present study provide insight into the sleep-related phenotype associated with *PAX6* haploinsufficiency. In addition, preliminary validation studies on age-appropriate tools to measure sleep in adolescents provided insight into the potential usefulness of self-reported sleep disturbance and sleep-related impairment measures. This study used a combination of physiological and patient-reported health measures, and although WAGR syndrome and isolated aniridia are rare disorders, describing the sleep-related phenotypes in this population will advance the knowledge of assessment and treatment of sleep disorders in general, facilitating their use in additional adolescent populations.

APPENDIX A: PROTECTION OF HUMAN SUBJECTS

DATE: July 9, 2013

TO: Joan C Han, M.D.
Principal Investigator

THROUGH:

FROM: Gilman Grave, M.D.
Chair, NICHD IRB

SUBJECT: IRB Review Amendment 05/30/2013 (12) for of Protocol 08-CH-0213: "WAGR Syndrome and Other 11p Contiguous Gene Deletions: Clinical Characterization and Correlation with Genotype"

Your Amendment 05/30/2013 (12) was approved without stipulations by the NICHD Institutional Review Board (IRB) at the June 26, 2013, meeting.

DISCUSSION:
Dr. Han was present for the discussion and explained that the amendment was aimed at improving the research results for the adolescents in the study. These subjects have decreased melatonin, which can affect the quality of sleep, and the amendment proposes to use a measuring device, an actigraph, which is worn like a watch and records sleep onset and physical movement, as well as the presence of light. In addition, Dr. Han explained that new shorter questionnaires would be introduced, which would be more appropriate to the adolescent subjects (the current questionnaires are designed for adults).

Asked about the research question that prompted the amendment request, Dr. Han explained that there is interest in whether and how much lack of melatonin correlates with sleep disturbances and daytime sleep. She added that the power calculation for this segment of the study indicated a requirement for 32 subjects, and the target is 35. Since there are 40 WAGR syndrome subjects and 12 aniridia subjects already enrolled, developing that subject cohort should not be a problem.

Research progress and rationale for continuation of the study: Research progress has been satisfactory and there has been no change in the rationale for continuing the study.

Changes in previously approved research: There have been no changes in the previously approved research.

Significant new findings: There have been no significant findings that would change the risk/benefit profile of the study

Summary of Adverse Events, Protocol Violations, Serious or Continuing Non-compliance and Unanticipated Problems: There have been no adverse events since the last review.

Protocol recruitment: As expected.

Summary of Adverse Events, Protocol Violations, Serious or Continuing Non-compliance and Unanticipated Problems: No adverse events reported.

Informed consent process is adequate and is appropriately documented (45 CFR 46.111(a)(4), 46.116 and 46.117):

Monitoring of data to ensure safety of subjects (45 CFR 46.111(a)(6)): continues as addressed in protocol

Privacy & confidentiality (45 CFR 46.111(a)(7)): no changes

Risks are reasonable in relationship to anticipated benefits/benefits, if any(45 CFR 46.111(a)(s): no changes

Stipulations/Conditions: none

Recommendations: none

IRB Decision and Vote (Executive Session): The IRB moved and seconded the motion that this Amendment be Approved.

Risk/Benefit Assessment: The Board agreed that the risk to which children with WAGR syndrome enrolled in this protocol will be exposed falls within Section 46.406 of 45CFR 46, Subpart D, namely, greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition. The Board agreed that the risk to which healthy controls enrolled in this protocol will be exposed falls within Section 46.404 of 45CFR 46, Subpart D, namely, not greater than minimal risk

Vote: Total # [9]: For [0], Against [0], Abstained [0], Absent [0], Recused [0]

INCLUSION CRITERIA: WAGR/ANIRIDIA PROTOCOL 08-CH-0213

For WAGR/11p deletion subjects:

1. Diagnosis of WAGR/11p deletion confirmed by prior genetic testing or clinical history consistent with WAGR syndrome (Wilms Tumor and/or genitourinary anomalies plus aniridia). Genetic diagnosis will be confirmed at the NIH, if not done previously
2. Medically stable (so that the patient can safely undergo planned testing); if history of Wilms tumor, must be > 6 months since completion of chemotherapy and must be considered in remission by primary oncologist caring for the patient

For aniridia subjects:

1. Diagnosis of aniridia confirmed by ophthalmologist
2. Medically stable, with no chronic medical or psychiatric conditions anticipated to affect results or impede study participation

For healthy control subjects:

3. No chronic medications. Use of as-needed and over-the-counter medications will be reviewed on a case-by-case basis by the Principal Investigator
4. No chronic medical or psychiatric conditions anticipated to affect results or impede study participation

EXCLUSION CRITERIA: WAGR/ANIRIDIA PROTOCOL 08-CH-0213

For WAGR/11p deletion subjects:

1. Anorexiant use in preceding 6 months
2. Greater than 2% body weight loss in preceding 6 months
3. Pregnancy
4. Individuals who have, or whose parent or guardians have, current substance abuse or a psychiatric disorder or other condition which, in the opinion of the investigators, would impede competence or compliance or possibly hinder completion of the study

For aniridia subjects:

1. Anorexiant use in preceding 6 months
2. Greater than 2% body weight loss in preceding 6 months
3. Pregnancy
4. Individuals who have, or whose parent or guardians have, current substance abuse or a psychiatric disorder or other condition which, in the opinion of the investigators, would impede competence or compliance or possibly hinder completion of the study

MEDICAL RECORD	**CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY** • Adult Patient or • Parent, for Minor Patient

INSTITUTE: National Institute of Child Health and Human Development

STUDY NUMBER: 08-CH-0213 PRINCIPAL INVESTIGATOR: Jack A. Yanovski, M.D., Ph.D.

STUDY TITLE: WAGR Syndrome and Other 11p Contiguous Gene Deletions: Clinical Characterization and Correlation with Genotype

Continuing Review Approved by the IRB on 02/26/14
Amendment Approved by the IRB on 06/12/14 (Q) Date Posted to Web: 06/24/14
Consent for Actigraphy

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Purpose

The purpose of this study is to learn about the role of a gene called paired box 6 (*PAX6*) in sleep patterns in people with WAGR/11p deletion syndrome or people with isolated aniridia.

Background

PAX6 may play an important role in the development of the pineal, a gland in the brain that produces melatonin, a hormone involved in sleep. This study is being conducted to assess sleep patterns in subjects with WAGR/11p deletion syndrome or people with isolated aniridia compared to healthy controls using actigraphy, which is an activity monitor worn on the wrist.

MEDICAL RECORD	**CONTINUATION SHEET for either:** **NIH 2514-1, Consent to Participate in A Clinical Research Study** **NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study**

STUDY NUMBER: 08-CH-0213 CONTINUATION: page 2 of 4 pages

Study Population
People who have WAGR syndrome or other deletions on chromosome 11, people who have isolated aniridia, and healthy people can participate in this study. Subjects who are enrolled in the main protocol will have the option of participating in this additional actigraphy study.

Inclusion Criteria
Participants may be eligible for this research study if:

1. Age is 10-20 years old.
2. For subjects with WAGR/11p deletion syndrome or isolated aniridia, if prior genetic testing has not previously been performed, then the subject will be required to enroll in the main 08-CH-0213 protocol and obtain the outpatient screening blood draw for genetic testing, but participation in the inpatient studies will not be required.
3. Control subjects must be taking no chronic medications or sleep aids, and have no chronic medical or psychiatric problems.

Exclusion Criteria
Participants may not be eligible for this research study if:

1. For control subjects, use of sleep medications or sleep aids in the last 6 months
2. For subjects with WAGR/11p deletion syndrome or isolated aniridia, inability to stop sleep aids for 1 week prior to study participation and for 1 week during the study.

Procedures

1. The participant will wear a portable wristwatch-like device that records movement. The device will be placed on the wrist and will be worn for seven days and nights.
2. The participant will complete a sleep diary recording sleep and wake times. Times when the device was removed, resting periods, or unusual sleep patterns will also be recorded.

Risks, Inconveniences and Discomforts
There may be risks or discomforts associated with participating in this research study. The risks and discomforts for all the tests performed as part of the research study are described below. Also, if during the evaluation at the NIH, we identify abnormalities that require other medically necessary tests and examinations, the diagnosis and treatment of the condition could be associated with additional risks and discomforts that will be discussed with you on an individual basis.

1. Wearing the wrist-watch sized activity monitor does not hurt. Some people do not enjoy wearing a strap on the wrist for an entire week.
2. There are no risks to these sleep studies, but the time involved may be inconvenient.

Anticipated Benefits
Participation in this project may result in no direct benefit to you or your child, but will contribute to our knowledge about the role of *PAX6* in sleep patterns in people with WAGR/11p deletion syndrome or people with isolated aniridia.

Right of Withdrawal and Conditions for Early Withdrawal
Participation is entirely voluntary. You may withdraw from the study at any time and for any reason without loss of benefits or privileges to which you are otherwise entitled.

Results From this Study
The information we obtain from this study will not provide information on your health. You will not receive any individual results, but we will inform you of the overall results of the study.

MEDICAL RECORD	**CONTINUATION SHEET for either:** **NIH 2514-1, Consent to Participate in A Clinical Research Study** **NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study**

STUDY NUMBER: 08-CH-0213 CONTINUATION: page 3 of 4 pages

Alternatives to Participation or Treatment

1. The alternative to participating in this study is not to participate.
2. You may choose not to participate in this study but to continue to receive standard care from your own physicians.
3. This study does not provide treatment and does not replace any therapy that your own doctor is providing.

Compensation

Participants will be compensated for research-related discomfort and inconveniences in accord with NIH guidelines.

_____I have received a copy of the signed consent form.

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient

STUDY NUMBER: 08-CH-0213 CONTINUATION: page 4 of 4 pages

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Jack A. Yanovski, M.D., Ph.D., Building 10-CRC, Room 1-3330, Telephone: (301) 496-4686. You may also call the Clinical Center Patient Representative at (301) 496-2626.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM FEBRUARY 26, 2014 THROUGH FEBRUARY 25, 2015.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____

PATIENT IDENTIFICATION	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY (Continuation Sheet) • Adult Patient or • Parent, for Minor Patient NIH-2514-1 (07-09) P.A.: 09-25-0099 File in Section 4: Protocol Consent

MEDICAL RECORD	**MINOR PATIENT'S ASSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY** • Attach to NIH-2514-2, Consent to Participate in a Clinical Research Study

INSTITUTE: National Institute of Child Health and Human Development

STUDY NUMBER: 08-CH-0213 PRINCIPAL INVESTIGATOR: Jack A. Yanovski, M.D., Ph.D.

STUDY TITLE: WAGR Syndrome and Other 11p Contiguous Gene Deletions: Clinical Characterization and Correlation with Genotype

Continuing Review Approved by the IRB on 02/26/14
Amendment Approved by the IRB on 06/12/14 (Q) Date Posted to Web: 06/24/14
Assent for Actigraphy

INTRODUCTION

We are health care providers at the National Institutes of Health (NIH). We would like to learn more about sleep and activity. We hope that what we learn will help us take care of people who may have problems with sleep. This paper tells what we want to do. Read this paper and ask us questions about anything that you do not understand. Then you and your parents decide if you wish to take part.

What We Are Asking You To Do

At home, we will ask you to wear a wristwatch activity monitor for seven days. This will track your rest and activity patterns. We will ask you to complete a sleep diary that records the time you went to sleep at night and when you woke up in the morning. We will also ask you to record any time you take off the watch. We do not expect the watch to cause you any discomfort.

You can decide to not take part in this study or to stop taking part at any time that you want to. You may ask any questions that you have at any time.

What You Need To Decide

We want you to understand what we will do and why we do it. Please ask us to explain whatever you do not understand. If you agree to participate as we have explained, we will ask you to write your name on this paper, beside the X. Writing your name on this paper is a way of showing that you agree. You are volunteering for this study. You do not have to do this if you and your parents do not want to. Don't write your name until you feel that you understand what will happen. Even if you agree now, you and your parents can change your minds later. Just tell us that you don't want to take part any longer.

I have had this study explained to me in a way that I understand, and I have had the chance to ask questions. I agree to take part in this study.

Signature of Minor Patient:_____ Date:_____

Print Name:_____

Signature of Investigator:_____ Date:_____

Print Name:_____

PATIENT IDENTIFICATION	**MINOR PATIENT'S ASSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY** NIH-2514-2 (10-09) P.A.: 09-25-0099 File in Section 4: Protocol Consent (23)

APPENDIX B: SLEEP QUESTIONNAIRES AND SCORING

PROMIS Item Bank v. 1.0 – Sleep Disturbance – Short Form 8b

Sleep Disturbance – Short Form 8b

Please respond to each item by marking one box per row.

In the past 7 days...

		Not at all	A little bit	Somewhat	Quite a bit	Very much
Sleep108	My sleep was restless..... ..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep115	I was satisfied with my sleep	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Sleep116	My sleep was refreshing	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Sleep44	I had difficulty falling asleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
Sleep87	I had trouble staying asleep.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep90	I had trouble sleeping..... ..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep110	I got enough sleep	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

In the past 7 days…

		Very poor	Poor	Fair	Good	Very good
Sleep109	My sleep quality was	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

PROMIS Item Bank v. 1.0 – Sleep-Related Impairment – Short Form 8a

Sleep Related Impairment – Short Form 8a

Please respond to each item by marking one box per row.

In the past 7 days...

		Not at all	A little bit	Somewhat	Quite a bit	Very much
Sleep10	I had a hard time getting things done because I was sleepy	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep119	I felt alert when I woke up	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Sleep18	I felt tired.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep25	I had problems during the day because of poor sleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep27	I had a hard time concentrating because of poor sleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep30	I felt irritable because of poor sleep.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep6	I was sleepy during the daytime.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep7	I had trouble staying awake during the day.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Scoring PROMIS Short Forms

Scoring Short Forms

Static short forms administered online can be scored using the same numerical scoring algorithm implemented in the Assessment Center software. The item response theory (IRT) model adopted in PROMIS (i.e., the graded response model) uses the pattern of item responses for scoring, not just the sum of individual item responses. Under paper administration of static short forms, however, it is inevitable to streamline the process and use some form of lookup tables based on summed scores, because the number of different response patterns will become unmanageably large to present in a lookup table as the test length increases beyond two or three items. Therefore, for a given collection of IRT calibrated items, we estimate the conditional distribution of each summed score as a function of the trait continuum. Taking into account the score distribution in the population of examinees from which our standardization sample was drawn, we then determine the optimal trait level for each summed score and populate a scoring table. A scoring table can be prepared in advance to map all possible summed scores to trait scores in one-to-on correspondence. Upon administering a static short form, the test administrator can add up the individual item responses reported by the examinee and map the summed score to a trait score using the scoring table provided for the form. All PROMIS short forms are designed to be scored from 1 to 5 (or higher depending on the number of response options). For instance, the PROMIS Depression short form is composed of 8 items and each item has five response options (i.e., Never, Rarely, Sometimes, Often, and Always). Thus, the minimum possible raw summed score for the form is 8 (8 times 1) and the maximum 40 (8 times 5). The scoring table for the form lists all possible raw summed scores ranging from 8 to 40 and the corresponding trait scores and standard errors. Because a scoring table is prepared for a fixed set of items, it can only be used when an examinee responds to all of the items in the set. *One or more missing responses will render such scoring tables unusable*.

Raw Score to T Score Conversion Tables

The following conversion tables allow a user to convert simple summed raw scores from PROMIS short forms into T-score values on an individual respondent or group of respondents. In all cases, these conversions only work accurately when all questions on the short form have been answered. T-Score distributions are standardized such that a 50 represents the average (mean) for the US general population, and the standard deviation around that mean is 10 points. *A high score always represents more of the concept being measured.* Thus, for example, a person who has a T-score of 60 is one standard deviation higher than the general population for the concept being measured. For symptoms and other negatively-worded concepts like pain, fatigue, anxiety, and sleep disturbance, a 60 is one standard deviation worse than average; for functional scores and other positively-worded concepts like physical or social function, a 60 is one standard deviation better than average, etc.

Instrument Name Revisions

Previous versions of Pain Interference were named Pain Impact. Previous versions of Sleep-related Impairment were named Wake Disturbance. Item and Instrument content are unchanged.

Scoring PROMIS Short Forms

Physical Function 10a *Short Form Conversion Table*		
Raw.Score	**T.Score**	**SE***
10	14.1	3.3
11	17	2.8
12	18.7	2.7
13	20.1	2.5
14	21.3	2.4
15	22.4	2.3
16	23.4	2.2
17	24.4	2.2
18	25.3	2.1
19	26.2	2
20	27.1	2
21	28	1.9
22	28.8	1.9
23	29.6	1.9
24	30.4	1.8
25	31.2	1.8
26	32	1.8
27	32.7	1.7
28	33.5	1.7
29	34.2	1.7
30	35	1.7
31	35.7	1.7
32	36.4	1.7
33	37.2	1.7
34	37.9	1.7
35	38.7	1.7
36	39.4	1.7
37	40.2	1.8
38	41	1.8
39	41.8	1.8
40	42.6	1.8
41	43.5	1.9
42	44.4	2
43	45.4	2
44	46.4	2.2
45	47.7	2.4
46	49.1	2.6
47	50.8	3
48	53	3.4
49	55.3	3.7
50	61.7	5.9

*SE = Standard Error

Sleep Disturbance 8b *Short Form Conversion Table*		
Raw.Score	**T.Score**	**SE***
8	28.9	4.8
9	33.1	3.7
10	35.9	3.3
11	38	3
12	39.8	2.9
13	41.4	2.8
14	42.9	2.7
15	44.2	2.7
16	45.5	2.6
17	46.7	2.6
18	47.9	2.6
19	49	2.6
20	50.1	2.5
21	51.2	2.5
22	52.2	2.5
23	53.3	2.5
24	54.3	2.5
25	55.3	2.5
26	56.3	2.5
27	57.3	2.5
28	58.3	2.5
29	59.4	2.5
30	60.4	2.5
31	61.5	2.5
32	62.6	2.5
33	63.7	2.6
34	64.9	2.6
35	66.1	2.7
36	67.5	2.8
37	69	3
38	70.8	3.2
39	73	3.5
40	76.5	4.4

*SE = Standard Error

Conversion Table applies only when ALL questions on the short form have been answered

Scoring PROMIS Short Forms

Sleep-related Impairment 8a *Short Form Conversion Table*		
Raw.Score	**T.Score**	**SE***
8	30	5.4
9	35.1	4.6
10	38.7	4.2
11	41.4	3.8
12	43.6	3.6
13	45.5	3.4
14	47.3	3.1
15	48.9	2.9
16	50.3	2.7
17	51.6	2.6
18	52.9	2.6
19	54	2.5
20	55.1	2.5
21	56.1	2.5
22	57.2	2.5
23	58.2	2.4
24	59.3	2.4
25	60.3	2.4
26	61.3	2.4
27	62.3	2.3
28	63.3	2.3
29	64.3	2.3
30	65.3	2.3
31	66.3	2.3
32	67.3	2.3
33	68.4	2.3
34	69.5	2.4
35	70.7	2.4
36	71.9	2.5
37	73.3	2.6
38	75	2.8
39	76.9	3.1
40	80	3.9

*SE = Standard Error

Pain Intensity 3a *Short Form Conversion Table*		
Raw.Score	**T.Score**	**SE***
3	30.7	4.5
4	36.3	3.1
5	40.2	3.0
6	43.5	3.0
7	46.3	3.0
8	49.4	2.9
9	52.1	2.8
10	54.5	2.9
11	57.5	3.1
12	60.5	3.1
13	64.1	3.8
14	67.4	4.2
15	71.8	5.0

*SE = Standard Error

Conversion Table applies only when ALL questions on the short form have been answered

APPENDIX

Cleveland Adolescent Sleepiness Questionnaire

Today's Date: (fill in) __ __/__ __/__ __

What is your age? (fill in years) _____ **What is your sex? (check one)** 1. Female 2. Male

We would like to know about when you might feel sleepy during a usual week. For each statement, mark the circle under the response that best fits with how often it applies to you. It's important to answer them yourself – don't have people help you. There are no right or wrong answers. For example, if we asked "I sleep with a pillow," and the response that best fit how often you sleep with a pillow was "often," you would mark the item as follows:

EXAMPLE	Never (0 times per month)	Rarely (less than 3 times per month)	Sometimes (1-2 times per week)	Often (3-4 times per week)	Almost every day (5 or more times per week)
I sleep with a pillow	○	○	○	⊗	○

Sleepiness Questions

	Never (0 times per month)	Rarely (less than 3 times per month)	Sometimes (1-2 times per week)	Often (3-4 times per week)	Almost every day (5 or more times per week)
1. I fall asleep during my morning classes	○	○	○	○	○
2. I go through the whole school day without feeling tired	○	○	○	○	○
3. I fall asleep during the last class of the day	○	○	○	○	○
4. I feel drowsy if I ride in a car for longer than five minutes	○	○	○	○	○
5. I feel wide-awake the whole day	○	○	○	○	○
6. I fall asleep at school in my afternoon classes	○	○	○	○	○

	Never (0 times per month)	Rarely (less than 3 times per month)	Sometimes (1-2 times per week)	Often (3-4 times per week)	Almost every day (5 or more times per week)
7. I feel alert during my classes	○	○	○	○	○
8. I feel sleepy in the evening after school	○	○	○	○	○
9. I feel sleepy when I ride in a bus to a school event like a field trip or sports game	○	○	○	○	○
10. In the morning when I am in school, I fall asleep	○	○	○	○	○
11. When I am in class, I feel wide-awake	○	○	○	○	○
12. I feel sleepy when I do my homework in the evening after school	○	○	○	○	○
13. I feel wide-awake the last class of the day	○	○	○	○	○
14. I fall asleep when I ride in a bus, car, or train	○	○	○	○	○
15. During the school day, there are times when I realize that I have just fallen asleep	○	○	○	○	○
16. I fall asleep when I do schoolwork at home in the evening	○	○	○	○	○

(Spilsbury et al., 2007).

Cleveland Adolescent Sleepiness Questionnaire
Score Sheet

Name: _____ **Date:** __ __ / __ __ / __ __

Once you complete the questionnaire, use the scoring keys below to determine your score for each statement. Then add the numbers together to get your total sleepiness score.

Sleepiness Statements

Statement #	Your Score
1.	_____
3.	_____
4.	_____
6.	_____
8.	_____
9.	_____
10.	_____
12.	_____
14.	_____
15.	_____
16.	_____

Scoring Key: Sleepiness Statements

1 = Never
2 = Rarely
3 = Sometimes
4 = Often
5 = Almost every day

Alertness Statements

Statement #	Your Score
2.	_____
5.	_____
7.	_____
11.	_____
13.	_____

Scoring Key: Alertness Statements

5 = Never
4 = Rarely
3 = Sometimes
2 = Often
1 = Almost every day

Total Score: _____

How sleepy are you? A higher score means that you are sleepy during the day and need to get more sleep on school nights. A higher score also could be a sign that you may have a sleep disorder called obstructive sleep apnea (OSA).
You should discuss your score with your parents and your doctor.

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