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Electrophysiological biomarkers of neurodevelopmental disorders: Discoveries from Dup15q syndrome

Author
Saravanapandian, Vidya

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Electrophysiological biomarkers of neurodevelopmental disorders: Discoveries from Dup15q syndrome

A dissertation submitted in partial satisfaction

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by

Vidya Saravanapandian

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ABSTRACT OF THE DISSERTATION

Electrophysiological biomarkers of neurodevelopmental disorders:

Discoveries from Dup15q syndrome

by

Vidya Saravanapandian

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2021

Shafali Spurling Jeste, Chair

Neurodevelopmental disorders (NDDs) show considerable heterogeneity, both in terms of genetic underpinnings and clinical presentation. Certain NDDs arise due to rare genetic etiologies, providing a valuable opportunity to explore possible mechanisms of cognitive and behavioral dysfunction. For instance, duplications of 15q11.2-13.1 cause Dup15q syndrome, an NDD characterized by intellectual disability, autism spectrum disorder (ASD), epilepsy, motor delays, sleep impairment and abnormal brain activity. Genes in the 15q region, particularly UBE3A and a cluster of $GABA_A$ receptor genes, are critical for neural development, synaptic protein synthesis and degradation, and inhibitory neurotransmission. During awake electroencephalography (EEG), children with Dup15q syndrome demonstrate increased oscillatory activity within the beta range (12-30 Hz) that likely reflects aberrant GABAergic neurotransmission. By rigorously investigating the properties of this EEG biomarker, this dissertation expands our understanding of Dup15q

syndrome pathophysiology and uses an innovative methodological pipeline to gather and process remote clinical EEG recordings from patients across the country, thus facilitating large scale studies across NDDs. Chapter 1 introduces NDDs, Dup15q syndrome and EEG biomarkers. Chapter 2 investigates the properties of beta oscillations in Dup15q syndrome, including their relationship to clinical symptomatology, stability over time, and reproducibility, both across analytic pipelines and across research and clinical EEG. Chapter 3 evaluates the presence of beta oscillations across brain states such as wakefulness and sleep and describes novel quantitative biomarkers of sleep disruption in children with Dup15q syndrome, including elevated beta oscillations in sleep and abnormal NREM sleep physiology. Chapter 4 explores the relationship between abnormal sleep physiology and the neurobehavioral phenotype in Dup15q syndrome. Chapter 5 discusses key next steps in further understanding the implications of genetic and brain circuit level changes that occur in Dup15q syndrome and considers whether pharmacological manipulation of the neural dysfunction can change outcomes.

Both beta oscillations and healthy sleep rhythms necessary for healthy cognitive development rely on GABAergic modulation. As such, elevated beta oscillations and the sleep disruptions reported in this dissertation both point towards GABAergic dysfunction in Dup15q syndrome. Therapeutic advances in Dup15q syndrome can include disease-modifying therapies that target GABA signaling. The EEG biomarkers described in this dissertation have the potential to serve as measures of drug target engagement or as a proximal outcome measures that precede behavioral responses to treatment. Ultimately, these biomarkers will help monitor treatment progress and change in clinical outcomes in individuals with NDDs.

This dissertation of Vidya Saravanapandian is approved.

Damla Senturk

Daniel Geschwind

Christopher S. Colwell

Peyman Golshani

Shafali Spurling Jeste, Committee Chair

University of California, Los Angeles

2021

DEDICATION

This dissertation is dedicated to my mother, Saroja Muthusamy, my husband, Saran Balasubramanian, and my son, Ishaan Saran, for their endless love and support, and to the memories of my brother, Vigneswaran Nellaiappan, and my father, Saravanapandian Nellaiappan, who both inspired my pursuit of science and helped me in all things great and small.

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VITA

EDUCATION AND WORK EXPERIENCE

Ph.D. Candidate in Neuroscience — Fall 2015 - Present
University of California, Los Angeles
(UCLA), Jeste Lab

International League Against Epilepsy (ILAE) Fellow — November 2020 - Present

Life Science Research Associate (LSRA), Palmer Lab — July 2011 – June 2015
Department of Neurosurgery, Stanford University

Genetic Researcher — July 2007 – July 2011
Histogenetics, LLC, New York

Master of Engineering in Biotechnology — September 2005 – June 2007
Birla Institute of Technology and Science, India, Biotechnology (GPA: 8.36/10.00)

Bachelor of Technology in Biotechnology — September 2001 – June 2005
Bharathidasan Institute of Technology, India, Biotechnology (GPA: 3.86/4.00)

HONORS AND LEADERSHIP

Dup15q Alliance Predoctoral Fellowship Recipient, Dup15q Alliance — 2018 – 2020

Neurobehavioral Genetics Predoctoral Fellowship Recipient, UCLA — 2016 – 2018

Graduate Programs in Biosciences Fellowship, UCLA — 2018

Brain research Institute Outreach Coordinator, UCLA — 2016 - Present

Brain Research Institute (BRI) & Semel Institute Travel Awards , UCLA — 2017 – 2018

Neuroscience Graduate Student Association Outreach Fellowship, UCLA — 2017

Dup15q Alliance Travel Award, National Dup15q Alliance — 2016

Teaching Assistant for Project Brainstorm — 2016 - 2017
Neuroscience Undergraduate Course, UCLA

2nd Best Poster Award, Stem Cells and Regenerative Medicine Annual Retreat — 2013
Stanford University

PUBLICATIONS

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Chapter 1. Introduction

1.1. Neurodevelopmental disorders

Neurodevelopmental disorders (NDDs) are a group of conditions that emerge in early childhood and are characterized by impairments in cognitive, motor, language, learning, and behavioral development (Thapar, Cooper, and Rutter 2017; Morris-Rosendahl and Crocq 2020). NDDs affect 1-2% of the general population (Blackmer and Feinstein 2016), and early identification of children at risk for developing NDDs can help design clinical interventions that can potentially modify the natural history of these conditions.

NDDs are etiologically and phenotypically heterogeneous. They result from complex genetic and environmental factors that disrupt essential neurodevelopmental processes such as transcriptional regulation, protein synthesis, and synaptic architecture (Sahin and Sur 2015; Stiles and Jernigan 2010; Parenti et al. 2020; de la Torre-Ubieta et al. 2016). Increased rates of comorbidities across etiologies suggest the possibility of shared underlying biological and cellular mechanisms (Gilman et al. 2011; Kilinc et al. 2018). Rapid advances in genetics have resulted in the identification of causative genetic etiologies, from single gene mutations to copy number variants (Gaugler et al. 2014; Schaefer and Mendelsohn 2013; Tammimies et al. 2015; Tărlungeanu and Novarino 2018) that may contribute to the phenotypic heterogeneity in NDDs, thus improving our understanding of the pathophysiology associated with these conditions. Despite this progress, diagnosis and treatment for NDDs rely primarily on the evaluation of behavior. In autism spectrum disorder (ASD), while the disorder is diagnosed based on behavioral symptoms during the second year of life or later, intervention studies have shown that early and timely intervention can help children with ASD overcome some of the associated core deficits and

positively change their developmental trajectory (Sacrey, Bennett, and Zwaigenbaum 2015; Novak and Morgan 2019). About 40-50% of NDDs have identifiable genetic etiologies (Li et al. 2016; Cardoso et al. 2019). Identification of quantifiable and objective brain-based biomarkers that may precede behavior symptoms, and that reflect specific genetic disruptions, can greatly improve clinical trials for these syndromes by serving as measures of early diagnosis, patient stratification, drug-target engagement, or as outcome measures that precede behavioral responses to treatment. One such genetic disorder, characterized by clinical features representative of many NDDs, including ASD, intellectual disability (ID), epilepsy, sleep impairment, motor delays, and abnormal brain oscillatory activity is the 15q 11.2-13.1 duplication (Dup15q) syndrome.

1.1.1. Dup15q syndrome

1.1.1.1. Genetics of the 15q11.2-q13.1 region

The 15q11.2-q13.1 critical region is implicated in three distinct genetic syndromes associated with intellectual disability and is highly penetrant for global developmental delay and ASD. Maternally derived deletions or uniparental disomy of this gene region result in Angelman syndrome, while paternally derived deletions or uniparental disomy result in Prader-Willi syndrome (Thibert et al. 2013; Boronat et al. 2015; Buiting, Williams, and Horsthemke 2016; Butler, Miller, and Forster 2019; Angulo, Butler, and Cataletto 2015). Duplications of the 15q11.2-13.1 region result in Dup15q syndrome, which is one of the most common copy number variations associated with ASD, accounting for about 1-3% of cases (Abrahams and Geschwind 2008; Sanders et al. 2015). Different types of duplications result in the syndrome. 1) the interstitial duplication or trisomy, results in one extra copy of the 15q region that lies on the same chromosome arm. Interstitial triplications, int trp (15) having two extra copies of the chromosome 15q region is also possible. The former is the more common pattern; and 2) the isodicentric duplication or Idic 15, or

tetrasomy, result in two extra copies of the region on a supernumerary chromosome (Finucane et al. 2016).

Based on allelic inheritance, several genes within the 15q critical region are overexpressed in Dup15q syndrome. *UBE3A,* a gene that encodes a ubiquitin protein ligase is imprinted in neurons (Albrecht et al. 1997; Yamasaki et al. 2003) and regulates synaptic development and function (Miao et al. (2013); (Smith et al. 2011; Dindot et al. 2008; Greer et al. 2010). As individuals with maternal duplications present with a more severe clinical phenotype than those with paternal duplications (Cook et al. 1997), *UBE3A* has been of particular interest to researchers and has been hypothesized to be a key contributor to the pathophysiology in Dup15q syndrome. There is a cluster of $GABA_A$ receptor genes *GABRA5*, *GABRB3*, and *GABRG3* that encode the α5, β3, and γ3 receptor subunits respectively, and are overexpressed in both paternal and maternal duplications. Mutations in the $GABA_A$ receptor genes have been shown to result in ASD and epilepsy phenotypes both in humans and in mouse models (Cook et al. 1998; Chen et al. 2014; DeLorey et al. 1998; Mesbah-Oskui et al. 2017; Moller et al. 2017; Nakatsu et al. 1993). Other genes in the 15q critical region include *ATP10A, CYFIP 1, NECDIN, SNRPN, HERC2* (Finucane et al. 2016). Data gathered from gene expression analysis from post-mortem brain tissues and dental pulp stem cells of Dup15q syndrome patients highlight the overexpression of many of these genes including the *CYFIP, UBE3A, HERC2,* and the *GABRB3* genes (Parikshak et al. 2016; Scoles et al. 2011; Urraca et al. 2018).

1.1.1.2. Clinical phenotype

Individuals with Dup15q syndrome have a high risk for ASD and ID, with a constellation of clinical features, including hypotonia resulting in motor delays, language impairments, social communication impairments, sleep impairments, and epilepsy (Battaglia, Parrini, and Tancredi 2010; Finucane et al. 1993; Conant et al. 2014). Individuals with interstitial duplications have a

milder clinical phenotype compared to those with isodicentric duplications, implying a gene dosage effect (Cook et al. 1997; Germain et al. 2014; Urraca et al. 2013). In studies comparing children with non-syndromic ASD and Dup15q syndrome, it has been shown that patients with Dup15q syndrome exhibited significant delays in motor skills and adaptive function compared to the non-syndromic ASD cohort and that Dup15q children with epilepsy showed more significant impairment in cognitive and developmental domains compared to those without epilepsy (DiStefano et al. 2016). Overall, there is considerable phenotypic heterogeneity in individuals with Dup15q syndrome. While the mechanisms underlying the clinical variability is unclear, variability in gene expression as observed from post-mortem brain samples of Dup15q syndrome patients, (Scoles et al. 2011; Parikshak et al. 2016) suggests that variable levels of overexpression of genes in the 15q region possibly may contribute to symptom severity.

1.1.1.3. EEG phenotype

Abnormal beta oscillations during wakefulness were first described in children with Dup15q syndrome by Urraca et al., in 2013. The EEG phenotype, characterized by increased resting state beta power (12 – 30 Hz), distinguishes patients with Dup15q syndrome from typically developing children as well as a non-syndromic ASD cohort (Frohlich et al. 2016). Interestingly, converging studies in both patients and mouse models have shown that administration of benzodiazepines increases beta oscillations. Benzodiazepines act as positive allosteric modulators of $GABA_A$ receptors and extend the duration of channel opening, thereby increasing the duration of the inhibitory postsynaptic current (IPSCs) through chloride transport. Moreover, spontaneous beta oscillations typically observed in human EEG reflect a state of central nervous system activation through a network of inhibitory interneurons, and this action is gated by $GABA_A$ receptor function (Kopell et al. 2000; Faulkner, Traub, and Whittington 1999). Blockade of $GABA_A$ receptors has

shown to result in loss of synchronization of high-frequency oscillations (beta and gamma oscillations), thus supporting the fundamental role of $GABA_A$ receptors in the generation of beta oscillations (Haenschel et al. 2000). The fact that individuals with Dup15q syndrome have duplications in a region of the chromosome that harbors $GABA_A$ receptor genes, and that we see an increase in spontaneous beta oscillations at rest, suggests a role for overexpression of the $GABA_A$ receptor genes in the generation of this EEG biomarker. A 2019 study further strengthened this hypothesis by showing that pharmacological $GABA_A$ receptor modulation in healthy adults showed an increase in beta power similar to what is seen in Dup15q syndrome (Frohlich, Reiter, et al. 2019), with the same peak frequency. Moreover, individuals with paternal Dup15q syndrome, where UBE3A levels are likely normal, also showed a similar increase in beta power (Frohlich, Reiter, et al. 2019), thus highlighting the critical role for GABAergic neurotransmission in the Dup15q syndrome EEG phenotype.

While an increase in beta oscillations is highly specific to individuals with Dup15q syndrome in these studies, the level of increase in beta power seemed to vary between individuals. Studies have shown that classic benzodiazepines have a stronger binding affinity to certain $GABA_A$ receptor subunits (e.g., the alpha 5 subunit). Thus, $GABA_A$ receptor subunit composition as well as the amount of time the channel is open may likely contribute to this variability. The change in $GABA_A$ receptor kinetics can also be captured by measuring peak beta frequency (the frequency at which we see the highest peak in the beta band). The duration of IPSCs mediated by $GABA_A$ receptors (through specific subunit composition) directly modulates beta oscillations (Buzsaki, 2006). Beta power and peak beta frequency therefore may represent promising biomarkers that reflect aberrant GABAergic function and therefore, may inform phenotypic heterogeneity in Dup15q syndrome.

1.2. Why are biomarkers important?

As stated previously, the expansion of genetic studies resulting in the discovery of large and recurrent CNVs, and the growing research in preclinical models of NDDs have significantly advanced our insights into the genetic basis and pathological mechanisms underlying various NDDs. Despite this knowledge, one of the biggest challenges that hampers successful clinical trial design and implementation in NDDs is the lack of robust quantifiable and objective biomarkers (Jeste and Geschwind 2014). The recent clinical trials in Fragile X syndrome (FXS) (Berry-Kravis et al. 2013; Erickson et al. 2017) are one example that highlights several factors that potentially undermine success in clinical trials in NDDs. These include lack of markers of drug-target engagement, despite having a clear biological, mechanistic target; lack of objective methods for patient selection for trials and therefore targeting broad developmental windows despite the biological and developmental heterogeneity; lack of outcome measures that are sensitive to short term changes or vulnerability to the placebo effect; and outcome measures chosen based on preclinical research that isn't translatable to humans. With insights gained from such studies, research efforts have recently focused on the identification and quantification of robust biomarkers that can serve as diagnostic markers helping with early detection, inform patient stratification and treatment compliance, predict treatment response and disease course, and serve as outcome measures for drug-target engagement and that is invulnerable to a placebo effect.

1.3. Role of electrophysiological oscillations as biomarkers in neurodevelopmental disorders

Given that NDDs represent a disruption of fundamental processes in early brain development such as protein translation, synaptic signaling, and excitatory and inhibitory neurotransmission (de la Torre-Ubieta et al. 2016), these changes may occur well before behavioral signs of developmental delay. These cellular alterations ultimately lead to abnormal circuit

development. A powerful technique that provides a window into the downstream physiological consequences of these aberrant cellular processes is EEG (Varcin and Nelson 2016).

EEG measures neuronal activity driven by somatic and dendritic currents in millions of synchronized cortical pyramidal cells and represents a read-out of the summation of all of the inhibitory and excitatory currents in the underlying network (Kirschstein and Kohling 2009). From a practical perspective, the motion tolerance and noninvasiveness of EEG facilitate the investigation of a wide developmental range, including populations that may not be able to follow basic instructions or sit still for a testing session (Varcin and Nelson 2016). As EEG measures neural activity patterns in real-time and tracks information processing with millisecond precision (Bosl et al. 2011; Langer et al. 2017), its temporal precision makes EEG a powerful tool to non-invasively study different levels of neural processing in children and adults with complex NDDs. Specifically, EEG can detect and quantify changes in neuronal synchronization, which may be spontaneous, or evoked by external stimuli. Spontaneous EEG may display signs of atypical cortical development as early as infancy. For instance, resting state EEG may be used to identify ASD risk as early as six months (Bosl et al. 2011; Duffy and Als 2012; Zeng et al. 2017; Tierney et al. 2012). Furthermore, mathematical techniques for estimating brain connectivity and signal sources increase the information yield of EEG. Brain connectivity measures help determine which brain regions are interconnected to form networks that underlie specific neuronal function (O'Reilly, Lewis, and Elsabbagh 2017; Cabral, Kringelbach, and Deco 2014; Lord and Opacka-Juffry 2016). Source localization techniques help estimate the cortical sources of EEG signals detected on the scalp, providing clues to plausible biological pathways (McLoughlin, Makeig, and Tsuang 2014).

Beyond clinical scalp EEG, intracranial electrophysiological recordings of single-unit activity from pyramidal neurons and inhibitory interneurons in the rodent cerebral cortex may parallel and complement human EEG studies and help investigate the biological mechanisms underlying these brain network changes. Such translational work would facilitate the investigation of brain network changes to potential treatments in individuals with genetic variations. Taken together, EEG is scalable and helps measure brain circuitry changes across model systems and ages, and when combined with genetic and mechanistic studies in animal models may ultimately inform syndrome-specific targeted treatments.

1.4. Validation of EEG biomarkers to inform its utility in clinical trials

As EEG can measure circuit-level treatment response before behavior changes can be observed, EEG biomarkers have the potential to address the challenges involved in pharmacological trials, as learned from the FXS trials (Berry-Kravis et al. 2018; Erickson et al. 2017; Berry-Kravis et al. 2013) discussed above. Depending on clinical and research demands, well-characterized and clinically relevant biomarkers should be: (1) highly sensitive and disorder-specific to distinguish the clinical population from typical development, (2) stable over time, across developmental age or experimental set-up with test-retest reliability, (3) stable or at least consistently variable with changes in brain-state (wakefulness and sleep), (4) scalable, (5) reproducible across different data collection sites (research and clinical settings) as well as different data processing methods, and (6) translatable from preclinical to clinical models and vice versa. While they may not directly reflect disease mechanisms, biomarkers are powerful when they do, offering more insights into treatment targets.

The large etiologic heterogeneity in NDDs has resulted in biomarkers being much more elusive in this field compared to other branches of medicine. However, advances in genetics have

greatly elucidated the underlying neurobiology of NDDs, which in turn has prompted the use of EEG to quantify convergent neurodevelopmental processes in NDDs. In the case of Dup15q syndrome, we have a quantifiable and mechanistic EEG biomarker that distinguishes individuals with Dup15q syndrome from those who do not have the syndrome and likely reflects the underlying genetic abnormality (Frohlich, Reiter, et al. 2019; Frohlich et al. 2016) and, therefore, if validated, can accelerate clinical trial design in Dup15q syndrome.

1.5. Sleep physiology as a putative biomarker for clinical trials in neurodevelopmental disorders

Sleep problems are highly prevalent in NDDs compared to the general population, and sleep physiological disturbances may reflect disrupted neural network activity associated with NDDs (Bruni et al. 2019; Heussler and Hiscock 2018; Wickboldt et al. 2012). Because the same objective measures can be studied in humans and animal models, sleep has high translational relevance and, if quantified, sleep physiology can represent a robust biomarker that sheds light on the etiology of cognitive impairment while serving as a surrogate endpoint in clinical trials.

Parents of children with Dup15q syndrome report behavioral sleep difficulties (Urraca et al. 2013). A retrospective, descriptive clinical overnight EEG study of children with Dup15q syndrome reported the presence of abnormal sleep patterns including electrical status epilepticus in sleep (ESES), alpha-delta patterns, and periods of high amplitude paroxysmal fast activity that impair sleep architecture (Arkilo et al. 2016). As discussed previously, children with Dup15q syndrome have an awake EEG biomarker, in the form of increased beta oscillations, that likely reflects abnormal GABA neurotransmission. Healthy sleep rhythms necessary for robust cognitive development (Tarokh, Saletin, and Carskadon 2016; Yaffe, Falvey, and Hoang 2014; Pace-Schott and Spencer 2015) are also highly dependent on GABAergic neurotransmission. If the Dup15q

syndrome EEG biomarker truly reflects underlying genetic variability, then from a biomarker standpoint, being able to evaluate the persistence of beta oscillations in sleep in Dup15q syndrome would facilitate the extraction and quantification of this biomarker from any routine EEG recording (wakefulness or sleep), thus enhancing scalability and feasibility. From a mechanistic standpoint, the persistence of beta oscillations and altered GABAergic neurotransmission may compromise healthy sleep physiology and would call for the need to quantify sleep structures and ultimately determine whether these sleep physiology measures relate to impaired cognition in children with Dup15q syndrome.

1.5.1 Developmental changes in sleep physiology

During the early months of life, as the human brain develops, patterns of sleep EEG develop dramatically. Newborns spend about 70% of each 24 hours sleeping, and by almost 6 months of age, distinct functional brain states coordinated by distinct patterns of neurophysiological oscillations emerge (Jiang 2019), including wakefulness and sleep. The maturation of sleep is one of the most important physiological processes occurring during the first year of life, and conversely, the development of distinct sleep states in a newborn is dependent on the maturation of the central nervous system (CNS) and is said to be an indicator of normal brain development (Curzi-Dascalova 2001, 1992; Holditch-Davis and Edwards 1998). Impaired sleep during early development may, therefore, have negative consequences that may alter the natural process of the maturing brain, including myelination (Kurth et al. 2015) and ultimately disrupt the overall development of a coherent neurophysiological network in the developing brain.

Sleep has been broadly separated into two main types: non-rapid eye movement (NREM) sleep, characterized by increasingly larger and slower brain waves and, rapid eye movement sleep (REM), characterized by dreaming and including mixed frequency brain activity similar to that

seen during wakefulness (Chokroverty 2010). NREM sleep is further divided into 3 stages: stage 1 NREM (N1), a period of relatively light sleep dominated by theta waves, stage 2 NREM (N2), a period of deeper sleep, dominated by low theta to delta range and characterized by presences of K-complexes and sleep spindles, and stage 3 NREM (N3), a period of deepest NREM sleep, also called slow wave sleep (SWS), dominated by high-amplitude slow waves. While sleep macrostructures are almost mature by the first few months, several sleep structural changes occur at critical periods during development, and by 6 months of age, neurophysiological patterns of sleep closely resemble that seen in adults (Bathory and Tomopoulos 2017; Jiang 2019). As we age, sleep architecture changes are also accompanied by changes in duration. The amount of deep sleep increases from birth through childhood and decreases over the lifespan. The amount of REM sleep is twice as much in newborns and decreases from birth to adulthood.

The control of sleep-wake cycles undergoes a substantial change in development during the first year of life. The circadian timing system located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus controls this regulation (Moore 2013; Rivkees and Hao 2000). The development of SCN and the associated changes to circadian rhythms occur during infancy, further emphasizing the critical role for early development. Therefore, pathological alterations in the development and maturation of these regulatory systems during infancy can induce negative effects on overall development and functioning. REM and NREM sleep deprivation during early development has been associated with loss of brain plasticity, with poor learning ability as well as long-term behavioral problems (Maquet et al. 1996; Maquet 2010). REM sleep is also a time for early neurosensory development which includes the visual and auditory systems, and REM sleep deprivation has resulted in the underdevelopment of these systems (Frank and Heller 2003; Ednick et al. 2009). Moreover, sleep microstructures including spindles and K-complexes play a critical

role in normal brain maturation (Dan and Boyd 2006). Any alterations in the age-related development of these structures may contribute to significant changes in cortical development as well as affect overall learning and memory.

1.5.2. Changes in sleep physiology in neurodevelopmental disorders

Sleep disturbances are one of the most common co-occurring conditions in children with NDDs. 50-95% of these children meet criteria for a sleep disorder (Polimeni, Richdale, and Francis 2005; Tan-MacNeill et al. 2020), compared to 10-30% of typically developing children (Souders et al. 2017). Disruptions in quantity and quality of sleep during early development could be a precursor to various medical conditions and, in fact, sleep disturbances are said to precede the onset of core symptoms in several psychiatric and NDDs (Tesler, Gerstenberg, and Huber 2013; Lunsford-Avery, Dean, and Mittal 2017). Sleep disturbances in NDDs result from a complex interaction between neurobiological, psychological, and environmental factors, and recent studies have shown that genetic vulnerability to major psychiatric disorders and NDDs have a strong association with sleep disturbances (Ohi et al. 2021). Most studies of sleep in NDDs have primarily focused on sleep behavior, typically quantified through caregiver questionnaires or polysomnography, with the most common complaints being delayed sleep onset, frequent nocturnal awakenings, and reduced sleep duration (Elrod and Hood 2015; Robinson-Shelton and Malow 2016; Veatch et al. 2017; Veatch, Maxwell-Horn, and Malow 2015; Mazurek et al. 2016)

Although limited, studies examining changes in sleep architecture in individuals with NDDs have been critical in highlighting dissimilarities in sleep EEG patterns between NDDs and typical development. These include: 1) fewer sleep spindles and K-complexes during stage 2, low levels of delta EEG activity and shorter duration of SWS during stage 3, and deficiency of REM sleep in ASD (Limoges et al. 2005; Limoges et al. 2013; Rochette et al. 2018; Lehoux, Carrier,

and Godbout 2019), 2) fewer and shorter spindles, and increased delta power in sleep, in Angelman syndrome (den Bakker et al. 2018; Sidorov et al. 2017) 3) presence of electrical status epilepticus during sleep (ESES), alpha-delta patterns, and periods of high amplitude paroxysmal fast activity in Dup15q syndrome, 4) poorly-developed spindles and K-complexes, and reduced SWS in Rett syndrome (Saby et al. 2020; Young et al. 2007; Johnston et al. 2014; Garofalo, Drury, and Goldstein 1988; Aldrich, Garofalo, and Drury 1990; Ammanuel et al. 2015), 5) reduced sleep time and efficiency, increased awakenings, increased levels of alpha power in stage 2 sleep, and decreased REM sleep in Tuberous Sclerosis Complex (Bruni et al. 1995; Cook et al. 2020), 6) reduced sleep time and reduced sleep spindles in Asperger's syndrome (Godbout et al. 2000; Lázár et al. 2010), 7) reduced sleep duration, increased REM latency and increased awakenings in Fragile X syndrome (Carotenuto et al. 2019), 8) decreased sleep efficiency, increased arousals and atypical age-related deceleration of SWS in Williams syndrome (Mason et al. 2011; Bódizs et al. 2014), and 9) reduced REM latency in Prader-Willi syndrome (Hertz et al. 1993; Weselake et al. 2014; Camfferman et al. 2008). Many of these studies also include findings on the abnormalities in sleep behavior supported by parent reports.

1.5.3. Neurodevelopmental consequences of abnormal sleep physiology

Loss of even a few hours of sleep can contribute to adverse changes in a variety of cognitive processes such as attention, decision making, reasoning, language, visuomotor performance, learning, and memory. Sleep microstructures, specifically spindles and SWS, are highly involved in learning and forming long-term memories (Fernandez and Lüthi 2020; Born 2010). Sleep spindles are time-locked to slow wave activity (SWA) during NREM sleep, with depolarizing up-state of SWA coupled to fast spindles (13-16 Hz) and hyperpolarizing down-state of SWA coupled to slow spindles (10-12 Hz) (Andrillon et al. 2011; Sitnikova, Grubov, and Hramov 2020).

Additionally, high-frequency oscillations (80-100 Hz in humans and 250 Hz in rodents), in the form of sharp-wave ripples (SWRs), arise within the CA1 region of the hippocampus, typically during NREM, and are said to be time-locked with spindles and SWA (Fernandez and Lüthi 2020; Oyanedel et al. 2020). During SWRs, neurons are activated in precise temporal sequence, rapidly replaying the same sequences of activation that occurred during wakefulness, but in a time-compression fashion, suggesting that SWRs could play a role in encoding or consolidating behaviorally relevant information. Therefore, disruptions to the temporal synchronization of hippocampal SWRs, thalamocortical spindles, and cortical slow oscillations may impair the maintenance and consolidation of memories during sleep.

Alterations in sleep physiology and severity in sleep disturbances have been associated with cognitive and behavioral impairments in NDDs. For example, disruption in sleep spindle characteristics, such as spindle density and changes in the amount of SWA during the night, have been highly associated with abnormal cognitive performance (Maski et al. 2015; Limoges et al. 2013; Tham, Schneider, and Broekman 2017; Christensen et al. 2014; Kopp, Rudolph, and Tobler 2004; Dijk 2009). Abnormal delta rhythms in sleep have been associated with insomnia and sleep apnea, and disruptions in REM sleep have been associated with impairments of procedural memory and problem-solving and increases in risk for anxiety and depression (Ackermann and Rasch 2014; Wickboldt et al. 2012). While there is extensive evidence suggesting that sleep impairments could be central to the clinical presentation in NDDs and that abnormal sleep could impact the maturation of CNS and overall functioning, it is surprising that only a handful of studies have examined whether abnormal sleep physiology relates to cognitive impairment and, in turn, whether modulation of this sleep physiology can improve cognitive function and overall development. Given the fact that most children with Dup15q syndrome and other NDDs that have

comorbid epilepsies undergo clinical overnight EEG, large-scale studies that investigate sleep physiological parameters and their relation to cognitive function are feasible and will provide us with potential opportunities for therapeutic interventions.

1.6. Overview of dissertation

Given the heterogeneity of NDDs, identification of biological and behavioral endophenotypes, specifically those that may precede behavior symptoms and clinical diagnosis could facilitate identification of underlying common genetic pathways. Identifying these endophenotypes in syndromes of known genetic etiology can bridge our understanding of genes and behavior and inform diagnosis, prognosis, and treatment targets. EEG has a long history in child development research, and advances in technological and neuroscientific methods have allowed us to utilize EEG measures to capture brain dynamics and explore EEG markers as predictors of treatment response. Thus, Dup15q syndrome, a genetic syndrome with clinical symptoms relevant to many NDDs, including autism, intellectual disability, and epilepsy, with abnormal brain oscillatory activity that may reflect underlying genetics, provides us with an opportunity to identify EEG biomarkers that potentially can serve as outcome measures or surrogate endpoints in pharmacological treatments. Chapters 2-4 of this dissertation reflect our understanding of the electrophysiological biomarkers during wakefulness and sleep in children with Dup15q syndrome. An abnormal electrophysiological pattern in the form of increased beta oscillations was first reported in children with Dup15q syndrome. This turned out to be a biomarker of clinical relevance as it was found to distinguish individuals with Dup15q syndrome from those who did not have the syndrome. Chapter 2 of this dissertation builds on this quantitative biomarker and evaluates key properties of the beta EEG biomarker which would inform its utility in clinical trials. Chapter 3 of this dissertation evaluates the presence of this biomarker during sleep and

further characterizes sleep physiology in Dup15q syndrome and describes, for the first time, EEG biomarkers of NREM sleep disruption in children with Dup15q syndrome. Chapter 4 explores the relationship between this abnormal sleep physiology and behavior in children with Dup15q syndrome. These chapters are archival records of manuscripts that have either been published or are currently submitted or in preparation for publication. Chapter 5 summarizes findings from these studies and discusses important next steps in further establishing the mechanisms underlying abnormal physiology and neural circuit development in Dup15q syndrome, which can be applied to the investigation of other NDDs.

Chapter 2: Properties of beta oscillations in Dup15q syndrome

2.1. Introduction

Genetic testing for neurodevelopmental disorders (NDDs) has become increasingly precise and clinically available. As a result, hundreds of causative genetic etiologies for NDDs have now been identified, from single gene mutations to copy number variants (de la Torre-Ubieta et al. 2016). Under a conceptual framework of precision health for NDDs, identification of mechanistic biomarkers that reflect specific genetic disruptions can greatly improve clinical trials for these genetic syndromes by serving as measures of drug target engagement or as outcome measures that precede more subtle, yet meaningful, behavioral responses to treatment (Berry-Kravis et al. 2013; Ferlini, Scotton, and Novelli 2013).

Recently, we quantified a robust electroencephalography (EEG) biomarker of the copy number variant syndrome caused by duplications of chromosome 15q11.1-q13.1 (Dup15q syndrome) (Frohlich et al. 2016; Frohlich, Reiter, et al. 2019; Urraca et al. 2013). Dup15q syndrome is highly penetrant for autism spectrum disorder (ASD), accounting for 1-3% of cases (Cook et al. 1997; Moreno-De-Luca et al. 2013). Individuals with this syndrome also have comorbid global developmental delay, intellectual disability (ID), hypotonia, and a high rate of epilepsy (DiStefano et al. 2016; Finucane et al. 2016; Urraca et al. 2013; Abrahams and Geschwind 2008). Based on allelic inheritance, the 15q region harbors several genes critical for brain development and synaptic function, particularly *UBE3A,* and a cluster of bi-allelically expressed gamma-aminobutyric acid type A receptor ($GABA_AR$) genes, *GABRB3*, *GABRA5*, and *GABRG3,* which encode β3, α5, and γ3 subunits, respectively (Finucane et al. 2016). *UBE3A* encodes a ubiquitin protein ligase and is maternally expressed (i.e., paternally imprinted) in most neurons

(Yamasaki et al. 2003; Dindot et al. 2008) while playing an important role in regulating synaptic development and function (Albrecht et al. 1997; Yamasaki et al. 2003). Functional loss of the *UBE3A* protein causes Angelman syndrome, another rare genetic NDD whose clinical features (e.g., ID and epilepsy) (Kishino, Lalande, and Wagstaff 1997) and etiology (e.g., *UBE3A* dysfunction) (Copping et al. 2017) partially overlap with Dup15q syndrome. However, the beta EEG biomarker found in cases of maternal Dup15q syndrome is also seen in paternal duplications with little to no impact on *UBE3A* (Frohlich, Reiter, et al. 2019), suggesting a crucial role for nonimprinted 15q genes, rather than *UBE3A*, in generating the beta EEG phenotype.

Different types of duplications result in Dup15q syndrome: 1) interstitial duplications generally result in one extra copy (i.e., partial trisomy) of the 15q region that remains on the same chromosome arm as the original copy. In some cases, interstitial triplications occur as two extra copies (i.e., partial tetrasomy) of the 15q region. 2) Isodicentric duplications, result in two extra maternal copies of the 15q region manifesting as a supernumerary chromosome (Finucane et al. 2016). Individuals with interstitial duplications tend to have a milder clinical phenotype and lower incidence of epilepsy compared to those with isodicentric duplications, implying a gene dosage effect (Cook et al. 1997; Germain et al. 2014; Urraca et al. 2013) on clinical outcomes.

Spontaneous, high amplitude beta (12-30 Hz) oscillations are an EEG biomarker of Dup15q syndrome (Frohlich et al. 2016; Frohlich, Reiter, et al. 2019; Urraca et al. 2013). This beta EEG phenotype was first noted in a comprehensive case series of children with interstitial duplications, based on clinical EEGs obtained for epilepsy monitoring. (Urraca et al. 2013; Al Ageeli et al. 2014). Our group then quantified this EEG phenotype in high-density research EEG recordings and found that beta oscillations significantly distinguish children with Dup15q syndrome from age matched typically developing children and age and cognitively matched

children with nonsyndromic ASD and ID (Frohlich et al. 2016). Evidence from clinical and preclinical studies demonstrates a crucial role for GABAergic neurotransmission in the generation of beta oscillations, thus implicating the GABAergic system in the Dup15q syndrome beta EEG phenotype. Positive allosteric modulators (PAMs) of $GABA_A$Rs (e.g., benzodiazepines) induce beta oscillations in human scalp recordings and intracranial recordings from rodents (Christian et al. 2015; van Lier et al. 2004; Faulkner, Traub, and Whittington 1999; Kopell et al. 2000). These pharmacological agents enhance the inhibitory chloride current through the $GABA_A$R when bound in the presence of GABA (Nutt and Malizia 2001). Conversely, blockade of $GABA_A$Rs results in desynchronization and diminished oscillatory power at high frequencies in the beta/gamma (12-80 Hz) range (Haenschel et al. 2000). Furthermore, cases of Angelman syndrome caused by deletions of 15q11-q13 (the genetic converse of Dup15q syndrome) demonstrate lower EEG beta power as compared with etiologies not involving the $GABA_A$ β3/α5/γ3 gene cluster (Frohlich, Miller, et al. 2019), also suggesting that beta power may serve as a biomarker of altered GABA neurotransmission.

Although an exact mechanism of beta oscillations has not been elucidated in Dup15q syndrome, increased gene dosage of *GABRB3*, *GABRA5*, and *GABRG3* (Scoles et al. 2011; Germain et al. 2014; Parikshak et al. 2016; Urraca et al. 2018) suggests dysfunctional GABAergic neurotransmission. While the foregoing genes are likely crucial to the presence of the beta EEG phenotype (Frohlich, Reiter, et al. 2019), *UBE3A* plays a critical role in the co-release of GABA in rodents (Judson et al. 2016), suggesting that it also affects beta oscillations. As further evidence of the intimate connection between *UBE3A* and GABA, the $GABA_A$ enhancers Gaboxadol and Ganaxolone have been shown to restore behavioral phenotypes in Ube3a knockout mice (Egawa et al. 2012; Ciarlone et al. 2017). Thus, *UBE3A* overexpression likely affects GABAergic

transmission (and downstream effects thereof on beta oscillations) in this syndrome.

As disease modifying therapies, particularly those that modulate altered GABA signaling, are developed and tested in Dup15q syndrome, the Dup15q syndrome EEG biomarker has the potential to serve as a measure of drug target engagement or as a proximal outcome measure. In order to facilitate and inform the use of this biomarker in clinical trials, we examined the following properties in a larger cohort of children with this syndrome: (1) relation to clinical features, including age, duplication type, epilepsy, (2) relation to behavior, namely those features that contribute most to the clinical heterogeneity of the syndrome (cognition and adaptive skills), (3) stability over time, and (4) reproducibility of the signal in clinical EEG. Given that this work spanned several years and projects, we also had the opportunity to compare results generated from different pre-processing pipelines, thus indirectly testing the reproducibility of the biomarker between analytic pipelines. To accomplish these goals and to adequately enhance our clinically representative sample size in this rare disorder, we partnered with a patient advocacy group, the Dup15q Alliance, and we collected EEG data at two consecutive national family meetings, as well as at our own institution. This study reflects an effort to improve clinical trial readiness in this genetic syndrome by comprehensively characterizing the aspects of this biomarker that would then guide its future use in treatment studies.

2.2. Subjects and Methods

2.2.1 Sites for data collection

Data were collected at the University of California, Los Angeles (UCLA) and at two national Dup15q syndrome family conferences. In order to ensure that there were no site differences in EEG outcome, a one-way analysis of variance (ANOVA) with post-hoc tests were

performed. There was no evidence of an effect of site on beta power ($F(2,38)=0.28$; $p=0.75$) or beta peak frequencies ($F(2,38)=1.10$; $p=0.34$).

2.2.2. Participants

Children (age < 18 years) were clinically referred through the Dup15q clinic at UCLA and the Dup15q Alliance. Combining data collected from the three sites, EEG recordings were analyzed from a total of n = 61 participants. The flowchart in Figure 2.1 shows the participant distribution for each parallel study described in this paper and reasons for exclusion from analysis.

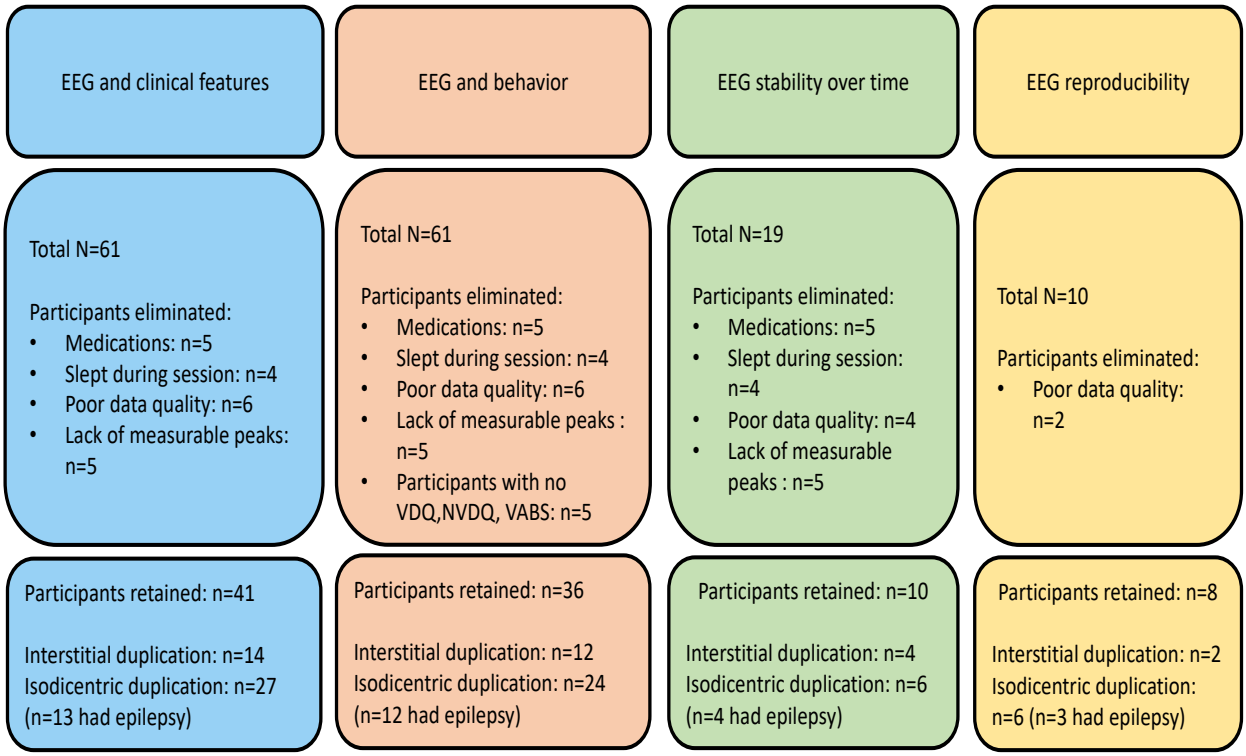

Figure 2.1. Flowchart showing participant distribution for each study

2.2.3. Behavioral assessments

Participants were administered the following measures: 1) The Mullen Scales of Early Learning (MSEL), which assesses general cognition and development (EM. 1995). The MSEL yields standard as well as age equivalent scores that measure receptive and expressive language, visual reception, and gross and fine motor skills. These scores were then utilized to calculate verbal and non-verbal cognition scores. Given that most of our children with Dup15q syndrome had significant delays in overall development, age equivalent ratio scores were used instead of standardized development quotient scores. 2) Vineland Adaptive Behavior Scale (VABS), a parent reported measure of adaptive behavior, yields standard and age equivalent scores for communication, daily living skills (DLS), socialization and motor skills (Scattone, Raggio, and May 2011).

2.2.4. EEG data acquisition and processing

All data were collected under protocols approved by the Institutional Review Board (IRB) (# 15-001565). High density research EEG data were acquired at a sampling rate of 500 Hz using 129 channel vertex-referenced Philips Neuro (Eugene, OR, USA) nets with Ag/AgCl electrodes. Full details of the research EEG acquisition are found in a previous publication (Frohlich et al. 2016). For the reproducibility analysis, we accessed overnight clinical EEG recordings that were collected at UCLA as part of routine epilepsy monitoring. These EEGs were collected at a sampling rate of 200 Hz using a 21-channel 10-20 montage data acquisition set up.

We eliminated data from participants with 1) medications that are known to pharmacologically induce beta oscillations (benzodiazepines or barbiturates), 2) poor data quality due to artifacts from non-neural sources, or 3) lack of measurable peak (i.e., local maximum) in the beta band of the EEG power spectrum. Our final cohort of children with Dup15q syndrome

yielded n = 41 participants. Details of age, sex, duplication type, epilepsy status, medications, and IQ can be found in Table 2.1.

Table 2.1. Dup15q syndrome participant characteristics (Cross-sectional study)

Age (months)	**Gender**	**Medications** (generic)	**Duplication Type**	**Epilepsy (active)**	**Verbal DQ**	**Nonverbal DQ**	**VABS_DLS**
9	M	None	Interstitial	No	66.67	61.11	106.00
18	M	None	Isodicentric	No	50.00	52.78	77.00
29	M	Oxcarbazepine	Isodicentric	Yes	15.52	20.69	51.00
38	M	Levetiracetam	Isodicentric	Yes	13.16	25.00	38.00
39	M	None	Isodicentric	No	30.77	32.05	60.00
42	M	None	Isodicentric	No	N/A	N/A	57.00
44	M	None	Interstitial	No	91.00	68.00	83.00
46	F	None	Isodicentric	No	16.30	2.17	53.00
47	M	None	Isodicentric	No	73.40	69.15	83.00
48	M	None	Interstitial	No	N/A	N/A	66.00
50	F	None	Isodicentric	No	63.00	46.00	73.00
51	M	None	Isodicentric	No	32.35	38.24	54.00
53	M	Lamotrigine	Isodicentric	Yes	9.43	19.81	57.00
54	F	None	Interstitial	No	85.19	97.22	81.00
54	M	None	Interstitial	No	8.33	24.07	48.00

57	M	None	Isodicentric	No	47.37	44.74	N/A
61	F	Valproic acid; Clobazam; Lacosamide; Perampanel	Isodicentric	Yes	9.02	13.93	50.00
62	F	Rufinamide; Valproic Acid	Isodicentric	No	50.00	30.65	57.00
64	M	Lamotrigine; Rufinamide	Isodicentric	Yes	10.16	15.63	45.00
65	M	None	Isodicentric	Yes	9.23	13.08	38.00
67	F	None	Interstitial	No	37.31	37.31	57.00
68	F	None	Interstitial	No	14.71	21.32	N/A
69	M	Valproic Acid Topiramate	Isodicentric	Yes	12.32	13.04	45.00
78	M	None	Interstitial	No	19.87	21.15	50.00
82	M	Valproic acid; Oxcarbazepine	Isodicentric	Yes	N/A	N/A	36.00
94	M	None	Isodicentric	No	54.00	39.00	N/A
96	F	None	Isodicentric	No	N/A	N/A	61.00
100	M	Carbamazepine; Levetiracetam	Isodicentric	Yes	54.00	64.00	66.00
102	F	None	Isodicentric	No	34.31	22.55	58.00

106	M	None	Interstitial	No	N/A	N/A	71.00
108	M	None	Interstitial	No	64.35	60.65	73.00
111	F	None	Interstitial	No	26.58	23.87	55.00
118	F	None	Interstitial	No	36.86	44.07	N/A
127	F	Topiramate	Isodicentric	Yes	22.83	20.47	47.00
134	M	None	Isodicentric	No	9.70	18.66	38.00
153	M	Rufinamide; Levetiracetam; Lacosamide; Epidolex	Isodicentric	Yes	24.18	22.22	N/A
156	M	None	Isodicentric	No	37.18	24.20	40.00
161	M	None	Interstitial	No	73.00	66.00	73.00
169	M	Rufinamide; Valproic Acid	Isodicentric	Yes	16.57	13.02	20.00
175	F	None	Interstitial	No	31.00	41.00	86.00
189	M	None	Isodicentric	Yes	40.00	36.00	82.00

Cognitive tests were not available for all the participants. N/A = not available.

Of the participants that had at least two research EEG recordings from multiple visits, 4 had recordings from three visits. We selected recordings that were at least 10 months apart to enforce this duration of time as a buffer between repeated observations. A total of 10 participants

were included in the longitudinal stability analysis. See Table 2 for details of age, epilepsy status during the visits, and duplication type. Dosages of medications were not confirmed with each participant's physician and, therefore, were not included in the table. A subset of our sample (n = 8) had additional EEG recorded in a clinical setting for epilepsy monitoring.

Table 2.2. Dup15q syndrome participant characteristics (Longitudinal study)

Participant	**Duplication type**	**Visit**	**Age (months)**	**Epilepsy (active)**	**Medications**
P1	Isodicentric	57	72	No	N/A
P2	Isodicentric	18	28	Yes (at second visit)	Vigabatrin (at second visit)
P3	Interstitial	51	78	No	N/A
P4	Isodicentric	110	134	No	N/A
P5	Isodicentric	84	100	Yes (at both visits)	Carbamazepine; Levetiracetam (unchanged at both visits)
P6	Interstitial	48	72	No	N/A

P7	Isodicentric	29	53	Yes (at second visit)	Lamotrigine (at second visit)
P8	Isodicentric	38	61	Yes (at both visits)	Valproate Urbanyl Lacosamide Perampanel (unchanged at both visits)
P9	Interstitial	44	68	No	N/A
P10	Interstitial	161	185	No	N/A

N/A not available. Dosages were not available for all the medications listed, hence not included in the table

Data collection for the described studies spanned several years, and data were processed at two different sites (site 1: Hoffmann-La Roche, Ltd., Basel, Switzerland; site 2: UCLA, Los Angeles, USA) with two different data processing pipelines. To confirm the consistency of the quantified biomarker between different analytic approaches, we compared beta power and peak frequency estimates from data that were processed through both pipelines. Results, as shown in the results section, showed strong reproducibility between analytic pipelines and encouraged us to use the same descriptions of EEG variables ("beta power" and "beta peak frequency") for output

from both pipelines despite differences in processing (see Results section for details). Of note, processing methods remained consistent within each study aim (i.e., data from different pipelines were not mixed within one analysis).

For studies 1 and 2 (relation to clinical and behavioral features), EEG data were analyzed at site 1 as an extension of recent work elucidating the mechanism of the EEG biomarker (Frohlich, Reiter, et al. 2019) using a combination of in-house tools and the MATLAB software toolbox Fieldtrip (Oostenveld et al. 2011). EEG signals were bandpass filtered 1 – 45 Hz using a finite impulse response filter (FIR). Sections of data containing gross artifacts and noisy channels were identified by visual inspection and excluded from analysis. Next, noisy channels were marked bad and excluded from subsequent independent component analysis (ICA), a statistical blind source separation technique, was implemented to remove physiological artifacts including eye blinks, saccades, ballistocardiogram, and muscle activity, using the Fast ICA algorithm (A 1999; Jung et al. 2000). Finally, rejected channels were spatially interpolated and data were re-referenced to average (in all studies, datasets were discarded when the number of bad channels exceeded the square root of the total number of channels). To derive spectral power estimates, logarithmically scaled frequencies with a spectral smoothing using Morlet wavelets were employed (2 to 45 Hz, 12 wavelets per octave) (Tallon-Baudry et al. 1997). Power was then averaged across successive overlapping temporal windows of continuous clean data after discarding time-points corresponding to artifacts. Next, power was normalized with respect to the $\log_2$(frequency), resulting in a power spectral density (PSD) with units of μV2/log2(Hz) (i.e., power per octave) rather than μV2/Hz, thus accounting for the logarithmic nature of electrophysiological signals (Buzsaki and Draguhn 2004). We reported beta power using trapezoidal integration in the 12 – 30

Hz band (MATLAB: trapz, absolute power integrated with respect to $\log_2$(frequency)). For further details of data processing see Frohlich and colleagues 2019 (Frohlich, Reiter, et al. 2019)

For studies 3 and 4, a separate data processing pipeline was applied using the EEGLAB software toolbox (Delorme and Makeig 2004) for MATLAB. In this pipeline, data were FIR filtered 1– 45 Hz. Sections of data containing gross artifacts and noisy channels were identified by visual inspection and excluded from analysis. Data were interpolated to a 25-channel montage before using ICA (infomax algorithm; EEGLAB: "runica") to remove physiological artifacts. Data were then re-referenced to an average of all channels. For each electrode, PSDs were computed according to Welch's method (Frohlich et al. 2016; WELCH 1967), with power normalized per Hz (yielding μV2/Hz). Beta power was reported as the sum of the absolute power in the 12 – 30 Hz band.

In order to extract peak frequencies within the beta band, power spectra computed using aforementioned methods were averaged across electrodes. Peak labeling was performed automatically using the local maximum in the beta band, as well as manually using visual inspection. Manual labeling was performed by two trained raters. Raters were blinded to epilepsy status. In instances of manual labeling, an average of the peak labeling values obtained from the two raters was used. For each participant, automatically and manually labeled peak frequencies were compared. When the automated peak labeling fell within 5% of the value of the manual peak labeling, values from automated labeling were used. Otherwise, values from manual labeling were used.

2.2.5. Data analysis

In order to ensure that there were no differences in the dependent variables between the two data processing methods, we compared recordings from the participants (n=8) that were processed using both pipelines, and intraclass correlation coefficients (ICC) were derived.

2.2.5.a. Studies 1 and 2: Relation to clinical and behavior features

To determine the relation between EEG and clinical features, simple linear regression models were performed using beta power and BPF as outcome measures, and duplication type, epilepsy status and age as separate predictors of beta power and BPF. Age was treated as a continuous variable, while duplication type and epilepsy were treated as binary variables. Next, to determine the relationship between EEG and behavior, variables of beta power and peak frequency were regressed on quantitative measures of cognition, as well as parent reported measures of social skills.

2.2.5.b. Studies 3 and 4: EEG stability and reproducibility

To evaluate stability of spectral power and peak frequency in the beta band across time points, ICCs were derived from EEG recordings of all participants with more than one research visit. To evaluate reproducibility of the EEG signature in Dup15q syndrome, we compared beta power between (a) high density research EEG recorded from participants while they were awake and resting, and (b) low-density clinical EEG collected for epilepsy monitoring, with data extracted from segments in which participants were awake and resting prior to entering sleep. Data from low density clinical EEG and high density research EEG was computed and compared through derived ICCs.

Because our studies were performed in parallel, with no primary analysis selected between the four, we did not correct for multiple comparisons within or across the studies. 95% confidence

intervals (CIs) and effect sizes (where applicable) are reported to allow the reader to better interpret the meaningfulness of each finding.

2.3. Results

The comparison of the two different pre-processing pipelines (site 1 and site 2) yielded an ICC of 0.93 (95% confidence interval: 0.67 - 0.99) for beta power and an ICC of 0.92 (95% confidence interval: 0.64 - 0.98) for BPF, indicating moderate to excellent correlation between the two data processing methods.

2.3.1. Study 1: Relation to clinical features (age, duplication type and epilepsy)

Neither beta power (R^2= 0.014, 95% CI: -0.41 - 0.19, p=0.47), nor peak frequency (R^2=0.005, 95% CI: -0.24 - 0.37, p=0.65) correlated with age (Figure 2.2A,B). There were no significant differences in beta power between duplication types (Figure 2.3A; $R^2=5 \times 10^{-5}$, 95% CI: -0.31 - 0.30, p=0.96). BPF did not differ significantly between duplication types. Individual beta band peaks derived from participants in the two duplication groups are shown in Figure 2.3B, and the average peak frequency for the isodicentric and interstitial duplication groups was 22.6 Hz and 23.1 Hz, respectively. Mean topographic distribution of power across the scalp at the mean peak frequency is shown for duplication type (interstitial, Figure 2.3C, isodicentric, Figure 2.3D). Both duplication types showed characteristics of excessive beta oscillations similar to that found in previous work (Frohlich et al. 2016; Frohlich, Reiter, et al. 2019).

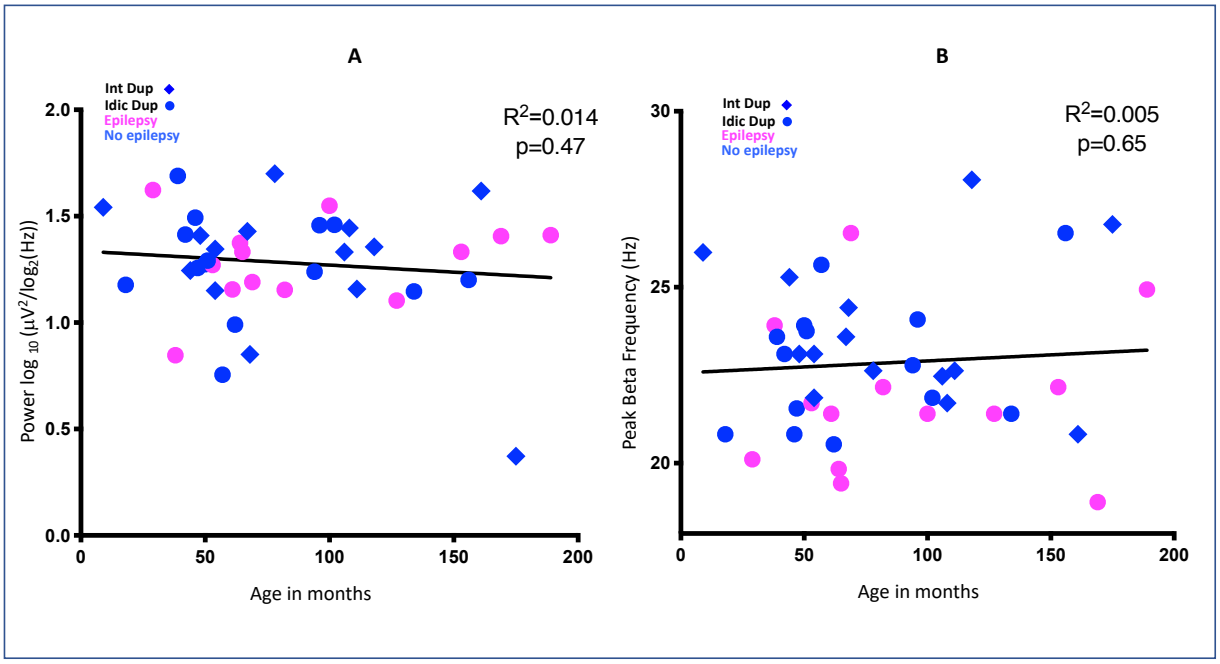

Figure 2.2. Age and beta power/beta peak frequency. *A) Age vs. beta power. B) Age vs. beta peak frequency. Participants with epilepsy are shown in pink and those without epilepsy in blue.*

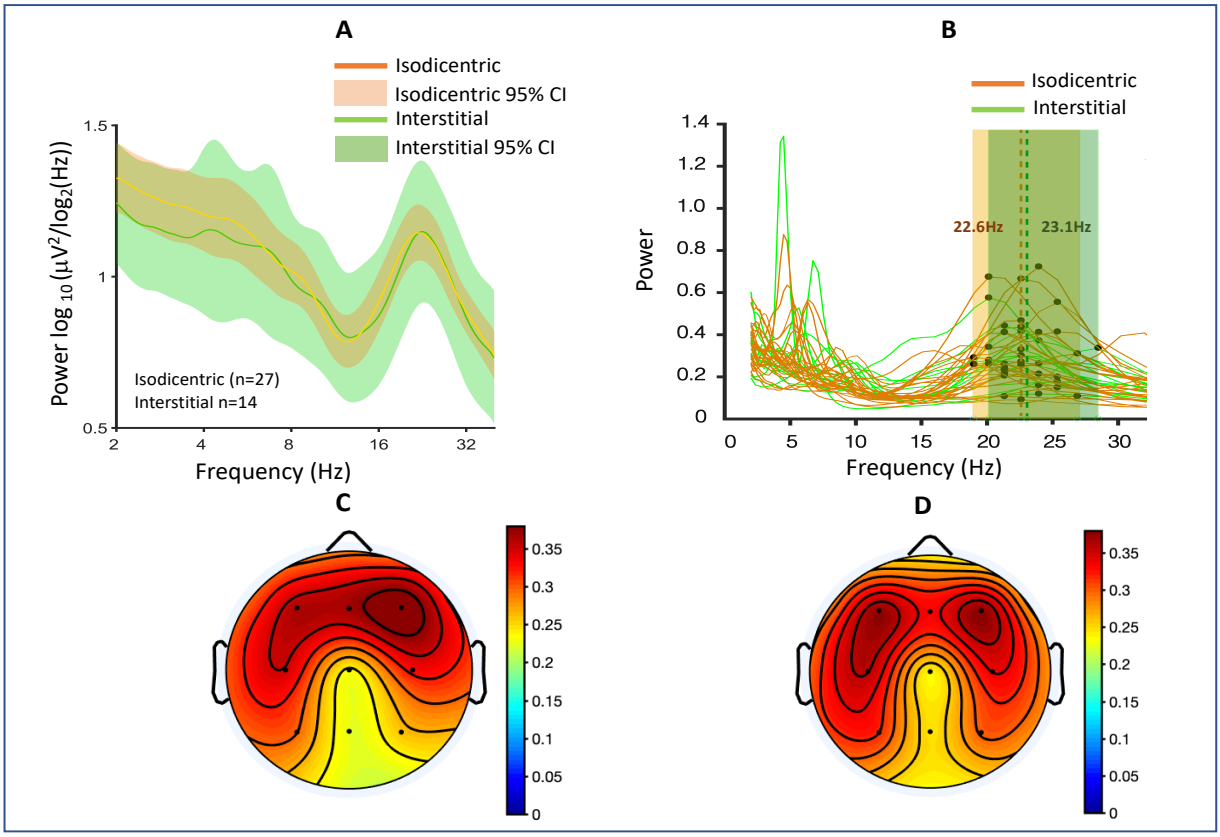

Fig. 2.3. Duplication type and beta power/beta peak frequency*. A) Spectral profiles of isodicentric (orange) and interstitial (green) duplication groups. PSDs are averaged across channels, log10 transformed, and then averaged across participants; colored highlights represent 95% confidence intervals. B) PSDs derived from isodicentric (orange) and interstitial (green) duplication groups. Beta peaks from each individual are labeled in black (group-level averages: isodicentric, f = 22.6 Hz; interstitial, f = 23.1 Hz.). C) Mean topographic scalp power (mean across participants at the group-level peak frequency, f = 22.8 Hz) for participants with interstitial duplications. D) Mean topographic scalp power (mean across participants at the group-level peak frequency, f = 22.8 Hz) for participants with isodicentric duplications.*

Beta power did not significantly differ between those with and without epilepsy (R^2=3 x 10^{-4}, 95% CI: -0.28 - 0.32, p=0.90, Figure 2.4A). However, BPF did significantly differentiate groups, with children with epilepsy showing a significantly lower peak frequency compared to those without epilepsy (correlation: R^2=0.11, 95% CI:-0.58 - -0.02, p=0.038; t-test: t=2.15, 95% CI: 0.08 - 2.86, d = 0.07). Mean topographic distribution of power across the scalp at the mean group-level peak frequency is shown for epilepsy and non-epilepsy groups in Figure 2.4C and Figure 2.4D. Individual peaks captured for each participant in the beta frequency range are shown in Figure 2.4B, with average peak frequency within the epilepsy and non-epilepsy groups being 21.8 Hz and 23.3 Hz, respectively.

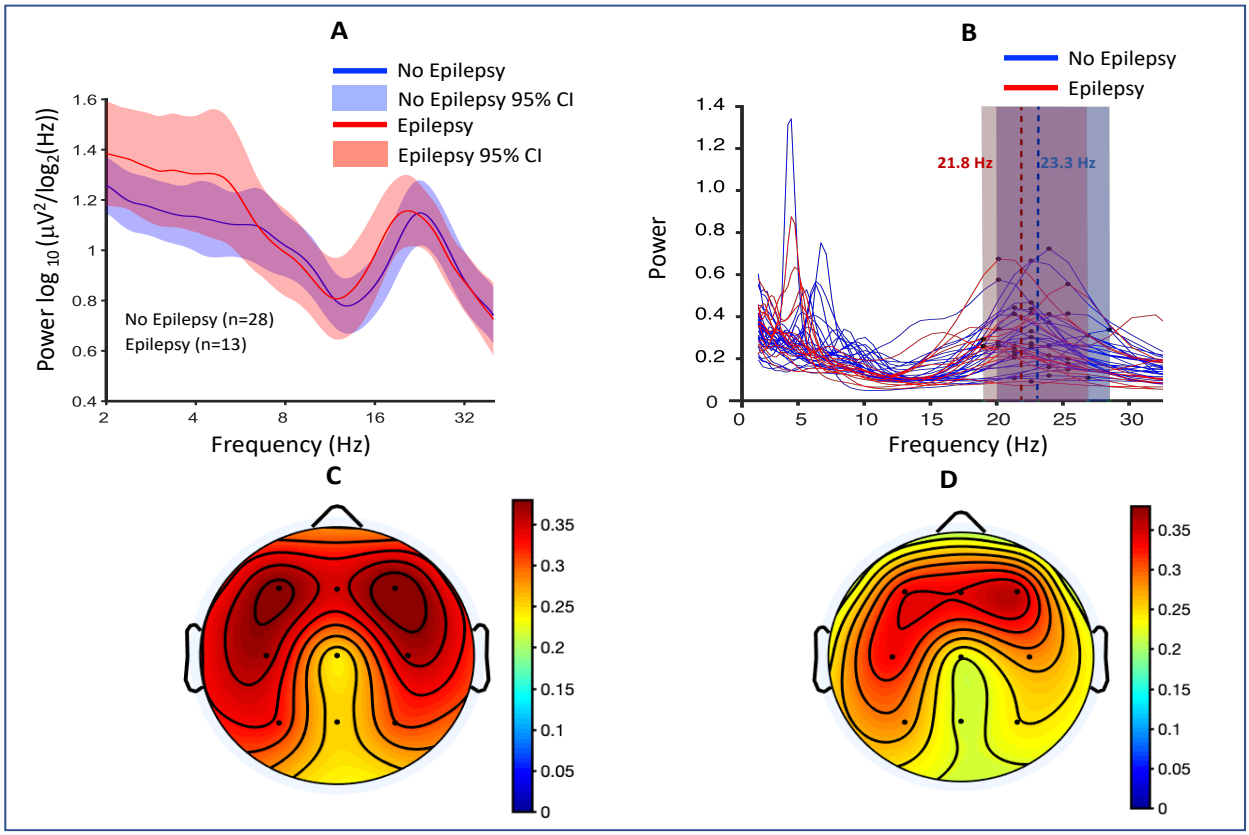

Figure 2.4. Epilepsy and beta power/beta peak frequency*. A) Spectral profiles of epilepsy (red) and non-epilepsy (blue) groups. PSDs are averaged across channels, log10 transformed, and then averaged across participants; colored highlights represent 95% confidence intervals. B) PSDs derived from epilepsy (red) and non-epilepsy (blue) groups. Beta peaks from each individual is labeled in black (group-level averages: epilepsy, f = 21.8 Hz; non-epilepsy, f = 23.3 Hz). C) Mean topographic scalp power (mean across participants at the group-level peak frequency, f = 22.8 Hz) in the epilepsy group. D) Mean topographic scalp power (mean across participants at the group-level peak frequency, f = 22.8 Hz) in the non-epilepsy group.*

2.3.2. Study 2: Relation to cognition and adaptive skills

Behavioral testing is summarized in Table 2.1. Regression models to investigate predictors of beta power revealed that verbal and non-verbal cognition, and DLS, were not predictors of beta power (VDQ: R^2=0.005, p=0.75, NVDQ: R^2=0.0002, p=0.94, DLS: R^2=0.0002, p=0.97).

We also performed regression models within the epilepsy and non-epilepsy groups separately and did not find meaningful associations (non-epilepsy group: VDQ: R^2=0.005, 95% CI: -0.34 – 0.46, p=0.75, NVDQ: R^2=0.0002, 95% CI: -0.42 – 0.39, p=0.94, DLS: R^2=0.024, 95% CI: -0.53 – 0.26, p=0.46; epilepsy group: VDQ: R^2=0.17, 95% CI: -0.21 – 0.80, p=0.18, NVDQ: R^2=0.11, 95% CI: -0.30 – 0.76, p=0.29, DLS: R^2=0.11, 95% CI: -0.30 – 0.76, p=0.30) (Figure 2.5A-C).

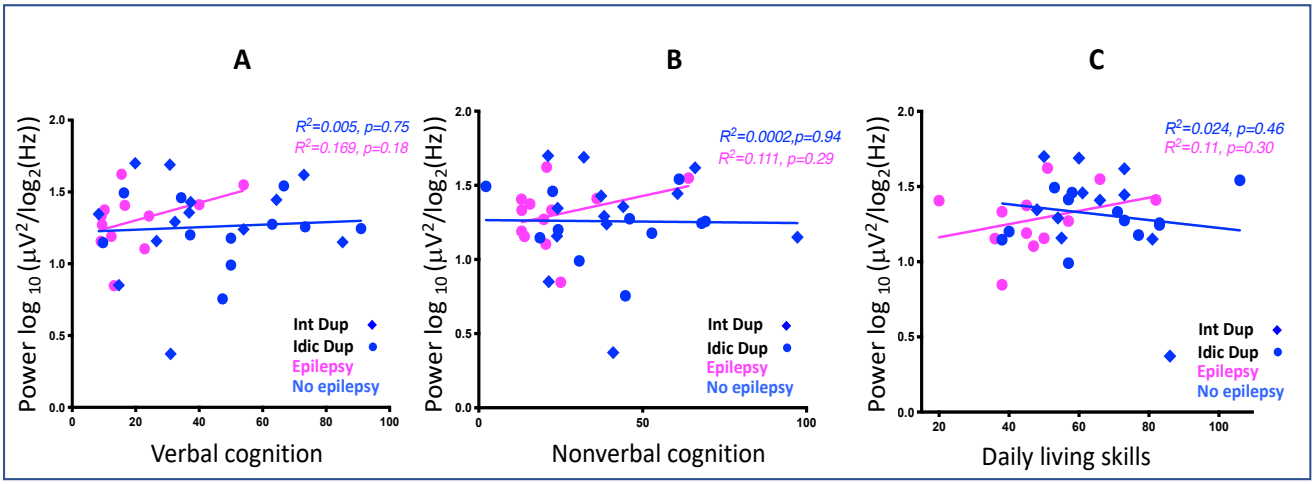

Figure 2.5. Cognition, daily living skills, and beta power*. Beta power vs. verbal (A) and non-verbal cognition (B), and DLS (C). Participants with epilepsy are shown in pink and those without epilepsy in blue.*

Neither verbal nor non-verbal cognition predicted BPF within the Dup15q syndrome cohort (VDQ: R^2=0.049, p=0.19, NVDQ: R^2=0.055, p=0.17). Upon performing regression models within the epilepsy and non-epilepsy groups separately, we found no significant relationship within the non-epilepsy group (non-epilepsy group: VDQ: R^2=0.002, 95% CI: -0.36 – 0.44, p=0.82, NVDQ: R^2=0.012, 95% CI: -0.31 – 0.49, p=0.61; epilepsy group: VDQ: R^2=0.035, 95% CI: -0.43 – 0.69, p=0.56, NVDQ: R^2=0.024, 95% CI: -0.46 – 0.67, p=0.63) (Figure 2.6A-B). A moderate correlation between BPF and measure of DLS was seen, in that participants with lower adaptive skills had significantly lower peak frequency (R^2=0.166 95% CI: 0.09 – 0.65, p=0.01). This relationship may be driven by individuals who have epilepsy, as participants with epilepsy have lower DLS scores

and lower BPF [epilepsy group: mean DLS score = 47.9, mean BPF = 21.8Hz] compared to those in the non-epilepsy group [non-epilepsy: mean DLS score = 65.0, mean BPF = 23.0Hz]. Nonetheless, BPF accounted for a similar proportion of DLS variance in the epilepsy subgroup as in the overall cohort (epilepsy group: R^2=0.17, 95% CI: -0.21 – 0.79, p=0.18; non-epilepsy group: R^2=0.08, 95% CI: -0.13 – 0.61, p=0.19), suggesting a correlation within the epilepsy subgroup that we were underpowered to detect (Figure 2.6C).

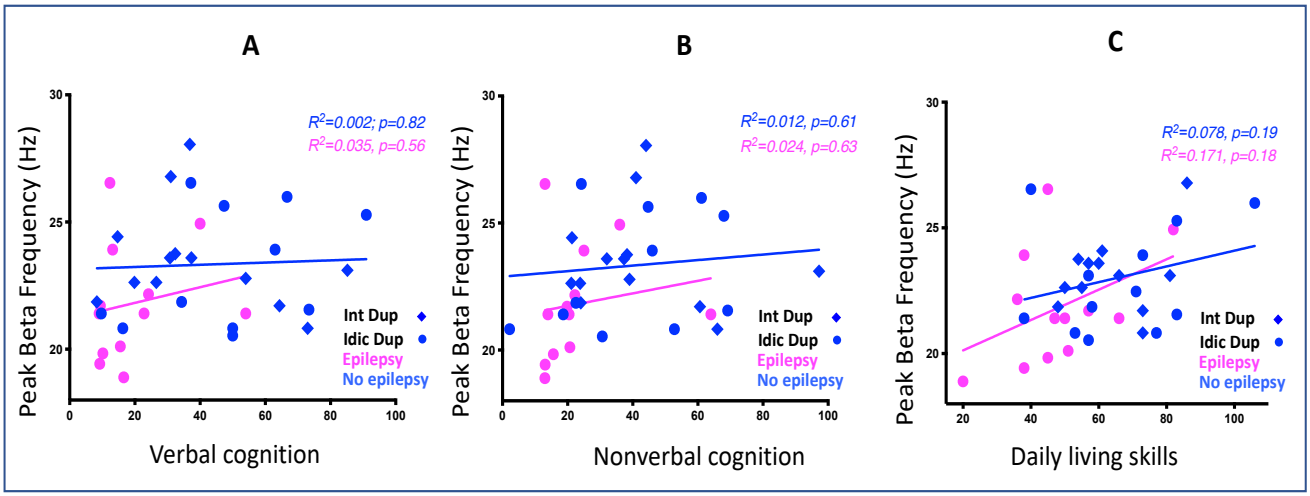

Figure 2.6. Cognition, daily living skills, and beta peak frequency*. A, B) Beta peak frequency vs. verbal and non-verbal cognition. C) Beta peak frequency vs. DLS. Participants with epilepsy are shown in pink and those without epilepsy in blue.*

2.3.3. Study 3: Stability over time

The ICC derived from beta power from participants that had at least two EEGs was 0.93 (95% CI: 0.63 - 0.98). ICC derived from peak beta frequencies from the same participants was

0.92 (95% CI: 0.64 - 0.98), indicating moderate to excellent stability of both beta power and BPF across multiple EEG recordings (Figure 2.7). Since two out of the ten participants developed epilepsy between visits, they were excluded from the ICC analysis.

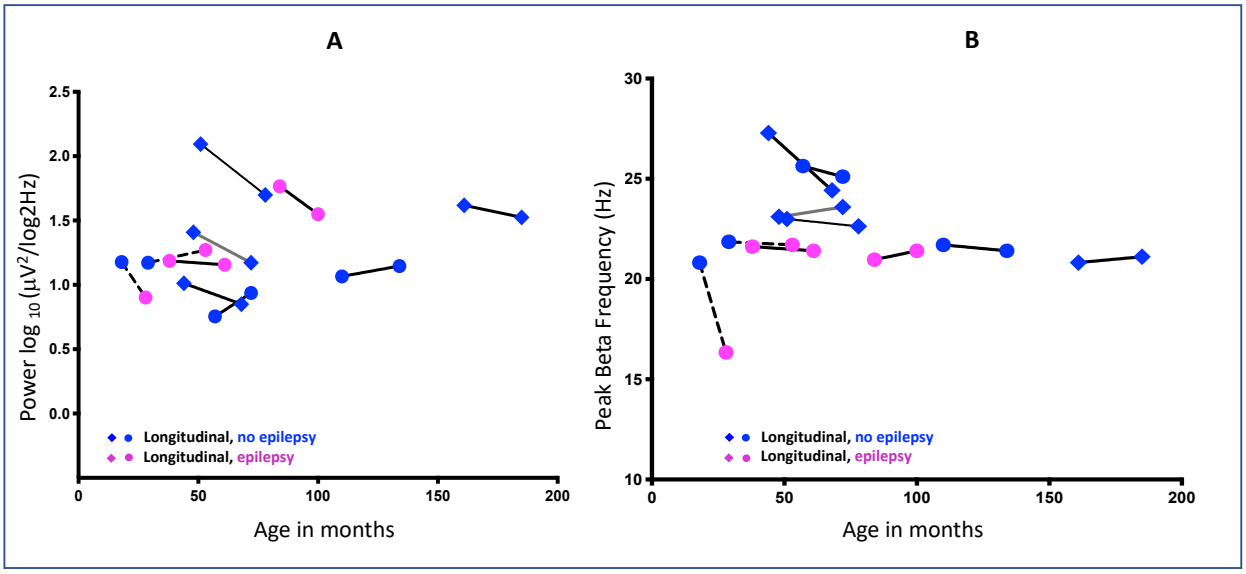

Figure 2.7. Longitudinal beta power and beta peak frequency*. A-B) Scatter plots of channel-averaged beta power and BPF derived from participants across multiple visits. Participants with epilepsy are shown in pink and those without epilepsy in blue. Longitudinal visits are connected by lines. Data connected with dotted lines represent participants that developed epilepsy between visits.*

2.3.4. Study 4: Reproducibility from research to clinical EEG

The ICC derived from spectral power values computed from participants with high density research and low density clinical EEG recordings was 0.94 (95% confidence interval: 0.60 - 0.98),

indicating moderate to excellent reproducibility of the biomarker from research to clinical recordings. Figure 2.8 shows PSD plots of research EEGs (top row) and clinical EEGs (bottom row) of two representative participants.

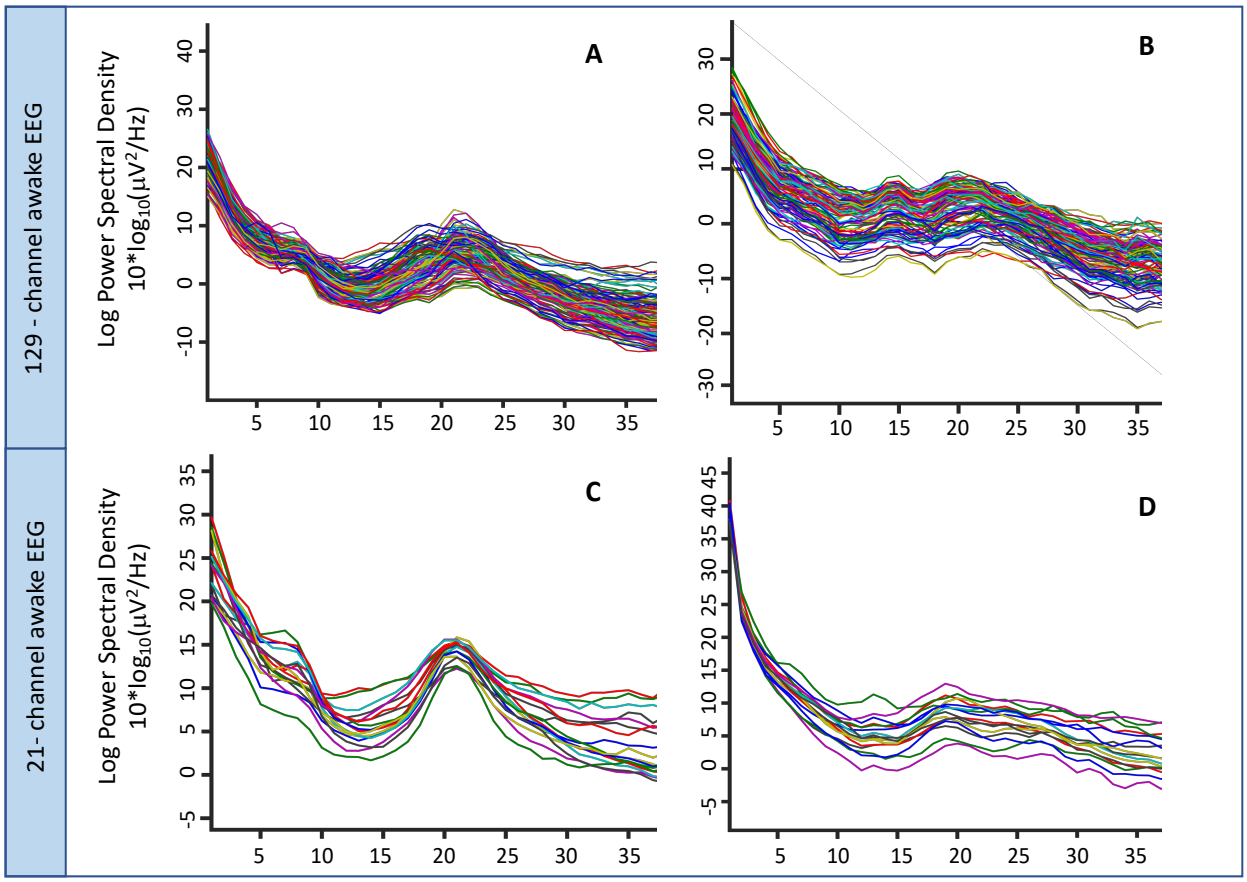

Figure 2.8. High- vs. low-density EEG PSDs. *PSDs derived from research EEG (top row) and clinical EEG (bottom row) from two participants are shown. a, c PSDs from research and clinical EEG respectively of first participant. b, d PSDs from research and clinical EEG respectively of second participant. Individual channels are shown in different colors.*

2.4. Discussion

Elevated beta band oscillations represent a robust, easily measurable biomarker of Dup15q syndrome, a genetic variant highly penetrant for NDDs and a promising target for future disease modifying therapies. Here, we extended our quantification of this biomarker by testing properties that would inform its use in future trials, namely its relation to clinical and behavioral features, stability over time, and reproducibility across data collection systems and analytic pipelines. Key results included: (1) differences in BPF between those with and without epilepsy, (2) stability over time based on consistency of signal between two EEG recordings at least 10 months apart, and (3) reproducibility between research recordings and clinical recordings obtained as part of a child's routine epilepsy monitoring, as well as reproducibility across two analytic pipelines that employed different frequency transforms and normalization.

2.4.1. Beta oscillations in Dup15q syndrome

Spontaneous beta oscillations typically observed in human EEG reflect a state of cortical activation (Barry et al. 2009) through a network of inhibitory interneurons and pyramidal cells (Jensen et al. 2005). The period (and thus frequency) of neural oscillations is determined in part by the time constants on postsynaptic receptors (i.e., faster time constants yields faster oscillatory frequencies), as is known to be true of gamma oscillations and $GABA_AR$s (Buzsaki and Wang 2012). To this effect, barbiturates such as phenobarbital and pentobarbital increase the duration of $GABA_AR$ channel opening (Barker and McBurney 1979; Study and Barker 1981), as does zolpidem, a benzodiazepine-like compound (described as a "benzodiazepine agonist" in older literature) (Mody et al. 1994; Shimono et al. 2000). Moreover, benzodiazepines decrease the frequency of beta oscillations (Shimono et al. 2000; Jensen et al. 2005), lending plausibility to the idea that increases in beta power observed with both pharmacological $GABA_AR$ modulation and

15q duplication result from shifting of fast oscillations towards a resonate frequency (i.e., the frequency the system prefers to oscillate at when energy is added) in beta, thus explaining the very large amplitude of beta seen in both contexts. This mechanism is highly speculative, and it remains unknown how it would interact with other factors (e.g., epilepsy and receptor properties altered by antiepileptic medication). Nevertheless, based on the hypothesized role of $GABA_AR$s in the generation of these oscillations, this EEG biomarker could enrich clinical trials by serving as a measure of drug target engagement or as a proximal outcome measure that precedes behavioral responses to pharmacological treatments that modulate GABAergic neurotransmission.

2.4.2. Beta oscillations and relationship to phenotype

We found no significant relationship between beta parameters and age, demonstrating that beta power and frequency are likely readouts of the fundamental disease pathology in Dup15q syndrome, that is unchanged with development. There is increased interest in identifying biomarkers of NDDs that relate to clinical symptomatology. We found that the strongest clinical predictor of the EEG signature in Dup15q syndrome was epilepsy, as BPF differed based on epilepsy status.

As in many syndromic NDDs, epilepsy in Dup15q syndrome is associated with greater functional impairment (Conant et al. 2014). We urge caution in not overinterpreting the relationship between epilepsy and beta oscillations, as without preclinical models to manipulate the underlying altered circuitry, we will not be able to prove directionality of the association. Epilepsy (both active seizures and interictal epileptiform EEG activity) causes significant changes in the EEG, such as slowing of background oscillations and spike/wave discharges, and these changes have been demonstrated in overnight EEG studies of patients with Dup15q syndrome (Arkilo et al. 2016). Antiepileptic medications also change oscillatory activity, with some

medications, such as $GABA_AR$ PAMs, causing further elevation of high frequency oscillations, while others, such as phenytoin or carbamazepine, increasing generalized cortical slowing. If and how seizures and/or their treatments slow the $GABA_AR$ time constant to produce a slower beta frequency is not fully understood. This question may be best addressed in preclinical models, in which various aspects of the circuits can be directly manipulated. However, from a clinical perspective, this association has tremendous promise, and future studies will assess whether BPF serves as a predictor of epilepsy early in development or as an informative marker of response to anti-epileptic drug studies.

We found no correlation between duplication type and beta power or BPF. Beta oscillations are an emergent network property that may non-linearly increase and then saturate based on GABA receptor expression. Therefore, it is feasible that subtle variability in overexpression may not lead to further increase or change in beta power. In addition, from a biophysical standpoint, there also may be an upper ceiling for beta power. The short duration of the beta cycle (33 – 83 ms) limits the number of neurons that can be recruited for the oscillation, thus putting a practical limit on the spectral power of beta oscillations (Buzsaki 2006); this is indeed the same reason that faster EEG oscillations generally show lesser power and spatial extent than slower oscillations. Future studies with computational models backed by experiments will be needed to assess changes of individual cellular biophysical parameters to network oscillation.

We found no correlation between behavior and beta power or BPF in Dup15q syndrome. Although BPF was associated with adaptive skills, this relationship is likely driven by epilepsy status, as children with epilepsy have lower overall adaptive skills compared to those without epilepsy (Berg et al. 2013; Papazoglou, King, and Burns 2010; Villarreal et al. 2014; Chapieski et al. 2005). It is possible that our behavioral measures lack sufficient sensitivity to capture the range

of clinical variability in individuals with Dup15q syndrome. However, our results build on findings in Angelman syndrome showing that children with 15q11-q13 deletions (deletion Angelman) have lower beta (23 Hz) power than children with other etiologies that principally affect *UBE3A* (nondeletion Angelman) (Frohlich, Miller, et al. 2019). In fact, deletion and nondeletion Angelman differ not only in beta power but also in clinical severity (Moncla et al. 1999; Lossie et al. 2001; Gentile et al. 2010; Minassian et al. 1998). Given that beta power and clinical severity covary in Angelman, it is possible that a similar relationship exists in Dup15q syndrome, but that either the severity of cognitive impairment in this population or other limitations to the psychometric properties of the tests used in this sample may limit our ability to capture subtle relationships between this biomarker and behavior. However, this lack of correlation with behavior does not undermine its potential for use as a marker of drug target engagement in clinical trials, as here we did not test whether change in beta oscillations predict or relate to change in clinical outcomes.

2.4.3. Stability and reproducibility of the EEG biomarker

Our data show stability across multiple recordings and reproducibility across data acquisition (research vs clinical EEG) methods. A fundamental question addressed by our study was whether this biomarker could be quantified from low-density clinical recordings performed outside of research study setting, of particular importance given that most of these children undergo clinical EEGs regularly as part of their clinical monitoring. One of the biggest challenges in EEG studies in NDDs is data collection itself (i.e., bringing in participants to sites to collect the research EEG). Future efforts that would bypass data collection in an expensive, structured research setting and quantify beta power and frequency in a repository of clinical EEGs would allow additional analyses to be far more clinically relevant, scalable, and statistically powered. In

fact, our study has already motivated these analyses in an ongoing clinical trial for epilepsy in Dup15q syndrome, with beta power being quantified through clinical EEG collected at baseline.

2.4.4. Biomarkers in neurodevelopmental disorders

Research in preclinical models has truly advanced our insights into the pathological mechanisms underlying various NDDs. Despite this knowledge, even presumably well-designed clinical trials have struggled to demonstrate significant effects in treatment groups compared to placebo (Erickson et al. 2017; Berry-Kravis et al. 2018). These challenges may reflect several gaps: 1) even when the treatment has a clear biological, mechanistic target, lack of measurement of drug target engagement limits the ability to determine if a drug could have an effect, 2) in the setting of the biological and developmental heterogeneity of these conditions, there are few objective methods for patient selection for trials, 3) outcome measures themselves often prove ineffective to capture the effect of a treatment because of insensitivity to short term change or vulnerability to reporting bias or placebo effect, and 4) outcome measures chosen based on preclinical research do not translate to meaningful or modifiable patient-centered outcomes. The identification and quantification of objective biomarkers can mitigate some of these challenges by facilitating patient stratification, measuring drug target engagement, and defining outcomes relatively resistant to the placebo effect.

As EEG can measure circuit-level treatment response before behavior changes can be observed, EEG biomarkers have the potential to address the challenges involved in pharmacological trials (Berry-Kravis et al. 2018). To that end, this study holds promise in identifying EEG biomarkers in a rare genetic population highly penetrant for NDDs. The hypothesis that we can quantitatively measure the impact of altered GABA signaling is particularly exciting in the field of NDDs, as dysfunction in the dynamics of cortical GABAergic circuitry may

be implicated in syndromes other than Dup15q syndrome. Furthermore, converging evidence from gene linkage studies suggest that point mutations in the $GABA_A$ β3/α5/γ3 gene cluster may also be implicated in other NDDs (Buxbaum et al. 2002; Cook et al. 1997; Menold et al. 2001). It is possible that elevated beta oscillations in other individuals with NDDs may herald other genetic causes of altered GABA neurotransmission, such as point mutations in the $GABA_A$ β3/α5/γ3 gene cluster. Future studies can therefore leverage existing electrophysiological data from children with NDDs and explore the utility of the EEG biomarker in Dup15q syndrome to predict genetic variants in children with NDDs and further our understanding of underlying circuit level pathology in a subset of these children.

2.5. Limitations, conclusions and future directions

Our work herein established the robustness and reproducibility of the EEG beta phenotype as a biomarker of Dup15q syndrome. Studies of rare genetic disorders are often limited by small sample number, and we also faced these sample size challenges, particularly in the studies of stability over time and reproducibility. Findings from our reproducibility study have led to the development of a new data acquisition and storage pipeline for clinical overnight sleep EEG recordings of children with Dup15q syndrome across the world, in partnership with the Dup15q Alliance. We will utilize these recordings to investigate the presence of beta oscillations in sleep EEG and to characterize sleep physiology in children with the syndrome. As more children with syndromic forms of NDDs undergo clinical EEG investigation, this pipeline will directly guide decisions to replace research EEG recordings with clinical ones, thereby facilitating larger scale studies of EEG biomarkers across syndromic NDDs.

Chapter 3: Abnormal sleep physiology in children with Dup15q syndrome

3.1. Introduction

Neurodevelopmental disorders (NDDs), such as autism spectrum disorders (ASD), intellectual disability (ID) and attention deficit-hyperactivity disorder (ADHD), affect 1-2% of the general population. Sleep problems are highly prevalent in NDDs (Lord 2019; Angriman et al. 2015), with 50-95% of children meeting criteria for a sleep disorder at a behavioral level (Polimeni, Richdale, and Francis 2005; Vaidyanathan, Shah, and Gayal 2016; Esbensen and Schwichtenberg 2016). Healthy sleep physiology plays an essential role in overall health and cognitive development (Krause et al. 2017; Stickgold 2013; Maski et al. 2015; Limoges et al. 2013; Tham, Schneider, and Broekman 2017; Becker et al. 2017), and sleep micro- and macrostructures -- particularly spindles and slow wave sleep (SWS), are critical for learning, memory consolidation and overall intellectual ability (Hahn et al. 2018; Diekelmann and Born 2010; Walker 2009; Fogel and Smith 2011; Manoach and Stickgold 2019). Abnormal spindle number and morphology are associated with epilepsy, as well as with neurodevelopmental and neuropsychiatric disorders (Ferrarelli and Tononi 2017; Wamsley et al. 2012; Limoges et al. 2005; Tessier et al. 2015; Christensen et al. 2014; Fernandez and Lüthi 2020). Deficits in SWS have been reported in genetic forms of NDDs such as Rett syndrome (Ammanuel et al. 2015; Johnston et al. 2014), as well as in nonsyndromic ASD (Arazi et al. 2020). Results on sleep microarchitecture in ASD, however, have been inconsistent likely due to the heterogeneity of the condition and differences in analytic techniques.

Maternally derived duplications of chromosome 15q11.2-13.1 collectively represent one of the most common copy number variants associated with NDDs (Sanders et al. 2015; Abrahams

and Geschwind 2008) and result in a clinical syndrome (Dup15q syndrome) that includes delays across developmental domains and epilepsy (Conant et al. 2014; Finucane et al. 2016; Frohlich et al. 2016; Urraca et al. 2013). Two primary duplications result in the syndrome: an interstitial duplication or trisomy, resulting in one extra copy of the 15q region that lies on the same chromosome arm, or an isodicentric duplication, resulting in two extra copies of the region on a supernumerary chromosome (Finucane et al. 2016). Several genes within the 15q critical region are overexpressed in Dup15q syndrome, notably: 1) UBE3A, a gene that encodes a ubiquitin protein ligase which is imprinted in neurons (Albrecht et al. 1997; Yamasaki et al. 2003) and regulates synaptic development and function (Dindot et al. 2008; Greer et al. 2010; Miao et al. 2013; Smith et al. 2011), and 2) a cluster of gamma-aminobutyric acid type A receptor ($GABA_AR$) genes, *GABRB3*, *GABRA5*, and *GABRG3,* which encode the β3, α5 and γ3 receptor subunits, respectively. Several studies have shown that both in humans and in mouse models, mutations in the $GABA_AR$ genes result in autism and epilepsy phenotypes (Chen et al. 2014; DeLorey et al. 1998; Mesbah-Oskui et al. 2017; Moller et al. 2017; Nakatsu et al. 1993).

On awake electroencephalography (EEG), children with Dup15q syndrome demonstrate increased beta band oscillations that distinguish them from typically developing children as well as from those with non-syndromic ASD (Frohlich et al. 2016; Saravanapandian et al. 2020; Frohlich, Reiter, et al. 2019). This EEG signature resembles the pattern seen in patients taking benzodiazepines or other positive allosteric modulators of $GABA_A$ receptors, suggesting that this biomarker reflects aberrant GABAergic neurotransmission (van Lier et al. 2004; Hambrecht-Wiedbusch et al. 2010; Christian et al. 2015). As a follow up to the quantification of these abnormal awake EEG oscillations, we asked if sleep physiology also was affected in Dup15q syndrome. We quantified the following metrics from overnight clinical EEG recordings: 1) beta band oscillations,

2) spindle density and 3) percentage of SWS and compared them with age-matched neurotypical (NT) controls. Disruptions in these sleep rhythms can significantly affect overall quality of life and functionality, while exacerbating the severity of existing developmental and cognitive problems associated with NDDs. We hypothesized that sleep physiology would be abnormal in children with Dup15q syndrome, and that this finding could lay the foundation for future investigation of the relationship between sleep EEG and cognition and the identification of potential pharmacological targets to improve not only sleep, but overall neurodevelopmental outcomes, in this syndrome.

3.2. Subjects and Methods

3.2.1. Study participants

The study consisted of 27 participants. Overnight clinical EEG data on 25 participants were collected at the University of California, Los Angeles (UCLA) Ronald Reagan Medical Center, and overnight polysomnograms were collected from 2 participants at the Boston Children's Hospital (BCH). 15 recordings were from children with Dup15q syndrome, aged 9 months – 13 years, and 12 were from age-matched NT children, aged 7 months – 14 years. The average ages for children in the two groups did not significantly differ, with 5.69 yrs. in Dup15q syndrome and 5.78 yrs. in NT controls. Children with Dup15q syndrome had a confirmed genetic diagnosis of the syndrome and were either clinically referred through the Dup15q clinic at UCLA or recruited through the UCLA Intellectual Disability and Development Research Center (IDDRC). Participants from BCH were referred by a health care provider to the BCH Pediatric Sleep Laboratory for the clinical indication of restless sleep and concern of sleep disordered breathing and periodic limb movements of sleep. Details of age, sex, duplication type, epilepsy status,

frequency of spikes and medications for all children with Dup15q syndrome in the study can be found in Table 1. The NT control group included those that were admitted to UCLA for EEG evaluation in the setting of concerning spells that were not seizures, and they had no history or current diagnosis of a neurodevelopmental disorder or epilepsy.

Table 3.1. Dup15q syndrome participant characteristics

This table describes the characteristics of participants in the Dup15q syndrome cohort. Details on age, gender, epilepsy status and medications were extracted from participant background questionnaire, and duplication type was extracted from participant genetic reports. The percentage of sleep occupied by spike-waves was quantified as spike-wave index.

Age (months)	**Gender**	**Duplication type**	**Epilepsy (active)**	**Spike-wave index in sleep**	**Medications (generic)**
105	Female	Isodicentric	No	<35%	Risperidone Melatonin
23	Female	Isodicentric	No	<35%	None
108	Female	Isodicentric	No	<35%	None
18	Male	Interstitial	No	<35%	None
35	Male	Isodicentric	No	<35%	None
54	Male	Isodicentric	No	<35%	None
68	Female	Isodicentric	Yes	45-50%	Clobazam Topiramate

137	Male	Isodicentric	Yes	40-45%	Topiramate
73	Female	Interstitial	Yes	<35%	Lamotrigine Guanfacine
19	Male	Isodicentric	Yes	35-40%	Vigabatrin Prednisolone
57	Female	Isodicentric	Yes	<35%	None
9	Female	Isodicentric	Yes	<35%	Levetiracetam Phenobarbital
55	Male	Isodicentric	Yes	65-70%	None
108	Male	Isodicentric	Yes	<35%	None
156	Male	Isodicentric	Yes	40-45%	None

Dosages were not available for all the medications listed, hence not included in the table

3.2.2. EEG data acquisition

All overnight EEGs were retrospectively identified in accordance with the Institutional Review Board. EEGs from UCLA were acquired from the Pediatric Neurophysiology Laboratory at the UCLA Ronald Reagan Medical Center, and they were recorded at a sampling rate of 200Hz, utilizing a standard 10-20 montage, 21 channel gold disc electrode placement recording set up, on a Neurofax Polysmith DMS 11.0 Build 8093 with 921 amplifiers (Nihon Kohden America Inc, Irvine, CA). Data from BCH were recorded at a sampling rate of 200Hz using XLTEK PSG system

and Natus SleepWorks software (Natus Medical Inc., San Carlos, CA). Data were extracted and converted into European Data Format (EDF) for analysis.

3.2.3. EEG data processing and analysis

Overnight clinical EEGs were reviewed for timestamps, and the recording between approximately 10pm and 5am was extracted. In the absence of formal sleep staging, this window was selected in order to maintain a comparable duration of potential sleep epochs between the two groups. Therefore, about 7 hours of continuous overnight EEG recording was included for all participants, and the duration of the recording was not significantly different between the two groups (Dup15q, 7.03h vs. NT, 7.06h).

Raw EEG data were processed using the EEGLAB (Delorme and Makeig 2004) software toolbox for Matlab. Data were high-pass filtered at 1.0 Hz and low-pass filtered at 50 Hz with zero-phase FIR filters and forward-backward filtering. EEG channels with poor signal quality were automatically removed and interpolated with the following criteria: (1) spectral power between 1-50Hz that was three standard deviations above or below that of other channels, (2) channels with flat signals (i.e. zeros) longer than 5 seconds, (3) channels that were poorly correlated ($r<0.7$) with their reconstructed versions based on adjacent channels, (4) channels with line noise power four standard deviations higher than their signals, using *clean_rawdata()* function in EEGLAB. The interpolated EEG data were then re-referenced to common average reference.

The power line noise (i.e. 60 Hz) was further removed using CleanLine in EEGLAB (Bigdely-Shamlo et al. 2015). Artifact subspace reconstruction (ASR) was applied using *clean_asr()* function ($\sigma=20$) (Chi-Yuan Chang 2020) to automatically remove and interpolate non-stationary, high-amplitude bursts such as eye blinks, eye movement activity, possible complex epileptiform activity as well as motion artifacts. Independent component analysis (ICA) was

performed, and an automatic independent components (IC) classifier, ICLabel (Pion-Tonachini, Kreutz-Delgado, and Makeig 2019), was used to separate and label ICs into seven categories. The ICs labeled as muscle, eye, heart, line noise, and channel noise with probability higher than 0.5 were rejected. The final cleaned channel signals were reconstructed using the remaining ICs. Time-frequency analysis was performed for each channel of the cleaned overnight EEG using *spectrogram()* function in Matlab with a Hanning window of 60-sec and a 30-sec overlap. The mean power at beta (12-30 Hz) and delta (1-4 Hz) band oscillations were further obtained for each epoch.

3.2.3.a. Spindle detection

Sleep spindles were quantified and visualized using YASA (Yet Another Spindle Algorithm), a Python-based toolbox for automated multi-channel spindle detection (Raphael Vallat May 2020). Spectral power in the spindle frequency range (11-16 Hz) was first obtained relative to the total power in the broadband frequency (1-30Hz) for all channels, using a 2-second window with a 200-ms overlap. Only the windows in which 20% of the signals' total power was contained within the spindle frequency range were kept, in order to avoid false detection due to artifacts (Raphael Vallat May 2020). The selected windows of spindle activity were then reviewed for morphological features. Spindles that were less than 500 ms apart were merged, and those that were <0.5sec or >2.0sec in duration were eliminated. In order to avoid double counting, spindles detected with an initiation interval of <300 ms were considered to be a single event. Throughout overnight recordings, spindles were identified and quantified for multiple epochs of at least 2 minutes and spindle density was calculated as the number of spindles per minute for each epoch and averaged across epochs for each subject.

Spindles were also quantified manually by a board-certified pediatric epileptologist who blind to diagnosis and group status was. Spindles were visually identified and quantified for multiple epochs of at least 2 minutes, which were spread throughout the same hours of 10pm and 5am. Similar to the automated quantification approach, spindle density was calculated as the number of spindles per minute for each epoch and averaged across epochs for each subject.

3.2.3.b. Slow wave sleep analysis

Non-rapid eye movement (NREM) sleep cycles are categorized into stages including: N1, the lightest stage of sleep characterized by low-amplitude mixed frequency activity; N2, a deeper sleep stage characterized by the presence of spindles and K-complexes; and N3 or slow wave sleep (SWS), the deepest stage of sleep, characterized by the presence of slower frequency and high amplitude signals (delta waves, 1-4 Hz). Delta power (1 – 4Hz) was computed for every 30sec epoch of the cleaned overnight EEG. SWS was automatically identified as epochs with higher delta power in relative to baseline periods. Specifically, the baseline periods were identified as 50% of all 30-sec epochs in the sleep recording with the lowest delta power. The mean and standard deviation of the delta power over the baseline periods were computed. For the whole sleep recording, "high-delta" epochs were identified in which delta power was higher than one standard deviation of the mean baseline delta power. To avoid false positives (e.g., from sporadic motion artifacts), the final SWS periods excluded all the epochs with less than 32 consecutive high-delta epochs (i.e., 16 min) with methods based on a prior study (Ammanuel et al. 2015). The percentage of the amount of time spent in high-delta epochs during the entire night was defined as percent SWS for the study. SWS also was manually quantified based on scoring criteria from the American Academy of Sleep Medicine (AASM), defined by 0.5-2Hz slow waves of at least 75 μV occupying at least 20% of consecutive 30 second epochs (Medicine). The duration of each N3 sleep epoch

was calculated across the 7 hours of overnight EEG. Percentage of SWS was calculated as the total amount of time each subject spent in N3 sleep during the entire sleep period.

REM sleep was not evaluated in this study given that electrooculogram (EOG) leads were not placed for clinical EEGs, thus limiting our ability to definitively score this sleep stage according to AASM criteria. All statistical analyses were performed using GraphPad Prism 8 software. Student's *t* tests were used to compare spectral power, spindle density and percentage of SWS between groups. In all the figures, the asterisk indicates $p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $**** p < 0.0001$.

3.3. Results

3.3.1. Clinical evaluation of sleep architecture

Clinical evaluation of sleep EEGs indicated that children with Dup15q syndrome demonstrated progression through NREM sleep cycles N1 and N2. Excessive beta oscillations were identified throughout the sleep recordings in all the children with Dup15q syndrome but fluctuated between sleep stages, notably with a qualitative drop in stage N2. Vertex waves were observed in stages N1 and N2 (Figure 3.1A). Sleep spindles and K-complexes emerged in stage N2 (Figure 3.1B). Two children with Dup15q syndrome, aged 54 months and 156 months, demonstrated markedly abnormal sleep spindles -- the former with frequent hemispheric asynchrony of sleep spindles (Figure 3.1C), and the latter with poor spindle morphology and attenuated spindle voltages. However, not all children entered stage N3, as they did not meet AASM frequency criteria for slow waves. Of those who achieved SWS (Figure 3.1D), half demonstrated fewer N3 cycles and reduced aggregate duration of N3 compared to NT controls.

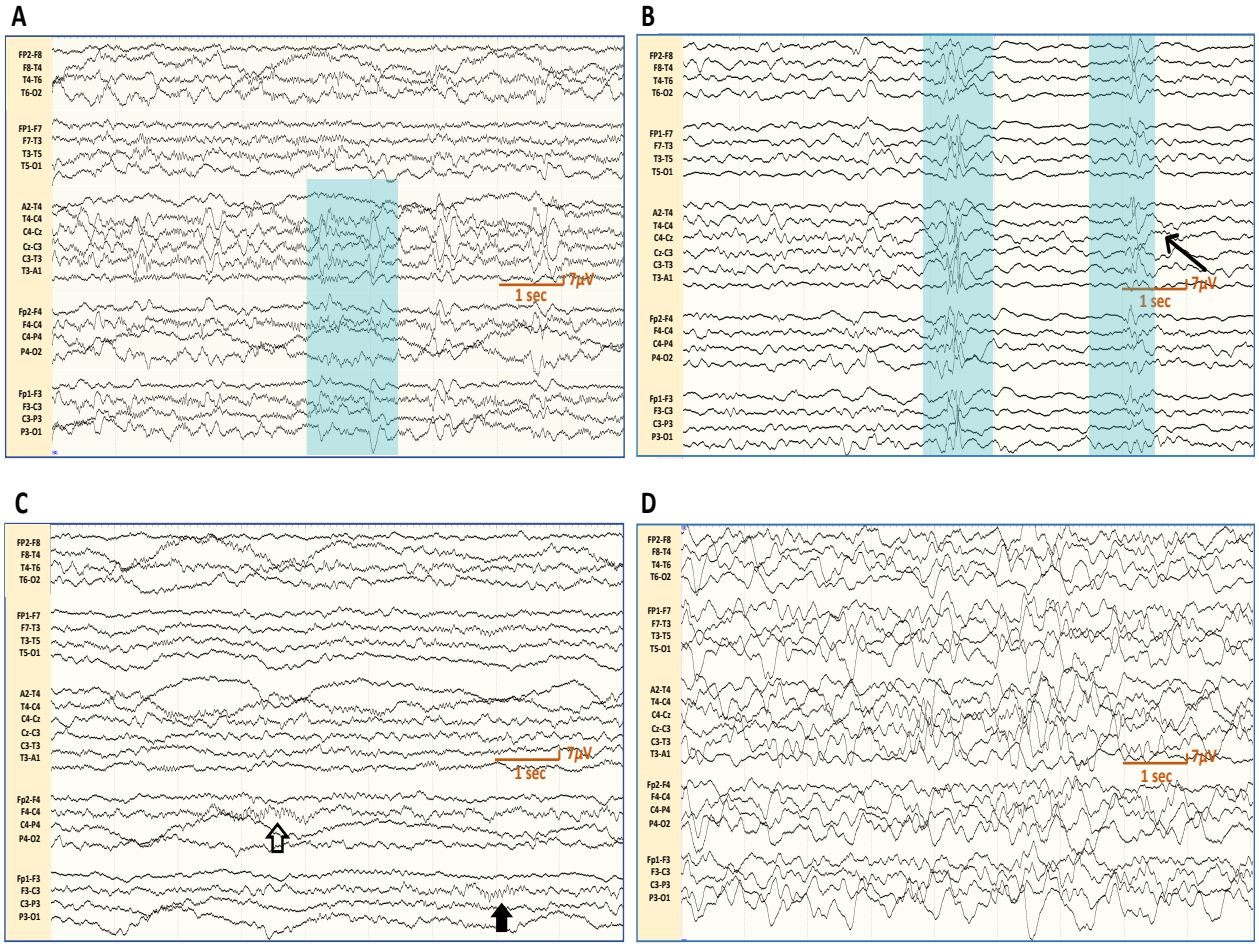

Figure 3.1: Sleep stages (N1 to N3) in children with Dup15q syndrome. *Representative 9-second traces of continuous sleep EEG recording from children with Dup15q syndrome depicting vertex waves (field highlighted by blue rectangle) during stage N1* ***(A)****, K-complexes (broad field highlighted by blue rectangles) during stage N2 juxtaposed with sleep spindles (arrow)* ***(B),*** *asynchronous spindles in the right (hollow arrow) and left (solid arrow) frontocentral electrodes during stage N2* ***(C)*** *and slow wave sleep during stage N3* ***(D)****.*

Epileptiform activity was noted in 9 children with Dup15q syndrome and in all of the children with dual diagnoses of Dup15q syndrome and epilepsy. Observed epileptiform activity varied widely between participants and included generalized spike-wave discharges; focal or multifocal spikes, sharp waves and spike-waves; and focal or generalized paroxysmal fast activity (PFA).

3.3.2. Beta oscillations in sleep

Time-frequency analysis of the overnight EEG recordings revealed that children with Dup15q syndrome had visible and quantifiable beta oscillations (12-30Hz) throughout sleep. Figure 3.2 shows examples of time-frequency plots from a child with Dup15q syndrome (Figure 3.2A) and a child in the NT control group (Figure 3.2B). Beta power was much higher and changed over time in the EEG recording from the child with Dup15q syndrome (Figure 3.2C) compared to the child in the NT control group (Figure 3.2D).

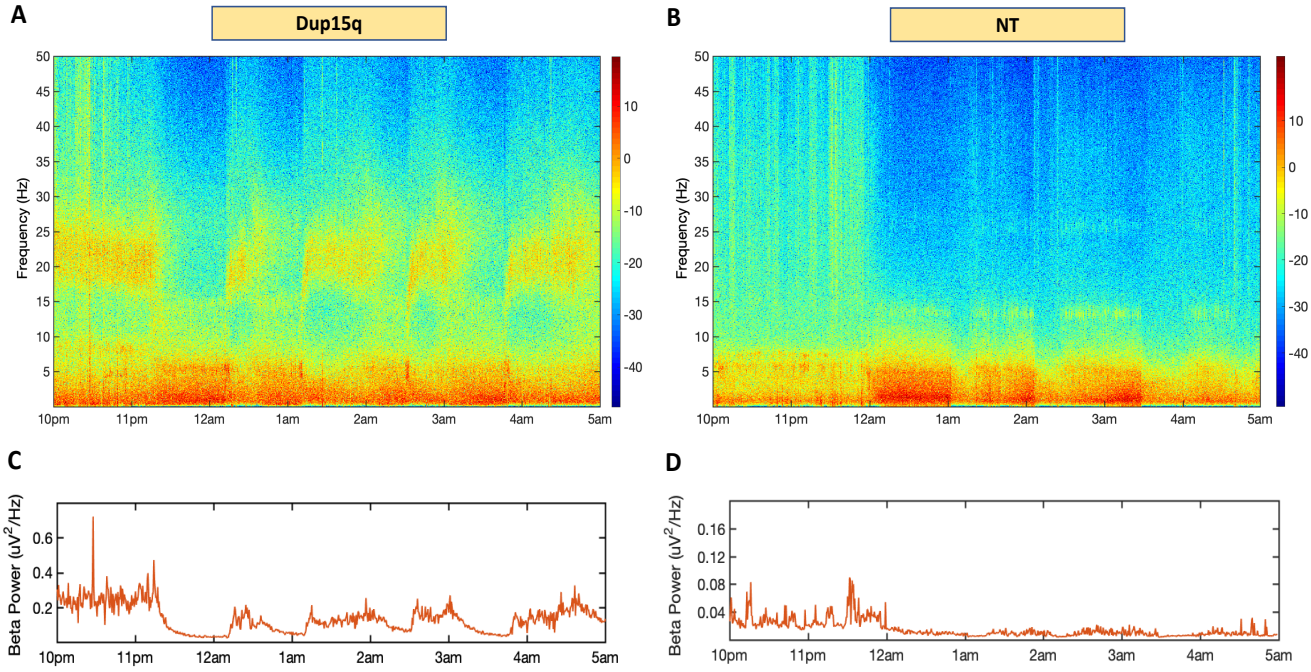

Figure 3.2**: **Persistent overnight beta oscillations in Dup15q syndrome.** Time-frequency plot derived from 7 hours of overnight sleep EEG from a 19-month old representative Dup15q syndrome participant **(A)** and a 19-month-old representative neurotypical (NT) participant **(B)**. Beta power (absolute power) dynamics plotted across the night in the 19-month-old participant with Dup15q syndrome **(C)** and in the 19-month-old NT participant **(D).

Mean beta power calculated across the overnight recording was significantly different between the groups. In all three spatial locations (Figure 3.3B), beta power was significantly higher (frontal: p=0.001; central: p=0.01; occipital: p=0.0009) in children with Dup15q syndrome compared to age-matched NT controls (Figure 3.3C). Within the Dup15q syndrome cohort, there were no differences in beta power based on the presence of epilepsy or between duplication types.

3.3.3. Sleep spindles

Both the automated spindle detection algorithm as well as manual spindle detection revealed that children with Dup15q syndrome had significantly fewer spindles ($p<0.0001$) compared to age-matched NT controls (Figure 3.4A-B). Spindle density did not correlate with age, and there were no differences in duration or amplitude of spindles between groups. Within the Dup15q syndrome cohort, there were no significant differences in spindle density based on the presence of epilepsy or between duplication types.

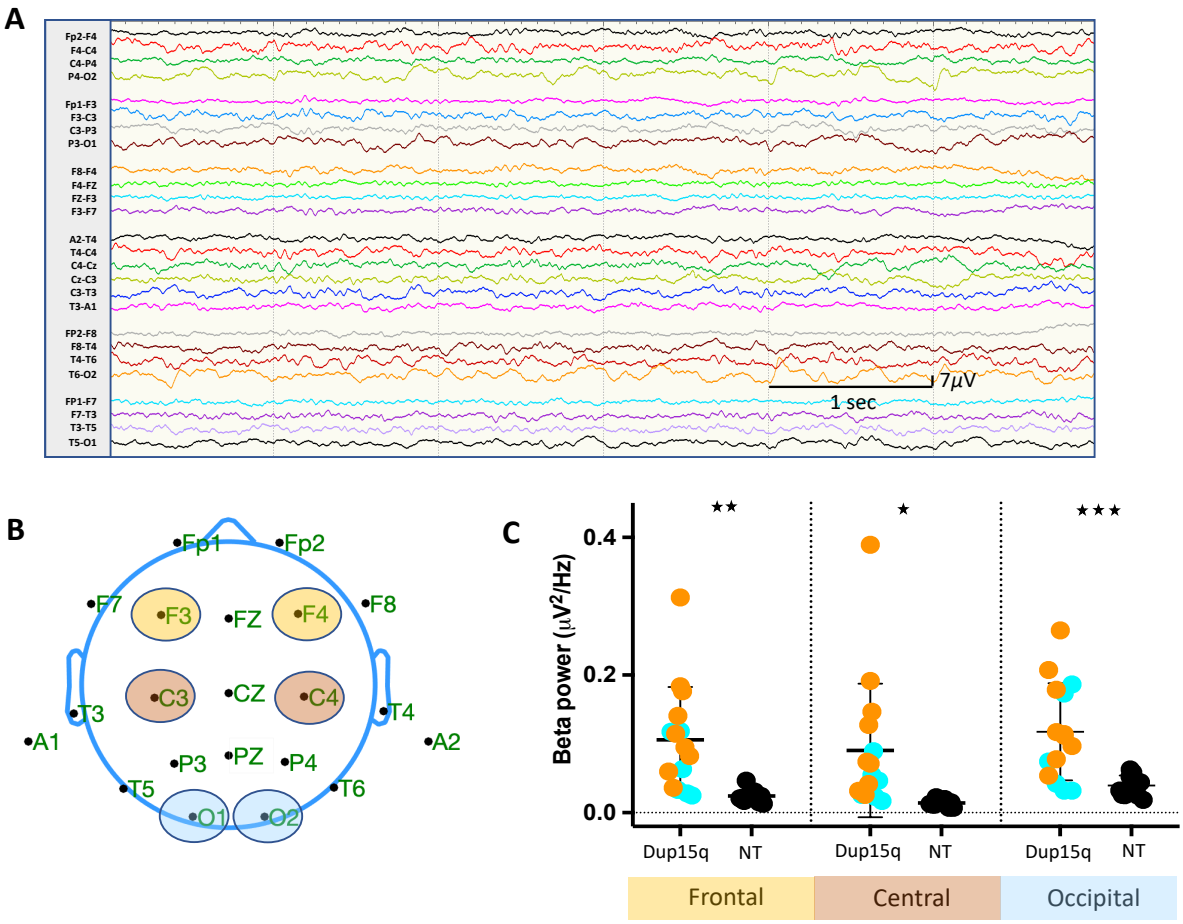

Figure 3.3. Elevated beta power in sleep in children with Dup15q syndrome*. 6 seconds of continuous sleep EEG recording from a 19th month old Dup15q participant* ***(A).*** *A scalp map showing standard 10-20 EEG electrode placements on the scalp, with channel groups of interest highlighted (frontal: yellow, central: red and occipital: blue)* ***(B).*** *Dot plots of absolute beta power (12-30Hz) averaged across overnight sleep EEG, in the Dup15q syndrome group (turquoise: participants with no epilepsy, orange: participants with epilepsy) and the NT group (black), plotted for each channel group* ***(C).***

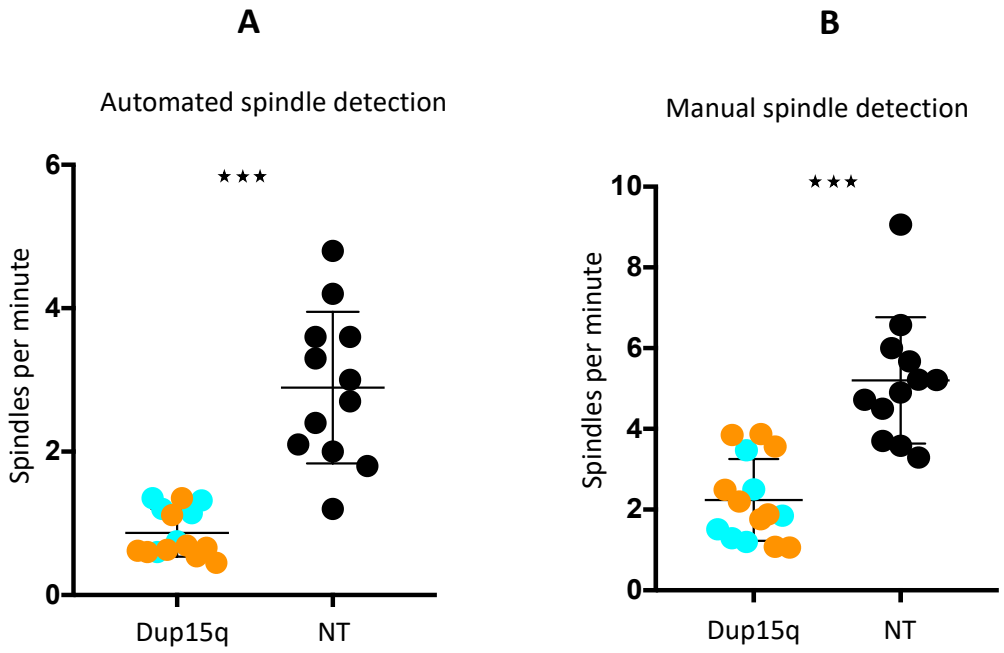

Figure 3.4. Reduced sleep spindle density in children with Dup15q syndrome. *Dot plots of average spindle density in participants in the Dup15q syndrome group (turquoise: participants*

*with no epilepsy, orange: participants with epilepsy) and the NT group (black), using automated spindle detection **(A)** and manual spindle detection **(B)** methods.*

3.3.4. Slow wave sleep

Quantitative analysis of SWS revealed markedly reduced SWS in children with Dup15q syndrome. There were significant group differences in the time spent in each discrete segment of SWS, the total amount of time spent in high delta cycles (Figure 3.5A) and the percentage of SWS in all three channel groups (frontal, $p<0.0001$; central, $p=0.0003$; occipital, $p=0.0005$), based on automated SWS detection. These differences indicated that children with Dup15q syndrome spent significantly less time in SWS compared to age-matched NT controls (Figure 3.5B).

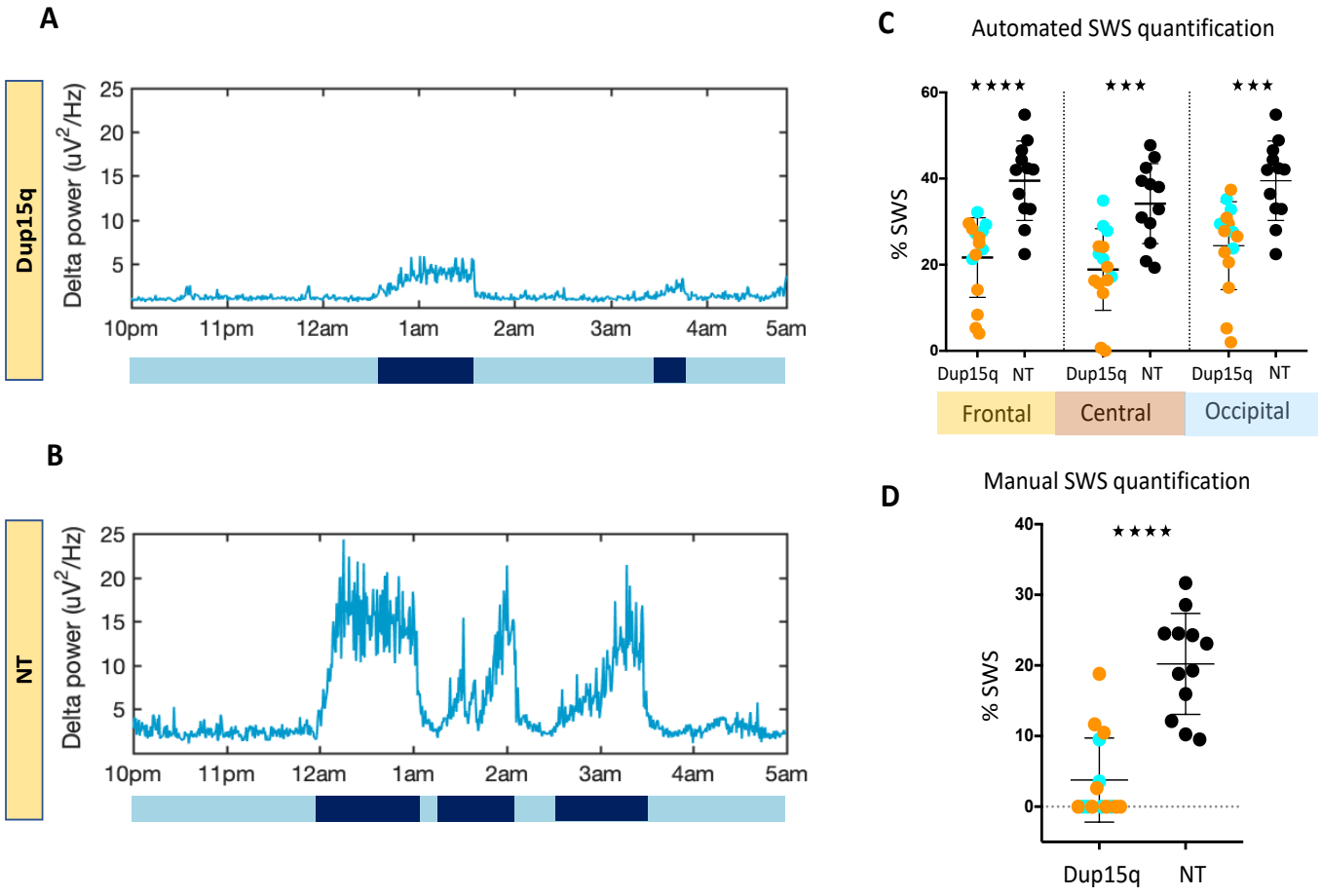

Figure 3.5. Reduced SWS in children with Dup15q syndrome. *Delta (1-4 Hz) power dynamics across 7-hours of overnight EEG from a 19-month-old Dup15q syndrome participant* ***(A)*** *and a 19-month old NT participant* ***(B)****, scored for high delta cycles (black) and low delta cycles (blue). Dot plots of percentage of SWS in participants in the Dup15q syndrome group (turquoise: participants with no epilepsy, orange: participants with epilepsy) and the NT group (black), using automated SWS quantification* ***(C)*** *and manual SWS quantification* ***(D)*** *methods. Different channel groups are highlighted in different colors (frontal: yellow, central: red and occipital: blue).*

Manual quantification of SWS was consistent with automated SWS detection, revealing that 9 out of 15 children within the Dup15q syndrome group did not demonstrate N3 SWS and that children with Dup15q syndrome showed significantly less time in SWS ($p<0.0001$) compared to the NT controls (Figure 3.5C-D). Within the Dup15q syndrome cohort, there was no difference in the percentage of SWS based on the presence of epilepsy or between duplication types.

3.4. Discussion

In this study, we quantified parameters of sleep physiology in children with duplications of 15q11.2-13.1, a genetic syndrome highly penetrant for NDDs and compared them to age-matched typically developing children. We hypothesized that elevated beta oscillations -- previously described in awake EEGs in children with Dup15q syndrome -- would persist in sleep, and that NREM sleep rhythms that are highly dependent on GABAergic synaptic transmission would be disrupted. Indeed, we found that sleep physiology is abnormal in Dup15q syndrome, characterized by excessive beta oscillations, reduced spindle density, and reduced or sometimes absent SWS. Given the fact that most children with Dup15q syndrome undergo clinical overnight EEGs for epilepsy monitoring, findings from this study could guide larger scale examination and quantification of sleep parameters and inform modifiable targets of intervention, particularly with pharmacological agents that modulate GABA neurotransmission.

3.4.1. Abnormal sleep physiology in neurodevelopmental disorders

Abnormal sleep rhythms have been reported in several neurodevelopmental and neuropsychiatric conditions. In a retrospective, descriptive clinical overnight EEG study of

children with Dup15q syndrome, electrical status epilepticus during sleep (ESES), alpha-delta patterns, and periods of high amplitude paroxysmal fast activity were described in approximately 1/3 of patients (Arkilo et al. 2016). Fewer and shorter spindles have been identified in children with Angelman syndrome (den Bakker et al. 2018), which has some genetic and clinical overlap with Dup15q syndrome due to the loss of neuronal expression of the maternally inherited *UBE3A* gene, usually due to a deletion of the 15q region. Poorly developed spindles and K-complexes, as well as altered SWS have been shown in Rett syndrome (Ammanuel et al. 2015; Garofalo, Drury, and Goldstein 1988; Aldrich, Garofalo, and Drury 1990). Deficits in sleep spindles and REM sleep have been demonstrated in children with ASD (Buckley et al. 2010; Tessier et al. 2015; Godbout et al. 2000; Limoges et al. 2013; Farmer et al. 2018) and abnormal spindle features including spindle density and amplitude also have been reported in Alzheimer's disease, Parkinson's disease, Huntington's disease, and schizophrenia (Purcell et al. 2017; Manoach and Stickgold 2019; Ferrarelli and Tononi 2017; Manoach et al. 2016; Christensen et al. 2015).

3.4.2. Role of GABAergic neurotransmission in healthy sleep physiology

Sleep is a complex and dynamic physiological process that is classified into distinct stages defined by neural oscillatory patterns that can be identified on EEG: Stage 1 of NREM (N1) sleep, a short period of relatively light sleep, dominated by theta waves; stage 2 of NREM (N2) sleep, a period of deeper sleep when background waves continue to slow into the low theta to delta range and are punctuated by bursts of thalamocortical activity known as K-complexes and sleep spindles; stage 3 of NREM (N3) sleep, also known as SWS, which is the deepest stage of sleep, dominated by high-amplitude slow waves; and REM sleep, a stage characterized by vivid dreaming, defined

by mixed frequency brain wave activity similar to what is seen during wakefulness with overriding eye movement artifact.

Brain state-specific patterns of neurons and brain state-specific neurotransmitters are either activated or inhibited in order to regulate wakefulness and sleep. The basal forebrain (BF), for instance, consists of cholinergic neurons that are active during wakefulness and REM, and a heterogeneous group of GABAergic neurons, some of which are active during wakefulness and REM, and others which are active during NREM sleep only (Jones 2017). The latter so-called "NREM-ON" neurons promote sleep through projections within the BF as well as through direct projections to the cortex (Szymusiak et al. 1998; Hassani et al. 2009). Additionally, levels of cortical GABA in the BF neurons are significantly higher during NREM sleep (Vanini, Lydic, and Baghdoyan 2012). GABAergic neurons in the ventral tegmental area regulate GABA neurotransmitter release and inhibit wake-promoting orexin/hypocretin neurons, thereby promoting NREM sleep (Chowdhury et al. 2019). Overall, neural pathways engaged in NREM sleep tend to be inhibitory, and therefore most sleep-promoting neuronal populations are GABAergic (Sherin et al. 1996; Anaclet et al. 2015).

In the lateral hypothalamus (LH), GABAergic neurons project to the thalamic reticular nucleus (TRN) where they inhibit local TRN GABAergic neurons. Optogenetic and lesion studies have shown that while activation of LH GABAergic neurons induces transitions from NREM to wakefulness, inhibition promotes NREM sleep and delta oscillations (Herrera et al. 2016; Venner et al. 2016). This TRN-mediated inhibitory mechanism is essential in the generation of synchronous thalamocortical oscillations , sleep spindles, thus giving the TRN its name "sleep spindle pacemaker" (Fernandez and Lüthi 2020). GABAergic neurons located within the medulla,

striatum and the hypothalamus are critical for the induction of SWS, which is generated within the thalamocortical system and cortically expressed by high amplitude oscillations occurring at a frequency of 0.5-2.0 Hz. During SWS, excitatory and inhibitory neurons throughout cortical layers engage into periods of depolarized “up” states, and hyperpolarized “down” states, the dynamics of which are regulated by the activation of $GABA_BR$ (Sanchez-Vives et al. 2020). In fact, most hypnotics such as the benzodiazepine receptor agonists suppress SWS (Wisor et al. 2006), and $GABA_B$ receptor agonists such as gamma hydroxybutyric acid increase SWS and improve sleep efficiency (Walsh 2009; Hindmarch, Dawson, and Stanley 2005; Foldvary-Schaefer et al. 2002).

To our knowledge there are no studies that have directly examined structure or function of the aforementioned brain regions in Dup15q syndrome, and more detailed functional anatomic investigations remain a necessary area of future study. However, the fact that GABAergic neurotransmission plays an essential role in the initiation, synchronization and maintenance of sleep spindles and SWS, and in the overall regulation of healthy NREM sleep, does support a plausible mechanism for the altered sleep features quantified in this study.

3.4.3. Beta oscillations in Dup15q syndrome

In children with Dup15q syndrome, the duplicated 15q11.2-13.1 gene region includes several genes critical for GABAergic neurotransmission, including UBE3A and three $GABA_AR$ genes. $GABA_AR$ agonists and modulators such as benzodiazepines induce patterns of beta oscillations very similar to what is observed in children with Dup15q syndrome (Domino et al. 1989; Mandema and Danhof 1992; van Lier et al. 2004; Visser et al. 2003; Kopp et al. 2004; Kopp, Rudolph, and Tobler 2004). Typically, the frequency of neural oscillations is determined by time constants on postsynaptic receptors, with faster time constants yielding faster oscillatory

frequencies (Buzsaki and Wang 2012). Benzodiazepines augment the action of GABA by increasing the frequency of $GABA_AR$ channel opening and decreasing the frequency of beta oscillations. This higher beta power seen in pharmacological $GABA_AR$ modulation and in Dup15q syndrome likely reflects shifting of faster oscillations towards the beta frequency. As beta oscillations are present to varying degrees irrespective of brain-state, these oscillations may potentially inhibit brain-state-dependent modulation of neural activity.

3.4.4. NREM sleep micro- and macrostructures in Dup15q syndrome

Changes in spindle activity during development have been closely associated with neural maturation. Ontogenesis of sleep spindles in typically developing children may begin at birth but often starts by 3-9 weeks post-term. Initially, spindles are seen in the rolandic regions, appear comb-like in morphology, demonstrate prolonged durations up to approximately 10 seconds, and occur asynchronously between the hemispheres with relatively low spindle density (Gruber and Wise 2016). Spindle length, morphology, synchronicity and density fluctuate over the first few years of life, becoming bifrontocentrally-predominant and synchronous by 2 years of age; from 3 years through early adolescence, spindle density increases (Gruber and Wise 2016; Purcell et al. 2017). Because of these well-established changes with age, spindles have been considered a potential index for neural maturation (Scholle, Zwacka, and Scholle 2007). Sleep spindles guard offline information processing by suppressing sensory perception of external noise and external stimuli during sleep (Dang-Vu et al. 2010), and sleep spindle features have been associated with greater resilience to external perturbation (Hennies et al. 2016) as well as cognitive abilities (Bang et al. 2014; Bódizs et al. 2005; Lustenberger et al. 2012; Cox et al. 2014). In fact, in healthy individuals, spindle density has been correlated with the ability to learn a given task (Gruber and Wise 2016).

In addition to an overall reduction in sleep spindle density in our Dup15q cohort, one child notably demonstrated sleep spindles with comb-like morphology that occurred both synchronously and asynchronously between the hemispheres -- a pattern found in typical development below two years of age or in developmental disorders associated with dysgenesis of the corpus callosum. Brain structural changes have been reported in postmortem studies of Dup15q syndrome, including abnormal neuronal growth and neuronal migration and altered cytoarchitecture (Wegiel et al. 2012; Wegiel et al. 2015). This cellular pathology could directly disrupt cortical, subcortical and hippocampal network connectivity and healthy sleep physiology. Future studies that investigate structural interhemispheric connectivity through magnetic resonance neuroimaging (MRI) and/or diffusion tensor imaging (DTI) tractography may be needed to help investigate the relationship between altered brain connectivity and abnormal NREM sleep physiology in Dup15q syndrome.

SWS is considered to be the most restorative sleep stage associated with sleep pressure and sleep quality (Dijk 2009). Slow oscillations during NREM sleep critically stimulate and synchronize other sleep phenomena. For example, physiological ripples ranging from 80-100Hz in humans arising within the CA1 pyramidal layer of the hippocampus have been shown to coordinate with SWS, and they are implicated in the replay of wake-related hippocampal learning activity. Moreover, about 50% of sleep spindles are time-locked to specific phases of slow oscillations (Fernandez and Lüthi 2020), resulting in a cross-frequency phase-amplitude coupling. While the role of spindle-slow oscillation coupling in different sleep stages is still under investigation, it is postulated that the coordinated synchrony between thalamocortical spindles, neocortical slow wave oscillations and hippocampal ripples is critical for brain communication and plasticity and promotes overall cognitive performance (Durmer and Dinges 2005; Krause et al. 2017).

Converging evidence shows that disruption in sleep spindle characteristics such as spindle density and changes in the amount of slow wave activity during the night are highly associated with abnormal cognitive performance (Gruber and Wise 2016; Limoges et al. 2013; Maski et al. 2015; Prehn-Kristensen et al. 2011; Tessier et al. 2015; Tham, Schneider, and Broekman 2017). In Dup15q syndrome, significant reduction in spindles necessary for nesting into specific phases of slow oscillations may disrupt the temporal coordination between spindles, slow oscillations and hippocampal ripples and, as a result, alter overall brain network communication and plasticity. Disruptions in NREM sleep parameters, therefore, may not only imply sleep fragmentation and increased day time sleep propensity, but also contribute to and exacerbate the neurodevelopmental disabilities seen in Dup15q syndrome.

3.4.5. Clinical implications

As targeted therapeutics emerge in genetically defined neurodevelopmental disorders, there arises a rather urgent need to identify quantifiable mechanistic electrophysiological biomarkers that can shed light on the etiology of cognitive impairment and also serve as a surrogate endpoint in clinical trials. In Dup15q syndrome, abnormal sleep physiology, likely attributable to pathological variants of the UBE3A and $GABA_AR$ genes, can be quantified as elevated beta oscillations in sleep, combined with changes in spindles and SWS. These changes may impair oscillatory synchronization across brain regions during NREM sleep and affect overall brain network function, and may precede or even exacerbate the profound cognitive deficits and behavioral challenges commonly diagnosed in these children.

3.5. Limitations and future directions

Behavioral sleep measures from parent report and neuropsychological testing were not available for the entirety of this cohort. However, these promising data have motivated a larger scale investigation of sleep EEG and behavioral phenotyping to be able to examine correlations between phenotype and electrophysiology. A larger cohort also would allow for the examination of the role of epilepsy, including severity of seizures, anti-epileptic medications, and developmental age that may uniquely impact sleep physiology and contribute to heterogeneity in clinical symptomatology. Moreover, because we relied on clinical EEG quantification, formal sleep staging as would have been performed with polysomnograms, was not possible. However, clinical polysomnograms are challenging to collect in this population and therefore, we may need to rely on routine overnight EEG recordings as a proxy for sleep monitoring. In fact, the ability to quantify these biomarkers in clinical EEG opens the door for larger scale studies in syndromic NDDs, where epilepsy is highly penetrant, as individuals with epilepsy undergo routine EEG monitoring on a regular basis. Leveraging access to these clinical data prevents the cost, time and stress of bringing children to research centers for additional data collection.

We speculated about mechanisms underlying these abnormal sleep patterns, but both clinical and pre-clinical studies could directly elucidate etiology. Clinically, combined MRI and EEG studies will allow us to examine brain structural abnormalities that may contribute to altered brain network connectivity. Quantification of sleep electrophysiological recordings in pre-clinical models of Dup15q syndrome, particularly those with and without the overexpression of $GABA_AR$ genes, will directly elucidate the effect of putative genes in the 15q region on altered sleep physiology and also will allow for both behavioral and pharmacological manipulations that could

improve sleep and learning mechanisms.

3.6. Conclusions

While research in EEG biomarkers has traditionally focused on oscillatory changes in the EEG during wakefulness, our findings suggest that studying sleep physiology in NDDs may be extremely valuable in helping identify quantitative biomarkers of sleep function. The quantitative methods used in this study could be applied to other NDDs. While sleep spindles and slow wave oscillations may be detected by qualitative measurements, subtle features may be difficult to capture clinically. Quantitative semi-automated measures can identify differences in sleep physiology and help identify biomarkers across syndromic NDDs. Insights gained from this study deepens our understanding of the pathophysiology in Dup15q syndrome and may lay the foundation for studies that investigate the relationship between sleep and cognition, with the ultimate goal of testing specific therapeutics to alter sleep physiology and potentially enhance cognitive development and overall clinical outcomes in children with Dup15q syndrome.

Chapter 4: Relation between sleep physiology and neurodevelopmental phenotype in children with Dup15q syndrome

4.1. Introduction

Despite the high prevalence of sleep disturbances and cognitive deficits in children with NDDs, very few studies have examined the relationship between altered sleep physiology and cognition in NDDs. This chapter describes an exploratory study of the relation between abnormal sleep physiology (elevated beta oscillations, reduced sleep spindles, and reduced SWS) and neurodevelopmental phenotype in children with Dup15q syndrome. A better understanding of the relation between impaired sleep and cognition in Dup15q syndrome will provide further understanding into the pathophysiology associated with the syndrome and offer insights into potential treatments that target sleep, and ultimately, improve cognitive and developmental outcomes in children with Dup15 syndrome.

4.2. Subjects and Methods

4.2.1. Study participants

Overnight clinical EEG recordings were gathered from 28 participants (13 male, 15 female) that were clinically referred through the Dup15q Alliance and have a genetically confirmed diagnosis of Dup15q syndrome. Recordings included those we reported previously in a study to characterize sleep physiology in children with Dup15q syndrome. Data were collected either locally, at the University of California, Los Angeles (UCLA) Pediatric Neurophysiology laboratory at the Ronald Reagan Medical Center, or from clinics across the country that treated

patients with Dup15q syndrome. Participant ages ranged between 9 months and 19 years (median: 4.5 years).

4.2.2. Behavioral assessments

All behavioral assessment data were collected as part of the UCLA IDDRC study. Participants were administered the following measures: 1) *Language and cognition*: The Mullen Scales of Early Learning (MSEL), which assesses general cognition and development. The MSEL yields standard as well as age-equivalent scores that measure receptive and expressive language, visual reception, and gross and fine motor skills. These scores were then utilized to calculate verbal and non-verbal cognition scores. Given that most of our children with Dup15q syndrome had significant delays in overall development, age equivalent ratio scores were used instead of standardized development quotient scores; 2) *Adaptive behavior*: Vineland Adaptive Behavior Scale (VABS), a parent-reported measure of adaptive behavior, which yields standard and age equivalent scores for communication, daily living skills (DLS), socialization and motor skills (Scattone, Raggio, and May 2011); 3) *Sleep behavior*: Child sleep habits questionnaire (CSHQ) (Owens 2000) is a 45-item parent questionnaire that has been used in many studies to examine sleep behavior in young children. The questionnaire comprises several key sleep domains that will be grouped into the following sleep behavior variables: 1) Bedtime resistance, 2) Sleep onset delay, 3) Sleep duration, 4) Sleep anxiety, 5) Night wakings, 6) Parasomnias, 7) Sleep-disordered breathing, 8) Daytime sleepiness. A total sleep disturbance score is calculated based on all the eight subscale items mentioned above.

4.2.3. EEG data acquisition

All clinical overnight EEGs, recorded for clinical monitoring, were gathered in accordance with the Institutional Review Board. EEG data were recorded at a sampling rate of 200Hz or 250Hz, with a standard 10-20 montage, 21 channel electrode placements, using one of the following data acquisition setups: a Neurofax Polysmith DMS 11.0 Build 8093 with 921 amplifiers with gold disc electrode placement (Nihon Kohden America Inc, Irvine, CA), an XLTEK PSG system and Natus SleepWorks software (Natus Medical Inc., San Carlos, CA) and a NicoletOne v32 amplifier acquisition system from Natus. Data were extracted and converted into European Data Format (EDF) for analysis.

We eliminated data from participants with 1) medications that are known to pharmacologically induce beta oscillations (benzodiazepines and barbiturates), 2) poor or insufficient data due to artifacts from non-neural sources, or insufficient recording, or 3) recordings that were compressed to only viewable formats. Our final cohort of children with Dup15q syndrome yielded n = 23 participants. Details of age, sex, duplication type, epilepsy status, and medications can be found in Table 1.

Table 4.1. Dup15q syndrome participant characteristics

This table describes the characteristics of participants in the Dup15q syndrome cohort. Details on age, sex, epilepsy status, and medications were extracted from participant background medical questionnaire, and duplication type was extracted from participant genetic reports.

Age in months	**Gender**	**Duplication type**	**Epilepsy status**	**Medications**
9	Female	Isodicentric	Yes	Phenobarbital, Levetiracetam
18	Male	Interstitial	No	
19	Male	Isodicentric	Yes	Vigabatrin, Zonisamide, Prednisone
23	Female	Isodicentric	No	
31	Female	Isodicentric	Yes	Valproate sustained release, Clobazam, Lacosamide, Perampanel
33	Female	Isodicentric	Yes	Topiramate, Valproate
35	Male	Isodicentric	No	
43	Male	Isodicentric	Yes	
48	Female	Interstitial	Yes	Levetiracetam, Rufinamide

48	Female	Isodicentric	Yes	Levetiracetam, Phenobarbital
54	Male	Isodicentric	No	
55	Male	Isodicentric	Yes	Lamotrigine
57	Female	Isodicentric	Yes	Levetiracetam, Zonisamide
57	Female	Isodicentric	Yes	Rufinamide
68	Female	Isodicentric	Yes	
73	Female	Interstitial	Yes	Lamotrigine, Levetiracetam
105	Female	Isodicentric	No	
108	Female	Isodicentric	No	
108	Male	Isodicentric	Yes	
109	Female	Isodicentric	Yes	
137	Male	Isodicentric	Yes	Rufinamide
156	Male	Isodicentric	Yes	
235	Male	Isodicentric	Yes	Levetiracetam, Zonisamide

4.2.4. EEG data processing and analysis

Overnight clinical EEG recordings from 10 pm to 5 am were extracted similar to methods described previously. Raw EEG data were processed using the EEGLAB (Delorme and Makeig 2004) software toolbox for Matlab. Data were high-pass filtered at 1.0 Hz and low-pass filtered at 50 Hz with zero-phase FIR filters and forward-backward filtering. EEG channels with poor signal

quality were automatically removed and interpolated with the following criteria: (1) spectral power between 1-50Hz that was three standard deviations above or below that of other channels, (2) channels with flat signals (i.e. zeros) longer than 5 seconds, (3) channels that were poorly correlated ($r<0.7$) with their reconstructed versions based on adjacent channels, (4) channels with line noise power four standard deviations higher than their signals, using *clean_rawdata()* function in EEGLAB. The interpolated EEG data were then re-referenced to common average reference.

The power line noise (i.e. 60 Hz) was further removed using CleanLine in EEGLAB (Bigdely-Shamlo et al. 2015). Artifact subspace reconstruction (ASR) was applied using *clean_asr()* function ($\sigma=20$) (Chi-Yuan Chang 2020) to automatically remove and interpolate non-stationary, high-amplitude bursts such as eye blinks, eye movement activity, possible complex epileptiform activity as well as motion artifacts. Independent component analysis (ICA) was performed, and an automatic independent components (IC) classifier, ICLabel (Pion-Tonachini, Kreutz-Delgado, and Makeig 2019), was used to separate and label ICs into seven categories. The ICs labeled as muscle, eye, heart, line noise, and channel noise with probability higher than 0.5 were rejected. The final cleaned channel signals were reconstructed using the remaining ICs. Time-frequency analysis was performed for each channel of the cleaned overnight EEG using *spectrogram()* function in Matlab with a Hanning window of 60-sec and a 30-sec overlap. The mean power at beta (12-30 Hz) and delta (1-4 Hz) band oscillations was further obtained for each epoch.

Sleep spindles and SWS were quantified and visualized using methods described in the previous chapter. Throughout overnight recordings, spindles were identified and quantified for multiple epochs and averaged across epochs for each subject. In order to quantify SWS, the deepest

stage of sleep, characterized by the presence of slower frequency and high amplitude signals (delta waves, 1-4 Hz), delta power (1 – 4Hz) was computed for every 30sec epoch of the cleaned overnight EEG. SWS was automatically identified as epochs with higher delta power, with methods based on a prior study (Ammanuel et al. 2015) and described previously.

To determine the relation between sleep physiology and behavior, beta power, spindle density and %SWS were regressed on quantitative measures of cognition, as well as parent reported measures of adaptive skills and sleep behavior.

4.3. Results

4.3.1. Qualitative evaluation of overnight sleep EEG

Similar to the findings from our previous study, qualitative evaluation of overnight sleep EEGs demonstrated that all our study participants with Dup15q syndrome showed the presence of excessive beta oscillations in sleep. They showed progression through sleep stages NREM N1, evaluated by the presence of vertex waves, and NREM N2, evaluated by the presence of spindles and K-complexes. However, as previously reported, not all participants showed the presence of persistent slow waves in the NREM N3 stage as we would expect to see in healthy sleep. Epileptiform activity was noted in 17 children with Dup15q syndrome and in all of the children with dual diagnoses of Dup15q syndrome and epilepsy.

4.3.2. Evaluation of spindle density, % SWS and beta power in sleep, by age

Regression analysis revealed spindle density did not correlate with the age of the participant (no epilepsy R^2= 0.005, p=0.74) at the time of sleep EEG recording (Figure 4.1A). % SWS significantly correlated with participant age (R^2= 0.37, p=0.002), with older participants spending less time in SWS compared to younger participants (Figure 4.1B). Regression models within the epilepsy and non-epilepsy groups separately revealed a significant relationship between

participants with epilepsy and %SWS (no epilepsy group: R^2= 0.04, p=0.70, epilepsy group: R^2= 0.44, p=0.004). Beta power in sleep did not correlate with age (R^2= 0.055, p=0.28) as shown in Figure 4.1C.

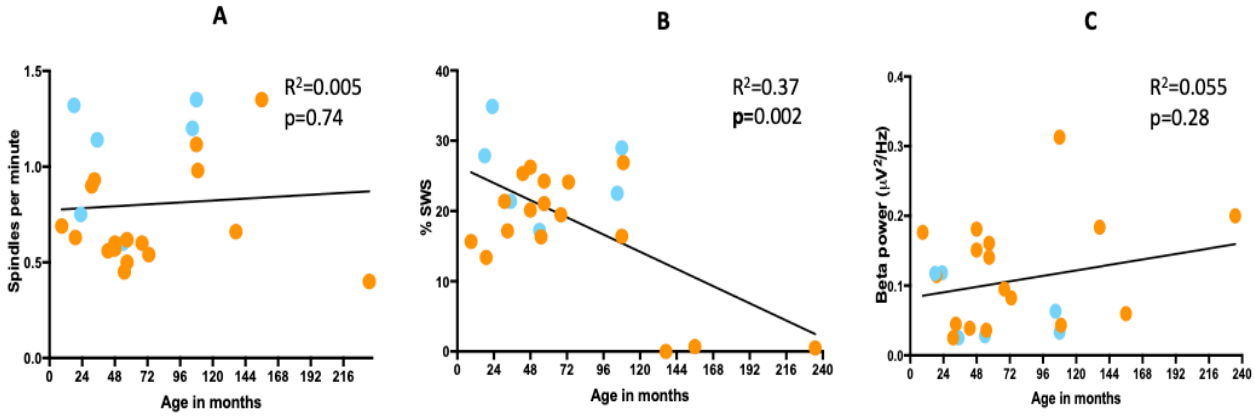

Figure 4.1. Spindle density, % SWS, beta power, and age. *A) Age vs spindle density. B) Age vs % SWS. C) Age vs. beta power. Participants with epilepsy are shown in orange and those without epilepsy are in blue.*

4.3.3. Relation between spindle density, cognition, and sleep behavior

Spindle density did not significantly correlate with VDQ or NVDQ. However, reduced spindle density showed a trend towards lower VDQ (R^2= 0.26, p=0.07)and NVDQ scores (R^2= 0.26, p=0.07) (Figure 4.2 A-B). There were no correlations between spindle density and daily living skills (R^2= 0.02, p=0.65) or daytime sleepiness (R^2= 0.03, p=0.61) (Figure 4.2C-D).

Regression models within the epilepsy and non-epilepsy groups separately did not reveal any significant relationships.

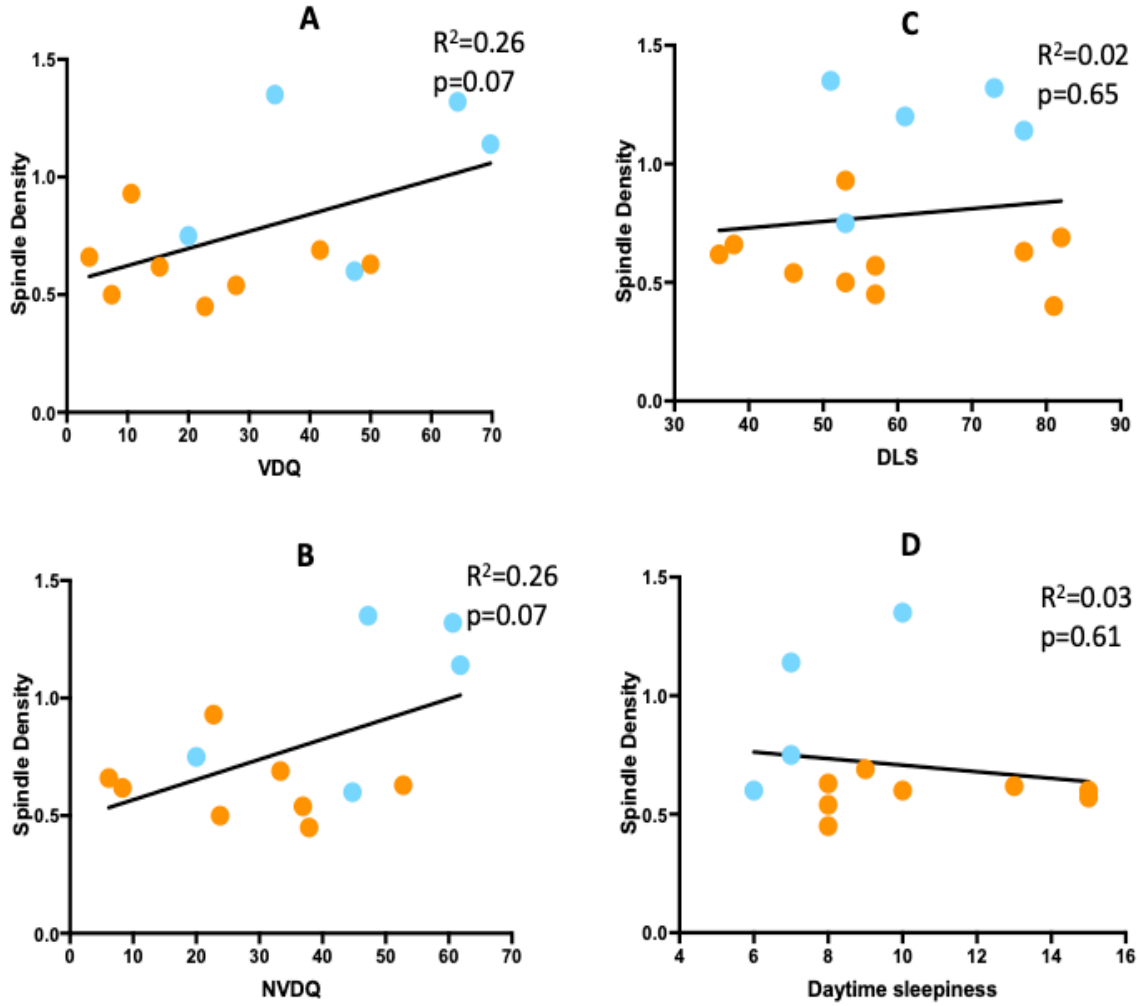

Figure 4.2. Cognition, daily living skills, daytime sleepiness, and spindle density. *A-B) Spindle density vs. verbal and non-verbal cognition. C) Spindle density vs. daily living skills. D) Spindle*

density vs. daytime sleepiness. Participants with epilepsy are shown in orange and those without epilepsy are in blue.

4.3.4. Relation between % SWS, cognition, and sleep behavior

% SWS significantly correlated with VDQ (R^2= 0.32, p=0.04) and NVDQ (R^2= 0.40, p=0.02) as shown in Figure 4.3 A-B. Regression models within the epilepsy and non-epilepsy groups separately also revealed significant relations between %SWS and verbal and non-verbal cognition among participants who had epilepsy (no epilepsy group: VDQ, R^2= 0.30, p=0.34, NVDQ, R^2= 0.30, p=0.34; epilepsy group: VDQ, R^2= 0.50, p=0.05, NVDQ, R^2= 0.30, p=0.34). % SWS did not correlate with daily living skills (R^2= 0.045, p=0.45) but did significantly correlate with daytime sleepiness (R^2= 0.36, p=0.04) (Figure 4.3 C-D).

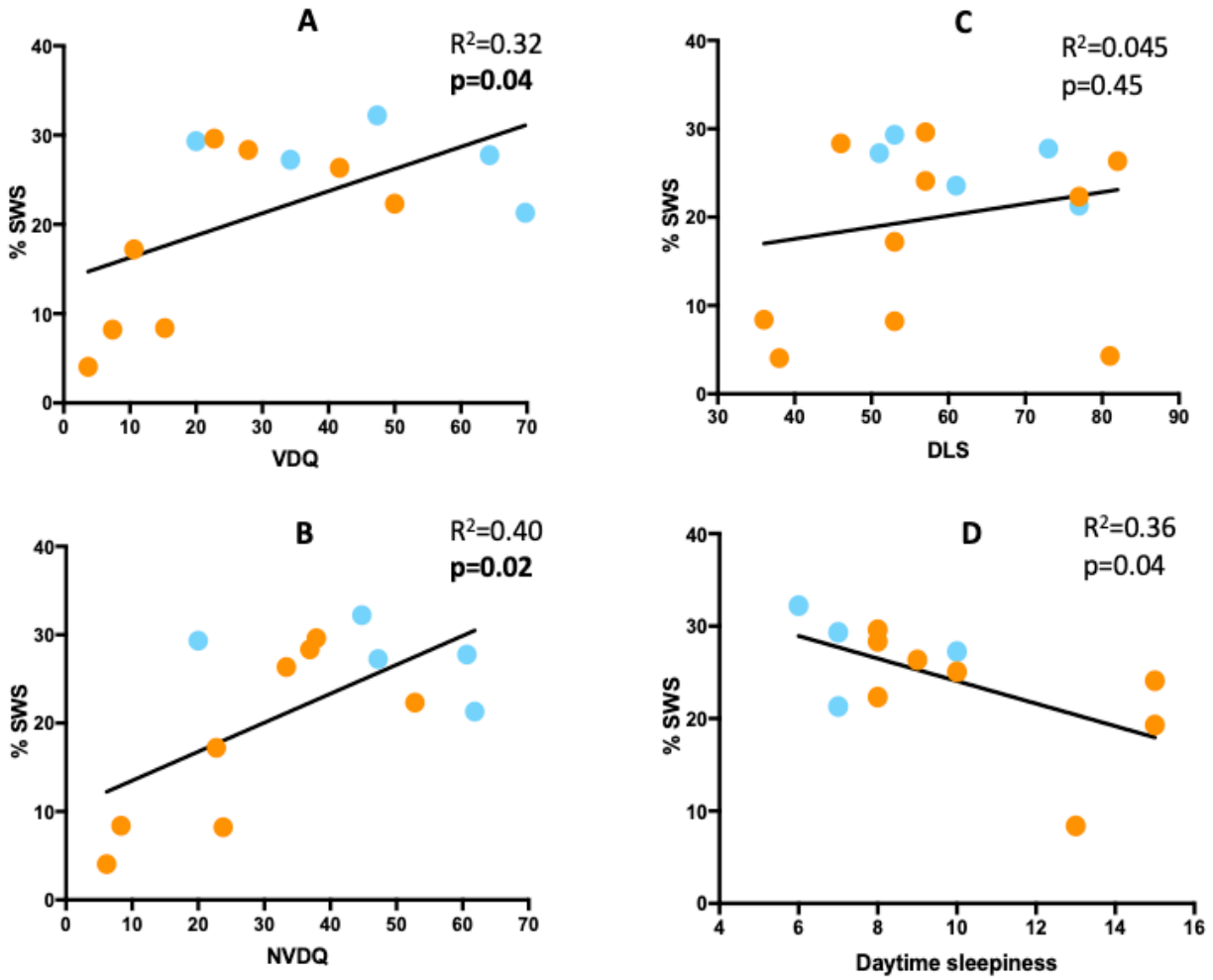

Figure 4.3. Cognition, daily living skills, daytime sleepiness, and % SWS. *A-B) % SWS vs. verbal and non-verbal cognition. C) % SWS vs. daily living skills. D) % SWS vs. daytime sleepiness. Participants with epilepsy are shown in orange and those without epilepsy are in blue.*

4.3.5. Relation between beta power, cognition, and sleep behavior

No significant correlations were seen between beta power and VDQ (R^2= 0.12, p=0.22) or NVDQ (R^2= 0.28, p=0.05) (Figure 4.4 A-B). Beta power did not correlate with daily living skills (R^2= 0.004, p=0.82) but significantly correlated with daytime sleepiness (R^2= 0.43, p=0.02)

(Figure 4.4 C-D). While there were no correlations between beta power and spindle density (R^2= 0.05, p=0.32) (Figure 4.4E), beta power in sleep strongly correlated with % SWS (R^2= 0.22, p=0.02) (Figure 4.4F). However, regression analysis within the epilepsy and non-epilepsy groups separately did not reveal any significant relations between beta power and other variables.

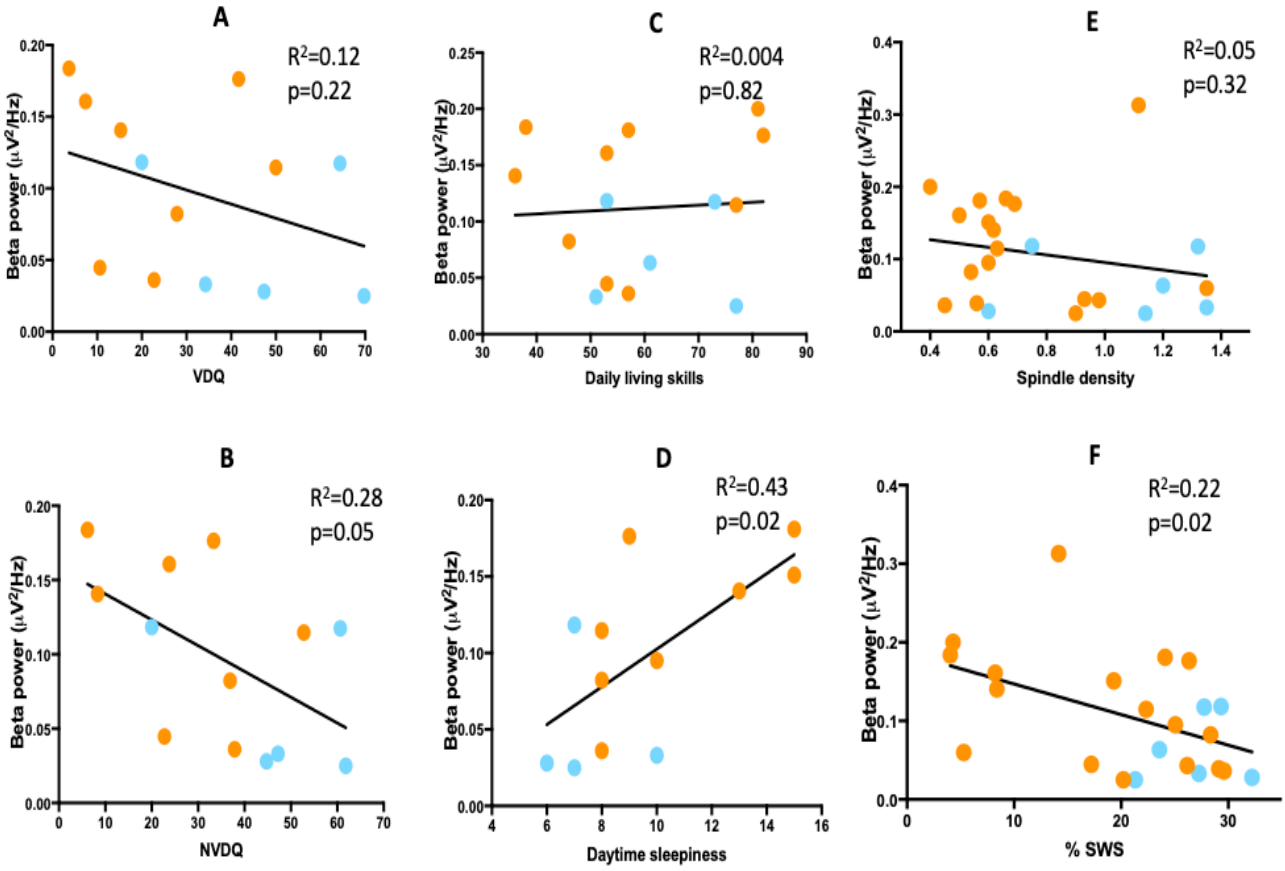

Figure 4.4. Cognition, daily living skills, and daytime sleepiness, compared with beta power, spindle density, and % SWS. *A-B) Beta power vs. verbal and non-verbal cognition. C-D) Beta power vs. daily living skills and daytime sleepiness. E-F) Beta power vs. spindle density and*

% SWS. Participants with epilepsy are shown in orange and those without epilepsy are in blue.

4.4. Discussion

In this study, we quantified sleep physiology in children with duplications of 15q11.2-13.1, a genetic syndrome highly penetrant for NDDs, and investigated the relationship between abnormal sleep physiology and behavioral phenotype. In a previous study comparing sleep physiology between children with Dup15q syndrome and age-matched neurotypical controls, we found elevated beta oscillations, reduced sleep spindles, and reduced % SWS in Dup15q syndrome. Given this finding, we hypothesized that changes in sleep physiology may correlate with behavioral phenotype. Indeed, we found that reduced spindle density in children with Dup15q syndrome correlated with adaptive skills, % SWS correlated with daytime sleepiness, and beta power in sleep significantly correlated with adaptive skills and daytime sleepiness. Additionally, elevated beta power in sleep also correlated with abnormal NREM sleep features, specifically reduced % SWS.

4.4.1. Altered physiology during wakefulness and sleep in Dup15q syndrome

Children with Dup15q syndrome demonstrate subjective clinical or behavioral sleep difficulties as evidenced by parent reports, as well as objective physiological changes in sleep, as seen from past clinical sleep studies (Urraca et al. 2013; Arkilo et al. 2016). A descriptive overnight clinical EEG study that focused on understanding epileptiform activity in sleep in individuals with Dup15q syndrome found prominent abnormalities, including higher incidences of electrical status epilepticus during sleep (ESES), and periods of high amplitude paroxysmal fast activity which disrupt the normal sleep architecture (Arkilo et al. 2016). Quantitative EEG studies from our group

showed abnormally elevated beta oscillations in children with Dup15q syndrome during wakefulness, which likely reflect aberrant GABAergic neurotransmission (Frohlich, Reiter, et al. 2019; Saravanapandian et al. 2020; Frohlich et al. 2016). As an extension of this study, we also recently discovered that elevated beta oscillations continue to persist in sleep and that NREM sleep physiology is disrupted in Dup15q syndrome. The presence of abnormal beta oscillations both during wakefulness and sleep emphasizes the fact that these oscillations may compromise brain-state-dependent modulation of network activity. Brain plasticity occurs both during wakefulness and during sleep (Facchin et al. 2020; de Vivo and Bellesi 2019; Tononi and Cirelli 2020). It is proposed that our waking experience triggers synaptic events that may be short-lived but are necessary to prime the brain circuits and synapses for further processing during sleep. These primed brain connections or synapses lead to structural changes that may be necessary for long-term memory consolidation in sleep. Therefore, a shift in network activity during sleep -- due to elevated beta oscillations -- may compromise neural network reactivations necessary for synaptic strengthening, thus compromising brain plasticity and long-term memory consolidation. Our findings indicate that elevated beta oscillations in sleep relate to parental reports of poor sleep behavior as well as worse outcomes concerning adaptive skills in Dup15q syndrome.

4.4.2. Relation between NREM sleep and cognition and behavior

NREM sleep features, spindles, and SWS play critical roles in healthy cognitive function (Nishida, Nakashima, and Nishikawa 2016; Siapas and Wilson 1998; Della Monica et al. 2018; Halassa et al. 2014). Sleep spindles are a hallmark of stage 2 sleep and are generated in the thalamic reticular nuclei (TRN) by changes in membrane potentials in the thalamocortical network of the brain (Murata and Colonnese 2019; Fernandez and Lüthi 2020). They are 11-16 Hz sinusoidal

cycles, lasting between 0.5 and 2 seconds. They are said to function in suppressing external stimuli and guard offline information processing by mediating consolidation of procedural and declarative memory during sleep. SWS is comprised of high-amplitude, slow oscillations that are cortically generated within the thalamocortical network. Delta activity during SWS promotes synaptic downscaling for the brain to renew its capacity to encode new information, thus playing a role in memory consolidation. SWS has also been characterized as a marker of homeostatic regulation of sleep and an index of sleep need, and through its role in synchronization in neuronal activity of networks, it is said to reflect synaptic efficacy. Furthermore, during NREM sleep, sleep spindles are time-locked to specific phases of SWS and this temporal coordination is important in transferring memories to the cortex and inducing synaptic plasticity and memory consolidation (Clemens et al. 2007). Alterations in spindle characteristics, including frequency, density, and amplitude, as well as abnormal SWS have been linked to several neurodevelopmental and psychiatric disorders. GABA has traditionally been described as a sleep-promoting neurotransmitter and GABAergic neurotransmission has a critical role in healthy NREM sleep (Chowdhury et al. 2019; Kodani, Soya, and Sakurai 2017).

Children with Dup15q syndrome have a constellation of clinical symptoms including intellectual disability, hypotonia, language impairments, social communication impairments, sleep impairments, and epilepsy. While the heterogeneity in clinical symptomatology may make it difficult to capture the complexities of sleep features, given that the genetics of Dup15q syndrome result in alterations in $GABA_AR$ functioning, and that NREM sleep is abnormal, it is not surprising that we see a relation between NREM sleep and cognition and behavior. The relation we found between impaired sleep physiology and daytime sleepiness is particularly interesting. This may

indicate that, even though children with Dup15q syndrome spend several hours on bed, and behaviorally may be sleeping, the fact that they have abnormal sleep physiology and increased daytime sleepiness may suggest that sleep may not be as restorative and may have implications for learning and adaptive functioning. These findings, however, should be interpreted with caution, as epilepsy and developmental age greatly impact sleep physiology and have functional implications. Additionally, our developmental assessments may not capture changes in specific cognitive domains such as attention and performance vigilance that may be likely impaired due to disrupted sleep. Therefore, large-scale studies that can better disentangle the role of age and epilepsy severity in conjunction with behavior assessments of sleep-dependent learning and memory during early development may help clarify these issues.

4.4.3. Interplay between sleep physiology and epileptic activity

Sleep deprivation is a strong activator of seizures (Carreño and Fernández 2016; Schmitt 2015; Peter-Derex et al. 2020). Changes in vigilance state, from wakefulness to sleep have been linked to the emergence of seizures and evidence suggests that regular sleep-wake times may confer benefits not only in day-time function but in seizure control. Epileptic discharges during sleep emerge most commonly during NREM sleep (Chan 2020; Mutti et al. 2020; Klimes et al. 2019). In fact, certain epilepsy syndromes are identified based on their relationship with NREM sleep. This is because the same thalamocortical circuit interactions that are involved in the generation of NREM sleep are pathologically activated during epilepsy. A large portion of the Dup15q syndrome population has seizures (Finucane et al. 2016). While limited by sample size in the no-epilepsy group, we found that the significant correlations between sleep physiology and neurobehavioral phenotype were driven by participants with epilepsy. Given that 74% of our

cohort have epilepsy, this finding calls for further investigation into the interactions between altered NREM sleep physiology, seizure frequency, anti-epileptic medications, and their relationship to behavioral phenotype.

4.5. Conclusions

We have found that altered sleep physiology in individuals with Dup15q syndrome relates to cognition and behavioral measures of sleep impairment. While research in EEG biomarkers has traditionally focused on brain activity changes during wakefulness, our findings suggest that studying sleep physiology in children with NDDs may be extremely valuable in helping identify quantitative biomarkers of sleep and cognitive function. Since children with NDDs, particularly those that are highly penetrant for epilepsy, undergo overnight clinical EEG assessments for epilepsy monitoring, large scale studies that examine and quantify sleep physiological parameters and investigate their relation to cognitive function are increasingly feasible and also critical in the identification of early interventions and potential treatments in NDDs.

Chapter 5: Summary and Conclusions

5.1. Summary

In summary, quantifiable electrophysiological biomarkers hold promise as measures of drug-target engagement and may help guide pharmacological interventions in NDDs. Elevated beta oscillations during wakefulness in children with Dup15q syndrome are quantifiable and likely reflect disrupted GABAergic neurotransmission. However, to use this EEG biomarker in clinical trials, we must evaluate the properties that would render it a robust clinical biomarker for the syndrome, including whether it 1) is stable over time and across developmental age, 2) is reproducible across different data acquisition setups, 3) relates to clinical symptomatology, 4) is translatable from preclinical to clinical models and vice versa, 5) reflects underlying disease mechanisms and 6) modulates with changes in brain state such as wakefulness and sleep. Given the role of sleep in cognition and the high rates of sleep disturbances in NDDs, quantifiable biomarkers of sleep are extremely valuable in providing new insights into mechanisms underlying the cognitive impairments and enabling effective therapeutics. In this broader context, this dissertation has demonstrated several key points:

1) Elevated beta oscillations in Dup15q syndrome represent a highly robust and reproducible biomarker: they are stable over time, and across development, scalable across different EEG recording modalities (research vs. clinical EEG), and are reproducible across data collected from multiple studies.
2) Elevated beta oscillations persist in sleep in Dup15q syndrome and are quantifiable in routine overnight clinical EEG recordings. The ability to

successfully quantify and reproduce the Dup15q syndrome EEG biomarker in clinical EEGs has led to the development of a remote clinical EEG repository that can inform future biomarker studies in syndromic and non-syndromic forms of ID.

3) Sleep physiology in children with Dup15q syndrome is abnormal, with elevated beta power in sleep, and abnormal NREM sleep features including reduced sleep spindles and reduced SWS that distinguishes children with Dup15q syndrome from age-matched neurotypical controls.
4) Abnormal sleep physiology in Dup15q syndrome relates to clinical features These sleep EEG markers may therefore be clinically relevant and useful as outcome measures in pharmacological treatments.

The impact of GABAergic neurotransmission on both the beta band EEG phenotype and NREM sleep physiology suggests that the EEG biomarkers of wakefulness and sleep we have found in Dup15q syndrome, both likely reflect genetic mechanisms mediated by changes in $GABA_AR$ functioning.

5.2. Limitations and future directions

While the findings described above are exciting and hold promise in advancing towards clinical trials in Dup15q syndrome, several limitations need to be taken into consideration as we think of the next steps towards potential treatment trials.

5.2.1. Large-scale studies across neurodevelopmental disorders

As with other studies of rare genetic disorders, we faced challenges with sample size. While we discovered that it is feasible to quantify the Dup15q syndrome EEG biomarker from recordings collected across different data acquisition methods, what we did not account for is the accessibility of collected data due to the way recording files are typically formatted before being handed over to families. We tried to mitigate this by utilizing software tools that helped extract raw EEG recordings from different file formats, but with limited success. Hence, future studies focused on large-scale analysis of EEG biomarkers should collaborate with patient advocacy groups (PAGs) and work with clinics and sleep data technicians to guide them through the study requirements and data transfer process. Just in the past decade several PAGs have been formed by families of individuals with rare genetic disorders, opening the possibilities for collaborations among PAGs and between PAGs, caregivers, clinicians, and researchers and for the development of syndrome-wide registries to collect and share data across syndromes. Utilizing large datasets across NDDs and mathematical tools such as machine learning can facilitate better approaches to identify physiological variability in large heterogeneous samples. Expanding our pipeline to gather clinical overnight EEG recordings not just across different Dup15q syndrome clinics but across different NDDs will help characterize sleep in these disorders and identify syndrome-specific biomarkers of sleep that can deepen our understanding of the pathophysiology associated with different genetic syndromes, and can serve as a treatment target for sleep and ultimately cognition.

5.2.2. Understanding the effects of developmental age and epilepsy

A larger sample size would allow for examination of other important factors that may impact sleep physiology, such as age and epilepsy. The presence of seizures adversely affects the quality and quantity of sleep. Furthermore, seizure severity, due to increased seizure frequency

and the impact of anti-epileptic medications can exacerbate this. Epileptiform discharges are activated by certain stages of sleep and propagate during NREM sleep. About 80% of seizures occur exclusively during sleep and sleep-related epilepsy emerges early in childhood. A large portion (70-80%) of the Dup15q syndrome population have comorbid epilepsy. It is therefore unclear whether treatments targeting sleep pathophysiology will have any functional impact, or if so to what extent, thus making it a critical area of investigation for future studies.

5.2.3. Using preclinical models to understand mechanisms

Different mouse models of Dup15q syndrome that are differentiated by overexpression of specific genes in the 15q critical region have been engineered. 1) A mouse model with a duplication of the homolog of the full 15q11.2-13.1 critical region (Nakatani et al. 2009), with a 6.3Mb duplication of the mouse chromosome 7 ("Takumi mouse model") exhibits different phenotypes based on whether the duplication is maternally or paternally inherited. While both show overexpression of the $GABA_AR$ subunits, Ube3a overexpression is only found in the maternally inherited models. Behavioral phenotypes, however, are seen only in the paternally inherited duplication mice, further emphasizing the fact that genes other than the maternally imprinted *UBE3A* gene may play a role in the cognitive deficits we see in the syndrome. The Takumi mice also demonstrated deficits in cerebellum-dependent motor learning and long-term depression (LTD) in parallel fiber-Purkinje cell synapses (a mechanism fundamental for motor learning). 2) A mouse model that overexpresses just *UBE3A* or specific Ube3a isoforms has been generated. These mice have been shown to exhibit core behavior phenotype associated with Dup15q syndrome including anxiety, learning impairments, defective social interaction, reduced seizure thresholds, as well as excitatory synaptic transmission (Copping et al. 2017; Smith et al. 2011). Thus, mouse models that overexpress individual genes (e.g., *UBE3A*) are useful for

characterizing pathology specific to those genes, and those that overexpress many 15q11-q13 gene homologs may help recapitulate many aspects of the Dup15q syndrome clinical phenotype. However, none of these studies has yet examined the EEG phenotype in Dup15q syndrome, characterized by increased beta oscillations.

High-density electrode array recordings collected from the aforementioned mouse models of Dup15q syndrome can help investigate the abnormal oscillatory activity of these different models and determine whether UBE3A alone, or $GABA_AR$ alone, or a combination of genes when over-expressed result in the excessive beta power phenotype seen in Dup15q syndrome patients. LFP recordings from hundreds of isolated single units will help determine the driver of these abnormal oscillations. Furthermore, as we hypothesize that the abnormal sleep physiology we found in Dup15q syndrome likely compromises the temporal coordination of NREM sleep rhythms and hippocampal ripples, mouse models can be utilized to record sleep physiology and investigate disruption of NREM sleep features in mice. Using simultaneous recordings in mouse models, we can also investigate how changes in spindles and SWS impact hippocampal/cortical interactions, the organization of neuronal circuitry during learning, and understand how specific genetic and cellular pathways may contribute to cognitive dysfunction in Dup15q syndrome.

Similar to mouse models, patient-derived human pluripotent stem cells (iPSCs) from individuals with Dup15q syndrome can be used to study organoid physiology and examine the neural activity profile of early developmental networks. Neural network organization in human cortical, subcortical and hippocampal organoids can help investigate the molecular mechanisms underlying network dysfunction associated with Dup15q syndrome.

5.2.4. Pharmacological modulation of GABAergic dysfunction

Given that the work described in this dissertation demonstrates that altered GABAergic signaling likely underlies the EEG signatures during wakefulness and sleep in Dup15q syndrome, these EEG phenotypes can be used as quantitative biomarkers that reflect GABAergic dysfunction in Dup15q syndrome. Beta power, spindle density, and %SWS can be used as measures to evaluate disease pathology, stratify patients, and estimate drug-target engagement and brain circuit-level changes that precede behavioral changes after pharmacological treatments. As these biomarkers show a relationship with neurobehavioral phenotype in children with Dup15q syndrome, they can be utilized as a surrogate endpoint in clinical trials. Furthermore, because slow wave activity and its coupling with other sleep features supports cognition, and there is a relationship between changes in SWS and cognition in children with Dup15q syndrome, future studies that investigate the enhancement of spindles and SWS in these children would be beneficial. Studies have shown that enhancement of SWS using pharmacological agents such as serotonin 2 receptor (5-hydroxytryptamine 2, 5-HT2) antagonists and $GABA_B$ receptor agonists (gamma-hydroxybutyric acid) improve sleep efficiency (Walsh 2009; Hindmarch, Dawson, and Stanley 2005; Foldvary-Schaefer et al. 2002) and promote cognitive function (Ferrero et al. 2017; Walsh 2009; Grimaldi et al. 2020; Malkani and Zee 2020; Zhang and Gruber 2019; Blackman et al. 2020). Furthermore, acoustic stimulation techniques have also been shown to increase slow-wave activity (Papalambros et al. 2017). These enhancements during sleep have resulted in changes in spindles as well as their coupling with other sleep features and have improved memory (Ngo et al. 2013; Antonenko et al. 2013; Westerberg et al. 2015). Therefore, administering techniques to enhance slow-wave activity in Dup15q syndrome map help improve sleep physiology and potentially have a positive impact on cognition.

5.3. Conclusions

While there is considerable heterogeneity both at the genetic and phenotypic levels in NDDs, rare genetic syndromes with known etiologies, such as Dup15q syndrome, provide an incredible opportunity to explore possible mechanisms of cognitive and behavioral dysfunction. In Dup15q syndrome, the EEG biomarkers in the form of increased beta oscillations and abnormal NREM sleep physiology have a mechanistic rationale for GABAergic dysfunction. The work in this dissertation is therefore a critical step in establishing mechanisms underlying abnormal physiology and lay the foundation for future studies that investigate abnormal brain network function, test therapeutics (using preclinical models) that can alter circuit-level changes, and inform clinical trial design in patients with Dup15q syndrome with the ultimate goal of improving their cognitive and developmental outcomes. As more children with syndromic forms of NDDs undergo clinical EEG investigation, quantification methods described in this dissertation, along with collaborations with patient advocacy groups, can facilitate remote data sharing and larger-scale studies of EEG biomarkers to inform our understanding of physiology and cognition in other NDDs.

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