MECP2 Duplication Syndrome Family Meeting

Meeting Summary

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Jan and Dan Duncan Neurological Research Institute
Texas Children's Hospital
Baylor College of Medicine

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Introduction

MECP2 Duplication Syndrome (MDS) is a devastating neurological disorder that is caused by the duplication of the genetic region spanning the *MECP2* gene. Current estimates place the prevalence of MDS at about 1:15,000 male births. The first clue that the isolated duplication of the *MECP2* gene may affect proper brain function came from a mouse model, known as the *MECP2*-TG1 mouse (Collins et al. 2004). Genetic studies in individuals with severe intellectual disability identified human patients carrying duplications spanning the *MECP2* gene (Lubs et al., 1999; Van Esch et al., 2005). As *MECP2* is located on the X chromosome, MDS typically affects boys. However, girls can also be struck by the disease in the event that X chromosome inactivation is random or skewed to favor the mutant allele due to translocations. MDS may be inherited from a carrier female, however, novel duplication events in the embryo are also possible, such that carrier status in moms is not a given.
Since the identification of MDS as a genetic disorder, several key discoveries have been made to advance our understanding of this disorder and move towards therapies:

i. The optimal dosage of *MECP2* is critically important: too little MeCP2 protein causes Rett syndrome, too much causes MDS
ii. Mouse models of MDS recapitulate many of the key neurological features seen in human patients
iii. Removal of the extra copy of *MECP2* in adult symptomatic mice using genetic tools reverses MDS phenotypes
iv. Using a therapeutic tool, antisense oligonucleotides (ASO) in adult symptomatic mice reverses MDS phenotypes

The 2020 MDS family meeting was broadly organized in three parts to provide attendees with updates on ongoing research efforts and insights from clinical experts:

1. Ongoing Research Studies
2. Next Steps in ASO Therapy
3. Clinical Management of MDS

1. Ongoing Research Studies

Tim Riley (formerly Chief Scientific Officer/Chief Business Officer at RSRT): New Interventions for MDS

The Rett Syndrome Research Trust (RSRT) is funding numerous projects to better understand *MECP2* disorders. Current or planned research supported by RSRT to identify intervention strategies for MDS include:

A. Silencing RNAs (Khvorova group, UMass): small RNA molecules that are specific to the *MECP2* RNA recruit proteins that can cleave the *MECP2* RNA thereby reducing the amount of RNA. This approach may serve as a stepping stone in the direction of a more permanent method to reduce MeCP2 levels, using gene therapy delivery of a 'microRNA'. Currently, gene therapy in the brain requires extensive research into optimal delivery and prevention of overdosing.
B. Gene editing (Cohn group, Sick Kids, Toronto): using the CRISPR/Cas9 system, the duplicated copy of *MECP2* can be permanently removed to normalize the underlying genetic cause of MDS. Currently, challenges in this field include optimal delivery and distribution of the editing system, as well as ensuring that only one copy is targeted for removal to prevent the emergence of Rett features if both copies are deleted.
C. MeCP2/NCoR interaction (Bird group, University of Edinburgh): NCoR is a protein complex that contributes to the execution of some of MeCP2's molecular functions. The idea here is to identify small molecule drugs that can disrupt the interaction between MeCP2 and NCoR thereby lowering the excessive execution of MeCP2 function in MDS.
D. Drug screening platform (Muotri group, UCSD): see below for Cleber Trujillo's talk.
E. Genetic screens to identify MeCP2 regulators (Zoghbi group, BCM): see below for Manar Zaghlula's talk.
F. ASO treatment of MDS (Zoghbi group, BCM): see next section.

RSRT is also supporting research to investigate better delivery of gene therapies. This includes novel adeno-associated viruses (AAVs)[1] for better brain distribution, 'back-up' AAVs that can be used for repeat delivery without eliciting an immune response, various nanoparticle strategies, and cell-based delivery strategies. Recent findings of toxicity in preclinical gene therapy studies in non-human primates highlight the importance of optimizing the delivery of gene therapy agents. Novartis has recently put its trials in Rett syndrome on hold until additional safety studies are completed.

[1] Specially designed viruses are used in the gene therapy field to introduce genetic material to a cell.

Cleber Trujillo (University of California, San Diego): Applications of Brain-Model Technology to Study MDS

Scientists use various model systems to gain a more thorough understanding of complex human disorders. One such system used in MDS is the generation and culture of neurons and brain-like models (also known as brain organoids or mini-brains) derived directly from patient skin cells. This works by 'reprogramming' the cells in the deep layers of skin to stem cells and then guiding these stem cells to become like the neurons in the brain, in this case, specifically, the brain cortex. This process recapitulates the molecular and cellular hallmarks of brain development and produces multiple different cell types and complex structures that can be maintained for years. Typically, brain organoids from patients are studied in comparison to those from neurotypical controls. Brain organoids can then be studied on a single cell basis or as a whole to give signatures such as EEGs. The study of this model shows that over time the cells connect and communicate mimicking human brain development.

Brain organoids from MDS patients show that there is an abnormally high number of connections (as seen in the MDS mouse). They are also larger in size than control organoids. Additionally, activity levels in these neurons are increased. A small-scale drug screen previously identified two compounds that may reverse some of these abnormalities. Indeed, one of the drugs corrected the high activity levels of the cells and reduced excessive connections. However, treatment of these drugs in mice did not show behavioral symptom improvement. Currently, an optimized organoid model is in use to screen more than 1,500 drugs to find additional candidates to correct features of MDS organoids.

Manar Zaghlula (Zoghbi lab, Baylor College of Medicine): Genetic Screens to Identify Regulators of MeCP2

MeCP2 protein levels must be maintained within a tight range for the brain to execute its functions properly. MeCP2 protein can be modified on the amino acid level, i.e. the individual chemical entities that make up the protein. Proteins that modify MeCP2 are known as 'regulators'. To identify regulators, a specially designed cell line was generated that tracks the levels of MeCP2 using fluorescent reporters. Then, individual genes in a subset of the genome are 'knocked out', i.e. removed, to determine whether they have an effect on MeCP2 protein levels. Those genes that, when knocked out, reduce MeCP2 levels are candidate drug targets for MDS.

One such target was identified that affects the rate of degradation of MeCP2. Degradation of proteins is an essential process in all cells to maintain an equilibrium of the amount of proteins present. Ubiquitin is a molecular tag that is added to proteins

such as MeCP2 to signal its degradation. On the flip side, there are enzymes that remove Ubiquitin molecules and stabilize the protein. Knocking out or inhibiting one such stabilizing enzyme proved to be effective in lowering MeCP2 levels in cells. Current efforts are aimed at studying this effect in the brains of an MDS mouse model. Additionally, a novel cell line was generated to expand the screen to the entire human genome. This is possible by using a much smaller cell type that can be sorted faster. The cell line also permits the identification of regulators of *MECP2* RNA, not just the protein, further expanding the pool of potential candidates. Ultimately, a large number of candidates will be crucial in selecting a small number of effective drug targets that are also *safe* to inhibit.

Sarika Peters (Vanderbilt University): Markers of Disease Progression in MDS
Studies that are performed in the laboratory setting (e.g. in mouse models) often fail when taken to human patients. Possible reasons why these 'translational' studies fail include:

i. a failure of animal models to predict efficacy in humans
ii. a lack of consensus outcome measures and study endpoints
iii. limitations of available measures to assess a sufficiently broad functional range
iv. the absence of direct observation measures
v. a lack of validated biomarkers.

It is therefore critical for a successful clinical study that standardized clinical measures and validated biomarkers are established.
To devise these outcome measures, an in-depth understanding of MDS is required. This is the goal of the natural history study (NHS). Thus far, 69 patients have been enrolled in the NHS and are being followed once per year. Ages of participating individuals range from 1 to 28 years. In-person evaluations include parental self-reporting as well as clinician-based assessments (which were found to correlate well with each other). The first visit serves as a baseline and every subsequent visit gives insight into progression of MDS.
The most salient features of MDS, based on NHS, are developmental delay in the first 6 months of age, hypotonia, vasomotor disturbances, constipation, drooling, bruxism (teeth grinding), recurrent infections (variable with age), and epilepsy. Regression was seen in 12 participants and starting at ~12yrs of age. 73% of patients with seizures experienced some form of developmental regression. It was also observed that patients have significant motor dysfunction past 10 years of age; and while functional skills appear to improve between 5-10 years of age, they become worse with increasing age. Parent reports also indicate that top concerns change with age. In patients younger than 5 years, a lack of effective communication was reported to be the primary concern. In

patients aged 5-10 years, communication and seizures are the top concerns, and seizures ultimately become the primary worry of parents with children aged > 10 years. Lastly, a study to examine diurnal cortisol levels was performed in 27 MDS patients. In 17/27 (63%) patients cortisol levels were observed to decline over the course of the day (Peters et al., 2016). Lower cortisol levels are associated with greater severity of MDS features and ongoing work is focused on determining the effects of lower cortisol levels on seizures, quality of sleep, and brain activity. Other ongoing work is also examining the contributions of larger duplications that span the *RAB39B* gene to disease severity in MDS patients. The gene may be of interest as it is highly expressed in the brain and duplications of the gene by itself have been reported in patients with developmental delay, microcephaly, and seizures (Peters et al., 2019).

Davut Pehlivan (Baylor College of Medicine): Genetic and Clinical Studies for Clinical Trial Readiness in MDS

Prior to initiating a clinical trial in MDS (with ASOs or any other pharmacological intervention) it is critical to collect detailed information about both disease progression on a population level as well as individualized genetic and molecular data. The success of a clinical trial hinges on a therapeutic intervention to be *safe* and *efficacious*. Population level studies are critical to develop clinical and neurophysiological outcome measures for efficacy. Individual level studies serve to identify reliable biomarkers that faithfully track changes in MeCP2 levels for safety.

As all preclinical studies are done in mouse models with controlled genetic backgrounds and *MECP2* as the only duplicated gene, a better understanding of the genetic complexity in human MDS patients is needed. To this end, a unique high-resolution testing platform is used to determine the complex patterns of genetic changes in each individual. The custom array CGH (comparative genomic hybridization) used at BCM (in collaboration with the Lupski lab) contains 275 probes for *MECP2* compared to 24 probes that are used in standard clinical tests. This is used to understand whether disease severity and abnormal genomic structure correlate with each other.

Additionally, the natural history study (see above) will give insight into the course of the disease and onset of individual symptoms. Lastly, a reliable severity scale must be developed. This is an important tool in assessing whether features of MDS are responding to an intervention and to what extent compared to baseline (no intervention).

2. Next Steps in ASO Therapy

Paul Goldberg (IONIS Pharmaceuticals): Rare Disease Clinical Development
While disorders caused by single genes are often very rare, they are also highly amenable to targeted genetic therapies. Antisense oligonucleotides (ASOs) are such a genetic intervention that are highly selective and target the primary genetic cause of a disorder such as MDS. Because ASOs are so specific and target the root of the problem, large effects are expected (as seen in spinal muscular atrophy using the ASO treatment Spinraza). However, rare diseases also face challenges to therapies, such as a low prevalence, under- or misdiagnosis, high variability of features among patients, a variable natural history of the disorder, which makes identifying outcome measures and endpoints (i.e. efficacy of the ASO) more difficult.
The key challenges in the ASO trial for MDS are two-fold:

i. disease progression and severity are highly variable from patient to patient requiring a thorough natural history study and reliable severity scale (efficacy)
ii. identification of biomarkers reflective of MeCP2 levels to prevent overcorrection resulting in features of Rett syndrome (safety) is crucial.

To address these challenges, IONIS is funding a longitudinal (24-month) study at Baylor College of Medicine/Texas Children's Hospital. This study will enroll 10 patients with MDS, 10 male patients with Rett syndrome, and 30 age-matched healthy individuals (age ranges 2-30 years) to identify clinical, neurophysiological, and molecular biomarkers. This study will also collect extensive data on fluids and neurophysiological biomarkers. Given this in-depth, precision medicine approach, the hope is to use expedited regulatory pathways to quickly advance ASO therapy to human MDS patients once clinical trial readiness studies are complete with reliable biomarkers and outcome measures/endpoints.

Yingyao Shao (Zoghbi lab, Baylor College of Medicine): ASO Safety Studies in a Mouse Model of MDS
The original mouse model of MDS, in which the reversal of symptoms was first shown using ASOs (Sztainberg et al. 2015), contains one copy of human *MECP2* and one copy of mouse *Mecp2*. ASOs are highly specific to the genetic sequence they target. Therefore, only the human copy of *MECP2* could be targeted in the 2015 study. However, this is not reflective of the use of ASOs in the clinical setting, where overdosing of ASO is possible, potentially lowering the levels of MeCP2 beyond safe limits, resulting in features of Rett syndrome. Therefore, a new mouse model was developed that carries two human copies of *MECP2* (but no mouse copy). This mouse model recapitulates the molecular and behavioral features of the original MDS mouse.

This study also employed acute bolus injection of ASO, a more representative and clinically relevant delivery method than chronic infusion of ASO using pumps. Acute delivery distributes the ASO widely in the brain and lowers *MECP2* levels in multiple brain regions. The levels of *MECP2* also decreased more dramatically with increasing doses of ASO. Importantly, the timing of the reversal was also mapped out in this study and revealed that while the molecular effects of ASOs start soon after treatment, it may take some time before overt symptomatic changes are detectable. Therefore, reliable biomarkers are critical to track changes in *MECP2* levels and avoid overdosing. Lastly, this study investigated whether lowering *Mecp2* beyond safe limits would result in features of Rett syndrome, and if so, whether these are reversible. To this end, healthy mice with normal MeCP2 levels were treated with ASO to mimic a Rett-like situation. Indeed, features of Rett syndrome emerged, but reversed over time (time range of reversal 3-16 weeks after ASO treatment depending on the feature).

Sameer Bajikar (Zoghbi lab, Baylor College of Medicine): Approaches to Identify Molecular Biomarkers Sensitive to *MECP2* Levels

Given the timeline for the effect of ASOs on various aspects of MDS features (see Yingyao Shao's talk), it is critical to identify early biomarkers that ideally change immediately after ASOs have reduced *MECP2* levels. Additionally, it is important to have a sense of the actual levels of *MECP2* in *each* patient. Isolated cells from patient blood samples indicate that MeCP2 protein levels vary more than in healthy control samples. To confirm this finding in a neuronal system, neurons were differentiated from patient skin fibroblasts (known as induced neurons, or iNeurons). Again, MeCP2 levels were found to be variable. Accordingly, studies are now focused on finding a MeCP2-responsive biomarker for the safety of a clinical trial by identifying key molecular changes that occur after ASO treatment. Potential candidates identified from these studies will further tested in MDS mice and iNeurons from patients.

Mirjana Maletic-Savatic (Baylor College of Medicine): Biomarker Discovery for Assessing Therapeutic Safety and Efficacy

To accurately assess the safety and efficacy of a treatment such as ASOs, we need not only a single biomarker, but *composite* biomarkers that reflects patient health on multiple levels. Such biomarkers would help monitor individual patients during treatment, give information about target engagement, identify candidate patients for a particular treatment, and measure outcomes. These include, but are not limited to,

i. Genetic evaluation (custom-designed CGH; see Davut Pehlivan's talk)
ii. Clinical examinations (e.g. neurological, gastrointestinal, immune, etc.)

iii. Molecular biomarkers from biofluids (blood, urine, CSF, saliva) and iNeurons (see Sameer Bajikar's talk)
iv. Small molecule biomarkers (metabolites, neurotransmitters, biogenic amines)
v. Brain circuitry biomarkers (pupillometry, EEG, sleep; see Matt McGinley's talk).

Small molecules, such as sugars, amino acids, lipids, and others, are essential for proper function of cells and tissues, as they provide their building blocks, energy, and the means to communicate among each other. Measuring these molecules, also called metabolites, in biofluids is critical to medical diagnostics. For instance, classic phenylketonuria (PKU), an inborn error of metabolism that causes accumulation of phenylalanine, is a devastating disorder that manifests with severe intellectual disability and seizures from birth. It is now diagnosed routinely by the newborn screen, and this early diagnosis and treatment are curative: children undergo normal development. But, finding specific and sensitive biomarkers that reflect disease pathology and prognosis is not easy.

Today, instead of focusing on discovery of a single molecule, we use sophisticated technologies to examine hundreds of small molecules in a sample at the same time, to elucidate composite changes that inform us about the clinical status of a patient at that particular time. The two complementary platforms are mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. While MS is excellent at detecting miniscule amounts of a metabolite, it is not easily employed to identify such metabolites. NMR, on the other hand, is an excellent identification tool, but it requires larger amounts of a metabolite in a sample in order to detect it. Therefore, both platforms are used in concert for small molecule biomarker discovery.

Our initial studies of plasma from mice with MeCP2 duplication treated with ASOs have identified two candidate biomarkers that appear to be sensitive to MeCP2 levels. The same two compounds were also abnormal in plasma from a small cohort of MDS patients, which is a promising first result. Ongoing studies aim to identify the relationship between these biomarkers and MeCP2 levels and to decipher whether they correlate with other clinical measures of the patients' disease stage. Importantly, more human samples are needed to confirm the validity of this finding.

Matt McGinley (Baylor College of Medicine): New Approaches to Assess Brain Function in MDS

In addition to the molecular and metabolic biomarkers (see Sameer Bajikar's and Mirjana Maletic-Savatic's talks), identifying physiological biomarkers will give a better

insight into changes in brain activity in response to ASO treatment. Assessing physiological biomarkers is typically minimally invasive (e.g. EEG, sleep study). Physiological biomarkers include:

1. Brain arousal: the ability of the brain to respond to moment-to-moment changes in behavioral and cognitive demands
2. Sensory evoked potentials: how does the brain respond to sensory stimuli, e.g. touch, sound, light
3. Sensory gating: the brain's ability to filter its response to focus on important stimuli

Brain arousal can be tracked closely by changes in the diameter of the pupils because pupils receive input from both the sympathetic ('fight or flight') and the parasympathetic ('rest and relax') nervous systems. These effects are driven by changes in levels of neurotransmitters in the brain. Studies in mouse models of *MECP2* disorders have validated differences in pupillary response to sound stimulation. The pupils can also provide a reliable measure of the brain's ability to filter salient information. This is done using a test known as prepulse inhibition (PPI). In PPI, a loud sound is played immediately after the quieter sound. Typically, a startle response is measured when the loud sound is played. This test can be modified to use pupillary response to assess sensory gating with improved sensitivity and resolution.

3. Clinical Management of MDS

Eric Marsh (Children's Hospital of Philadelphia): Seizures in MDS

Epilepsy is characterized by the development of recurrent, unprovoked seizures (per definition, two or more). Seizures can be provoked, among others, by genetic defects, brain bleeds/strokes, high fevers, infections, and drug and alcohol use. Seizures may either be clinically visible or subclinical, i.e. only detectable by EEG.

MDS patients may experience all of the classic categories of seizures:

i. Partial seizure: starts in one part of brain and spreads to other specific parts
ii. Secondarily generalized seizure: starts in one part and rapidly spreads to the whole brain
iii. Primarily generalized seizure: seizure activity occurs across the brain at once

Partial seizures typically do not affect consciousness, may be accompanied by involuntary movements, and at times can lack clear signs of a seizure. Absence seizures (staring spells) may be very brief episodes and therefore at times cannot be clearly distinguished from non-seizure activity. Generalized seizures, on the other hand, involve loss of consciousness, convulsions (rhythmic jerking of the body), shallow breathing and drooling, changes in muscle tone, and possible loss of bowel or bladder contents. Seizures may be:

i. myoclonic, i.e. brief muscle contractions that may affect certain muscles and involve one or both sides of the body
ii. tonic, i.e. stiffening or posturing of both sides of the body
iii. atonic, i.e. loss of tone resulting in a drop of the head and trunk.

Many MDS patients experience epilepsy that overlaps with features of Lennox Gastaut Syndrome, i.e. childhood onset (3-8 years), infantile spasms (very rare in MDS), atonic, tonic-clonic, atypical absence, and myoclonic seizures, that are often unresponsive to medications.

Seizure triggers can be varied, but may include emotional stresses, sleep deprivation, hormonal changes, interactions between medications, missed meals or specific food and drink items, nutritional deficiencies, and specific stimuli (flashing lights, hyperventilation, sudden loud noises). Approximately 50-70% of MDS patients suffer from epilepsy with an average age of onset of 8-9 years. MDS patients often do not respond to medications well and multiple different epilepsy drugs may be tested by neurologists (trial and error). Generally, treatment for epilepsy in MDS should avoid 'polytherapy', meaning seizure control with 3 or more drugs at a time. Regarding medical-grade marijuana in the form of EPIDIOLEX, it appears that some MDS patients may be responsive, however, no patients have become seizure free. Therefore, it may become another type of medication to be tried by neurologists, but is not a 'game changer'. EPIDIOLEX may also interact with other seizure medications, such as Clobazam and Depakote. (For clinical data on the use of cannabidiol in Lennox-Gastaut syndrome, refer to Thiele et al., 2018.)

Importantly, not all unusual events are seizures. It is also possible that abnormal EEG is recorded, but no visual signs of a seizure are present. Often video EEG can help with the visual confirmation of the presence of seizures, which will also inform the treatment plan. Indications that an episode is *not* a seizure may be eye contact, non-rhythmic, non-reproducible movements, and returning to one's self quickly after.

Some ways to help with seizures and seizure tracking: seizuretracker.com; keeping an epilepsy journal (duration, time of day, type of seizure); keep a seizure first aid kit.

<u>Daniel Glaze (Baylor College of Medicine, Texas Children's Hospital):</u> Sleep disturbances in MDS

Sleep is necessary for a child's optimal functioning and development. Estimates of children with neurological problems suffering from sleep disturbances range from 44-83%. Poor sleep may cause irritability, accidental injuries, behavioral problems (e.g. poor impulse control, hyperactivity), cognitive problems (e.g. inattention, learning problems), impaired cardiovascular, immune, and hormonal function.

Sleep disturbances can be treated and may be preventable. First, sleep problems need to be identified and characterized. It is important to identify factors contributing to the sleep disturbances, aside from the genetic MDS diagnosis (e.g. medications). Parental attitudes are critical too, as each plan of action has to be customized for each family. Medical problems that may affect sleep include epilepsy, gastrointestinal problems (e.g. GERD), medications that may either interfere with sleep or make patients too sleepy, additional psychiatric problems (e.g. behavioral problems).

Tools to evaluate quality of sleep include:

i. Sleep questionnaire, BEARS: bedtime problems, excessive daytime sleepiness, awakenings during the night, regularity and duration of sleep, snoring
ii. Sleep diary: 2-week recording of child's sleep time during each 24-hour period; also record what happens at night. This is a powerful tool to share with your child's physician.
iii. Actigraphy: a wristband/watch that can evaluate movements during sleep; the more movement during the night, the poorer the quality of sleep. This is a non-invasive method that provides lots of information.
iv. Pediatric Polysomnography: this method is performed in the clinic and acquires information about many modalities during sleep, including brain activity, breathing, chest and abdominal movements, oxygen and CO_2 levels, heart rate, leg movements. This is helpful to identify specific sleep problems and may ultimately serve as a biomarker in clinical studies.

Sleep problems in MDS include:

i. Insomnia: difficulty initiating and maintain sleep; early morning awakenings
ii. Wake/Sleep scheduling problems: delayed sleep onset; early morning awakenings; irregular schedules
iii. Sleep-disordered breathing: obstructive sleep apnea, i.e. air is not flowing due to blockage
iv. Parasomnia: a state between wakefulness and sleep; often characterized by sleep walking, night terrors, bruxism, and periodic limb movements.

Sleep problems may be managed by treating underlying medical problems, managing daytime behavioral problems, changing medications as needed, and exercising proper sleep hygiene. Good sleep hygiene:

i. age/developmental stage appropriate
ii. a set routine that is initiated 20-30 minutes prior to the desired sleep time
iii. put child to bed awake, but drowsy
iv. bedroom should be dark, cool, safe, and electronics-free environment
v. pre-bedtime meal, no caffeine
vi. routine exercise 3-4 hours prior to bedtime

vii. appropriate light cues: bright light in the morning promotes earlier sleep onset, whereas bright lights late in the day delays onset of sleep

To tackle insomnia, relaxation techniques can be helpful, e.g. massage therapy and weighted blankets. Medications may be used if necessary (however, none are approved for the pediatric population only), e.g. melatonin, clonidine, risperidone, zolpidem, trazadone, and gapapentin.

Night-time awakenings should be of concern if they occur more than 3-4 times per week, are longer than 20 minutes in duration, and require parental involvement to be resolved.

Sleep-disordered breathing can be managed using various techniques, e.g. T&A, CPAP, adjusting sleep side positioning, weight management, and ultimately medications.

Wake/sleep scheduling problems may be targeted by light therapy and melatonin in low doses 3-4 hours prior to bedtime to help set the internal clock.

Bernhard Suter (Texas Children's Hospital): Motor Dysfunction in MDS

Motor dysfunction is progressive in MDS patients. 82% of MDS patients experience infantile hypotonia. This early hypotonia often precedes subsequent spasticity, which is more pronounced in the lower limbs and may develop into mild to severe contractures at the hips, knees, and ankles. If tone is increased (dystonia), muscles contract uncontrollably. This is often seen at the feet and ankles and may require medication and/or orthoses for treatment. Kypho-scoliosis is the excess curvature of the spine at the chest (thoracic) region of the spine (kyphosis) together with a side-to-side flexing at the hip region (scoliosis). Early physiotherapy might improve or delay onset of kypho-scoliosis, which is seen in about half of MDS patients by age 22.

Gait problems in *MECP2* disorders, MDS and Rett, appear to track closely with disease progression, making them potentially useful as clinical biomarkers in clinical trials. MDS patients have a delayed median time to walking of 4 years of age. About 1 of 5 MDS patients is unable to walk without support. Some may also develop an ataxic gait, i.e. impaired balance and coordination. Typically, MDS patients have a characteristic crouch gait with flexion at the knees and a broad-based stance. Gait can be analyzed in a specialized gait lab, in a secured environment, to prevent injuries.

Additionally, there are 2 major forms of extraneous movements in MDS: stereotypies and choreiform movements. Stereotypies are repetitive, simple movements that often tend to be rhythmic, with a fixed pattern (e.g. hand flapping, waving, rotating, wringing) and regular frequency. Stereotypies can usually be stopped by distractions, such as calling the individual's name. 89% of MDS patients have stereotypies that appear around school age and persist in some form throughout life. Choreiform movements are

intermittent, spontaneous writhing movements of the arm, hands, and fingers that may also involve the head and tongue. They can also be described as flowing movements, e.g. from one arm into the other. Tremors have not been observed in MDS patients.

Conclusion

Many significant advances have been made since the discovery of *MECP2* Duplication Syndrome a mere 15 years ago. Most importantly, vigorous mouse studies suggest that symptoms of MDS can be reversed by lowering the excess levels of *MECP2* in the brain. Therefore, several studies in adult symptomatic mice are underway to treat MDS pharmacologically or genetically, with the goal to ensure a better quality of life to the patients afflicted by this devastating disorder.
Importantly, antisense oligonucleotide therapy, a method to *directly* lower *MECP2* RNA levels, is currently the most advanced and disease-specific treatment strategy. However, prior to administering ASOs in patients, comprehensive safety studies are requisite considering the consequences of lowering MeCP2 below healthy levels resulting in features of Rett syndrome. These safety assessments are in part performed in mouse models to better understand dosing, but also call for the establishment of a *composite biomarker* made up of genetic, molecular, metabolic, neurophysiological, and clinical data that closely track with changes in MeCP2 levels. Additionally, a successful clinical trial will hinge on identifying study endpoints that heavily rely on a detailed understanding of the natural history of MDS.
The 2020 MDS Family Meeting concluded with a positive outlook and a clear vision for the near-term requirements to initiate a successful clinical trial with ASO therapy, and to continue the ongoing research efforts into therapeutic intervention and clinical management of *MECP2* Duplication Syndrome.