7th INTERNATIONAL ANGELMAN SCIENTIFIC CONFERENCE

15-17 September 2022 Vienna AUSTRIA

Conference Summary

&

Abstracts of the Lectures

Association for the Research on Angelman Syndrome Austria

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INTRODUCTION

Dear parents, December 2022
dear friends,

the **7th International Scientific Angelman Syndrome Conference**, which took place at the medical University in Vienna from 15th to 17th September 2022, was a complete success.

More than 130 people from 23 countries all over the world came together, fruitful discussions between scientists, pharmaceutical companies, and parents could take place in a beautiful ambiance and will be remembered for a long time. Many new ideas could be developed and taken away.

7th international Scientific Angelman Syndrome Conference

The focus of the first two days (Thursday, Friday) has been the scientific exchange between scientists and medical professionals. On Saturday (Parents' Day), the program has included an overview of scientific and clinical research. The winners of the ASA Awards were able to present their project proposals. Lectures on the topics of communication and cannabidiol (CBD) has provided practical help for everyday life.

This document starts with a **summary** kindly provided by Angelman Center Munich, followed by the **abstracts** of the speakers, structured by thematic areas:

- UBE3A, mouse models, cellular pathways
- small molecules
- links to other disorders
- quality of life study

- ASA research grants 2021
- biomarkers
- clinical trials update
- networks & collaborations
- Parents' Day: Augmentative Alternative Communication (AAC), Cannabidiol (CDB) and sleep.

Notes taken during the talks were added to the abstracts.

Furthermore, the main points of the two **plenary sessions** were summarized and can be used for future activities and tasks of ASA.

For reference the conference program is included in the Appendix.

The local organizing committee of 7th international Scientific Angelman Syndrome Conference:

- Thomas Schramm, Chairman of Association for the Research on Angelman Syndrome
- Sandra Martinz, Deputy of Association for the Research on Angelman Syndrome
- Pia Schlögl, Association for the Research on Angelman Syndrome
- Martine Schramm, Association for the Research on Angelman Syndrome
- Roland Spielhofer, Association for the Research on Angelman Syndrome
- Mirella Karoly Association for the Research on Angelman Syndrome
- Nicole Scheickl, Association for the Research on Angelman Syndrome
- Dr. med. Christel Kannegießer-Leitner, Association for the Research on Angelman Syndrome
- Univ. Prof. Dr. Harald Sitte, Medical University Vienna, Chairman of the scientific advisory board Angelman Syndrome Alliance

With this in mind, we wish you interesting reading and hope it will be a good memory for the next ASA activities.

Best wishes,

Association for the Research on Angelman Syndrome Austria

Notice:

This report has been prepared to the best of our knowledge and belief. Nevertheless, we cannot guarantee the timeliness, accuracy, and completeness of the information.

CONFERENCE SUMMARY

by Angelman-Center Munich

Dear parents,

In September, as members of the team at the Angelman Center Munich, we had the pleasure of attending the 7th International Angelman Congress in Vienna. In the following we would like to give you a short overview of the current research projects and results.

We would like to start with news about basic molecular research on cell lines and mouse models, which are essential for understanding the pathomechanisms in Angelman syndrome (AS) and important for the development of therapies. While it is known that loss of the protein UBE3A leads to AS with severe neurodevelopmental delay, the exact function of UBE3A in neurons is not yet understood. In a cell experiment conducted by a research group at the University of Aveiro, it was shown that different UBE3A subtypes exist, which are important to control the development of excitatory and inhibitory synapses and to establish a balance between them[1]. In another research group at the University of Lisbon, it was possible to generate and study cerebellar organoids from pluripotent stem cells carrying a UBE3A mutation or deletion on the 15q11-13 locus. These showed smaller size, delayed neuronal maturation, and impaired functionality in contrast to control organoids. This could explain the ataxia present in AS patients, due to a lack of cerebellar functionality[2]. In addition to cell line research, various mouse models are being used. Most mouse models used to date are based on a UBE3A mutation, not a deletion, and therefore do not address expression changes of other genes on the 15q11-13 locus, which are also relevant in AS. Characterization of a mouse line by the Roche company with a mutation affecting the AS-PWS predisposition center showed that these mice exhibit similar deficits to the UBE3A mutation mice[3]. Furthermore, a study on AS mice was presented by the company PTC Pharmaceutics, in which an adeno-associated viral vector was applied to the mice as gene replacement therapy of the UBE3A gene into the cerebrospinal fluid or hippocampus. Hereunder, motor functions, total neurological scores, and anxiety of the AS mice improved. UBE3A protein was also detected in mice 6 months after injection.

In addition to exploring molecular mechanisms at the cell line and mouse model levels, it is important to establish clinical biomarkers to measure changes in AS patients after pharmaceutical intervention. Biogen reports that they have developed an assay to reliably detect UBE3A protein in healthy AS patients and in AS patients who have very low concentrations of UBE3A in their CSF. The concentration of UBE3A protein in CSF could be a valuable biomarker for ongoing and future intervention studies.

Also, the FREESIAS study presented by Roche set out to establish feasible and appropriate end points for measuring meaningful changes in children with AS. For this, a wide range of clinical outcome assessments, nocturnal EEGs, and digital health technologies were tested. Very good completion rates were shown for the clinical outcome assessments, whereas only about 1/3 of the nocturnal EEGs could be performed (presumably due to the COVID pandemic). The feasibility of digital health technologies (e.g. sleep mat or Actigraph) varied widely. In addition, the sleep behavior of patients with AS was evaluated using a sleep diary, a sleep mat positioned under the mattress, polysomnography, and a clinical sleep scale. Highly abnormal sleep structure and physiology was found in individuals with AS[4].

In a clinical study of sleep behavior in children with AS at the Angelman Center in Rotterdam, colleagues Bindels de Heus et al. reported that sleep problems could be improved by a behavioral therapy intervention involving psychoeducation, sleep evaluation by a behavioral therapist, and subsequent individual counseling. Children in the intervention group showed improvement in total sleep time with positive and long-lasting effects on sleep hygiene and quality of life[5]. Another prospective observational study conducted in Rotterdam aims to show whether and how genotype, epilepsy, sleep problems, cognitive developmental level, autistic traits, and emotional behavioral problems influence the child's health-related quality of life and parental stress situation. Deletion genotype and older age were found to be associated with lower quality of life. Sleep problems and emotional behavior problems are related to parental stress[6].

Other presentations covered cannabidiol and augmentative communication.

Finally, Roche reported that the first part of the ASO clinical trial TANGELO and recruitment have been completed. Results will not be analyzed and published until the second part of the study is completed. The clinical ASO study HALOS by Ionis was also presented by the company, however, there are also no results on the effect of ASO therapy in AS patients yet.

In summary, we as "clinicians" were able to learn a lot about basic research on stem cells and mouse models. This is important to gain insight into basic molecular science as a clinician as well. Likewise, the exchange with the Angelman Center in Rotterdam about current research results on sleep and behavior was very interesting and inspiring for our daily work in Munich. The collaboration with pharmaceutical companies is essential for the development of a therapeutic option for AS children. At the congress, we were able to learn about the latest approaches in personal discussions. The close contact and the interdisciplinary exchange in a nice ambience with all attendees have increased our knowledge about AS and will be incorporated in our daily work with your children. Thank you very much for your support.

Your Angelman Center Munich, Lena Manssen and Christine Makowski

Sources: Presentations during the 7. International Angelman Congress in Vienna by Ype Elgersma, Ugo Mayor, Rajini Rao, Stormy Chamberlain, Ben Distel, Hanoch Kaphzan, Ramiro Almeida[1], Gaia Novarino, Evgenia Bekman[2], Simao Rocha[2], Ilaria Tonzzini, Laura Borancelli, Karen Bindels[5], Marie Claire de Wit, Christel Kannegiesser Leitner, Michaela Zöbl, Hanna Vihma, Ramanathan Narayanan[3], Sebastian Camillo Holst4, Rob Komorowski, Matteo Fossati, Carin Maranga, Mia Tichy, Doesjka Hagenaar[6], Cesar Ochoa-Lubinhoff, Alberto Velez van Meerbeke, Brenda Vincenzi, Ifode Ajari, Becky Crean.

BASIC MOLECULAR RESEARCH ON CELL LINES AND MOUSE MODELS

Ype Elgersma, PhD

Erasmus MC, Department of Neuroscience, Rotterdam, Netherlands

Novel mouse mutants to address emerging questions

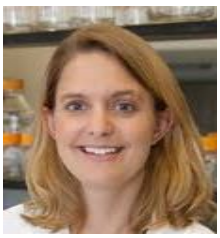
Key words: novel mouse models, importance of the UBE3A in the nucleus, striatum

In my presentation, I reviewed how we have been able to successfully use AS mouse models to understand the mechanisms underlying Angelman Syndrome and to test therapies. This has led to a large increase of our knowledge of how loss of UBE3A results in problems with neuronal function. In particular, we showed the **importance of UBE3A in the nucleus of the cell.** We further showed that most UBE3A missense mutations as found in AS patients result in the inability of UBE3A to go to the nucleus.

AS mouse models also demonstrated the importance of the brain region called **striatum**. We showed that UBE3A is important for the development of the striatum in mice. In human, malfunctioning of the striatum could underly many of the core phenotypes of Angelman syndrome.

In addition to providing insight into the mechanisms causing AS, mouse studies have facilitated the development of therapies such as ASO therapy. The key to that success is that AS mice recapitulate the human disorder very well (e.g., problems with motor coordination, epilepsy, behavioural problems).

I also indicated that there is a weakness with all our mouse studies, since all our mice carry a UBE3A mutation. In AS patients, only 15% of the patients have a UBE3A mutation. Hence, now we also investigated whether mice that mimic the UPD and imprinting (ICD) mutations that are present in some AS patients. Last but not least, we have now made **a novel mouse model** that mimics **the large 15q11-13 deletion** that is found in most AS patients. This will allow us even better to model the human disease, and to identify better treatments.



Stormy Chamberlain, PhD

Roche

Coordinated Regulation of UBE3A-ATS and UBE3A

No abstract available.

Notes

Stormy Chamberlain is an associate professor, and her laboratory uses induced pluripotent stem cells (iPSC) to model and study human imprinting disorders. In her presentation she talked about the investigation of the following research questions.

1. How is the imprinting in AS happening? 15q11-q13 is a complex locus. Coding and non-coding genes are regulated in an allele-specific manner. DNA methylation imprint at PWS-IC is established in the maternal germline and maintained in all somatic cells – Leads to expression of several coding and non-coding genes exclusively from the paternal allele. Imprinting of UBE3A occurs only in mature neurons and occurs due to neuron specific expression of UBE3-ATS.

2. How preventing the collision of the two trains UBE3A-ATS and UBE3A: How do ASOs unsilenced UBE3A? If ASOs cut RNA that has already been transcribed, how does this prevent the "collision"?
    - Does UBE3A activation in AS neurons require disengagement of RNA Pol II?
    - Designed an ASO to cut immediately upstream of SNORD 109B
    - Hypothetically would prevent XRN2 from disengaging RA Pol II.

Hanoch Kaphzan, PhD

University of Haifa, Sagol Department of Neurobiology, Israel

Angelman syndrome, learning from bioinformatics analyses and the role of redox homeostasis in early brain development

Keywords: ROS, Angelman syndrome, neurodevelopmental, mitochondria.

Angelman syndrome (AS) is a genetic neurodevelopmental disorder occurring in approximately 1:15,000 live births, caused by the loss of the maternal copy of the UBE3A gene. AS is characterized by developmental delay, lack of speech, motor dysfunction, epilepsy, autistic traits, and a unique behavioral profile of happy demeanor. Despite the apparent association between UBE3A expression and neurodevelopmental disorders, up to date, very little is known about the cellular pathways connecting UBE3A expression with AS traits in general and during neurodevelopment in particular. The present study aimed to address the functional effects of Ube3a loss during embryonic neurodevelopment. We found that AS embryonic neural precursor cells exhibited altered apoptotic capacity and mitochondrial-related metabolism aberrations, like Reactive oxygen species (ROS). ROS plays a significant role in signaling pathways and regulating physiological functions and development. Since the mitochondria are one of the primary sources of endogenous ROS, we studied how the alteration in the expression level of UBE3A leads to mitochondrial abnormalities that affect various cellular processes (such as apoptosis, proliferation, oxidative stress, and ROS accumulation). This study demonstrated that UBE3A dysregulation during the late embryonic stage leads to elevated reactive oxygen species levels subsequently affecting cellular processes such as apoptosis and proliferation that are required for normal brain development.

Ramanathan Narayanan, PhD

Roche

Characterizing mICD mouse model for AS

Key words: mICD mice, UBE3A protein, modelling, mouse models

Angelman Syndrome (AS) is a severe debilitating neurodevelopmental disorder with an estimated incidence of 1 in 20,000. Individuals with AS show strong deficits of fine and gross motor skills, absence of speech, intellectual disability and abnormal behavior (Williams et al., 2006). Additionally, 80% of the patients have epilepsy and problems with sleep (Williams et al., 2006; Bindels-de Heus et al., 2019). Currently, only symptomatic treatment is available, which is predominantly aimed at reducing seizures and improving sleep. AS is caused by the absence of functional maternally derived UBE3A protein. This is due to deletion in the 15q11-q13 region [DEL, >75% of the AS patients], imprinting defects affecting the AS-imprinting center (ICD), paternal uniparental disomy of chromosome 15 (UPD), and mutations specifically affecting the UBE3A gene (Buiting et al., 2016). Current mouse models used in AS research are UBE3A-centric and do not address the expression changes of other genes in the 15q.11-13 locus on the pathophysiology of AS. This limits the potential to dissect differences in therapeutic responses for current UBE3A-targeting strategies and hampers identification of novel therapeutics/co-therapeutics.

Here we studied a mouse line that harbors a mutation affecting the AS-PWS imprinting center (Lewis et al., 2019), hence modeling the mICD and UPD AS mutation. The mICD mice displayed robust deficits as previously reported for Ube3a mice (Jiang et al 1998; Sonzogni et al., 2019) such as increased body weight, reduced brain weight, impaired rotarod performance, reduced marble burying, nest building and increased immobility, hindlimb clasping in the tail suspension test. These behavioral abnormalities were accompanied by a loss of UBE3A protein in the cortex and bi-allelic expression of Ube3a-ATS, Mkrn3-Snord115 gene cluster. Subsequent experiments are planned for reinstating the expression of UBE3A in neonatal mICD mice by applying antisense oligonucleotides (ASOs) targeting Ube3a-ATS and to assess behavioral/molecular phenotypes in adulthood.

Such a comprehensive analysis of mouse models covering all AS subtypes will enhance the success rate of translating pre-clinical findings to the clinic by assessing differences in dosing, efficacy and guiding non-Ube3a-targeting therapeutic strategies.

Ugo Mayor, Dr.

University of the Basque Country UPV/EHU, Department of Biochemistry, Spain

UBE3A-induced ubiquitination changes in the brain elucidated by proteomics

Angelman Syndrome (AS) is a neurodevelopmental disorder with complex symptomatology caused by the loss of maternal allele expression of one single gene in the brain the ubiquitin E3 ligase UBE3A. The underlying genetic basis of AS and the phenotypes observed in both humans and in animal models of AS have previously been extensively described. However, the molecular mechanisms regulated by UBE43A ubiquitination in the brain remain highly elusive.

Previous studies have reported a number of proteins whose abundance or activity are altered in AS models implicating various signaling pathways in the physiopathology of AS. But the identified pathways could well be altered further downstream of UBE3A ubiquitination events. Here we provide the first proteomic report of Ube3A-mediated ubiquitination events in a mammalian brain. For this we have used both our bioUb mouse model and a new mouse strain that only slightly elevates UBE3A levels. Several proteins known to be involved in the trafficking and maintenance of neurotransmitter receptors as well as proteins relaying the signals of these synaptic receptors are shown here to be ubiquitinated by UBE3A. The identified proteins have roles in higher mental function long term potentiation seizures and neurodevelopmental disorders being involved in the BDNF RAS/ERK and TSC/mTOR signalling pathways. A reduced ubiquitination of these proteins is expected when UBE3A levels are lower in Angelman patients so their identification could be key to opening novel therapeutic strategies for treating AS. Further work will be required to characterize how UBE3A orchestrates each of these multiple regulatory events in the human brain.

Ramiro Almeida, PhD

Institute of Biomedicine, Department of Medical Science, University of Aveiro, Portugal

The role of UBE3A in synapse formation and function

The ubiquitin-proteasome system (UPS) has been widely shown to have a crucial role in neuronal development, maintenance and function. Recent studies show that control of protein turnover by the ubiquitin-proteasome system (UPS) occurs locally at synapses. In fact, ubiquitinated proteins are highly enriched at the Drosophila NMJ, with aggregates of ubiquitin (Ub) conjugates surrounding the active zone. Moreover, the presynaptic ubiquitinated proteome includes both structural and signaling proteins as well as proteins with known roles in synaptogenesis. We have recently demonstrated that proteasome inhibition induces the formation of new presynaptic terminals. We showed that inhibiting the proteasome specifically in axons leads to an increase in the number of presynaptic terminals. Moreover, these newly formed synapses are functional. Finally, we observed that in axo-dendritic synapse formation the proteasome is locally inhibited, and this precedes the formation of presynaptic terminals. These results demonstrate a crucial role for a localized action of the proteasome and more importantly demonstrate that poly-ubiquitinated proteins regulate the formation of presynaptic boutons. UBE3A, codes for a ubiquitin ligase, a protein that normally tags substrates with ubiquitin moieties determining its fate. At the molecular and cellular level AS is characterized

by a delayed window of synapse formation and poor refinement of synaptic connectivity. Mice engineered to lack expression of the maternal Ube3A copy display impaired long-term potentiation, impaired excitatory synaptic transmission and decreased experience-dependent maturation of neuronal circuits. Overall, brain function is highly compromised early in development, thus further suggesting that initial wiring of neuronal circuits does not occur properly. However, the role of Ube3A in synapse formation is poorly understood. We observed that depletion of Ube3A leads to a decrease in the number of synapses and Vglut1 puncta, suggesting that Ube3A is required for the formation of presynaptic clusters. These results unmask a new on-site Ube3A-related mechanism controlling the formation of presynaptic sites. Future studies will disclose the role of Ube3A in synapse formation.

Mattijs Punt
Department of Neuroscience, Ben Distel Lab, Rotterdam Netherlands

The effect of UBE3A on the function of the proteasome

Mattijs Punt[1,2], Isabell a Zampeta[1,2], Thomas van Nes[1,2], Ben Distel[1,2], Ype Elgersma[1,2]

[1]Department of Clinical Genetics and Dept. of Neuroscience, Erasmus MC, 3015 GD Rotterdam, The Netherlands, [2]*ENCORE* Expertise Center for Neurodevelopmental Disorders, Erasmus MC, 3015 CN, Rotterdam, The Netherlands

Every cell in the human body is equipped with a mechanism to get rid of proteins that are no longer needed. This mechanism is referred to as the ubiquitin-proteasome system. The UBE3A protein has a very important role in this system because it creates a signal (we call this signal ubiquitination) on proteins that need to be degraded, such that the rest of the system recognizes these proteins. Proteins that got this signal from UBE3A are transported to a large complex in the cell (the proteasome) where those proteins are broken down. We have known for a long time now that UBE3A creates these ubiquitin signals on only a subset of proteins, and that if UBE3A cannot perform this action, those proteins will not (or less efficiently) be broken down. However, we do not fully understand the consequences and we believe that we have yet to discover all the proteins that UBE3A ubiquitinates, so a lot is still uncertain! In our lab, we have found some pieces of evidence that show that the amount of the parts that make up the proteasome—the complex responsible for the breakdown of ubiquitinated proteins—are different in cells that do not have any UBE3A from cells that do. Based on this observation, we started to consider the possibility that—since cells without UBE3A (like the cells from AS patients) have different levels of these proteasome parts—maybe this would lead to changes in the speed by which (ubiquitinated) proteins are broken down in these cells (by the proteasome). We will test this hypothesis using a specific reporter protein, of which we can measure the levels over time to get an idea of how quickly this is broken down. These experiments will teach us more about the efficiency of the proteasome and the ways in which UBE3A has an effect on it. Altogether these experiments will be the first of many more to come, where the effect of UBE3A on the proteasome will be the central theme.

Short talks

Matteo Fossati, *Humanitas Research Hospital, Rozzano, Italy*

UBE3A-dependent regulation of synaptic development: implications for the pathogenesis of neurodevelopmental disorders

The UBE3A gene codes for an E3 ubiquitin ligase and is critical to ensure a proper brain function. Genetic defects of UBE3A result in pathological phenotypes. Loss of UBE3A causes the Angelman syndrome (AS), a severe neurodevelopmental disorder characterized by intellectual delay, motor deficits and seizures, while overexpression or increased activity of UBE3A are associated with autism. Although considerable efforts have been put to dissect the molecular underpinnings of UBE3A function in neurons, the pathogenic mechanisms of these neurodevelopmental disorders are still poorly understood. For this reason, current therapies only aim at mitigating symptoms. In this project, we study the effects of UBE3A loss (thus mimicking the genetic alterations of the Angelman syndrome) on the regulation of synaptic development in vivo. To this aim, we combine cortex-directed in utero electroporation to inactivate UBE3A in sparse pyramidal neurons with confocal and super-resolution microscopy to investigate the role of UBE3A on the formation, maturation and functional organization of excitatory and inhibitory synapses up to the nanoscale. As already reported by other groups, our results confirm that UBE3A loss affects the formation of excitatory synapses. In addition, our data suggest that UBE3A critically regulates the assembly and the maturation of specific subtypes of inhibitory synapses in a cell-autonomous manner. Importantly, the inutero replacement of endogenous Ube3a with individual human UBE3A isoforms indicates that the development of individual subtypes of synapses is selectively controlled by distinct isoforms (which are localized in different subcellular compartments). Together, our preliminary results suggest that the individual isoforms of UBE3A may regulate different aspects of excitatory and inhibitory synapse development through cell-autonomous mechanisms, ultimately contributing to set the equilibrium between excitation and inhibition at the single-cell level.

Carina Maranga, *Universidade de Lisboa, Portugal*

Modelling megadeletion cases of Angelman Syndrome using stem-cell based technology

Angelman Syndrome (AS) is a neurodevelopmental disorder with no cure caused by the absence of functional UBE3A protein in neurons, which is translated in pronounced developmental delay, speech impairment, ataxia, and epilepsy. Patient-derived induced pluripotent stem cells (iPSCs) offer an opportunity to generate human-based in vitro models of the disease, suitable for studying molecular causes and for testing therapeutic approaches. The main goal of our work is to contribute to a better understanding of AS using these stem cell models with a current focus on the most common genetic cause of AS, a large deletion of chr15q11-q13 deletion, aka megadeletion. Our works concentrates in generation of regionalized brain organoid models, while enlarging the current portfolio of patient-derived iPSCs to increase the robustness of our findings using in vitromodeling of this syndrome. We focused our attention on cerebellar organoid models as we postulate that the cerebellum could be affected in AS since ataxia is one of the consistent features of AS. We have generated cerebellar organoids from two megadeletion patient-derived iPSC lines that recapitulated normal cerebellar development and UBE3A imprinting. Megadeletion-derived cerebellar organoids consistently exhibited reduced size and expanded rates of cerebellar neuroepithelium, and a decreased expression of

progenitors for excitatory and inhibitory cerebellar neurons. Functional analysis by calcium imaging and electrophysiological studies demonstrated functional defects of cerebellar neurons with high degree of immaturity and an excitatory phenotype. These data provide the first demonstration in a humanized model that cerebellar dysfunction may contribute to the disease symptomatology. To substantiate these findings, we have generated and characterized a new iPSC line derived from a female infant with AS harboring a megadeletion. We show that this new iPSC line expresses pluripotency markers, is capable of trilineage in vitro differentiation, and retains epigenetic fidelity at the chr15 imprinted locus. This new cell line, available to the scientific community will contribute to increase the number pre-clinical stem cell models of AS and foster stem-cell based research for therapeutic advancement in the context of Angelman syndrome.

Eugenie Suter, *Roche*

Development of clinician-reported and caregiver-reported global impression scales for Dup15q Syndrome

AIMS: Clinical outcome measures for Dup15q Syndrome are required to support the development of new therapies. The objective was to design standardized clinician-reported and caregiver-reported global impression scales that assess the severity of and changes in clinically relevant symptoms and functional impairments in Dup15q Syndrome.

METHODS: The Dup15q clinician rated severity (CGI-S) and change (CGI-C) scales as well as the companion caregiver rated CaGI-S and CaGI-C were modified from the Angelman Syndrome (AS) clinician global impression scales (SAS-CGI) and Caregiver-reported Angelman Syndrome Scale (CASS). Although these are distinct syndromes, the overlap in neurodevelopmental and neurological manifestations in AS and Dup15q Syndrome allowed modification of the SAS-CGI and CASS scales for Dup15q Syndrome. Core symptoms and impacts were compared to a draft conceptual model of Dup15q developed by a representative of the Dup15q Alliance and an expert clinician. Clinical experts were consulted to ensure that the instruments captured relevant symptoms and associated impacts on functional abilities.

RESULTS: The new scales retained the same symptom domains as the SAS-CGI and CASS, but their clinical and behavioral descriptions were adapted to reflect the range of symptom manifestations of Dup15q Syndrome. The Dup15q CGI and CaGI assess: seizures, expressive communication, fine motor skills, gross motor skills, cognition, activities of daily living, social interaction, maladaptive behaviors, and sleep; A global item was included to assess overall Dup15q severity. Standardized clinical ratings of severity anchors were developed to optimize inter-rater reliability and ensure an equal degree of clinical meaning for 1-unit changes across the scale. The recall period was adapted from 2 weeks in the SAS-CGI scales to 7 days in the Dup15q CGI scales.

CONCLUSION: The Dup15q CGI and CaGI scales are the first instruments designed to capture clinically relevant concepts of Dup15q Syndrome. The scales will be used in interventional studies to evaluate their measurement properties and to determine further refinement and validation steps.

Adriana Vieira, *iMM-JLA, Portugal*

Correction of a UBE3A mutation in patient-derived iPSCs as an isogenic control to study cerebellar dysfunction in Angelman Syndrome.

Angelman syndrome (AS) is a rare neurodevelopmental disorder that features, among others, developmental delay, speech impairment, epilepsy, and ataxia. AS is caused by the lack of UBE3A expression in the brain. UBE3A is an imprinted gene that undergoes silencing on the paternal copy upon neuronal maturation, thus expression of this gene is exclusively maternal in neurons. 10 to 20% of AS cases are consequence of mutations within the maternal UBE3A allele.

Patients with UBE3A mutations present a comparatively milder phenotype, particularly when compared with patients bearing megadeletions in which 12-16 genes, besides UBE3A, are deleted, being hemizygotic and only expressed from the paternal allele. A comparison between UBE3A mutations and megadeletion cases of AS will allow to understand which phenotypes are due to the sole loss of UBE3A or haploinsufficiency of the whole chr15q11-13 deleted region.

Recent advances in induced pluripotent stem cell (iPSC) technology and in vitro neuronal differentiation have emerged as promising in vitro models, allowing detailed research of molecular and cellular aspects of AS in a faithful and accessible manner. The use of patient-derived iPSC lines makes possible to obtain patient-specific disease-relevant neuronal cells.

We took advantage of AS-Δ3X iPSC line (Stanurova et al. 2016), harbouring an UBE3A mutation to study AS-specific phenotypes in cerebellar organoids. AS-Δ3X line was derived from a female patient with a 3-nucleotide deletion in exon 4 of maternal UBE3A producing a non-functional protein that lacks amino acid G538. AS-Δ3X-derived cerebellar organoids present a smaller size compared to controls across differentiation, however this finding did not correlate with altered cell proliferation or death. UBE3A mutation-derived organoids also displayed a reduced expansion rate of cerebellar neuroepithelium, defects in cerebellar commitment, delayed neuronal maturation, and impaired functionality.

We were able to unveil relevant differences between AS and control-derived cerebellar organoids. Nevertheless, these lines were not isogenic and we were unable to control for the effect of genetic heterogeneity in the aforementioned phenotypes. To circumvent this problem, we generated an isogenic control line by correcting the disease-causing 3-nucleotide deletion in the maternal UBE3A allele using CRISPR/Cas9 technology. After screening, 15 corrected clones were obtained (5.7% efficiency of HDR).

We will present the updated status of characterization and validation of these lines. Since the corrected lines share the same genetic background as the AS-Δ3X line, they will facilitate the identification of AS disease phenotypes upon cerebellar differentiation and unravel potential new therapeutic targets for Angelman syndrome.

This research work was accomplished with the financial support of Fundação Amélia de Mello through the “Pedro Maria José de Mello Costa Duarte” 2019 grant for Angelman syndrome research.

SMALL MOLECULES: THERAPEUTIC POTENTIAL OF UNCILENCER 53

Hanna Vihma

Neuroscience Center, Department of Cell Biology & Physiology
UNC School of Medicine, Cell Biology and Physiology, Chapel Hill, USA
Member of Ben Philpot's lab

Treatment Strategy for Angelman Syndrome

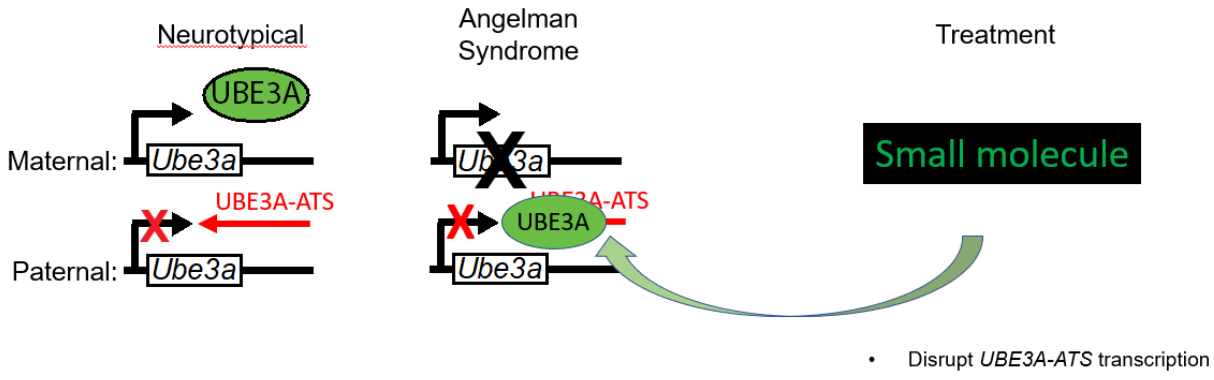
Abstract: Angelman syndrome (AS) is a neurodevelopmental disorder caused by maternal allele mutations or deletions of the ubiquitin protein ligase E3A (human/mouse gene UBE3A/Ube3a). Because the paternally-inherited copy of UBE3A is intact but epigenetically silenced in most mature neurons through a long non-coding antisense (UBE3A-ATS), maternal allele loss of functional UBE3A largely depletes UBE3A protein in the brain. This unique epigenetic biology suggests that activating the dormant paternal allele of UBE3A could provide a transformative treatment for AS. We previously demonstrated the feasibility of this approach by showing that topoisomerase inhibitors, such as topotecan, can successfully reactivate the paternal Ube3a allele in neurons from AS model mice. However, challenges with toxicities and central nervous system bioavailability have limited the potential for topoisomerase inhibitors to treat AS, raising a quest to identify novel small molecule Ube3a unsilencers. In our efforts to discover and develop new potential AS therapeutics, we discovered a potential unsilencer named UNCilencer53 from a high-content screen. We showed that like topotecan, UNCilencer53 significantly increases paternal UBE3A mRNA and protein levels, and downregulates Ube3a-ATS in mouse primary neurons derived from AS model mouse. However, UNCilencer53 does not act through inhibition of topoisomerases, thus it differs from previously identified unsilencers such as topotecan and offers a novel treatment strategy. Furthermore, we demonstrated that non-invasive peripheral delivery of UNCilencer53 potently unsilences paternal Ube3a in mouse neurons and produces brain-wide expression of UBE3A protein in AS model mice, in the absence of observable toxicity. Finally, to demonstrate the clinical relevance of our findings, we have shown that UNCilencer53 effectively unsilences paternal UBE3A in AS patient-derived neurons, thus demonstrating the translational potential of our approach. Thus, our findings have the potential to advance a small molecule treatment for AS that is safe, non-invasively delivered, and capable of producing brain-wide unsilencing of paternal UBE3A and, hence, meaningful improvements for individuals with AS.

Notes

The objective of this research is to investigate the therapeutic potential of UNCilener 53 as a future treatment for AS.

Background: Genetic Imprinting of UBE3A leads to loss of UBe3A in AS.

Gene reactivation strategy to treat Angelman Syndrome by small molecules as treatment. Could take small molecules or CRISPR –Cas. Small Molecules disrupt UBE3A-ATS transcription. The advantage of the unsilencing approach is that you do not have to be concerned about UBE3A overexpression.



Ube3a unsilencers version 1.0: Topoisomerase inhibitors unsilence Ube3a in neurons

Example: *In vivo unsilencing of paternal Ube3a by topotecan (by intrathecal injection in the spinal cord as described in Zylka et al., Neuron, 2008)*

1 year after Topotecan treatment, quite toxic (e.g. neutropenia), moderate CNS penetrance, active extrusion from CNS, longevity in cortex unknown.

Needs: Really a need for improved CNS delivery, safer CNS compounds, mechanistic insights.

Ube3a unsilencers version 2.0: Identification of a novel Ube3a unsilencer

Like topotecan, UNCilencer53 potently unsilences paternal Ube3a. UnCilencer53 increases paternal UBE3A protein levels. UNCilencer53 increases paternal UBE3A protein levels. UNCilencer53 decreases Ube3a-ATS levels.

Can systemic delivery of UNCilencer53 unsilence paternal Ube3a in vivo?

- Systemic delivery of UNCilencer53 produces broad unsilencing of paternal Ube3a in AS model mouse model. :hippocampal unsilencing.
- Systemic delivery of UNCilencer53 produces broad unsilencing of paternal Ube3a in AS model mouse model. :cortical unsilencing
- Systemic delivery of UNCilencer53 produces broad unsilencing of paternal Ube3a in AS model mouse model. :cerebellar unsilencing
- Systemic delivery of UNCilencer 53 produces a rapid and broad increase in paternal Ube3a mRNA levels in AS model mice.

Have not found toxicity in vivo… do not know the mechanism → in the dark, but it does not work like Topotecan

Future Studies:

Does systemic delivery of UNCilencer53 rescue physiological and behavioral deficits in AS model mice? --< Will test nest building, open field and rotarod

Therapeutic potential for Angelman Syndrome?

UNCilencer53 downregulates the *Ube3a-ATS* and increases paternal allele *UBE3A* mRNA in neurons derived from AS individuals

UNCilencer53 downregulates the *Ube3a-ATS* and increases paternal allele *UBE3A* mRNA in neurons derived from AS individuals

Link to Stormy Chaimberlain: Have also received the IPSc cells from Stormy Chamberlain

Conclusions:

- We have identified a novel Ube3a unsilencer UNCilencer53 using high-content screening assay
- UNCilencer53 downregulates Ube3a-ATS and increases paternal allele Ube3a mRNA and protein levels in vitro
- Systemic delivery of UNCilencer53 produces broad unsilencing of paternal Ube3a in the brain of AS model mouse
- UNCilencer53 downregulates the Ube3a-ATS and increases paternal allele UBE3A mRNA in neurons derived from AS individuals

Future directions:

- To identify the mechanism of action by which UNCilencer53 unsilences paternal Ube3a
- To identify the duration of unsilencing of paternal Ube3a in AS model mice by UNCilencer53
- To test the hypothesis that peripheral delivery of UNCilencer53 will rescue physiological and behavioral deficits in AS model mice

Notes:

- Have not found toxity in vivo
- Do not know the exact mechanism, but it does not work like Topotecan
- Mechanism --< in the dark, we know it comes to mind.
- In the future: Will test on behavior, nest building, open field

Link to Stormy Chaimberlain: Have also got the IPSc cells from Stormy Chamberlain.

ANGELMAN SYNDROME AND LINKS TO OTHER DISORDERS

Rajini Rao

Johns Hopkins, Physiology, Johns Hopkins Medicine, Baltimore, USA

Is Angelman Syndrome a disorder of organellar ion homeostasis? Insights from Christianson Syndrome.

Christianson Syndrome (CS) is a rare genetic disorder with striking similarities with Angelman Syndrome (AS). Patients exhibit a happy demeanor, cognitive defects, increasing cerebellar degeneration and ataxia. Other shared symptoms include sleep disorders and pain insensitivity. Indeed, CS was originally referred to as Angelman-like Syndrome, before being named after the physician who first described it. This presentation built upon the similarities of these two disorders with the hope of gaining new insights on shared cellular and molecular pathways. CS is caused by loss of function mutations in NHE6 (gene name SLC9A6), a sodium/hydrogen exchange protein in endosomes that is critical for establishing the pH balance inside the secretory and endo-lysosomal pathway. Intriguingly, loss of Ube3a (the gene defective in AS) has also been reported to cause pH imbalance in the secretory pathway. Other similarities with Ube3a and NHE6 include defects in protein glycosylation and sialyation. These observations raise the possibility that disruption of organellar pH may underlie some of the symptoms in AS. This could be therapeutically relevant as there are drugs that can alter pH inside compartments. In conclusion, it would be worth looking into organellar pH defects in cell models of AS.

Gaia Novarino

Institute of Science and Technology, Vienna, Austria

Studying cortical development through the lens of autism spectrum disorders

De novo loss of function mutations in the ubiquitin ligase-encoding gene Cullin3 (CUL3) lead to autism spectrum disorder (ASD). In mouse, constitutive Cul3 haploinsufficiency leads to motor coordination deficits as well as ASD-relevant social and cognitive impairments. However, induction of Cul3 haploinsufficiency later in life does not lead to ASD-relevant behaviors, pointing to an important role of Cul3 during a critical developmental window. Here we show that Cul3 is essential to regulate neuronal migration and, therefore, constitutive Cul3 heterozygous mutant mice display cortical lamination abnormalities. At the molecular level, we found that Cul3 controls neuronal migration by tightly regulating the amount of Plastin3 (Pls3), a

previously unrecognized player of neural migration. Furthermore, we found that Pls3 cell-autonomously regulates cell migration by regulating actin cytoskeleton organization, and its levels are inversely proportional to neural migration speed. Finally, we provide evidence that cellular phenotypes associated with autism-linked gene haploinsufficiency can be rescued by transcriptional activation of the intact allele in vitro, offering a proof of concept for a potential therapeutic approach for ASDs.

QUALITY OF LIFE STUDY - ERASMUS MEDICAL CENTER ROTTERDAM - ENCORE EXPERTISE CENTRUM

Doesjka Hagenaar

PhD candidate
Child and Adolescent Psychiatry/Psychology & General Paediatrics
ENCORE center of expertise, Rotterdam, Netherlands

Child characteristics associated with Child Quality of Life and parenting stress in Angelman Syndrome (ENCORE Expertise centrum)

It is not much known about the quality of life in children with AS. Previous studies have shown that parenting stress is heightened in AS. However, which factors influence a child's quality of life and parenting stress/impact? The current study aims to investigate the association of children with AS regarding genotype, epilepsy, sleep problems, cognitive developmental level, autistic features, and emotional and behavioral problems.

Methods: The study data has been collected as part of standard care from 2011-2020 in the outpatient clinic of the ENCORE Expertise Center for AS. 73 AS patients aged in the range 2-18 were part of the study.

The selected predictors were:

- Genotype (deletion/non-deletion)
- Epilepsy (yes/no)
- Sleep problems (Sleep Disturbance Scale for Children - SDSC)
- Cognitive developmental level (Bayley cognition scale)
- Autistic features (Autism Diagnostic Observation Schedule - ADOS)
- Emotional/behavioral problems (Child Behaviour Checklist - CBCL for children 1,5-5 years)

The outcomes were measured by:

- Child health-related quality of life (short Infant and Toddler Quality of Life Questionnaire – ITQOL)
- The impact of the child's syndrome on the parent (short Infant and Toddler Quality of Life Questionnaire– ITQOL)
- Parenting stress (Parenting Stress Index - PSI)

Conclusion: Internalizing problems in children with AS are associated with lower quality of life. Also having the deletion genotype and higher age is related to lower quality of life. Sleep problems and emotional (behavioral problems in children with AS are related to parenting stress and have a high impact on the parent. Cognitive developmental level, autistic features, and epilepsy were not significant predictors of a child's quality of life and parenting stress/impact.

Clinical implications: Interventions aimed at increasing child quality of life and decreasing parenting stress should focus on:

- Child emotional/behavioral problems
- Child sleep problems.
- Directly targeting the stress

ASA RESEARCH GRANTS 2021

Two research projects were selected to be funded by the ASA research grants 2021. The Layman summaries were provided by ASA.

Simão Da Rocha
Evguenia Bekman
Instituto Superior Tècnico, University of Aveiro, Portugal

"Stem cell toolkit for modelling cerebellar dysfunction in Angelman Syndrome"

The project of researchers Simão Teixeira da Rocha and Evguenia Bekman from the Institute for Bioengineering and Biosciences, tests the hypothesis that cerebellar dysfunction plays a major role in Angelman symptomatology, namely in ataxia and other features. The team is using a stem cell-based approach to generate cerebellar organoids, or miniature cerebella, from patient-derived induced pluripotent stem cells (iPSCs) and genetically matched healthy controls. Previous work of the team uncovered important signs of impaired neurodifferentiation and neuronal function, consistent with clinical observations in AS. To identify the causes of this dysfunction, the team will enlarge the portfolio of preclinical cellular models of AS by creating new iPSCs from Angelman patients and healthy relatives. The use of these new cell models will help to unravel disease-associated features and to identify potential therapeutic targets. These models then be used as a platform for drug screening to develop new therapies for AS.

Layman Summary

Angelman syndrome (AS) is a neurodevelopmental disorder caused by loss of function of the maternal UBE3A gene in neurons. Lack of UBE3A causes dysfunction of several parts of the central nervous system including forebrain, hippocampus and cerebellum. In particular, the role of cerebellar dysfunction in the cognitive and motor phenotypes associated with AS remains unresolved.
With the generation of induced pluripotent stem cells (iPSCs) and the derivation of organoids from these cells, personalized preclinical disease models have emerged. Patient-derived iPSCs can be used to generate organ-in-a-dish models and are becoming systems of choice to model diseases with a developmental cause such as AS. We have expertise in

creating patient-derived iPSCs, including from Angelman patients, as well as in ensuring the genetic and epigenetic fidelity of these models. iPSCs provide an alternative approach to the use of mouse models for studying cerebellar development and function. Indeed, important differences in cerebellar development between mouse and humans hamper the translation of mouse preclinical models to human diseases with relevant cerebellar dysfunctions. Previously, our team streamlined a protocol for the generation of iPSC-derived cerebellar organoids. We detected impaired neurodifferentiation in cerebellar organoids from Angelman iPSCs with maternal mega deletions, consistent with the clinical observations hinting at cerebellar dysfunction associated with AS. However, to identify the cause of this dysfunction, we need to enlarge our portfolio of preclinical cellular models of AS. Thus, we plan to create new iPSCs from female and male Angelman patients with mega-deletions and familiar healthy individuals and additionally, CRISPR/Cas9-edited isogenic human embryonic stem cells. The use of these new cell models will help to reduce inter-individual variability and highlight disease-associated features. Using these models, we will analyze global gene expression at critical steps of differentiation, recurring to two techniques, bulk and single-cell RNA sequencing. These techniques will provide a general understanding of when the neurodevelopmental defects of AS cerebellar organoids first emerge and further dissect the cellular composition and level of neuronal maturity in Angelman versus control cerebellar organoids. The establishment of these organoid systems will be instrumental to elucidate the contribution of the cerebellar dysfunction in AS, will help to identify potential therapeutic targets and also will potentially be used as a platform for drug screening for developing new therapies for AS.

Ilaria Tonazzini
Laura Baroncelli
Nanoscience Institute - Consiglio Nazionale delle Ricerche, CNR @ NEST, National Enterprise for nanoScience and nanoTechnology
Neuroscience Institute – Consiglio Nazionale delle Ricerche, CNR
Pisa, Italy

"Innovative brain-targeting nano-tools and imaging methods for therapeutic development in Angelman Syndrome (InnovAS)"

The research project **Innovative brain-targeting nano-tools and imaging methods for therapeutic development in Angelman Syndrome (InnovAS)** has been funded by Angelman Syndrome Alliance (ASA), a partnership of organizations from around the world that are focused on supporting people with Angelman Syndrome.The long-term goal of InnovAS project is to improve the therapeutic pipeline in Angelman Syndrome (AS). The project will devise a brain-delivery strategy based on biocompatible nanoparticles for the non-invasive intranasal administration of antisense oligonucleotides (ASOs) targeting Ube3a, and will assess the validity of non-invasive imaging methods as unbiased biomarkers for monitoring brain function in AS.

The research project is led by CNRnano (Pisa) in collaboration with CNR-IN (Pisa) and IRCCS Fondazione Stella Maris (Pisa). The project will be carried out in Pisa (Italy), by a team characterized by a combination of different skills, in which nanobiotechnologists (Dr. I. Tonazzini),

neuroscientists (Dr. L. Baroncelli), and clinicians (Prof. R. Battini) collaborate together. Dr. Ilaria Tonazzini and Dr. Laura Baroncelli, PIs of the project.

Layman Summary

The approaching therapies with Antisense oligonucleotides (ASOs) to reactivate the paternal Ube3a allele represent the most promising drug strategy for Angelman Syndrome (AS). This treatment has created enormous expectations and is going to represent an important breakthrough for the AS community. However, the how this therapy would be administrated, monitored and dosed is also important, and can impact not only the AS patient quality of life (e.g. invasive intrathecal injections, side effects) but also the outcome of the therapy itself. However, ASOs do not pass the brain blood barrier, which protects and isolates our brain, and the only administration route available today (and in the next future) for the AS-ASOs (i.e., they need to get into the brain) is via intrathecal injection (i.e. injection in the spinal canal). This administration route unfortunately presents a weakness point with related drawbacks, such as high invasiveness, risk of infection, need of hospitalization/anaesthesia, low power in terms of brain targeting and more importantly difficulty in predicting the amount of the administered dose that will reach the brain. The dream would be to be able to administer the AS-ASO in a non-invasive and controlled way, but the challenge is formidable. At the same time, the AS community is lacking evaluation tools with high translational power for the follow-up of AS patients and for monitor the efficacy of the therapeutic treatments, both at the preclinical and at the clinical level (i.e., mice and humans).

The final goal of our project is to develop innovative therapeutic tools for the delivery and evaluation of the ASO therapy in AS, with two main aims: 1) to develop and demonstrate biocompatible nanoparticles, made of a biodegradable and biocompatible material, as nanocarriers for the selective delivery of AS-ASOs to neurons into the brain via a non-invasive administration route, the intranasal one; 2) to demonstrate the validity of imaging methods as unbiased biomarkers of brain function for AS follow-up, in mouse and human models.

The project will be carried out in Pisa (Italy), by a team characterized by a combination of different skills, in which nanobiotechnologists, neuroscientists, and clinicians collaborate together to fight against AS.

The future benefits of such nanodelivery and biomarkers strategies will be higher quality of life for AS patients, and likely higher therapeutical effects.

BIOMARKERS

Rob Komorowski

Ionis Pharmaceuticals

Detecting UBE3A in Cerebral Spinal Fluid of AS Patients

Advances in the understanding of the molecular mechanisms that underly the silencing of the UBE3A gene which causes Angelman syndrome (AS) have led to the emergence of multiple pharmacological strategies which aim to increase levels of the UBE3A protein in neurons. However, there are currently no available biomarkers which can measure UBE3A protein in the central nervous system (CNS). Developing a biomarker is of great value for any drug development program and could serve as a direct or indirect measure of target engagement. This could inform on treatment-related changes in clinical phenotypes, as well as drug dose levels and frequency. Cerebrospinal fluid (CSF) is an important pathway to the CNS, which bathes the brain and could contain measurable levels of UBE3A in AS. Here we report that Biogen has developed an assay which can detect UBE3A protein in CSF samples acquired from healthy individuals with high specificity and good reproducibility in replicate analyses by liquid chromatography mass spectrometry. To determine the sensitivity of this assay, individuals with AS were enrolled in two independent CSF collection natural history studies. The first study was funded by Biogen and Roche recruiting 20 AS individuals in the US (NCT04103333). The second study, funded by Ionis, recruited 11 individuals with AS. The results reported here combine data from both studies (n=31). The results demonstrated UBE3A was detected in 96.8% of the samples. Further research will be needed to determine the source of CSF UBE3A, but these results demonstrate that this assay has sufficient sensitivity to detect very low levels of UBE3A protein in prospectively acquired AS CSF samples and may be a valuable biomarker in ongoing and upcoming interventional trials.

Cesar Ochoa-Lubinoff

Associate Professor & Division Chief of developmental-behavioral pediatrics at the Rush University Medical Center
Director of Rush Angelman Syndrome Clinic, Chicago, USA

Clinical Trial in Individuals with AS to enable Endpoint Development for interventional trials

Angelman syndrome (AS) is a neurodevelopmental disorder caused by absence of functional UBE3A, characterized by severe cognitive and motor impairment, lack of speech and other symptoms. Currently, there are no established feasible and suitable endpoints to holistically measure meaningful change in AS. FREESIAS aimed to identify such measures.

Methods. We followed 55 individuals with AS (<5 years: n=16; 5-12 years: n=27; >18 years: n=12; deletion: n=40, non-deletion: n=15)) and 20 typically developing children (1-12 years) in FREESIAS, for one year. The study tested a wide range of clinical outcome assessments (COAs), overnight electroencephalography (EEG), and digital health technologies (DHTs) across key AS characteristics, performed in-clinic and at-home.

Results. The completion-rate for the majority of COAs was high with >94% at the first and >80% at the second clinic visit. Adherence to and feasibility of the different DHTs varied by assessment. Only 85 (of 210) overnight-EEGs were performed, largely related to the Covid-pandemic. COA results were compatible with previously published natural history data, showing minimal developmental gain between the 5-12 and >18 year groups on the Vineland-3 and Bayley-III, and better overall performance on virtually all measures in the non-deletion group. The AS EEG phenotype of excess delta-band power was found in the home EEG and fell well within the range of previous reports.

Conclusions. FREESIAS data will support measure choice to holistically measure change in future interventional clinical trials in AS. The study identified suitable COAs, overnight EEG and DHTs, yet simultaneously illustrated the need for improved measures to assess change in AS.

CLINICAL TRIALS UPDATE FROM ROCHE, IONIS AND PTC THERAPEUTICS

Brenda Vincenzi
Roche

Roche: AS research on therapy & study status

Seven-year Angelman syndrome clinical program history:

In 2017 disease concept model started together with A-BOM, 2018-2019 FREESIAS non-drug observation study with families and scientific community in partnership with IONIS-Biogen, TANGELO Phase 1 started in 2020, Completion of FREESIAS in 2021, in 2022: Completion of first part of TANGELO, Start of TANGELO LTE.

How does rugonersen work? Rugonersen activates the father's UBE3A gene to produce UBE3A protein in the brain. The drug Rugonersen increases UBE3A protein in cells and monkeys.

The primary objective of FREESIAS was to collect information about the fasibility and value of novel endpoints and find biomarkers to create clinical trial design for TANGELO. Study has competed in May 2021 and was run only in the US. The main study results are currently being prepared for publication. Assessment tools have been questionnaires, sleep mat and sleep diaries and EEG assessments.

Brenda Vincenzi explained the traditional steps of drug development process. In rare diseases Phase II and III could be merged. Next step will be Phase III. After Phase 4 if everything was ok and FDA says yes, you can bring the drug to the market. Tangelo is an open-label, multicentre study to investigate the safety, toelability of rugonoersen in participants with AS.

TANGEOLO Phase 1 study overview:

Participants up to 74 (M/F), 1-12 years with mutations and deletions, 4 countries and 10 sites. The study duration will take approx. until 2023. The objective is to identify the highest safe dose that can be administered to patients → Higher doses will lead to longer intervals between lumbar punctures.

Initially they gave very low doses. The Purpose of TANGELO Part 1 was to find the highest safe and well-tolerated doses that will be tested during a longer treatment period. The long-Term Extension part has started now exploring chronic dosing with long intervals. It allows refinement.

Roche ask for understanding and is not sharing data while the trial is ongoing. They want avoid that expectations can influence the studies outcome. In TANGELO everybody receives the drug but positive (Placebo effect) or negative (Nocebo) beliefs about the drug can influence the results.

PET (Positron emission tomography scan) study in healthy adults is an imaging technique and allows us to see in the body → Imaging of radio-labeled rugonersen. After rugonersen is injected, the PET is performed. Then we look at the images to see where the rugonersen distributes in the CNS. Study started in April 2021. Data acquisition is ongoing.

Advice:

Ask all the questions: What is the trial design, what is my role as caregiver, try to understand the burden, what are potential risks, during the trial don't share information with the community to avoid expectation bias.

Biomarkers:

We learned a lot about biomarkers in the FREESIAS study. We are looking into biomarkers. We include the EEG (looks at the brain activity) and sleep characteristics as biomarkers in TANGELO. Roche is trying to quantify UBE3A and other proteins in the CSF. Other digital biomarkers aim to improve the collection of data.

What about other countries in the clinical trial? Based on feasibility, Roche might include new countries and sites in the next phase of development. The community will be informed the list of countries and sites will be published on clinicaltrial.gov.

Sebastian Camillo Holst

Senior Scientist, Roche Pharma

Assessments of the sleep abnormalities in AS from the FREESIAS observational study

Keywords: Sleep impairment, sleep mat, sleep spindles, sleep quality

Abstract: Angelman syndrome (AS) is a rare neurodevelopmental disorder caused by the absence of functional UBE3A in neurons. Excess low-frequency oscillations and disrupted sleep have been identified as characteristic features, but systematic investigations of sleep physiology and behavior are missing.

Methods. We followed 55 individuals with AS (<5 years: n=16 ; 5-12 years: n=27; >18 years: n=12) and 20 typical developing children (<13 years) in the non-drug study "FREESIAS", for one year. Sleep was assessed using several complementary means, including: I. Daily collection of a caregiver reported sleep diary; II. Continuous sampling via an under-mattress sleep mat that provides daily, effortless and objective sleep information; III. Polysomnography (PSG) at up to three home visits; and IV. a clinical sleep scale (SNAKE).

Results. Compared to controls, analysis of the sleep diary data revealed sleep impairment in AS. These were observed both in terms of variability of sleep and in the number of awakenings at night. Objective parameters of sleep extracted from the sleep mat correlated well with parent reports from the sleep diary. The highly abnormal EEG renders sleep scoring of the PSG data challenging. Nevertheless, PSG analysis show that sleep structure and physiology is highly abnormal and heterogeneous among individuals with AS.

Conclusions. We provide a holistic assessment of sleep in AS based on subjective and objective data. Whereas sleep disturbances and abnormal sleep patterns are a part of AS, large variability between patients is observed.

Notes

- 1-4 years, 5-12 years age groups, groups should have the same size, you see different patterns if you change the medication, epilepsy:
- Recommendations from another study: Keep the room cool, Sleep is really important for development.
- AS sleep duration not very different from other children, but the sleep quality (no REM phases).
- Have developed a sleep mat that replaces the diary
- Sleep spindles are important for cognitive development.

Becky Crean
Ionis Pharmaceuticals, USA

Update on the Ionis clinical trial of HALOS for Angelman syndrome

Abstract: Drug discovery of a potential therapeutic to get it to the clinic for testing is long and involved. The process is to determine if the drug safe and effective in people is equally long. Clinical trials are the 'Development Phase' of drug development and there are several phases of clinical trials that the drug must advance through to determine if it is safe and effective. There are 3 main phases of clinical trials, each with specific goals and objectives, and each trial, each phase of development, is meant to answer specific questions about the drug. Clinical trials in rare diseases, like Angelman syndrome, can sometimes take a different approach than what is typically done in other studies, such as being able to do fewer, and faster clinical trials, but which also means these studies are small and only include a limited number of participants. Designing these clinical trials to determine if the drug is working or not can be complex, and factors such as the placebo effect can cause studies top fail. Finally, an update Ionis's HALOS trial, which is an early phase clinical trial with our ASO, ION582, will be discussed.

Notes

Each phase of a clinical trial has a different goal. Clinical trials in rare diseases can be done faster, fewer. Early studies can enroll only a small number of patients. Placebo is a treatment that has not an active component. Placebo piece and a blinding piece (nobody knows whether the patient is receiving a placebo or the drug).

The challenges of social media in Clinical Trials: Researchers have been understood how strong the impact of social media can be on data. This does have impact.

Update on the HALOS Phase 1-2 clinical trial: Clinical Study Design: ION582 as an ASO treatment for AS, in corporation with Biogen, currently ongoing, released it early this year. (Ages 2 to 50 years), small trials, 44 patients. Study progressing well.

Phase 1 1st trial to study the effects of a drug in healthy volunteers (20-40). The primary goal is to provide safety information and determine how the drug distributes in the body. Short duration.

Phase 2 enrolls a larger group of patients (50-100) and tests of the drug provides clinical benefit and continues safety tests. Longer treatment duration with multiple doses.

Phase 3: Proves information needed for agencies. Treatment duration could take several years.

However, for rare diseases, it is allowed to combine phase 1 and phase 2 for drug development: The main objectives are patient safety and clinical benefit. How do we assess if a drug is improving the patient? By endpoints (or outcome measures). A successful treatment of AS should show improvements in both objective and subjective endpoints and be meaningful to patients and families. Objective: i.e. How far can the patient walk? How many seizures did the patient have last week?

There is also a flexibility in placebo in the first years. Placebo-controlled clinical trials are the gold standard for testing if a drug is safe and effective. Patients on a placebo could get the opportunity to transit to active treatments in some studies.

HALOS Clinical Study Design:

ION582 is also an antisense oligonucleotide (ASO) increasing production of UBE3A protein. The study is currently recruiting. The ASO is administered intrathecally with a lumbar puncture. AS patients will be given sedation. IONIS plan to test children and adults (2 to 50 years). Different age groups enter the study at different times. The study aims to enroll a minimum of 44 patients. Parents/caregivers will fill out questionnaires at clinic visits and at home. This is a Phase 1-2 and the treatment includes several doses of the drug every 4 to 8 weeks for 3 months and no placebo.

Goal of the first study will be to assess safety and tolerability of the drug as well as measuring changes in areas like communication, cognition, motor, sleep, seizures and everyday functioning.
Global trial, 8 or 9 countries, 13 sites most of USA. Transition of much longer time → is also planned.

Questions: Long-term evaluation → must be finished before going to the next stage? Becky Crean means that it is possible to summarize it.
How far to set up a study could also have an impact on the study itself.

Ifode Ajari

Ionis Pharmaceuticals, USA

Gene Therapy for the Treatment of Angelman Syndrome: A Preclinical Update of PTC Therapeutics

Abstract: Angelman syndrome (AS) is a rare, neurogenetic disorder that occurs by disruption of the UBE3A gene on the maternal allele of neurons. The paternal allele in neurons is epigenetically silenced and thus any complications on the maternal allele will result in a loss of expression of the Ubiquitin Protein Ligase E3A (UBE3A) globally in the brain. Patients often exhibit a lack of speech, ataxia, severe developmental delays, and seizures.

PTC-AS is an adeno-associated viral vector designed and being developed by PTC Therapeutics as a gene replacement therapy for the treatment of AS. Here, we describe studies performed in rodents and non-human primates (NHPs) evaluating the distribution, expression, and efficacy of PTC-AS after administration to the cerebrospinal fluid or directly to the hippocampus. Studies evaluated good distribution and measured dose-dependent increases in the vector DNA (qPCR), RNA (RT-qPCR), protein (both ECL sandwich immunoassay and IHC).

Mouse and rat models of AS were utilized. These models show a milder phenotype than the human syndrome but effectively recapitulate several etiologies. Studies with functional behavior endpoints included 15-20 animals/group. Administration of PTC-AS at PND 0 improved the phenotype in both the AS mouse and rat models. Improvements were observed in parameters covering a range of phenotypes directly correlating to those observed in the human including motor function, overall neurological score, and anxiety. Improvements were observed with gene therapy treatment at PND 0 as well as in the studies with rodents injected as adults.

Further, administration of PTC-AS to the cerebrospinal fluid of rats at PND 0 or to peripubertal NHPs resulted in measurable UBE3A-specific vector DNA and UBE3A-mRNA. Longer term studies in the rat showed detectable levels of UBE3A protein through 6 months. These studies strongly support continued development of PTC-AS.

Notes

- PTC Company: in 15 countries worldwide
- Ongoing work with PT-AS (adeno-associated viral vector) as a gene replacement therapy for the treatment of AS.
- Studies performed in rodents (Nagetiere) and non-human primates evaluating the distribution, expression, and efficacy of PTC-AS after administration (cerebrospinal fluid or hippocampus)
- Mouse and Rat Models utilized
- UBE3A Protein distribution depends on Age: The younger the better distribution
- Ongoing consultations with patient advocacy organizations, Caregiver focus groups and advisory board.
- More preclinical studies are ongoing.

Images:

- PTC-AS rescues cortical dendrites deficits in ICV injected AS mice.
- UBE3A Protein changes are detectable

Conclusions:

- PTC-AS leads to broad transgene DNA distribution/transduction and protein levels in multiple species
- PTC-AS distribution depends upon route of administration and age of administration
- PTC-AS expression is long-lasting and shows no overt toxicity
- PTC-AS can recover multiple phenotypes in two rodent models

The question from plenary about a potentially overexpression for UPD genotypes could not be answered at this time.

PLENARY SESSION 1: SCIENTISTS: WHAT DO WE NOT KNOW ABOUT AS?

A. Unanswered questions – Molecular

- What are the relevant substrates of the UBE3A isoforms? (And do we really need to know them)?
- What is the level of evidence we need to call it a substrate?
- How can drugs targeting single targets work so well if there are so many targets affected?
- Why does Ube3a bind to the proteasome?
- Why does overexpression of UBE3A not result in cytosolic labeling if PSMD4 binding is required sufficient for nuclear targeting?
- Why is UBE3A imprinted?
- What is the meaning of a hyperactive Ube3a mutation?
- How good is the evidence that overexpression of UBE3A is sufficient to be pathogenic?

B. Unanswered questions – Neuro

- How much UBE3A is needed to function normally?
- In what circuits is UBE3A important for neurotypical development?
- Is there evidence for a role ofUBE3A outside the brain?

C. Unanswered questions – Clinical

- What are the earliest phenotypes of AS?
- What biomarkers change with UBE3A reinstatement?
- What direct or proxy readouts are there for UBE3A expression?
- Do AS individuals have sleep or circadian deficits, and to what extent are these separable? To what extent do they exacerbate AS symptomology?
- Is sensory perception affected?
- What can single gene disorders that phenocopy AS inform us about shared pathophysiology?

PLENARY SESSION 2: DOCTORS: WHY START AN AS CLINIC? WHICH SPECIALIZATIONS NEEDED? WHICH FACILITIES NEEDED? PROGRAM?

Chaired by Karen Bindels-de Heus and Marie-Claire de Wit (Erasmus ENCORE)

~35 attendants

Agenda

- Introduction round
- Present ENCORE, advice on how to start an expertise center
- Discuss potential for European AS clinical network
- Discuss potential for European database based on LADDER

Introduction round

all attendants introduce themselves and talk on their background.

Observations:

- There are currently 28 AS clinics in USA
- Number of patients per clinic: 60 – 150
- In Germany, an additional AS center for children is planned in Leipzig

Presentation of Erasmus ENCORE in Rotterdam

- start in 2010 as an initiative of the Nina-Foundation
- publications, involved in current research
- for patients: annual check by speech therapist (Logopedics), neurologist, psychologist, nursing staff
- behavior and development assessment at the age of 3, 7, 11 and 15

Why start an AS center?

- AS is rare
- experience can be used in addition to daily care that is organized regionally
- multidisciplinary setting very valuable for parents
- Parents meet each other through the clinic, at the center
- knowing the patients with AS already provides basis for research like ASO trials
- working together internationally with other centers
- developing future therapies (ASO) needs centralized care

How to start an AS center?

What disciplines should ideally be involved?

- pediatric neurology, neurology (for epilepsy, motor problems, sleep, feeding problems, growth, puberty reflux, constipation, scoliosis)
- logopedics
- physiotherapy
- child and adolescent psychiatry (for development and behavior)

- dietetics (for feeding problems and growth)
- clinical genetics (for counseling)
- adult care physician
- orthopedics, ophthalmology
- administrative staff and AS nursing staff

What facilities are needed?

- outpatient clinic
- EEG
- cooperation with parent organization
- database with clinical date (genotype, epilepsy, growth parameters, …)
- interchange with local treatment team
- beneficial: facilities to participate at trials
- optional: a team for ketogenic diet, possibility for interventions under general anesthesia

Important

- clinic coordinator – a person that is the “glue” between clinic and patients and coordinates all questions

Other observations

- US experience: clinics start mostly from one single doctor, mostly neurologists, that have at least a few AS patients – ASF approaches these doctors and can offer funding
- in Israel, the starting point for the AS center was an existing Rett-center, plan is that physiotherapists of AS center are visiting patients at school and instruct local therapist on how to work with patients
- Questions on practice/daily business at center:
    - how many days is one stay? — 2 days at Erasmus ENCORE, 5 days in AS center Munich
    - how looks the schedule of a visit? – Erasmus ENCORE Schedule is presented

Goals of a European AS clinical network

- every AS patient has access to an expertise center
- improve care taking and daily life – learn from each other
- collaborate on data collection
- prepare for future therapies

Discussion on potential for European database based on LADDER

- The current status of LADDER, a US database is presented
- Two potential database systems exist for implementing a European database
- comments from GER/FRA that for data protection reasons, only the RedCap system will be allowed in the future

NETWORKS & COLLABORATION, LADDER DATABASE & LEARNING NETWORK

Anne Wheeler
RTI International, USA
University of North Carolina at Chapel Hill

Elizabeth Jalazo
ASF, USA

LADDER database & learning network
For Patients, For Providers, Together, Toward the Cure.

The LADDER network has four essential functions:

- To connect patients to care,
- Connects providers to one another,
- Supports Clinical Trials
- Operates LADDER Database

Ladder database linking Angelman & Dup15q Data for Expanded Research

- To accelerate knowledge about phenotypes and family needs
- To identify and promote effective treatment guidelines
- Make easier outcome measure development and validation for use in clinical trials.

Use of LADDER data for Clinical Decision making

- Use of clinical needs study to inform common and effective treatments
- Why? Combining datasets gives us more power to answer critical questions.
- Critical guidance, which information are helpful. This data will be summarized and distributed to the LADDER Learning Network.
- Differentiation from Global registry: The registry gives us an idea.

For further information please consult: **www.laddertotreatment.org.**

Announcement Ultragenyx:

Ultragenyx is a biopharmaceutical company and has announced it has acquired GeneTx Biotherapeutics in the summer of 2022 to advance the development of GTX-102. Ultragenyx currently has clinical studies ongoing and is open for collaboration.

Contact details: **https://www.ultragenyx.com/.**

PRACTICAL HELP FOR EVERDAY LIFE

AAC – AUGMENTATIVE AND ALTERNATIVE COMMUNICATION

Michaela Zöbl
Logopädin
Autorisierte Referentin nach Standard der GesUK e.v.
GesUK Regio Leitung Österreich

UK-Unterstützte Kommunikation (in German)
(see English translation below)

„Jeder Mensch hat viel zu sagen, aber was ist, wenn man nicht sprechen kann?“

- Frage: Was ist Kommunikation? Nur wenn wir eine gemeinsame Kommunikation (gemeinsame Zeichen, gemeinsame Bedeutung der Zeichen) haben, können wir miteinander kommunizieren. Kommunikation ist ein Grundrecht.
- Was ist UK? UK ist der deutsche Fachbegriff und kommt aus dem englischen und steht für „Augmentative and Alternative Communication (AAC)“

Stufen der Kommunikationsentwicklung:

- Nichtintentionale Kommunikation: Phase des sozialen Lachens, Blickkontakt
- Auf dem Weg zur intentionalen Kommunikation,
- Intentionale Kommunikation:
- Symbolische Kommunikation,
- Explosion des Vokabulars.

Das „Über Mich-Buch“ ist wichtig für Bezugspersonen und trägt den momentanen Stand des Wissens zusammen. Regelmäßige Aktualisierung erforderlich. Inhalte: Meine Daten. Das mache ich gerne. So nehme ich meine Umwelt wahr. Rituale und Routinen werden so auch für Vertretungskräfte umsetzbar.

Intention: Die Person weiß, dass sie Menschen und Dinge beeinfluss kann und beginnt zwischen Personen zu unterscheiden. Phase: Ich bewirke etwas. Kind, das lernt, dass etwas bewirken hat

Es gibt spezielle Apps, wo wir merken, ich kann etwas erreichen. „Jede Wahl ist ein Treffer“.

Später haben wir Taster oder einfach Sprachausgabegeräte.

- Interaktives Bücherlesen: Bücher mit wiederkehrenden Textpassagen. Wiederkehrende Teile mittels sprechender Tasten betätigen. Beispiele: Die kleine Raupe Nimmersatt, Von Kopf bis Fuß, Vom kleinen Maulwurf, der wissen wolle, wer ihm auf den Kopf gemacht hat.
- Partizipationspläne anbieten.

Intentionale Kommunikation: Kind hat Ursache Wirkung, Situationsverständnis, weiß auch dass Objektive existieren, auch wenn sie nicht da sind.

- Wir unterscheiden Dynamische (Gebärden) und statische Symbole (Zeichnungen/Piktogramme, Fotos, Wörter, Schriftsprache, reale Gegenstände, Miniatur Objekte). Gebärden gehen der Lautsprache voraus.

Symbolische Kommunikation: Die Person weiß, dass sie mit Personen über Symbole (Sprache, Bilder, Gesten) kommunizieren kann. Bei uns in Österreich setzt sich Metacom durch.

Weitere Ideen: Raumbeschilderungen, Beschriftungen, Auswahlkarten

Materialien mit Symbolen:

- Münz- und Fotoalben
- Klapp Bilderrahmen
- Plexiglasrahmen
- Tischsets
- Magnet-/Filzleisten
- Mappen/Ordner

Köln hat einen Lehrstuhl für Unterstützte Kommunikation.

TEACCH ist eine Strukturierungsmethode, die unsere Kinder brauchen, um besser zu verstehen. Beispiel: Tisch decken. Struktur des Tisches, um mir leichter zu tun, eine Aufgabe zu erledigen.

- Erst – Dann Pläne.

AAC-Augmentative and Alternative Communication (in English)

"Everyone has a lot to say, but what if you can't speak?"

Question: what is communication? Only when we have common communication (common signs, common meaning of signs), we can communicate with each other. Communication is a basic right.

What is UK? UK is the German technical term and comes from the English and stands for "Augmentative and Alternative Communication (AAC)"

Stages of communication development:

- Non-intentional communication: phase of social laughter, eye contact.
- Towards intentional communication,
- Intentional communication:
- Symbolic Communication,
- Explosion of vocabulary.

The **"About Me Book"** is important for caregivers and compiles the current state of knowledge. Regular updating is required. Contents: My data. This is what I like to do. This is how I perceive my environment. Rituals and routines become feasible for substitutes.

Intention: The person knows that he or she can influence people and things and begins to differentiate between people. Phase: I make a difference. Child learning that something has an effect.

There are special apps where we realize, I can make a difference. "Every choice is a hit".

Later we have push buttons or just voice output devices.

- Interactive book reading: Books with recurring passages of text. Operate recurring parts using talking buttons. Examples: The Little Caterpillar Neverending, From Head to Toe, Of the Little Mole Who Wanted to Know Who Hit Him on the Head.

- Offer participation plans.

Intentional communication: child has cause-effect, situational understanding, also knows that objects exist even if they are not there.

- We distinguish dynamic (signs) and static symbols (drawings/pictograms, photos, words, written language, real objects, miniature objects). Signs precede spoken language.

Symbolic communication: the person knows that he/she can communicate with people through symbols (speech, pictures, gestures). In our country, Austria, Metacom is catching on.

Other ideas: Room signs, labels, choice cards.

Materials with symbols:

- Coin and photo albums
- Folding picture frames
- plexiglass frames
- placemats
- Magnetic/felt strips
- Folders/Folders

Cologne has a chair for augmentative and alternative communication.

TEACCH is a structuring method that our children need to understand better. Example: setting the table. Structure the table to make it easier for me to do a task.

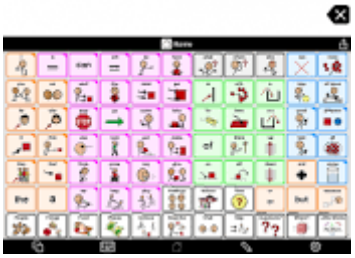
AAC Case report Colombia

ALBERTO VELEZ VAN MEERBEKE, M.D. M.Sc.
Medical Doctor of the Universidad del Rosario in Bogota, Colombia, Pediatric Neurologist of the Universidad Autónoma in Madrid Spain and Master in Epidemiology of the Universidad CES, Medellín, Colombia.

Augmentative Alternative Communication as a voice for patients with Angelman syndrome: Case report

Augmentative Alternative Communication (AAC) are all communication devices, systems, strategies, and tools that replace or support natural speech. AAC improves and supports communication and learning barriers for people with communication needs. However, professionals treating Angelman Syndrome do not yet consider AAC as an essential therapy method. People working and relating with children with Angelman Syndrome tend to use traditional language acquisition strategies to foster communication, even after finding that individuals with this condition struggle or cannot develop efficient oral communication skills.

There are three types of AAC modalities mentioned in the literature. AAC without technology, including handwriting and natural gestures (facial or manual signs). AAC with technological aids, including using image and symbol-based communication systems (communication system image exchange) or electronic and computerized voice generation devices, and multimodal AAC.



We present the case of a 12-year-old girl with Angelman Syndrome. She is non-speaking. Her Inclusion in the general education classroom has been facilitated by making AAC her primary communication tool. She currently attends fifth grade at a private bilingual international school and has consistently used the “Proloquo2Go” app to communicate for the last two years; however, she started her AAC journey when she was four.

Even though she is assertive in communicating with emerging signs, pointing, and other communication gestures, using AAC is her main educational goal. Her Inclusion program consists of a modified curriculum, accommodations, and differentiated instruction. She successfully participates in the classroom with partner-teacher support, prompting, and guidance. This girl is motivated to use her AAC device to communicate her favorite activities, wants, and needs. She can answer questions that expect one-word responses and requires support navigating most AAC application folders.

AAC has become her voice; this is an example of how this communication system supports and facilitates Inclusion. Children with Angelman Syndrome can benefit from AAC implementation, improving their quality of life, behavior, and learning and social skills. Children should be immersed early in AAC interventions to be more effective, as this type of communication does not have prerequisite skills. Fostering AAC skills should be considered more efficacious than traditional language acquisition therapy.

Notes

The 12-year-old girl has deletion as genotype.

CANNABIDIOL (CBD)

Christel Kannegiesser-Leitner

Doctor, Rastatt, Germany, Association for the research on Angelman Syndrome, Austria

Cannabidiol in the anticonvulsant treatment of Angelman Syndrome - Hype or realistic opportunity? Experiences of the last years

In Angelman syndrome, various symptoms, e. g. epilepsy, are triggered by neurotransmitter dysbalance. In particular, a shift in the GABA glutamate balance towards GABA deficiency and a glutamate overflow can be observed. When the inhibitory GABA neurotransmitter value decreases and, possibly, the excitatory glutamate neurotransmitter increases at the same time, an epileptic seizure may be triggered. For that reason, some anticonvulsants target to boost GABA and reduce glutamate. Undoubtedly, this appears to be beneficial. On the other hand, it is precisely the majority of these drugs that triggers a number of side effects. Even if the side-effect profile of newer anticonvulsants is more beneficial than the one of previous ones, side effects must not be neglected. This presentation will describe experiences of people with Angelman syndrome responding excellently to this CBD medication. However, there will also be reports of patients in whom CBD does not have the desired effect. The question is whether this is caused by a dosage that has been too high for years and, correspondingly, by the so-called bell-shaped dose-response curve of CBD. Or because patients were administered up to 5 medications at the same time. In this context, experiences with a Cannabis DNA Test will also be presented. With this test it can be determined how CBD is processed by the organism and how indications for the correct dosage can be reseat. Furthermore, measures accompanying the CBD medication will be presented.

Notes

- CBD is GABA-erg and effective as a glutamate blocker.
- Have not fully understood to this day if there are habituation effects with CBD.
- Children have habituated faster with synthetic CBD or it has then not worked as desired.
- CBD is not a miracle drug. CBD can be used to reduce antiepileptic drugs.
- Please do not order CBD over the Internet, but through the pharmacy.

SLEEP IMPROVEMENT

Karen Bindels-de Heus
Marie-Claire de Wit
Erasmus MC, ENCORE Expertise Center, Rotterdam, Netherlands

Information about the Rotterdam Expertise Centre "Encore" & information on how to improve sleep

Background: Angelman syndrome (AS) family studies report lowered child Health-Related Quality of Life (HRQoL) and heightened parenting stress. Little is known on the factors influencing child HRQoL and parenting stress. The current study investigates whether and how genotype, epilepsy, sleep problems, cognitive developmental level, autistic features, and emotional/behavioural problems of children with AS are associated with child HRQoL and parenting stress/impact.

Methods: We collected data prospectively through clinical assessments of AS patients (N = 73, mean age = 9.2 years, range = 2-18 years) at the ENCORE Angelman expertise centre (Erasmus MC Sophia). A linear regression analysis was conducted for the following dependent variables: 1) child HRQoL (child scales of the short Infant Toddler Quality of Life questionnaire – ITQoL); 2) the impact of the child's handicap on the parent (parent scales of the ITQoL), and; 3) parenting stress (Parental Stress Index – PSI). Independent variables were genotype (deletion versus non-deletion), epilepsy (yes/no), sleep problems (Sleep Disturbance Scale for Children – SDSC), cognitive developmental level (Bayley-III-NL cognition), autistic features (Autism Diagnostic Observation Schedule – ADOS), and emotional/behavioural problems (Child Behaviour Checklist 1,5-5 years – CBCL). Covariates were gender, age, and socioeconomic status (SES).

Results: Results show that the CBCL score was the strongest and a significant predictor of the ITQoL child scales, indicating that more emotional/behavioural problems were related to lower HRQoL. Additional exploratory analysis showed that the CBCL subscale 'internalizing problems' was driving this effect. Genotype and age were also significantly related to the ITQoL child scales. Having the deletion genotype and higher age was associated with lower HRQoL. SDSC score was a significant predictor of the ITQoL parent scales, illustrating that more sleep problems related to a higher impact of the child's handicap on the parent. Finally, CBCL score was a significant predictor of PSI score, indicating that more emotional/behavioural problems relate to higher parenting stress. Epilepsy, ADOS-2 score, and Bayley-III-NL score were not a significant predictor of ITQoL or PSI scores.

Conclusion: Internalizing problems in children with AS are associated with lower quality of life. In addition, having the deletion genotype and higher age is related to lower quality of life. Sleep problems and emotional/behavioural problems in children with AS are related to parenting stress and have a high impact on the parent. Cognitive developmental level, autistic features, and epilepsy were not a significant predictor of child HRQoL and parenting impact/stress.These results suggest that interventions aimed at increasing child HRQoL and decreasing parenting stress in AS should focus on child emotional/behavioral problems and child sleep problems. In addition, interventions may directly target the stress a parent perceives and

use a systemic approach. Future studies should consider measuring additional variables that could impact HRQoL and parenting stress, such as communication skills.

Notes

- Almost 90% of the children have/had sleeping problems.
- Melatonin is not a sleep medication but tries to regulate the rhythm.

Karen Bindels-de Heus, PhD
ENCORE Expertise Center, Rotterdam, Netherlands

Sleep problems in children with AS: The effect of a behavioral intervention program

Abstract: Sleep problems are highly prevalent in children with Angelman Syndrome (AS) and an important unmet clinical need rated by parents. There is limited data on the effect of behavioral sleep therapy.

We performed a randomized controlled single-blinded intervention trial. Children with genetically confirmed AS, aged 2-18 years with confirmed sleep problems were randomized stratified for age to either a behavioral intervention program for 6 weeks with a booster session at 8 and 10 weeks or to a control group. At baseline, after 12 and 26 weeks sleep was assessed with sleep diaries and video registration. Also, the calculated sleep index (CSI), sleep hygiene, quality of life and parental stress was measured with questionnaires. Intervention was performed by a behavioral therapist, who visited the parents at home twice and had weekly phone contact. Treatment was based on a standardized behavioral intervention program and included psycho-education, sleep hygiene, feedback on the observations of the video and specific behavioral treatment techniques (bedtime fading and gradual extinction). Primary outcome were sleep duration and CSI. Secondary outcome were parental sleep duration and nightly visits, sleep hygiene, daytime behavior, parental stress and quality of life of child and parents. Statistical analyses included Mann-Whitney, Wilcoxon Signed rank test and Non-Overlap of All Pairs (NAP).

Parents of 18 children (9 intervention and 9 control) participated. Children slept 30-60 minutes less based on baseline video registration compared to diaries. The groups did not differ at baseline except for a shorter sleep onset latency in the intervention group. A significant reduction of wake after sleep onset duration and improvement of total sleep time with a persistent effect for wake after sleep onset duration was seen in the intervention group (primary outcome). Also, a positive and persistent effect of intervention on sleep hygiene and some domains of quality of life of both child and parent (secondary outcome) was found. No difference was seen on the CSI. No effect on any sleep parameter was found within the control group.

These results indicate that behavioral intervention can improve sleep problems in children with AS. We advise a step-up approach to sleep problems in children with AS. This should include psycho-education on sleep for all parents, sleep evaluation by a behavioral therapist with video

registration followed by individual advice and the use of specific behavioral techniques for children with sleep problems.

Notes

- Sleep problems in AS are highly prevalent (50-90%)
- Mostly in younger children, no genotype difference, large impact on the family.
- Biological and physical factors but also behavioral aspects seen to play a role.

Take aways: Sleep education & advice

- Sleep hygiene
- Use AAC for day and night concept
- Bedroom dark & cool, no double function as playroom
- Sleeping bag (not too warm!)
- More than one pacifier in the bed
- Tent or high fence bed
- Camera
- When awake: Don’t get the child out if its bed/bedroom, use dim light, low voice, no food (all of course if possible!)
- Unravel the function of the problem behavior, e.g. related to anxiety, separating anxiety, attention seeking?
- Look for what works for you!

CONFERENCE PROGRAM

Day 1 – Thursday

13:00
Registration

13:40
Meet and Greet over a cup of coffee/tea

14:00
Welcome note from the organizers

14:10–16:10
Novel mouse mutants to address emerging questions
Ype Elgersma

Coordinated Regulation of UBE3A-ATS and UBE3A
Stormy Chamberlain

Assessments of the sleep abnormalities in AS from the FREESIAS observational study
Sebastian Camillo Holst

16:10–16:30
Refreshments

16:30–18:00
Recapitulating Angelman Syndrome using iPSC derived cerebellar organoids
Evguenia Bekman

Stem cell toolkit for modeling cerebellar dysfunction in Angelman Syndrome
Simao Da Rocha

Innovative brain-targeting nano-tools and imaging methods for therapeutic development in AS
Ilaria Tonazzini and Laura Baroncelli

18:00
Closing Remarks

Social Programme
Vienna City Tour with street car

19:10

Dinner at a „Heuriger" (wine tavern) „Zum Martin Sepp"

Day 2 – Friday

09:00

Opening by Organizers

09:10–10.30

Angelman syndrome, learning from bioinformatics analyses and the role of redox homeostasis in early brain development

Hanoch Kaphzan

Angelman syndrome biomarkers and treatment opportunities

Hanna Vihma

10:30–10:50

Coffee/Tea break

10:50–12:10

The effect of UBE3A on the function of the proteasome

Mattijs Punt

Studying cortical development through the lens of autism spectrum disorders

Gaia Novarino

12:10–13:00

Lunch

13:00–15:10

Short talks I

(10 minutes + 8 minutes Q&A at the end)

Matteo Fossati, Carina Maranga, Eugenia Suter, Adriana Vieira

Characterizing mICD mouse model for Angelman Syndrome

Narayanan Ramanathan

Is Angelman Syndrome a disorder of organellar Ion homeostasis? Insights from Christianson Syndrome

Rajini Rae

UBE3A-Induced ubiquitination changes in the brain elucidated by proteomics

Ugo Mayor

15:20–15:40

Refreshments

15:40–18:00

The role of UBE3A In synapse formation and function
Ramiro Almeida
Sleep Problems In children with AS: The effect of a behavioral intervention program
Karen Bindels-de Heus

Detecting UBE3A In Cerebral Spinal Fluid of AS Patients
Rob Komorowski

Clinical Trial in Individuals with AS to enable Endpoint Development for interventional trials
Casar Ochoa-Lubinoff

Short talks II
(10 minutes + 8 minutes Q&A at the end)
Doesjka Hagenaar, Alberto Velez-van-Meerbeke, Amanda Moore, Betty Willemsen

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18:10
Dinner on location: Traditional Austrian food + Cheese & Wine

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Three plenary sessions in parallel:
1. Scientists: What do we NOT know about AS? - closed Session
Ype Elgersma will chair this discussion focused on emerging research questions that need to be addressed concerning UBE3A function, AS pathophysiology and development of treatments.

2. Doctors: Why start an AS clinic? Which specializations needed? Which facilities needed? Program?
Chaired by Karen Bindels-de Heus and Marie-Claire deWit

3. ASA members: Member Meeting - closed session

20:30

Drinks at Stiegl Ambulanz in .Altes AKH'

Day 3 – Saturday

08:30
Registration to Conference
Organisers

09:00
Welcome from the Organizers

09:10–10:30
Overview on the latest AS research presentations
Harald Sitte

Layman explanation of new research project
Simao Da Rocha, Evguenia Bekman
Layman explanation of new research project
Ilaria Tonazzini, Laura Baroncelli

10:30–10:50
Coffee/Tea break

10:50–12:10
Roche: AS research on therapy and study status
Brenda Vincenzi

Ladder database & learning network
Anne Wheeler / Elizabeth Jalazo

Augmentative and Alternative Communication
Michaela Zöbl

12:10–13:00
Lunch

ASA Meeting II
For ASA members

13:00–14:20
Update on the Ionis clinical trial of HALOS for Angelman syndrome
Becky Crean

CBD anticonvulsant - Hype or real possibility
Christel Kannegiesser Leitner

Industry Update of AS Syndrome program PTC Therapeutics
Ifode Ajari

14:20–14:50
Refreshments

14:50–15:50
Information about the Rotterdam Expertise Centre “Encore” and Information on how to improve sleep
Karen Bindels-de Heus and Marie-Claire de Wit

15:50
Closing Remarks