1st International Scientific Meeting on ZC4H2 Deficiency.

Summary for parents

Dubai, 21-22 Sept 2018

1. What?

On the 21st and 22nd of September, a meeting was organized in Dubai to gather the foremost global experts in ZC4H2 and disorders related to ZC4H2 mutations. During the meeting various presentations were given and the extent of the current knowledge about ZC4H2 was discussed. Furthermore the next steps for potential future research were determined.

2. Why?

The meeting was organized by Cathy & John Paul, the parents of Milou, a 3 year old girl with a pathogenic mutation in the ZC4H2 gene, who live in Dubai. They decided to start a foundation to promote scientific research on ZC4H2 with the aim of finding a cure for this condition. The foundation, called "ZC4H2 Research Foundation" is a private, non-profit foundation incorporated in their native country, The Netherlands.

Cathy & John's dream is to create a positive impact on the lives of all patients with a condition similar to their daughter. With this in mind they felt it necessary to start a scientific discussion in order to understand what is the current knowledge on ZC4H2, what could possibly be needed to spearhead word-class scientific research on this condition and what could possibly be achieved in terms of finding viable treatments and ultimately a CURE for this devastating condition.

3. How?

Cathy & John partnered with the Orphan Disease Center (ODC) led by Dr. Jim Wilson, from the Perelman School of Medicine, University of Pennsylvania, USA. With the greatest support of the ODC starting early 2018, they selected the most experienced scientists worldwide in the field of ZC4H2 research and gather them to attend the meeting in Dubai in September 2018.

The scientific meeting was funded by the ZC4H2 Foundation and organized in conjunction with the ODC. It took place at the Al Jalila Children's Hospital, the prime pediatric hospital in the United Arab Emirates.

4. Who?

The attendees are detailed in the brochure of the meeting, which you can access and download from our website www.zc4h2foundation.com

5. What was discussed?

5.1. Summary of biology of ZC4H2:

- The *ZC4H2* gene is expressed in all human tissues. This gene is transcribed into 5 different RNAs or isoforms (isoforms are alternative messages of the gene which are translated into protein) with one of them being the main isoform. The main isoform is translated into the largest protein which is composed of 224 amino acids.
- It is known that in mouse the *Zc4h2* gene is expressed in various brain regions and the spinal cord. Expression appears to be the highest in the embryo, declining after birth (what may suggest an important function during development). In zebrafish *zc4h2* is primarily expressed in brain cells called "myelinating oligodendrocytes", with decrease in expression as these mature into another type of brain cells. This may suggest a function of ZC4H2 in myelination –which has not been studied to date.
- On a cellular level, overexpressed (transiently expressed ZC4H2 constructs transfected into cultured cells or neurons) ZC4H2 protein is located in: cytoplasm, nucleus, cell junction/synapse/postsynaptic cell membrane (excitatory).
- It is believed that the ZC4H2 protein is involved in nervous system development, neuromuscular junction development and spinal cord motor-neuron differentiation.
- As seen in experiments with embryos (zebrafish and mice at the moment), mutations in the Zc4h2 gene result in defects in the neural tube (the precursor of the brains and spine) and two types of neurons called V2b interneurons & V2a interneurons (a type of neurons directly involved in the brain/muscle communication).
- A study in a zebrafish model was presented where the mutation of *zc4h2* resulted in hyperactive movements of fins and jaw (may be correlated to the impaired muscular tone seen in humans?) and absent eye movements (may be correlated to the oculomotor apraxia seen in humans?).
- It has been observed that complete absence of functional *Zc4h2* gene and protein in mice is lethal in one strain background, while using another mouse strain showed that heterozygous females are viable. This may suggest that the genetic background (the natural genetic make-up of each individual) may affect the presentation (thus another cause of the large clinical variation observed among patients could be their own genetic make-up).
- Results from a current study show that the ZC4H2 protein interacts with an important cellular ion channel called TRPV4. The TRPV4 channel plays a crucial role in many physiological functions, including neurological and neuromuscular functions. These findings are important for the consideration of future research directions and therapeutic possibilities.
- Inheritance: pathogenic ZC4H2 mutations have been described as ''X-linked recessive". However, there was consensus that it is correct to refer to X-linked recessive inheritance only in families where the

mutation is passed through a female carrier. In de-novo sporadic cases, the correct inheritance pattern is X-linked dominant.

5.2. Summary of clinical knowledge on pathogenic ZC4H2 mutations:

- Male presentation is usually more severe but less variable than female presentation (however there is recently a case of a mildly affected boy carrying a ZC4H2 mutation)
- Primary symptoms observed in all affected known cases* to date (in different degrees) are: contractures in limbs, fingers and hands (in all cases diagnosed as AMC), proximal insertion of thumb and first toe, issues with muscle tone, delayed motor development (mobility), orthopedic problems (including but not all present in each case: dislocations specially of hips, bone malalignments or deformities).
- Secondary symptoms present in most patients (in different degrees) are: Dysphagia at least in early development, respiratory complications at least early in life, spinal complications, apraxia of speech, delayed cognitive development, oculomotor apraxia.
- There have been other symptoms described is some patients. The variability of these symptoms is large and includes: epilepsy, cortical visual impairment, vascular and cardiac problems, GI problems.
- We know that in females with an inherited missense variant some are unaffected and others are mildly affected (learning disabilities). In contrast, clinical presentations of females with a de novo deletion or de novo missense variant is highly variable, ranging from mild to severe.
- What is likely to affect the phenotype (clinical presentation) is: Mutation type, location of the mutation in the gene, X-inactivation, and possible mosaicism in some patients.
- Although X-inactivation is theoretically considered at least partly responsible for the variation observed in females, in previous studies, the X-inactivation pattern tested in different cell types (lymphocytes, fibroblasts, saliva) did not allow to predict the clinical outcome of the affected patients.
- Is the pathogenic phenotype **progressive**? The original publication from Wieacker points out that in this family affected males showed a slowly progressive distal muscle atrophy. However, most males were old age at the time of examination (!). The current literature merely points out to a single family with late onset (adulthood) of primary symptoms in female carries with an inherited pathogenic mutation. On the question whether “deterioration” would be the result of primary phenotypic progression or rather the result of secondary development (due to lack of appropriate care), there is definitely no consensus at this point.
- Primary limiting factor in diagnostics is access to sequencing: as explained, the ZC4H2 gene is transcribed into different RNA isoforms (alternative messages of the gene which are translated into protein). It has been noted that the major isoform is not included in all sequencing panels / data analysis and this may lead to a lack of diagnosis for some potential patients. In other words, not all potential patients get a correct diagnosis.
- There is no current understanding or consensus on the effect of care on outcomes. However, it has been observed that early and consistent supportive therapies result in improvement. There is case-based evidence that some contractures can resolve with careful PT attention.
- Specialists usually seen: CP clinic, AMC clinic, Geneticist, Neuropediatricians.

- Care: boys likely to end up in CP clinic and not receive progressive PT.
- Mortality: association of *ZC4H2* pathogenic mutations with mortality or life-span are largely unknown; there are known cases of death due to respiratory complications in teenage children.
- Very often information given by clinicians to patients is poor, incomplete or erroneous. In the absence of central and appropriate information about ZC4H2-related conditions, most clinicians base their knowledge on past publications or incomplete/faulty public online resources.

**Cases reviewed to date by Cathy Paul-Fijten –not from publications.*

5.3 What are the therapeutic objectives for patients with a pathogenic mutation of ZC4H2?

- Stop decline/deterioration
- Regain of function
- Increase developmental capacity
- Seizure control
- Stabilize/enhance pulmonary function- main cause of death (current understanding)
- Osteopenia and other orthopedic challenges
- Metabolic regulation
- Gastro-intestinal complications

6.1 What was concluded?

6.1 Function of ZC4H2

There is some understanding and consensus on aspects of molecular and protein function but more work is needed to confirm current findings as well as to find new aspects of the function of the *ZC4H2* gene and its protein. There was general agreement that currently known ZC4H2 mutations cause a loss or partial loss-of gene function and that at this moment there is no evidence for pathogenic mutations which would result in a gain-of gene function (for example a gene duplication of ZC4H2).

Research tools (antibodies and animals models) are critically needed for further understanding of the basic science and functions of the *ZC4H2* gene and its protein.

6.2 Natural history studies

Unfortunately, there is no information available on natural history, nor studies on natural history currently ongoing for pathogenic ZC4H2 mutations.

Knowledge of Disease Natural History is an essential element in the scientific foundation of any clinical development program.

Natural History Studies are studies that track the course of a disease or condition over time. These studies aim to identify demographic, genetic, environmental and other variables that correlate with the condition as well as outcomes in the absence of treatment. Data from natural history forms a reliable base upon which drug or therapy development programs can be built. In addition, the data collected throughout natural history studies can be used to establish best practices for patient care, to identify research priorities, to develop "centers of excellence" and to prepare for clinical trials.

To progress to the creation of natural history studies for ZC4H2, we need to:

- Build clinical consensus and publish (clinicians must access more and updated knowledge about this condition)
- Organize the community clinically (clinical centers, clinical Key Opinion Leaders, consideration of biomarkers)
- Aggregate clinical data across studies (Will need collaboration amongst groups)
- Collect data among patients
- Create a patient registry or patient portal
- Build a Clinical Working Group (key clinicians that can work collaboratively on studies)
- Organize new clinical-data collection

6.3 Nomenclature

There is general consensus on erroneous nomenclature of the clinical presentation of a pathogenic mutation of the *ZC4H2* gene as "Wieacker Wolff Syndrome", "Miles Carpenter Syndrome", "Intellectual disability-developmental delay-contractures syndrome", or any other descriptive denomination currently based on nomenclatures loosely adopted by previous publications or utilized without corresponding publication in some public resources.

Erroneous "descriptive" names for this condition have important consequences for patients and their families: not all the patients present the same symptoms, hence grouping of such a variable condition under one single descriptive name (often utilized to describe severe cases) can wrongly define the condition for some patients (think of consequences for social perception, acceptance and inclusion, accessibility to education, insurance, dispensation of appropriate resources, etc).

The group agrees to maintain a causal nomenclature rather than a descriptive one. Future publications from the group will adopt appropriate nomenclatures. For now it is recommended to utilize the general terminology "ZC4H2 (pathogenic) mutation" until upcoming publications with an agreed denomination.

7. What are the next steps?

As mentioned earlier the ZC4H2 Research Foundation has partnered with the Orphan Disease Center and under their supervision and advice, the Foundation has committed to provide initial funding for 7 independent research projects for the duration of 1 year, planned to start in 2019. The projects will be executed by different academic research groups selected following an international Request For Application (RFA). The 7 research projects are:

1. Discovery of the protein function of ZC4H2
2. Molecular characterization of ZC4H2 and the effect of mutations on molecular and protein function
3. Validation of a druggable target or pathway for ZC4H2 treatment.
4. Development of ZC4H2 antibodies
5. Development of a viable animal model
6. Characterization of iPSC lines
7. Establishment of Clinical guidelines

In addition the Foundation will work closely with the scientific community to encourage and support the publication of a Literature Review paper which aims to include a summary of the current knowledge on ZC4H2 and current consensus. This paper is aimed to be published in Q2 2019.

A scientific advisory board has been established which will advise the ODC and the foundation on the merits of the received grant proposals.

The ZC4H2 Research Foundation will fund the research which is being paid for by Cathy & John personally but it is the hope that the foundation will be able to raise funds to finance ongoing and future research.

8. Why this letter?

Cathy & John want to share with all "ZC4H2 parents" any news related to the condition as they feel it is important to create awareness and generate more interest in order to bring positive change. The larger the active community the more impact can be achieved. Specifically one goal that can currently be addressed is creating a database of all existing patients and details of their conditions.

Cathy has been working on capturing data from each known case for a while, even before the establishment of the foundation, as a personal effort to 'map out' all the known information about the children and share the knowledge among all parents, which will serve as a 'guide' for current and newly diagnosed families. Without pretending to be a diagnostic or prognostic tool, gathering patient information is incredibly important for parents and caregivers, to learn about the possible symptoms and possible outlook –especially when information is almost inexistent and there is only a small bunch of diagnosed patients in the world.

In addition, it has become clear that for a small disease group like ZC4H2, without this type of background knowledge, there is no research possible. So, there is an urgent and real need to capture and organize the data

in a formal and structured way, with the permission of each parent to share the data for the benefit of scientific research. This will help researchers and clinicians alike.