ASF Research Updates

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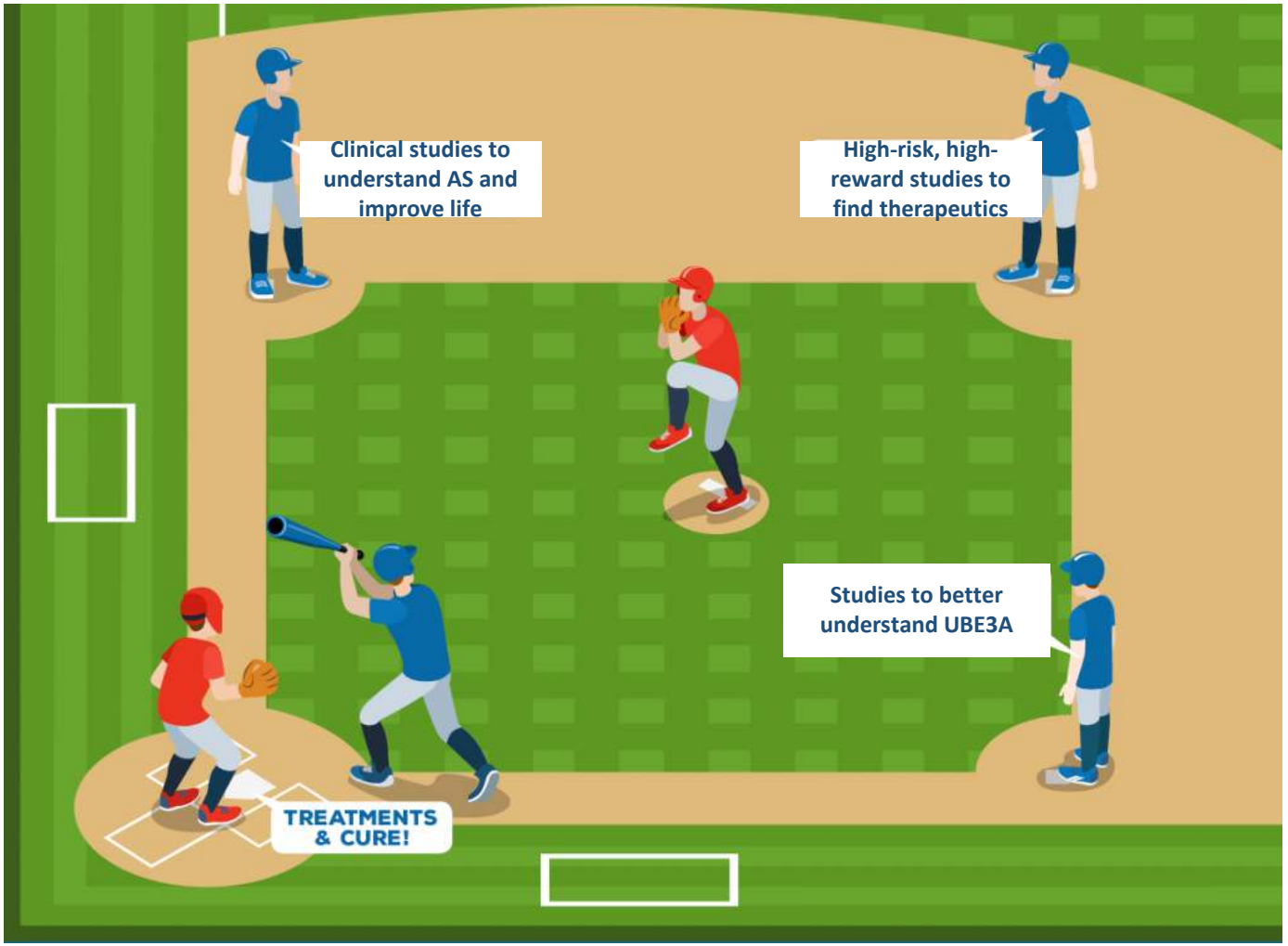
Associate Professor of Genetics and Genome Sciences
University of Connecticut, School of Medicine
Chair, Scientific Advisory Committee, Angelman Syndrome Foundation

Our Research Goal

- To improve the lives of individuals with Angelman syndrome

ASF's research funding philosophy



How we fund

- Seed funding to generate preliminary data
- Promising studies can then be further funded by NIH, SFARI, pharma

e.g. $200,000 to Dr. Mark Zylka in 2016 for his CRISPR studies resulted in a $2.8 million grant to him from the NIH in 2019

Peer review

- Any scientist or clinician can submit a grant application to the ASF
- Proposals are reviewed twice a year by the scientific advisory committee

Scientific Advisory Committee (SAC)

- 19 member committee comprised of scientists, clinicians, and parents
- Review grants individually, and then meet by phone to discuss and score

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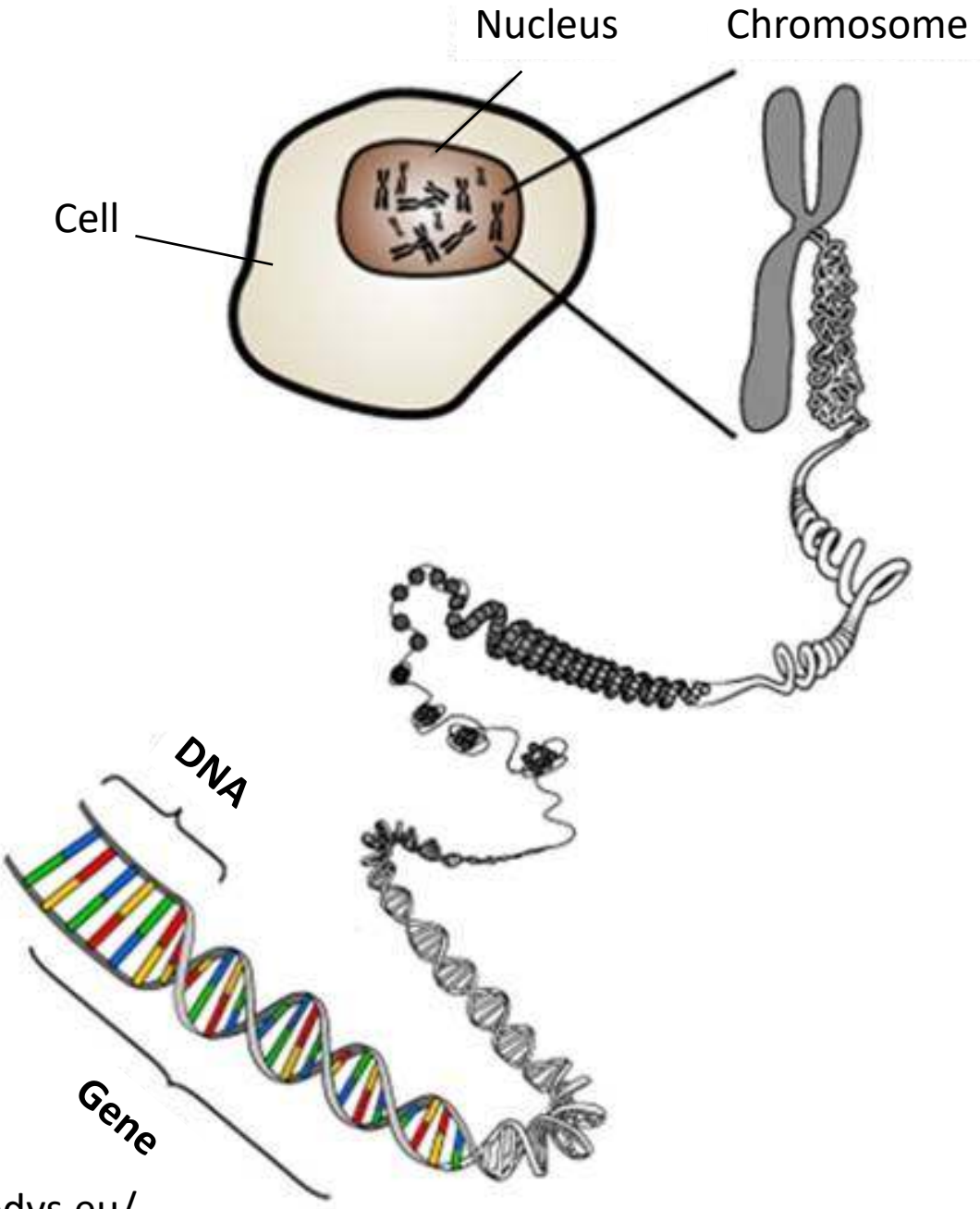

http://www.gencodys.eu/

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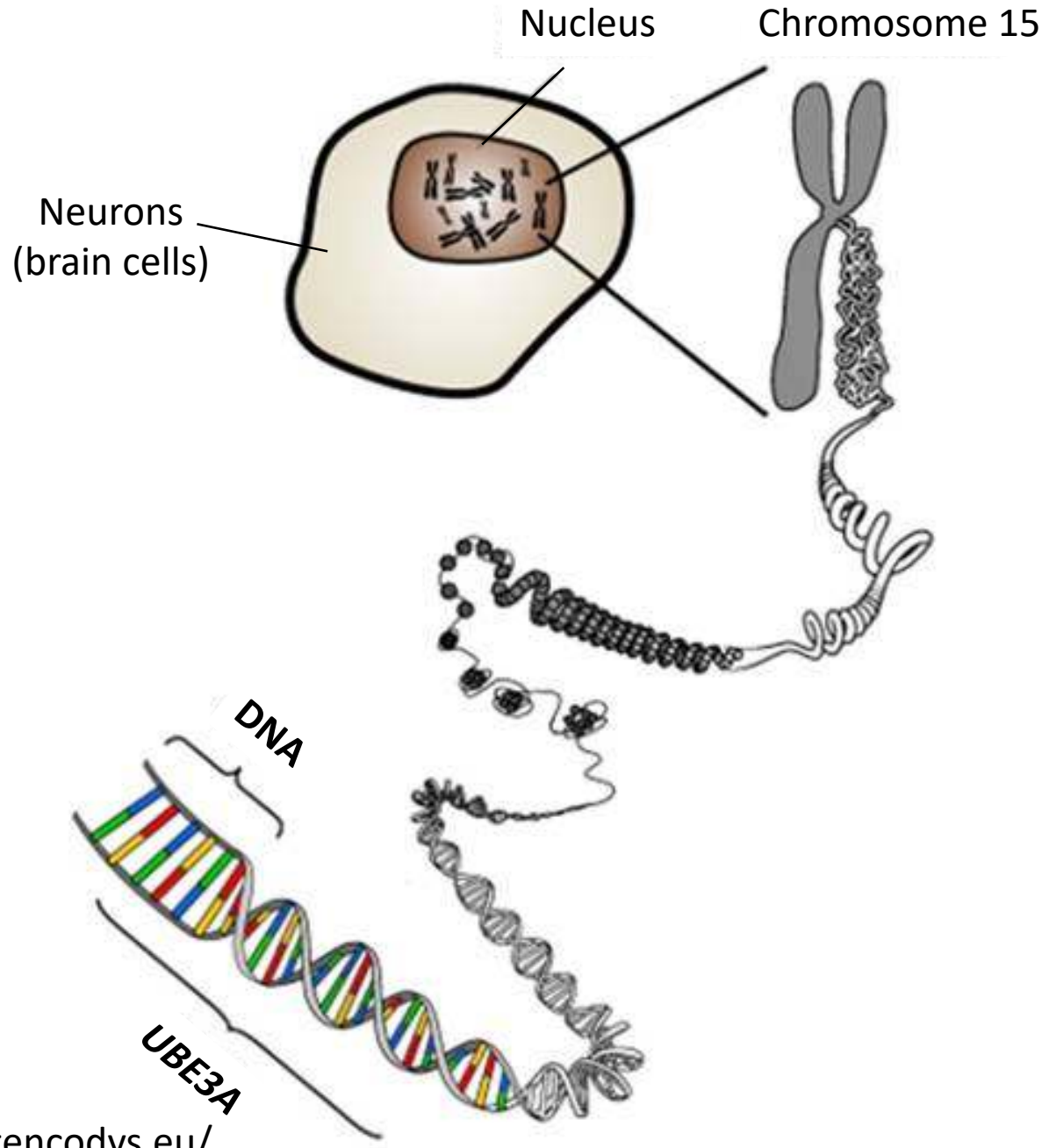

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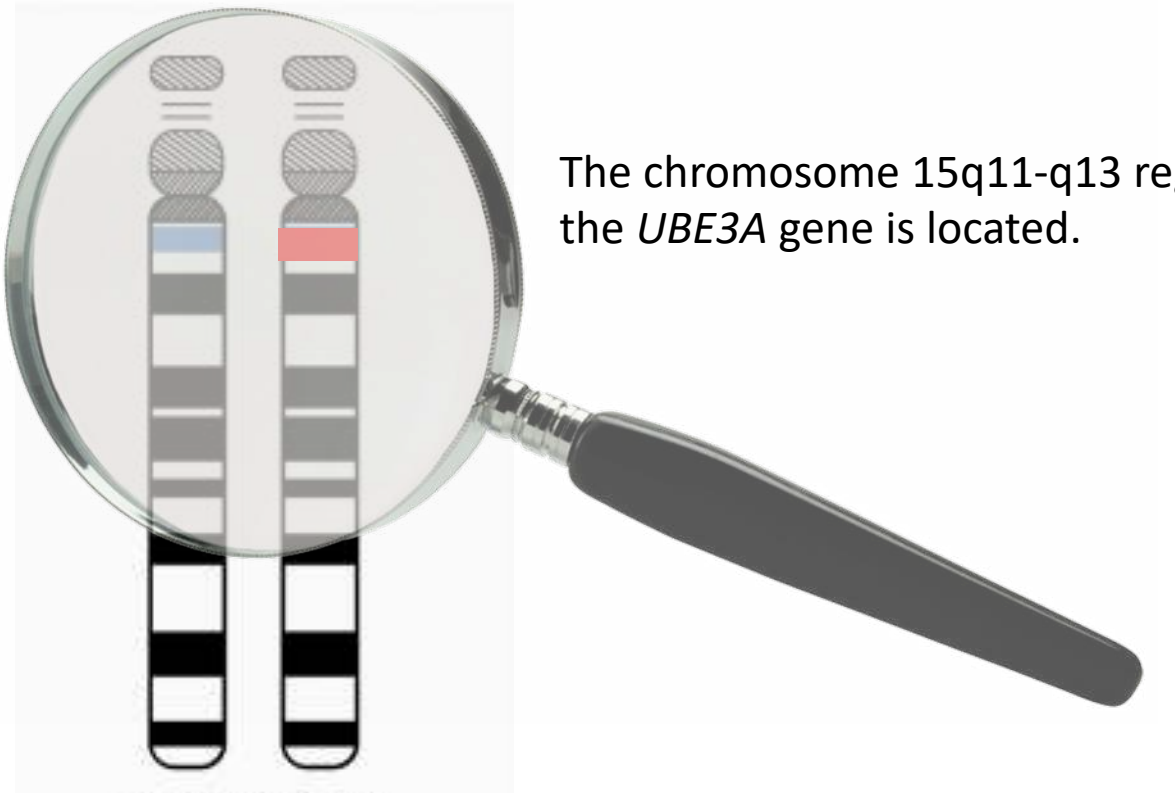
Unlike most chromosomes, mom's and dad's copies of chromosome 15 are different from one another



The chromosome 15q11-q13 region is where the *UBE3A* gene is located.

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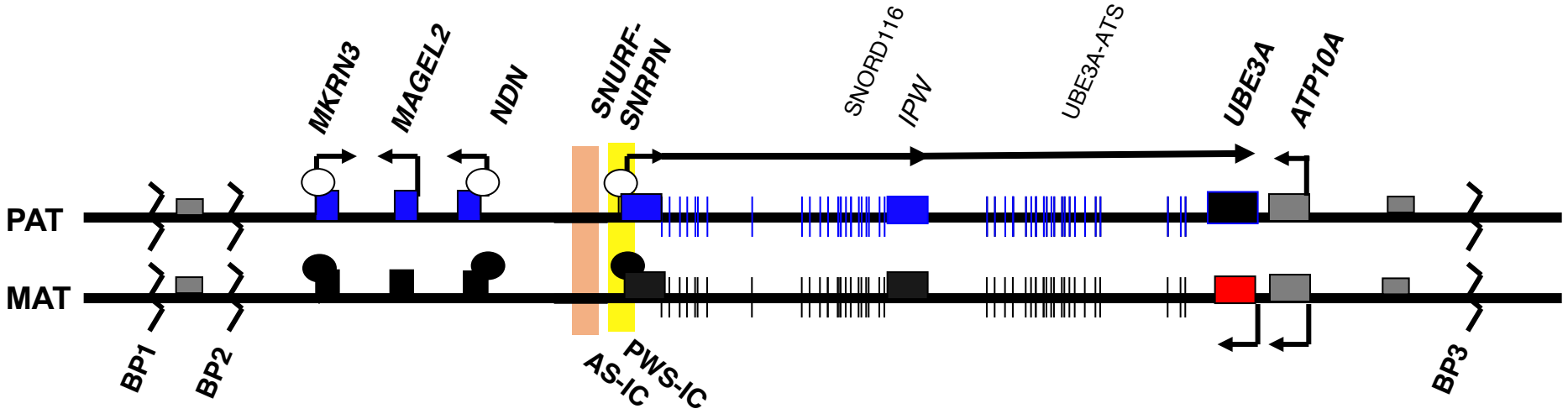
Some genes on chromosome 15q11-q13 are made from mom's copy and some are made from dad's copy



* DNA methylation determines the difference between mom's and dad's chromosomes 15.

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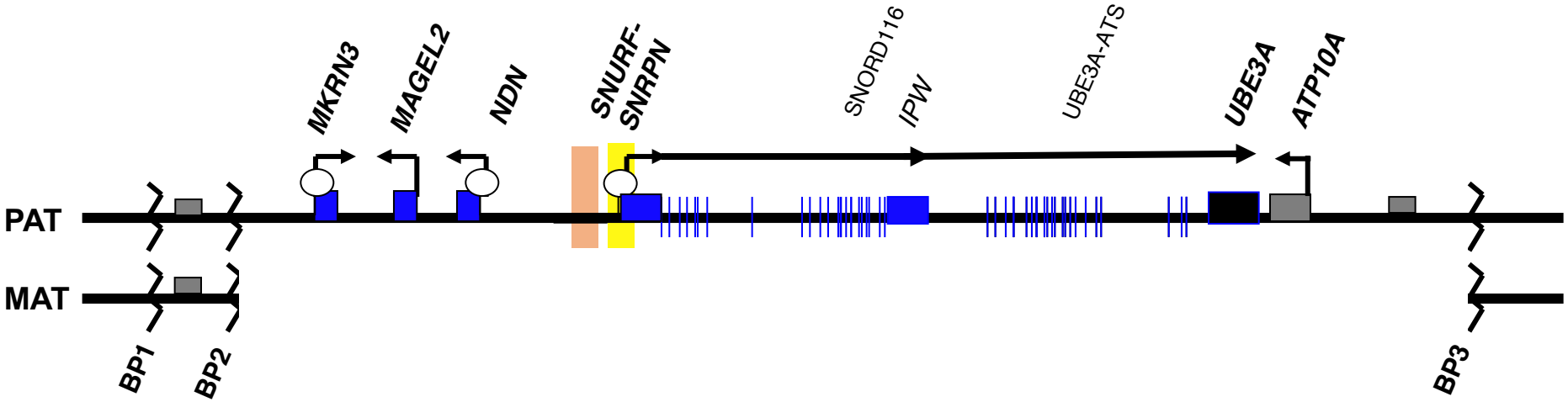
When mom's copy is missing, UBE3A is not made in neurons



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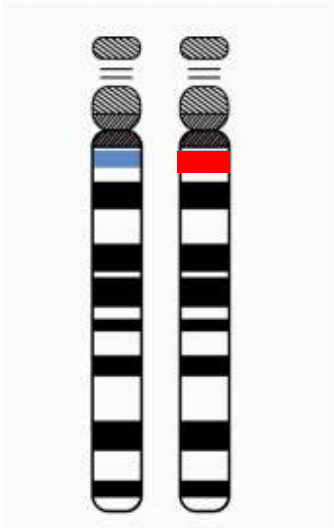
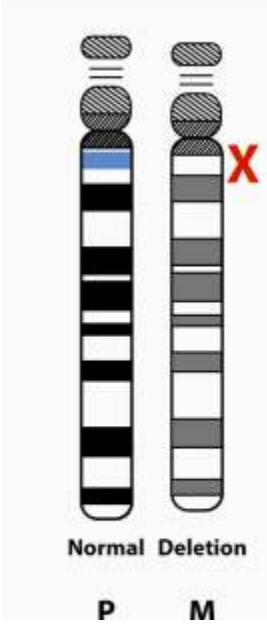
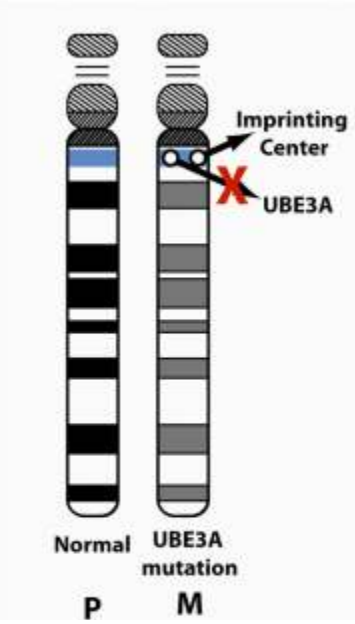
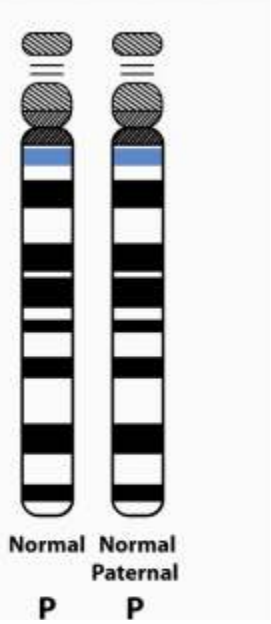
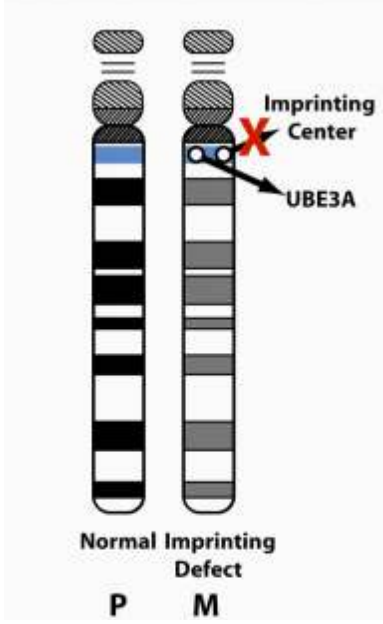
There are four “common” ways to lose mom’s copy of UBE3A











Typical

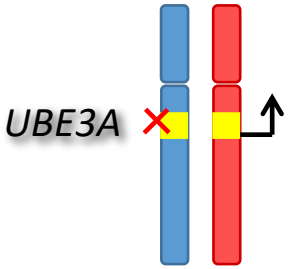
Deletion (~70%)

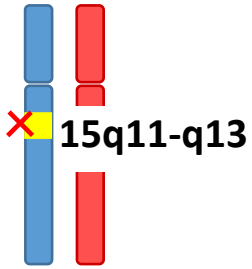
UBE3A mutation (~10-15%)

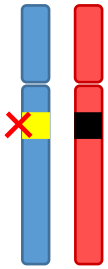
UPD (~3-5%)

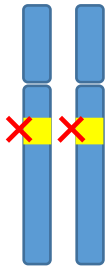
Imprinting defect (~2-5%)

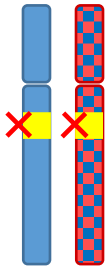
pat. mat.











ASF co-funded research to develop newborn screening

Dr. David Godler, MD
Murdoch Children's Research Institute,
Australia

- Developed an inexpensive methylation test ideal for use in newborn screening for AS.
- Partnered funding with Foundation for Prader-Willi Research and Dup15q Alliance.

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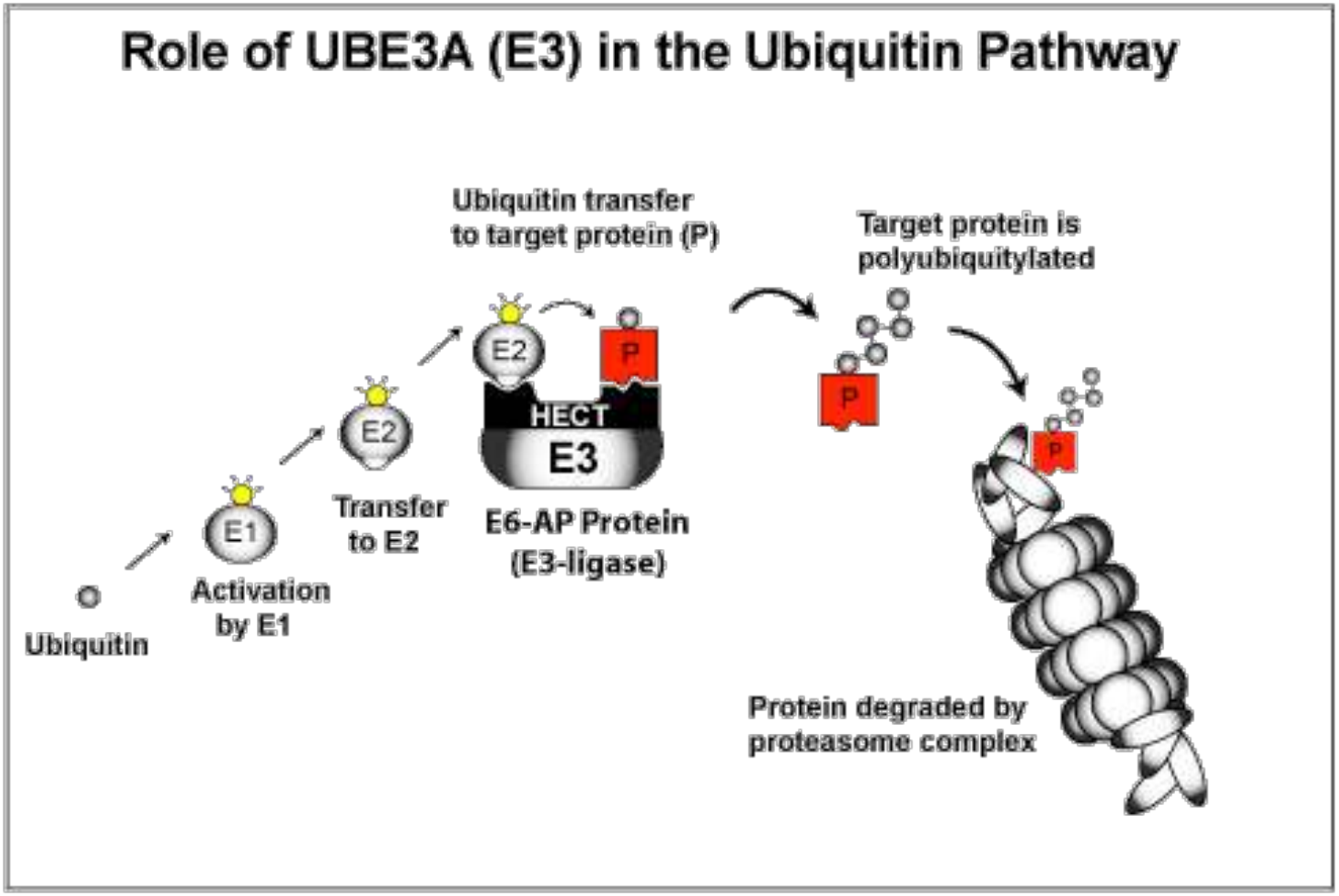
What does UBE3A do?



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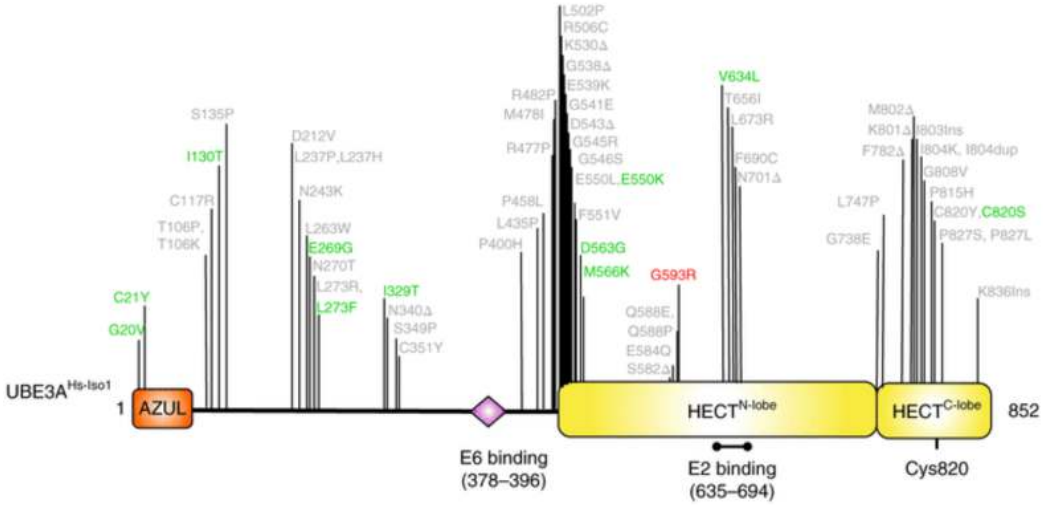
How do mutations in UBE3A cause AS?

Dr. Jason Yi, PhD
Washington University, St. Louis



- Catalogue all of the UBE3A mutations that cause AS and determine how they disable UBE3A protein function.
- Identify areas where mutations have unusually large impact on UBE3A function.
- Will aid in interpretations of genetic tests for diagnosis of AS.

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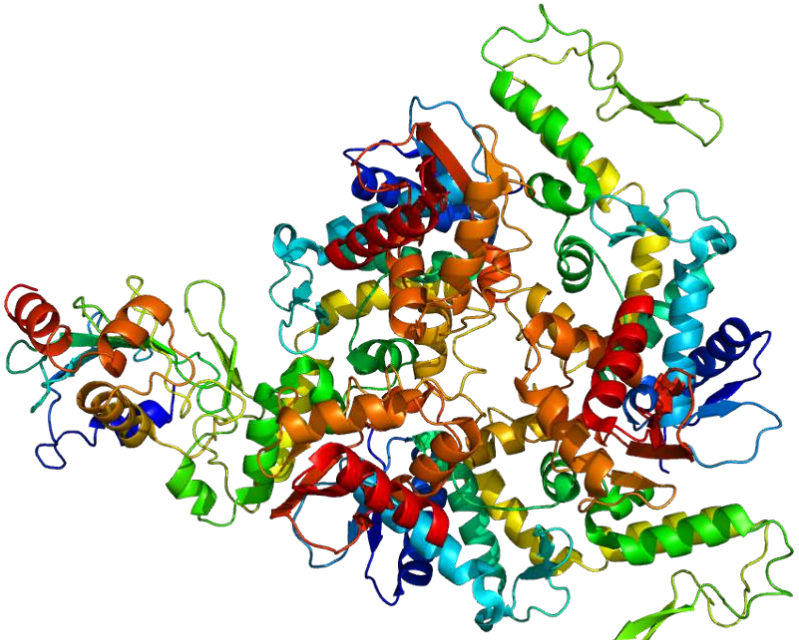
What is the full structure of UBE3A protein?

Dr. Gilles Trave, PhD
European Center of Research and Biology, Illkirch, France



https://en.wikipedia.org/wiki/UBE3A

- Determine the 3D structure of full-length UBE3A with and without HERC2
- Determine other proteins that interact with UBE3A +/- HERC2
- Important for understanding how UBE3A works with and without HERC2 to impact brain development

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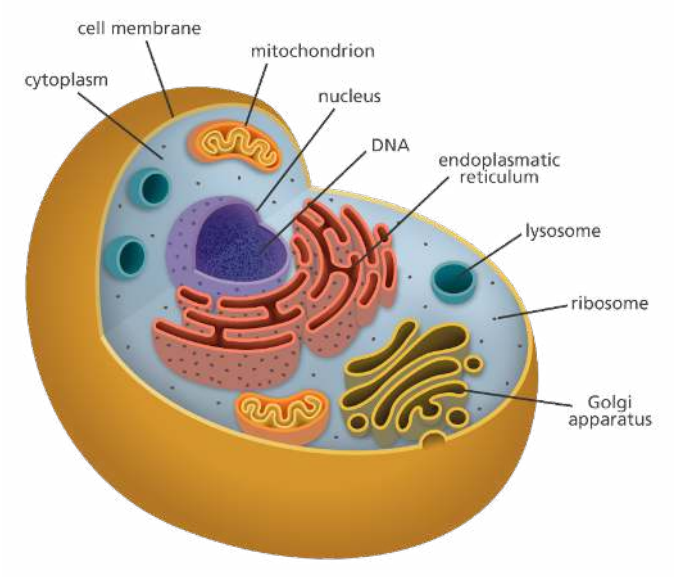
Where in the cell is UBE3A?

Dr. Ype Elgersma, PhD
Erasmus Medical Centre, Rotterdam



- Two different “forms” of UBE3A in mouse may be located in different parts of the cell. Short form is located in the nucleus, long form is located in the cytoplasm

- Short form accounts for ~80% of total UBE3A protein

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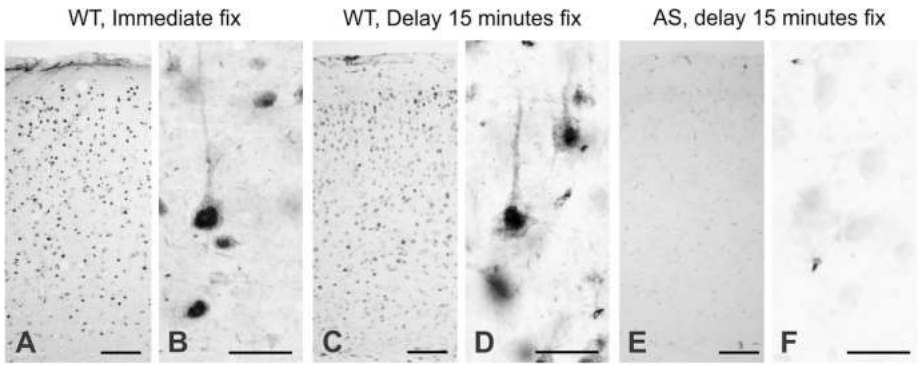
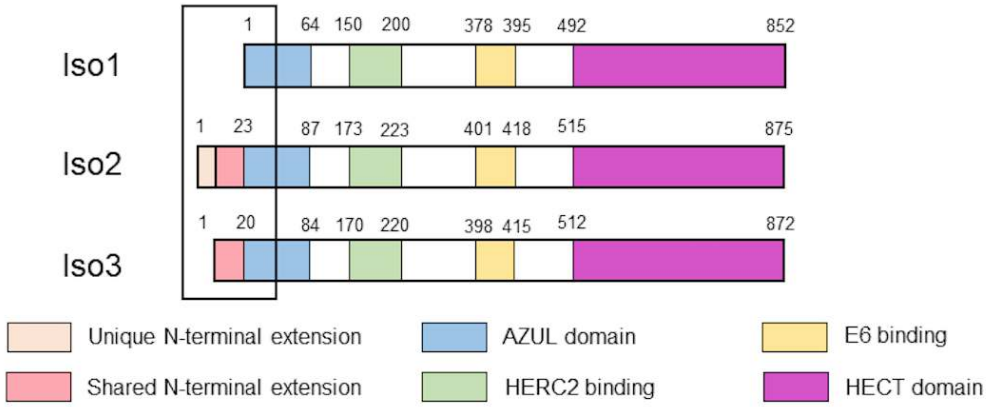
Where in the cell is UBE3A?

Dr. Ben Philpot, PhD
University of North Carolina

Dr. Stormy Chamberlain, PhD
UConn Health





- Overall, human UBE3A protein looks similar to that in mouse
- Humans have three different “forms” and their location may differ from mouse forms.

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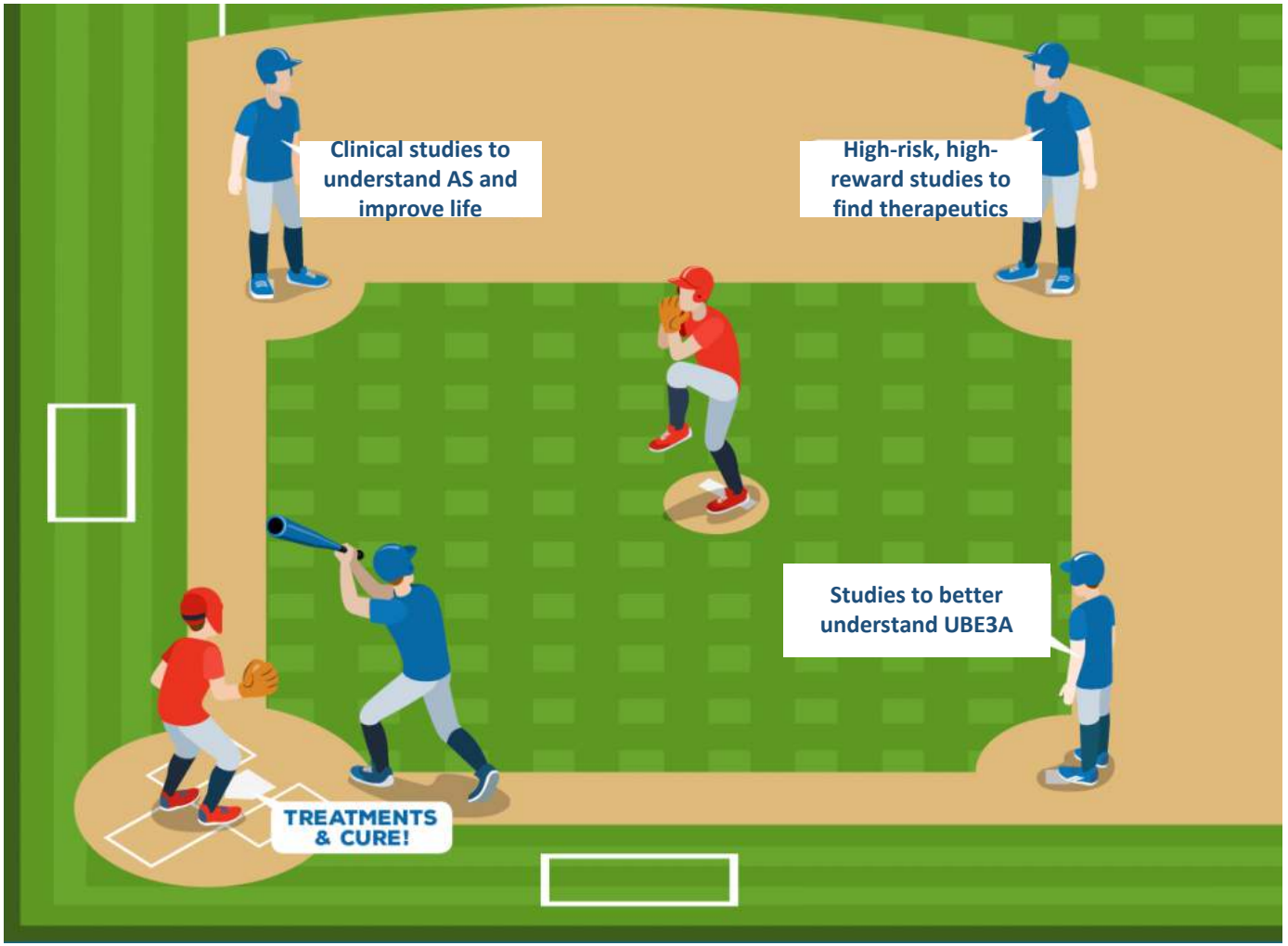
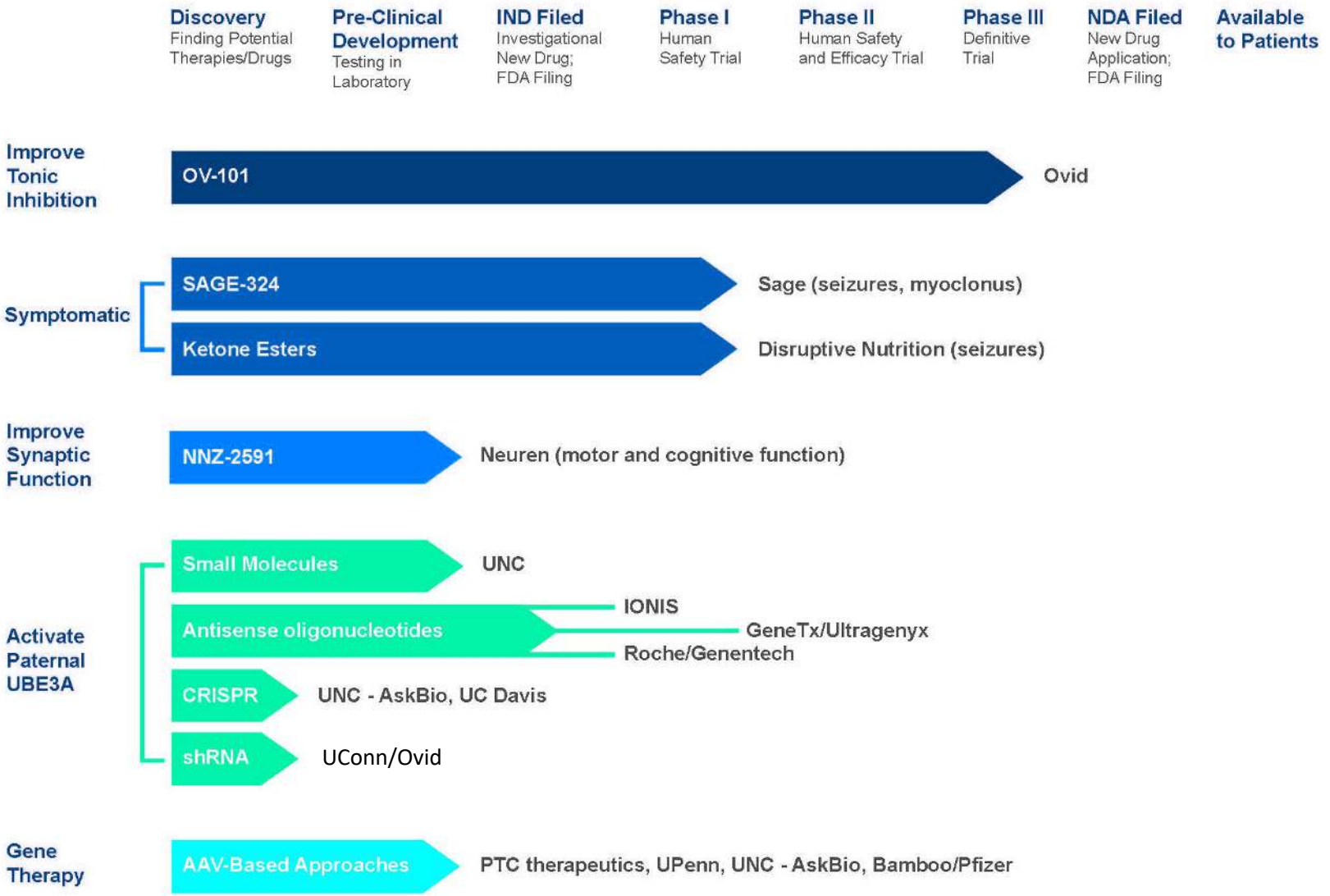
ASF's research funding philosophy



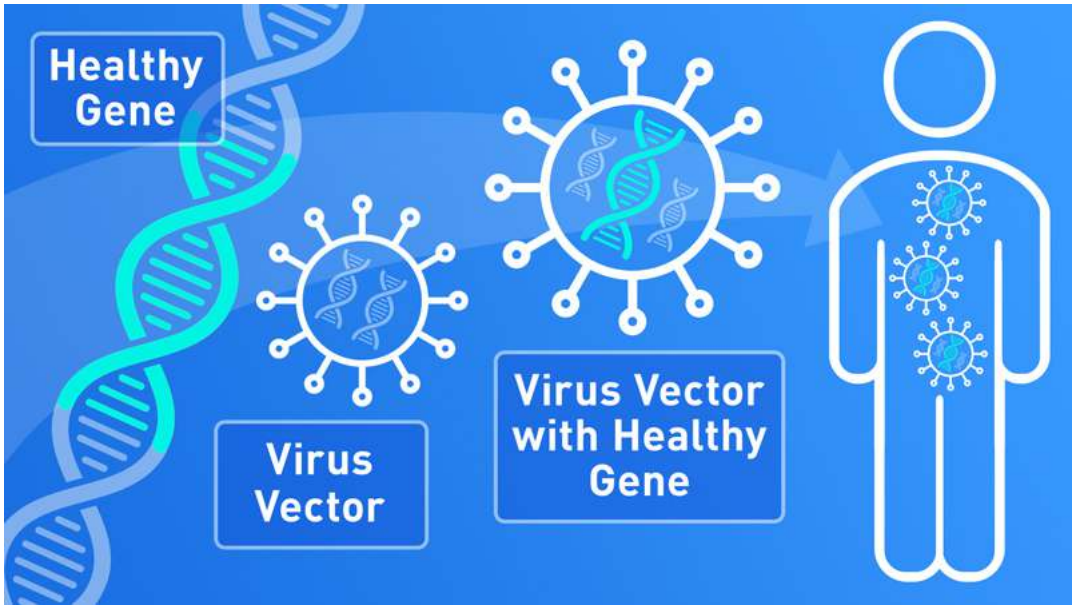
ANGELMAN SYNDROME THERAPEUTIC PIPELINE



https://www.angelmanclinicaltrials.com/drug-development

How can we restore UBE3A? Gene Therapy

Dr. Steve Gray, PhD
University of Texas, Southwestern Medical Center



- ASF funded the first studies of gene therapy back in 2005 and 2007.
- More recent study (2017) was a collaboration with Dr. Ben Philpot at University of North Carolina and is being developed by AskBio.
- Approach is being pursued by PTC therapeutics, UPenn Orphan Disease Center and potentially others.

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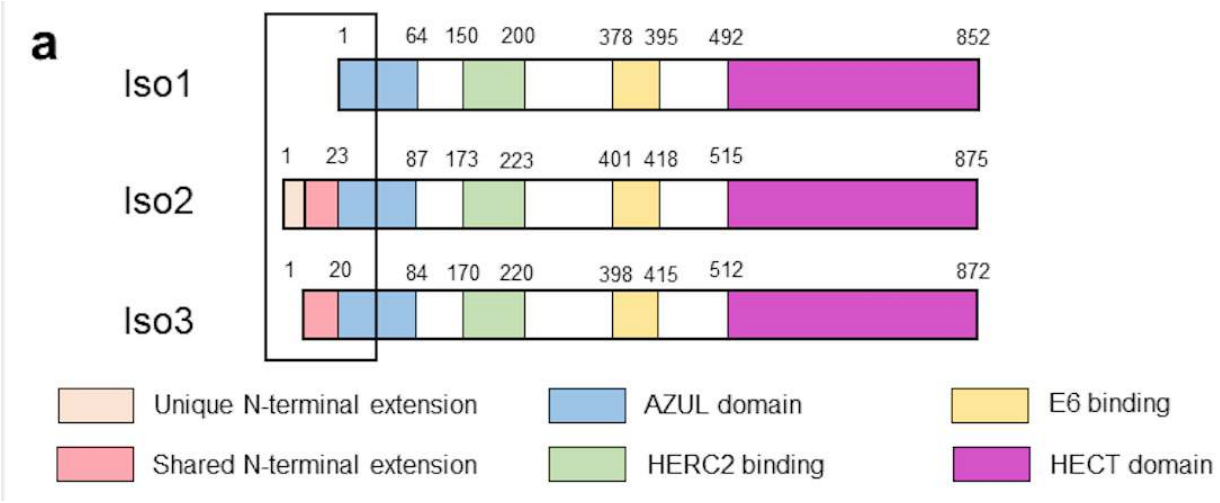
How much UBE3A is needed?

- Anjali Sadhwani and Wen-Hann Tan (Boston Children's Hospital) identified two families with a mutation that disrupts the short "form" of UBE3A protein.
- The three kids in these families are less strongly affected—individuals have speech and improved motor skills.
- Studies from Elgersma and Chamberlain labs suggest that these individuals have ~12-18% of typical UBE3A levels.



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Is too much UBE3A bad?

- Kids with Dup15q syndrome have 1 or 2 extra copies of UBE3A.
- Early studies and genetics suggest that excess UBE3A is causing many aspects of this disorder.
- Ben Philpot's lab has made mice with extra copies of UBE3A to test this in a mouse model.
- ASF collaborates closely-–and shares a research conference-–with Dup15q Alliance.

Dup15q Alliance

FOR 15Q 11.2–13.1 MULTIPLICATIONS

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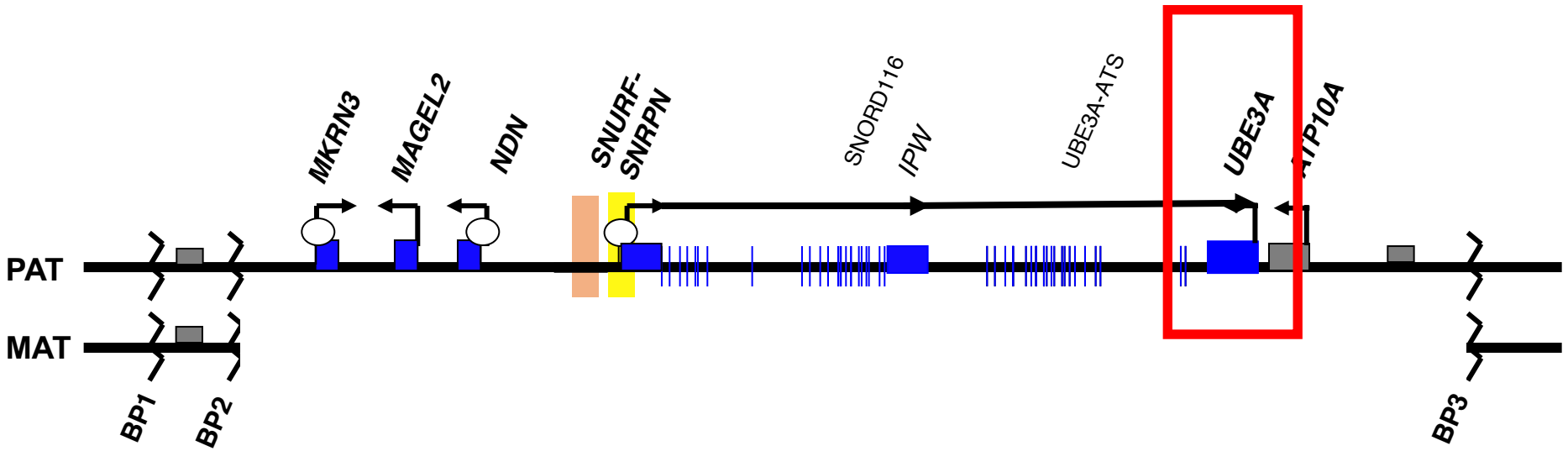
Every child with AS has an intact copy of *UBE3A*, but it is silenced



Activation of paternal *UBE3A* is an attractive therapeutic strategy

- ASF funded a study in Ben Philpot's lab (2009) that first showed paternal UBE3A could be activated.

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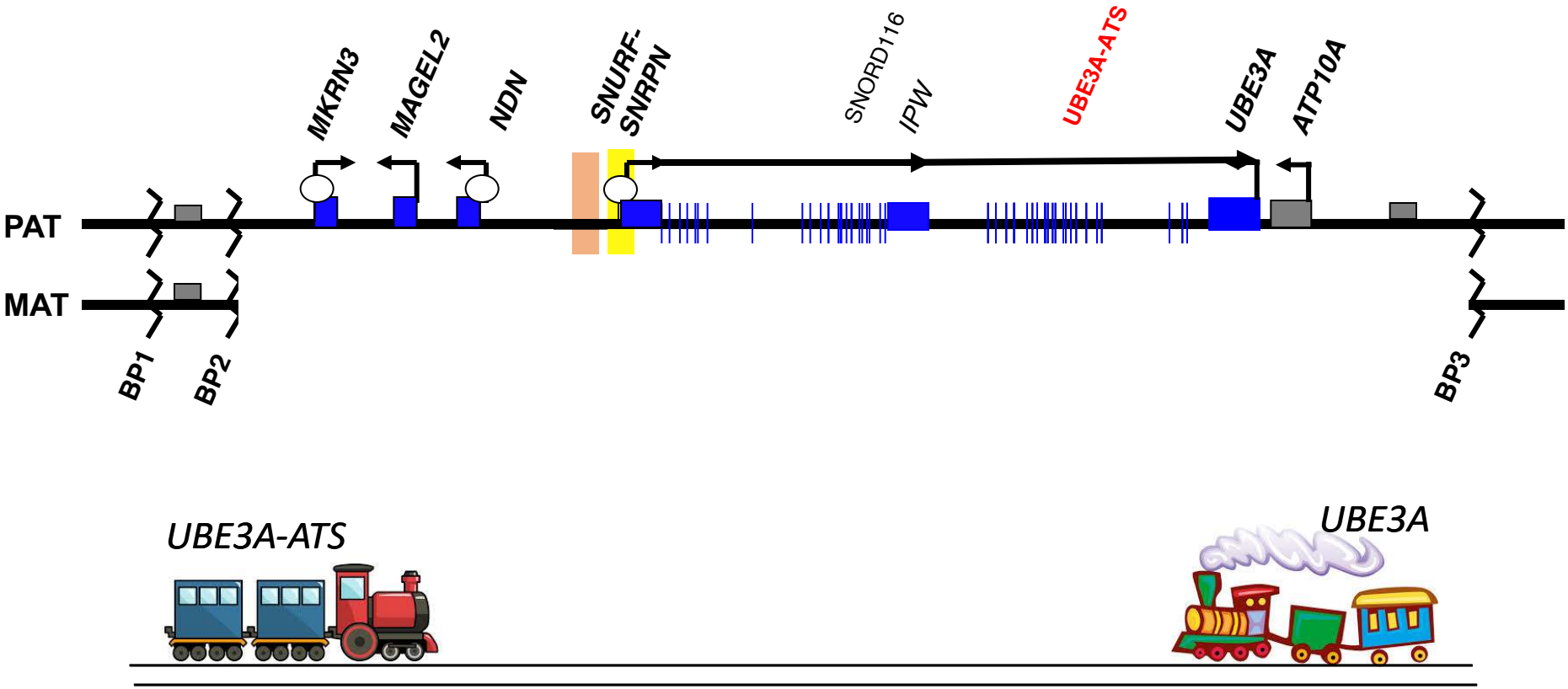
UBE3A-ATS silences paternal UBE3A



- ASF funded Dr. Art Beaudet (2011) to better understand how this happens.

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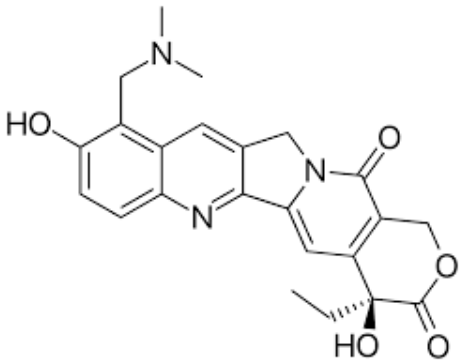
Small molecule activators prevent the UBE3A-ATS from being produced

Dr. Ben Philpot, PhD
University of North Carolina



Topotecan

- Topotecan was first example.
- ASF funded a recent study (2017) of additional small molecule activators that work by an unknown mechanism.
- Licensed by Pinnacle Hill and being developed in collaboration with Ben Philpot.

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CRISPR strategy stops UBE3A-ATS

Dr. Mark Zylka, PhD
University of North Carolina

- CRISPR has a small RNA that guides a larger protein to a specific spot on the DNA where it stops UBE3A-ATS.
- CRISPR does not need to cut the DNA = safer therapy.
- Can be delivered by AAV, even before birth, if necessary.

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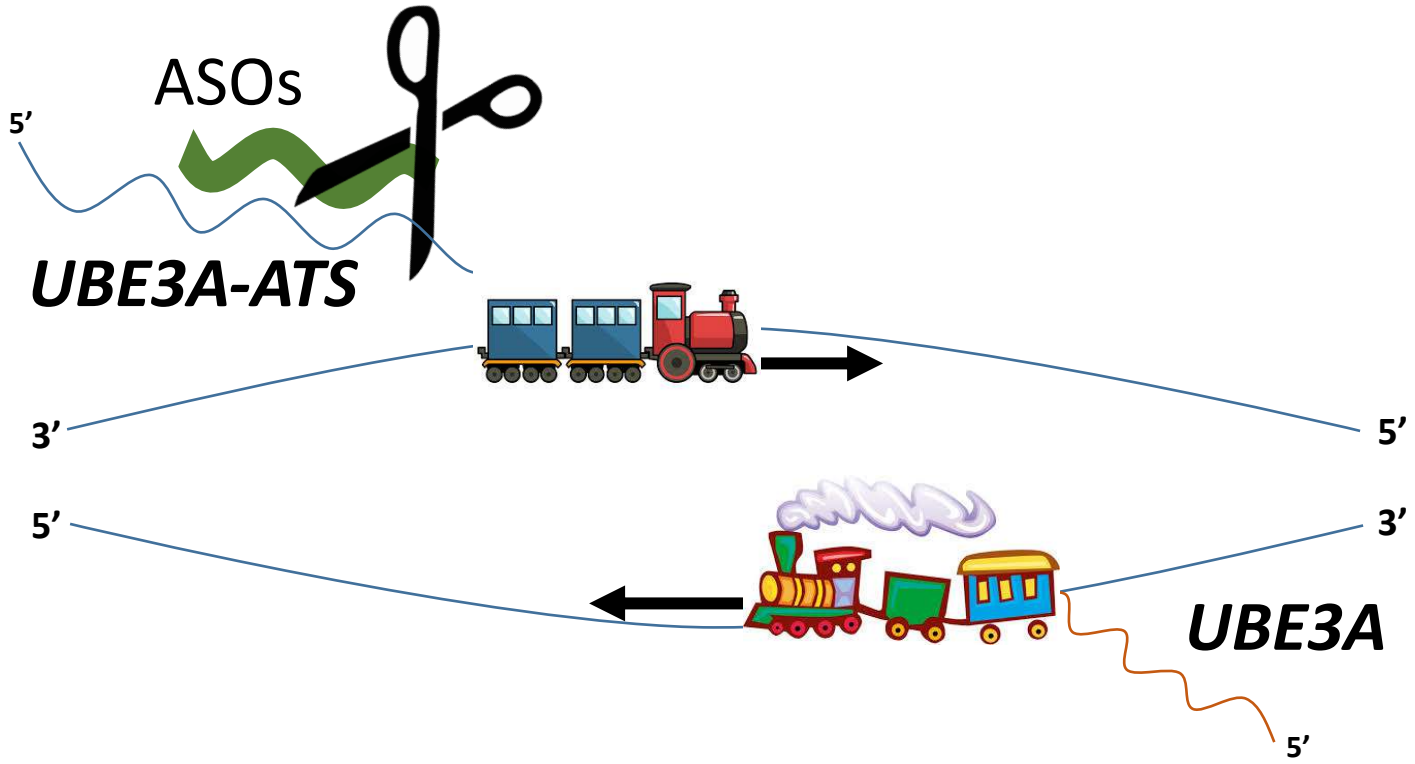
ASOs cut UBE3A-ATS, which derails the train



- ASF funded Art Beaudet to investigate this in mouse and Stormy Chamberlain to investigate this in human neurons.
- This is how therapeutics being developed by Genetx, Roche, and Ionis work.

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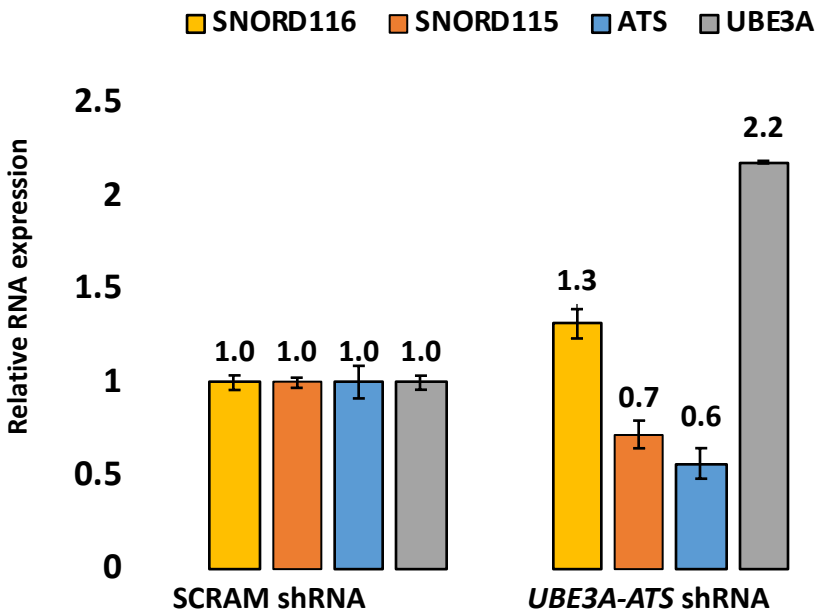
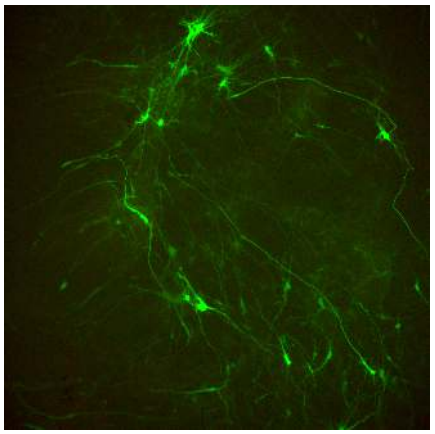
Other methods to cut UBE3A-ATS may also work

Dr. Noelle Germain, PhD
UConn Health
Joseph Wagstaff Fellow





- Short hairpin RNAs (shRNAs) cut RNA in specific spots.
- shRNAs designed to cut UBE3A-ATS activate paternal UBE3A
- shRNAs can be delivered by AAV for one-and-done treatment
- Being developed by Ovid Therapeutics

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Questions remaining about restoring UBE3A

- Which form of UBE3A is best for maximal benefit?
- What are expectations for recovery based on intervention age?
- Do we need to target the whole brain or is one region sufficient?
- What percentage of neurons must be infected?
- What are dangers of too much UBE3A, and how do we limit chances of this?

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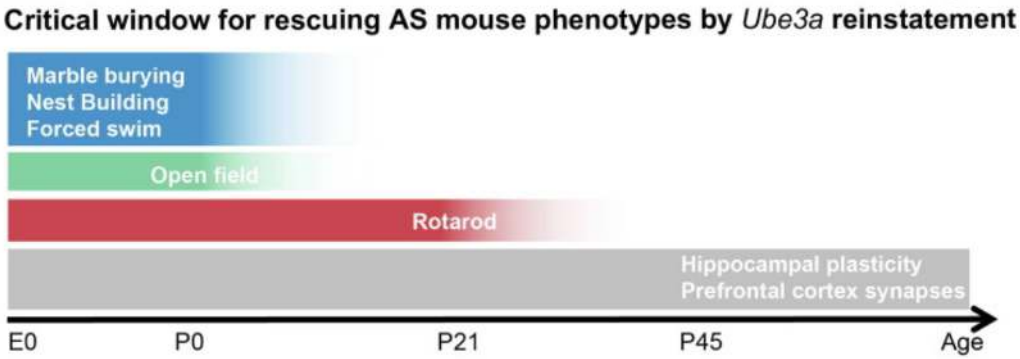
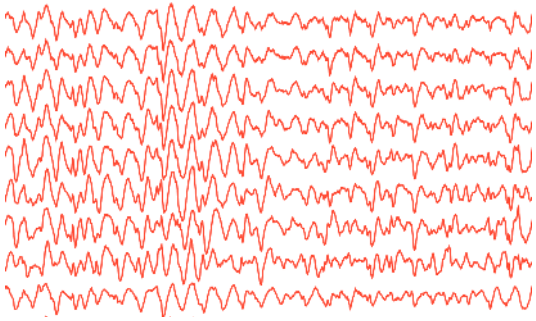
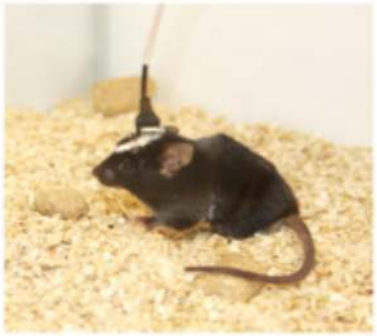
Enabling clinical trials

- ASF funded work on a mouse model that allows UBE3A to be restored at different times during development to determine the best time to restore for best therapeutic potential. (Elgersma)



- ASF funded work to study an EEG biomarker in AS mice and humans with AS. Biomarkers allow us to determine whether therapeutics are working. (Philpot and Hazlett)





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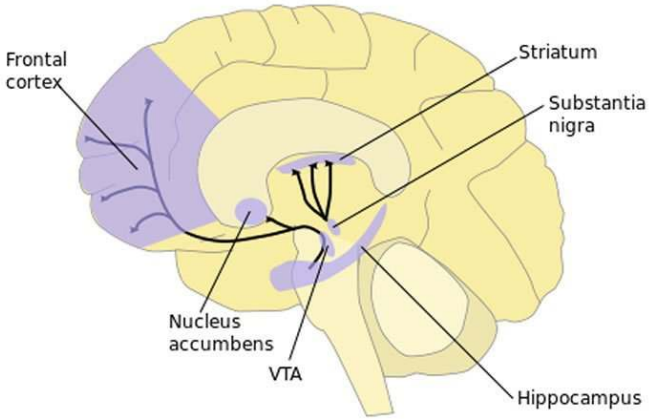
Which cells in the brain make/need UBE3A?

Dr. Ype Elgersma, PhD
Erasmus Medical Centre, Rotterdam



- Striatum is involved in motor learning, speech, emotional regulation, and cognition.
- Does loss of UBE3A in the striatum cause many of the mouse symptoms of AS?
- Important to understand the specific brain functions disrupted by AS, so we know where therapeutics need to be delivered.

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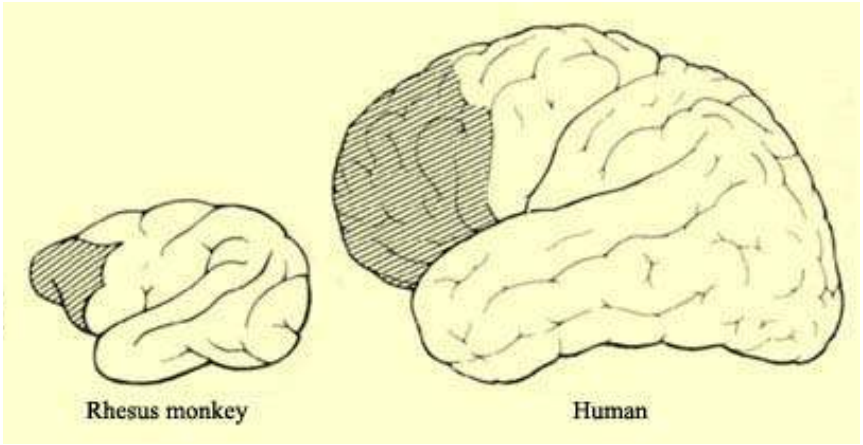
Which cells in the brain make/need UBE3A?

Dr. Ben Philpot, PhD
University of North Carolina



https://thebrain.mcgill.ca/

- Already collected brain samples from hon-human primates (*Rhesus macaque*) across different stages of life from embryo to adulthood.
- Where is UBE3A found—brain regions and cell types?
- Important to understand where therapeutics need to reach (biodistribution) to best treat AS.

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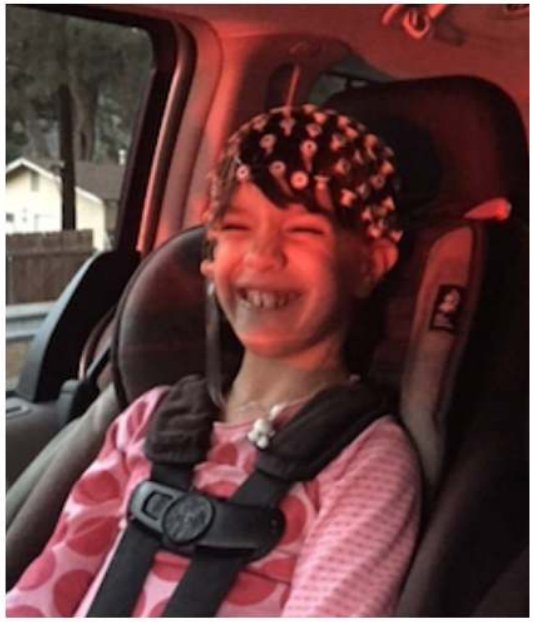
Biomarkers of language in AS



Dr. Charlotte DiStefano, PhD and Dr. Shafali Jeste, MD
UCLA Semel Institute for Neuroscience

- Need to identify biomarkers of language function in AS.
- Study will measure brain responses to different aspects of language processing in AS.
- Important to identify objective ways to measure improvement in language.

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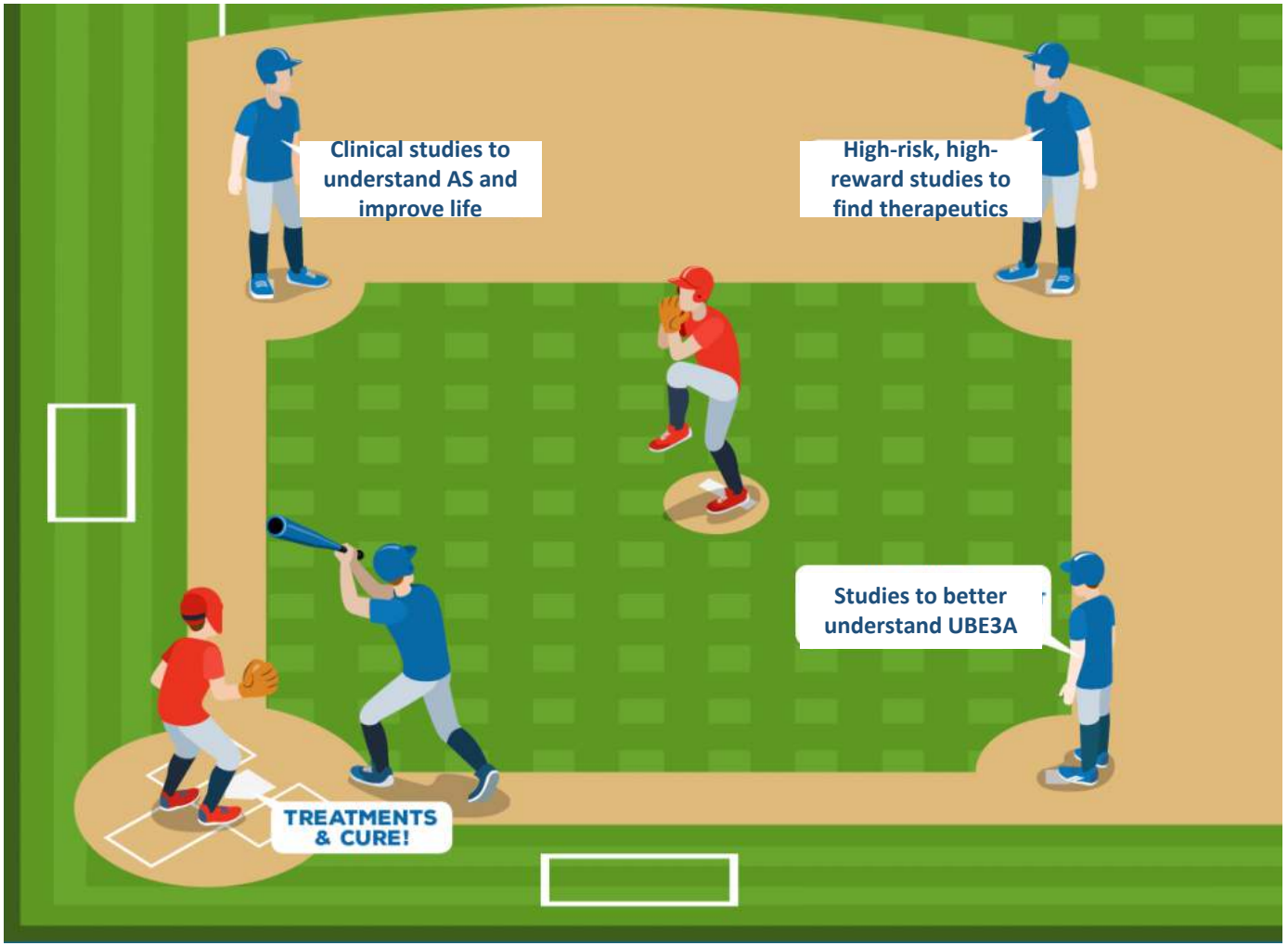
ASF's research funding philosophy



Improving the quality of life for individuals with AS

Neurological functions	
Cognitive	Severe delay
Motor	Severe
Language/ Vocalization	Absent
Behavioral	ASD and Anxiety
Seizures	Severe
EEG	Specific EEG: Increased delta activity Persistent theta activity Spikes and sharp waves
Sleep disturbances	Severe

Rotaru, Mientjes, and Elgersma, 2020

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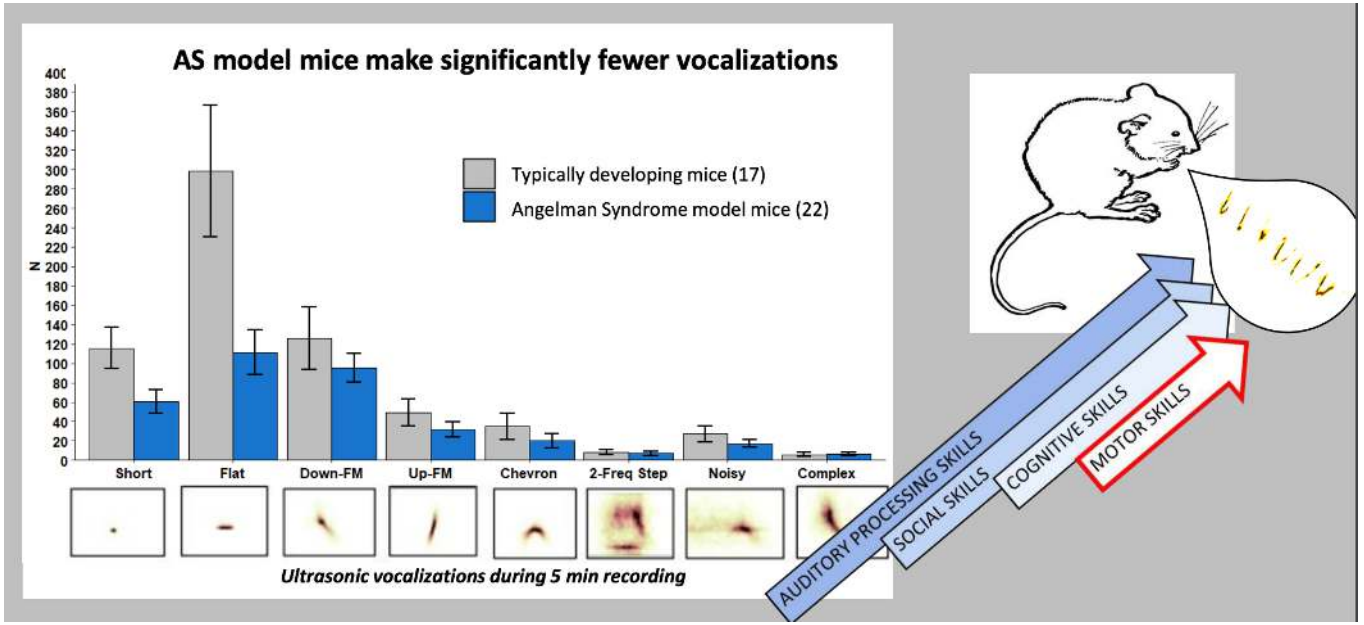
Can we study language in AS mice?

Dr. R. Holly Fitch, PhD
University of Connecticut



- Determine the brain circuitry required for reduced communication in AS mice—motor circuitry?
- Important to understand the brain region involved in speech/language disorder in AS and the therapeutic window for improving it.

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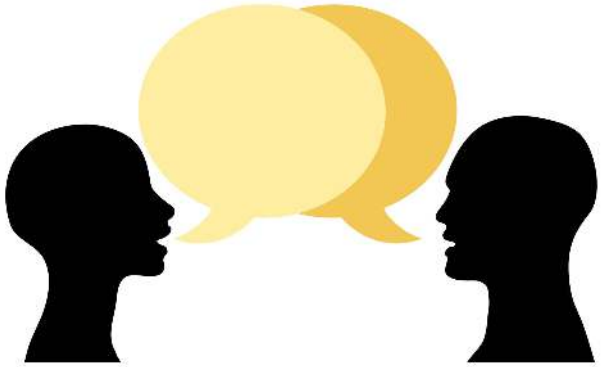
Approaches to improve communication in AS

Dr. H.A. Moll, MD
Erasmus Medical Centre, Rotterdam

Dr. Samuel Sennott, PhD
Portland State University



- High intensity versus low intensity training of parents and caregivers to use/model AAC.
- Determine best ways to improve communication.
- Create a successful communication training program that families can replicate at home.

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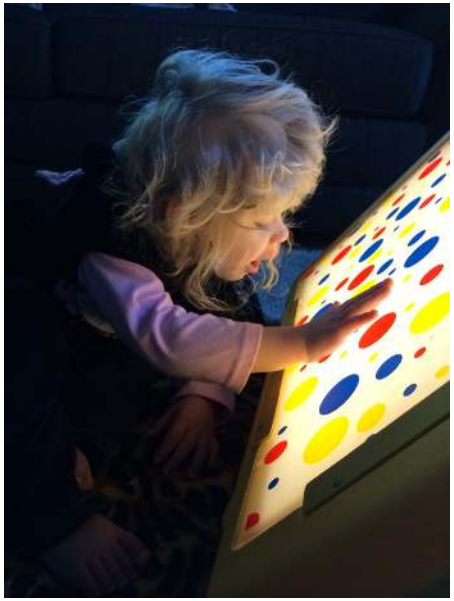
Do individuals with AS have cortical visual impairment?

Dr. Karen Erickson, PhD
University of North Carolina



https://cvi.aphtech.org/

- Cortical visual impairment (CVI) is a type of vision impairment that may affect balance, walking, communication, and behavior.
- Determine the prevalence and severity of CVI in AS.
- Determine whether CVI has an effect on communication in AS.

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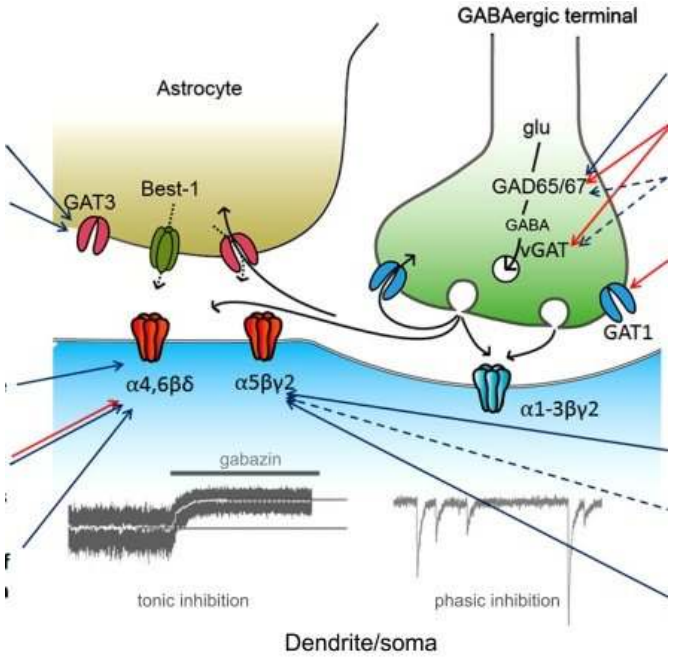
Therapeutic development for neuron dysfunction

Dr. Kiyoshi Egawa, PhD
Hokkaido University
Graduate School of
Medicine, Japan



- AS neurons have deficits in the mechanisms that keep neurons responsive to signals from other neurons –tonic inhibition.
- OV101, also called Gaboxadol, corrects this.
- Further studies to understand the brain regions and processes impacted by this deficit

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Testing CBD in a mouse model to inform future clinical trials

Dr. Paul Carney, MD
University of North Carolina

- Cannabidiol (CBD) provides relief from seizures (and other?) for some individuals with AS.
- CBD reduced seizure severity in mouse model, but at high doses causes a mild sedative effect.
- Testing CBD carefully is important for determining parameters for future clinical trials.

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15q Clinic Network provides an important research platform

LADDER database links multiple sources of clinical information for research

- Linking Angelman and Dup15q Data for Expanded Research
- Combines data from 15q Clinics Network, the Angelman Syndrome Natural History Study, ASF and Dup15q Alliance registries, and the Global Angelman Syndrome Registry
- Led by RTI International and supported by ASF, Dup15q Alliance, and industry partners

Our Research Goal

- To improve the lives of individuals with Angelman syndrome