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Webinar 2024

The genetics on Angelman syndrome unraveled

Chaired by

Ellen Koekoeckx, FAST and Samantha Eisenhauer, FAST France

ITHACA, PAB and WG T&T

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October 8, 2024

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Welcome – Technical points

- **Webinar being recorded**

- **Thank you for**
  - Turn off your microphone and disconnect your camera
  - Raise your hand at the time of the questions and discussions
  - We will answer the questions sent in the registration form
  - A satisfaction survey will be sent to you :

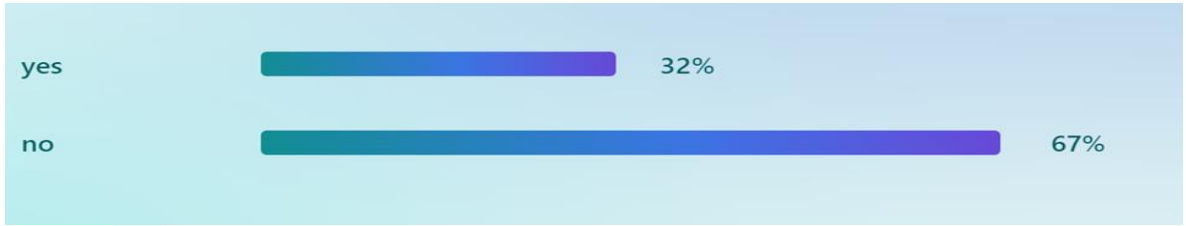
- **Webinars will be available on ITHACA's Website**

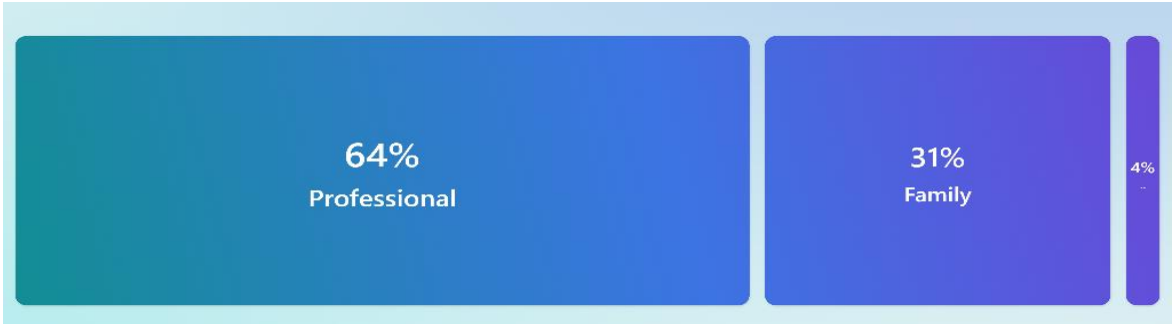
https://ern-ithaca.eu/documentation/educational-resources/

- **Anne Hugon Project Manager ERN ITHACA - anne.hugon@aphp.fr**

Registration Survey

- + 212 Registrations
- Proportion ERN ITHACA 32%
- 66 famillies/147cliniciens/30 countries





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Welcome and Introduction

- **Public:** Clinical geneticists, genetic counselors, neurologists, pediatricians, AS caregivers

- This webinar is the **first of a webinar series dedicated to Angelman syndrome**, a neurogenetic disorder, where we will delve into the genetic foundations of Angelman syndrome. This session will highlight the latest advancements in genetic testing, offering a comprehensive understanding of the various genotypes of AS and reoccurrence risk, shedding light on how treatment strategies are informed by the genetics of AS.

- Chaired by **Ellen Koekoeckx** on behalf of **FAST** (Foundation of Angelman Syndrome Therapeutics)

Agenda

- **Welcome & Introduction (6 – 6.05 pm)**
  - Ellen Koekoeckx, FAST (Foundation of Angelman Syndrome Therapeutics)
- **Genetics of Angelman syndrome (6.05 – 6.15 pm)**
  - Dr. Sofia Ourani, Clinical Geneticist-Paediatric Neurologist, Clinical Genetics Unit – Paediatric Department, Hospital Archbishop Makarios III, State Health Services Organization, Nicosia - Cyprus
- **Recommended genetic testing (6.15 – 6.25 pm)**
  - Dr. Sofia Ourani, Clinical Geneticist-Paediatric Neurologist, Clinical Genetics Unit – Paediatric Department, Hospital Archbishop Makarios III, State Health Services Organization, Nicosia - Cyprus
- **Genotypes of Angelman syndrome and reocurrence risk (6.25 – 6.40 pm)**
  - Niki Armstrong, MS, CGC, Vice-President, Genetic services and education; FAST - Certified Genetic Counselor in USA
- **Therapeutic pathway based on the genetics of Angelman syndrome (6.40 – 6.45 pm)**
  - Niki Armstrong, MS, CGC, Vice-President, Genetic services and education; FAST - Certified Genetic Counselor in USA
- **Discussion time (6.45 – 7.00pm)**

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Genetics of Angelman syndrome

Dr. Sofia Ourani

October 8, 2024

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What is Angelman syndrome?

Angelman syndrome (AS) is a rare, monogenic, neurologic disorder that affects approximately 1 in 15,000 individuals or ~ 500,000 individuals worldwide[1-4]







1. Buckley R, et al. Am J Med Genet. 1998; 80: 385-390.
2. Kyllerman M. Am J Med Genetics. 1995; 59(3): 405.
3. Steffenburg S, et al. Pediatr Neurol. 1996; 14(2): 131-136.
4. Mertz LGB, et al. Am J Med Genet A. 2013; 161(9): 2197–2203.

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Clinical manifestations of AS are severe, with lifelong impact on the patient and their caregivers

Symptoms of AS

- **Universal lack of speech**
- **Sleep disturbances**/severe insomnia
- Life-threatening/debilitating **seizures**
- Severe developmental delays
- Ataxia/incoordination
- Apraxia/Dyspraxia
- Feeding issues/GI issues
- Unique behaviors
- **Unable to live independently**
- **Significant clinical unmet need**

Impact on Family

- **Inability to maintain employment**
- **Loss of sleep**
- Anxiety
- Depression
- Stress
- Social isolation
- Impact on family relationships
- Difficulty caring for other children/home
- Chronic Fatigue
- Excessive costs

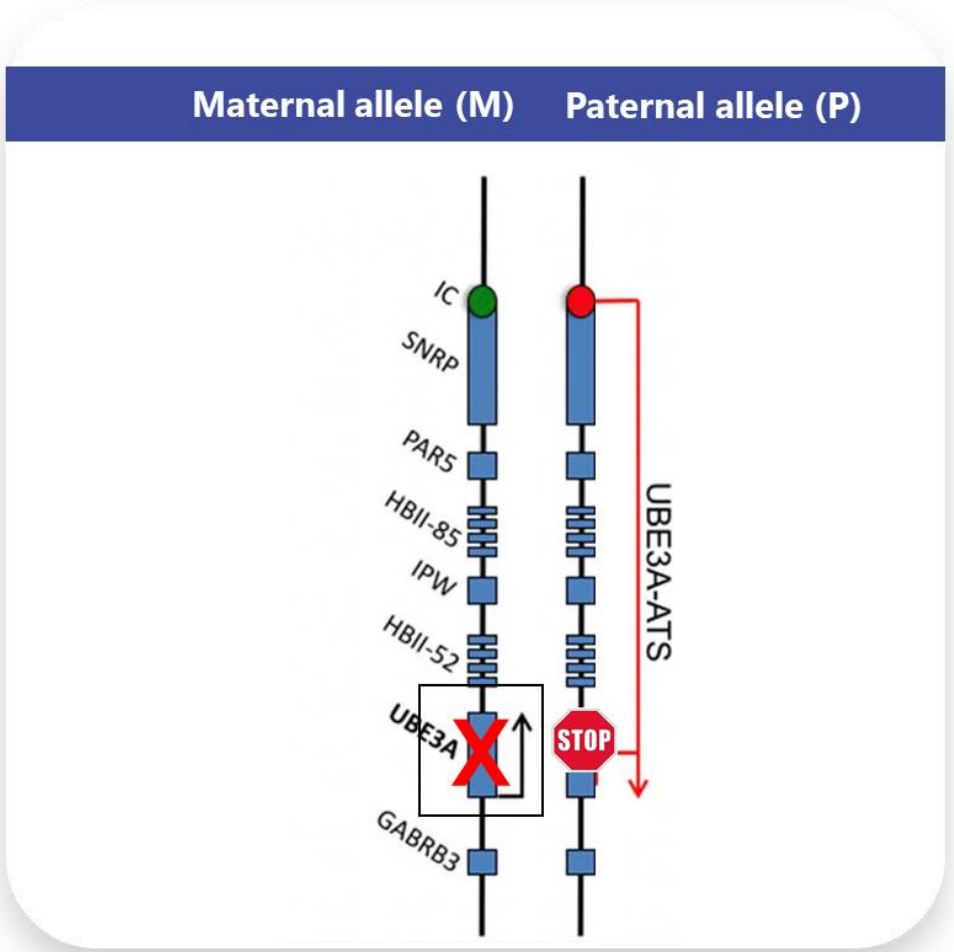




Genetics of AS

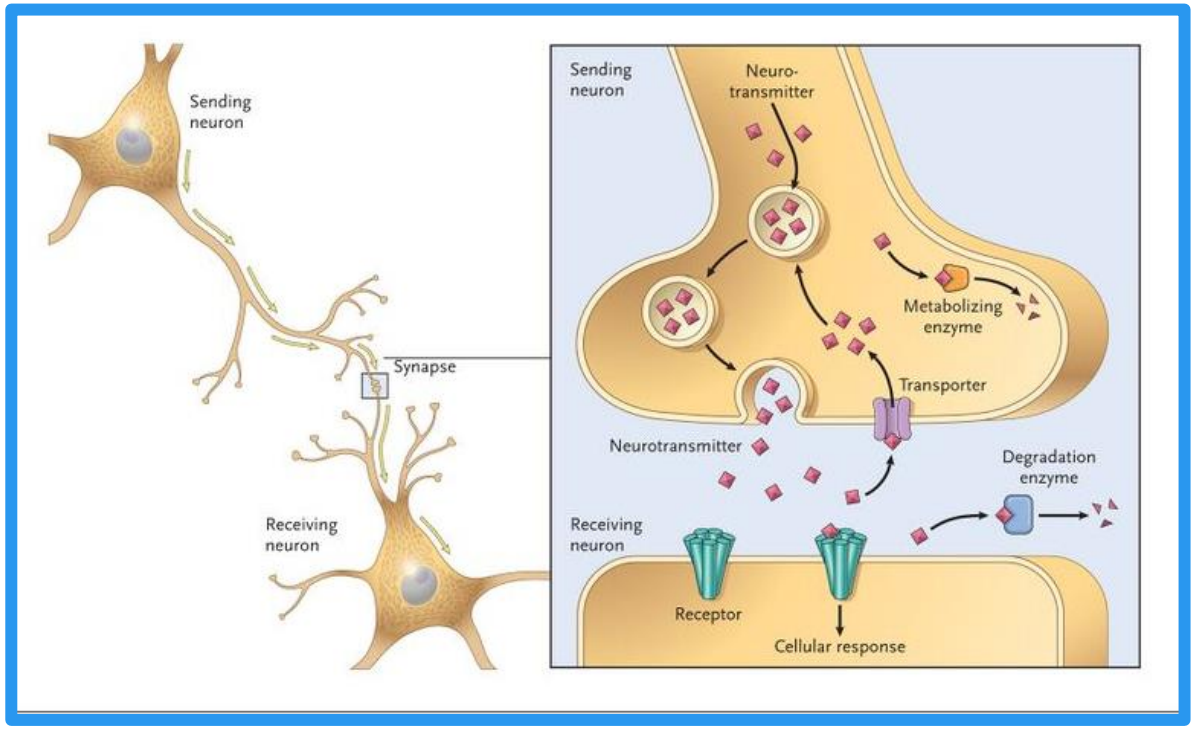
AS is caused by a reduction in expression of the *UBE3A* gene in the maternal copy of Chromosome 15

Imprinted Gene=Paternal silenced



N. Khatri et al. *Front. Mol. Neurosci.* 2018

The role of UBE3A



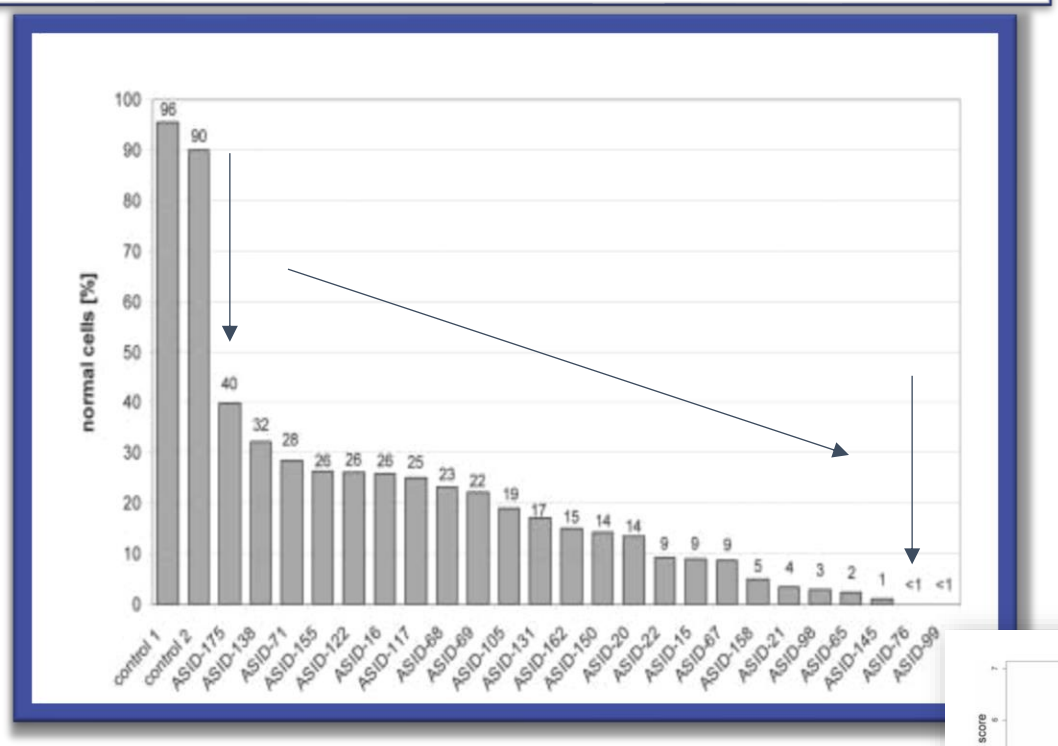
- Ubiquitin protein ligase E3A
- UBE3A is an enzyme that targets other proteins by tagging with ubiquitin to indicate degradation is needed
- Absence of UBE3A→ Dysregulation of proteostasis
  - Accumulation of excitatory signals and a failure of TONIC INHIBITION → difficult for the neurons to communicate
- UBE3A is also a transcriptional coactivator
- 3 isoforms

1. Sell et al. Front Neurosci. 2015; 9:322.
2. Martinez-Noel et al. J Mol Biol. 2018; 430(7): 1024-1050.
3. Greer et al. Cell. 2010; 140(5): 704+716
4. Gustin et al. Neurobiology of Disease. 2010; 39, 283–291
5. LaSalle et al. Epigenomics. 2015. 7(7): 1213–1228.

How much UBE3A do humans need?

Human Molecular Genetics, 2004, Vol. 13, No. 21
doi:10.1093/hmg/ddh296
Advance Access published on September 22, 2004

Somatic mosaicism in patients with Angelman syndrome and an imprinting defect



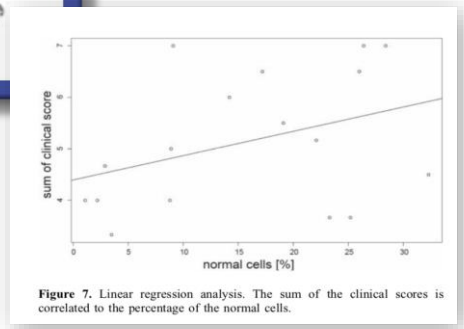


Figure 7. Linear regression analysis. The sum of the clinical scores is correlated to the percentage of the normal cells.

- **1-5%** *UBE3A* → few-no seizures, ambulatory, some ataxia, some speech
- **~20%** *UBE3A* → no seizures, ambulatory, minimal to no ataxia and speak in sentences
- **>40%** may not be symptomatic

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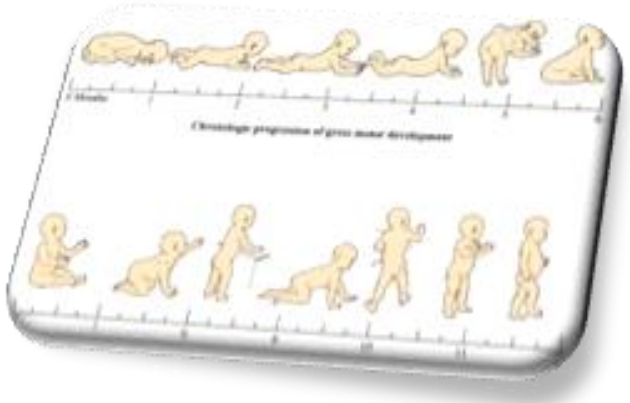
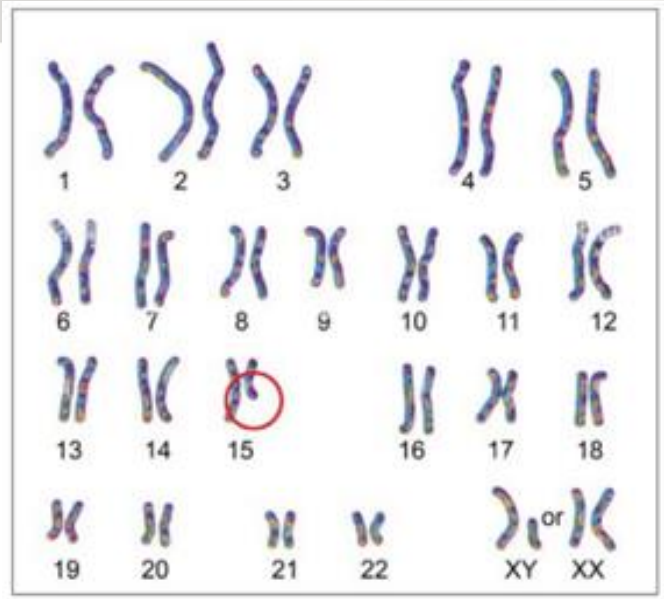
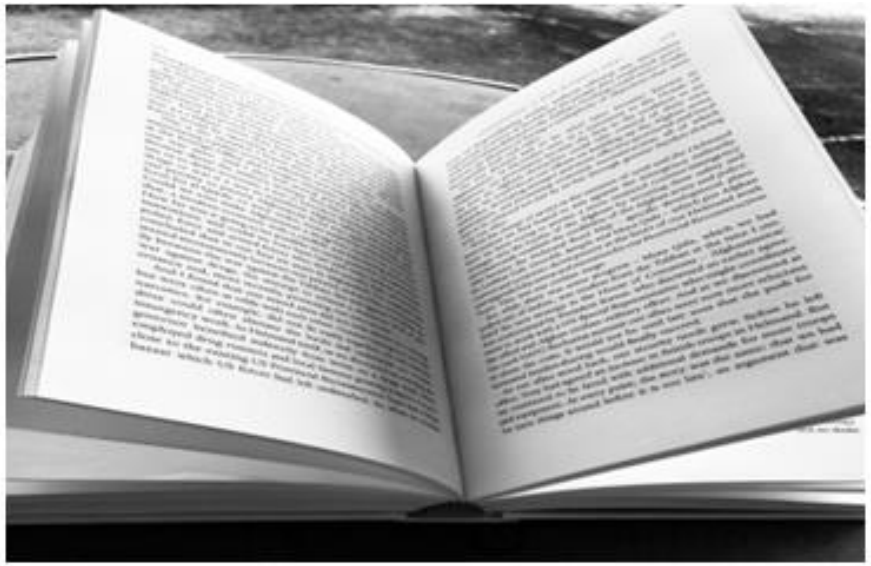
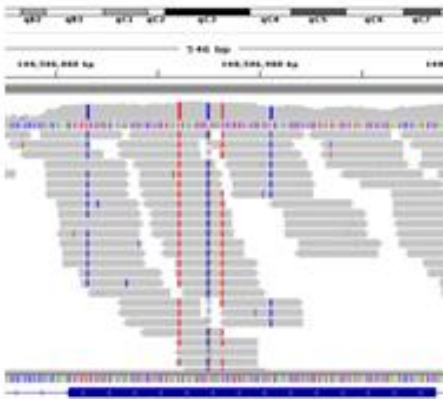



SPEECH
DELAY



https://www.sciencephoto.com/media/1127811/view/karyotype-of-prader-willi-syndrome-illustration





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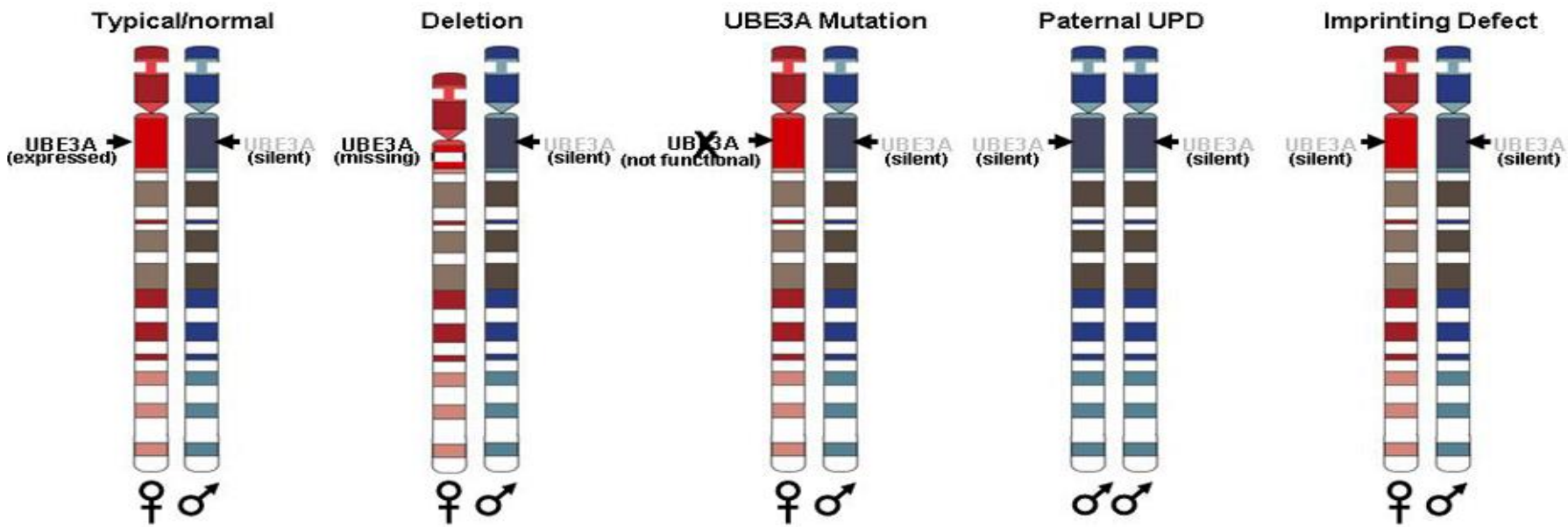
Angelman Syndrome Genotypes



1. Dagli A, Buiting K, Williams C. Molecular and Clinical Aspects of Angelman Syndrome. Mol Syndromol 2011; 2:100-112.
2. Dagli A, Mueller D, Williams C. Angelman Syndrome. GeneReviews, 2017. Editor, Adam. Seattle, WA. [https://www.ncbi.nlm.nih.gov/books/NBK1144/]
3. Williams C, Driscoll D, Dagli A. Clinical and genetic aspects of Angelman syndrome. Genet Med. 2010; 12(7): 385-395.

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Recommended genetic testing

Dr. Sofia Ourani

European Commission

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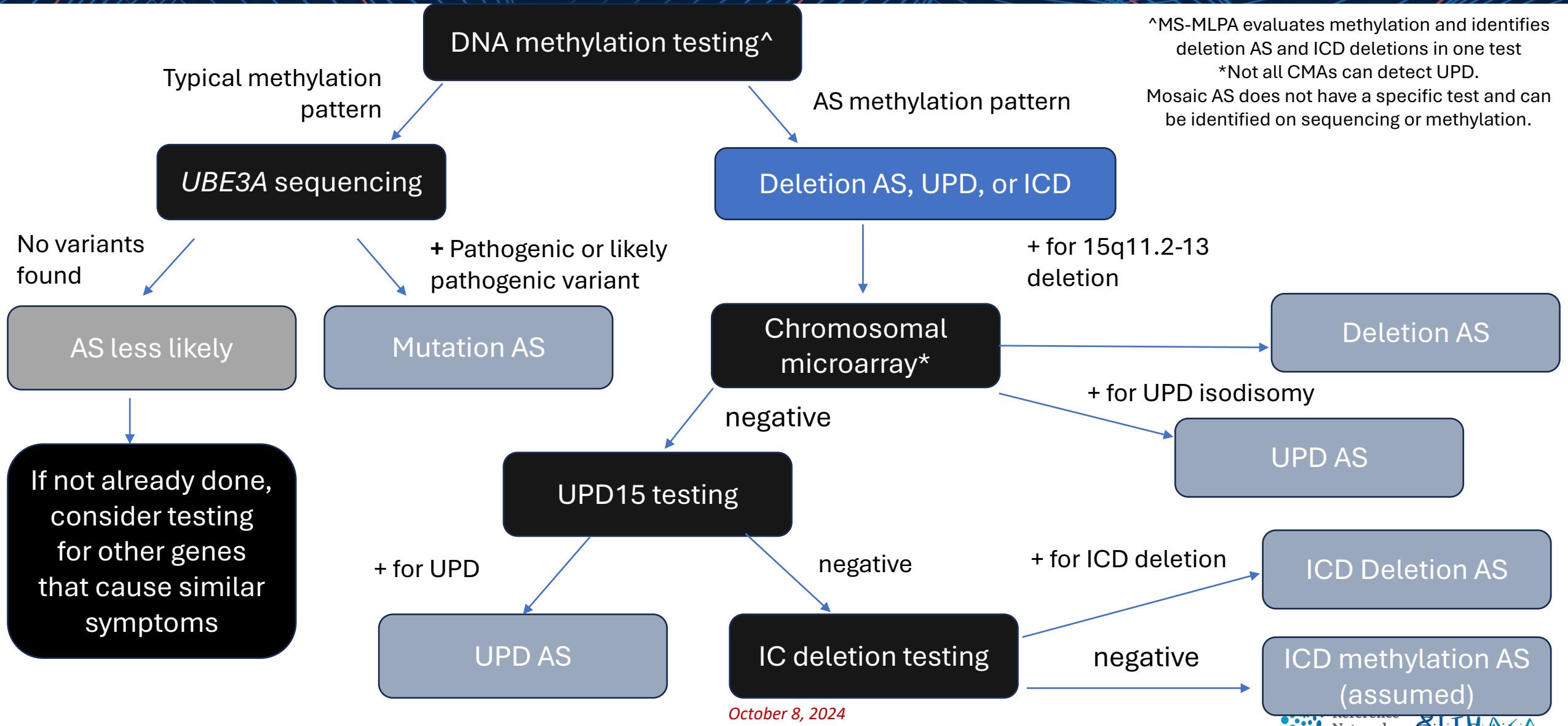
Diagnostic testing for Angelman syndrome (when AS is strongly suspected)



^MS-MLPA evaluates methylation and identifies deletion AS and ICD deletions in one test
*Not all CMAs can detect UPD.
Mosaic AS does not have a specific test and can be identified on sequencing or methylation.

Current genetic diagnosis

- Less common for genetic testing for specific diagnoses to be ordered as a first-tier
- More common to see individuals diagnosed after:
  - Chromosomal microarray
    - DNA methylation analysis needed to confirm Angelman syndrome versus Prader-Willi
  - Epilepsy or developmental delay gene panel
  - Whole exome or whole genome sequencing

IMPORTANT: AS from UPD (heterodisomy) and ICD are not identified on standard sequencing and CMA! DNA methylation testing is needed to rule out these genotypes.

Common methodologies for AS genetic testing and international availability

Method	Genetic Mechanism Detected [1]					Total Proportion of Probands Detectable by Method [2]
	15q11.2-q13del	UPD	Imprinting defect	*UBE3A* sequence variant	*UBE3A* deletion/ duplication	
DNA methylation analysis [3, 4]	X	X	X [5]			~80%
MS-MLPA [6]	X	X	X			~80%
FISH [7]	X					~68%
CMA [8]	X	X [9]				~70%-75%
UPD analysis [10]		X				~3%-7%
AS imprinting center deletion analysis [11, 12]			X			<0.3%
UBE3A sequence analysis [13]				X		~11%
UBE3A gene-targeted del/dup analysis [11, 14]					X	Rare

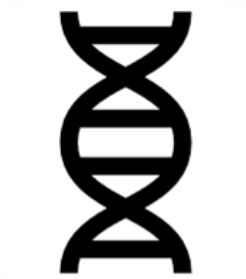
CMA = chromosomal microarray analysis; del/dup = deletion/duplication; IC = imprinting center; MS-MLPA = methylation-specific multiplex ligation-dependent probe amplification; UPD = uniparental disomy

About 10% of individuals with the presumptive clinical diagnosis of AS have normal results for all testing methods described in this table [Williams et al 2010].

EXOME SEQUENCING (=WES, NGS) IS NOT a 1st TIER DIAGNOSTIC TEST

Why is knowing the AS genotype important?

While each individual living with AS is unique, individuals with the same genotype may have certain characteristics in common.



Each genotype has a different chance to be inherited.

Many clinical trials have genotype as a criteria.

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Genotypes of Angelman syndrome and recurrence risk

Niki Armstrong

European Commission

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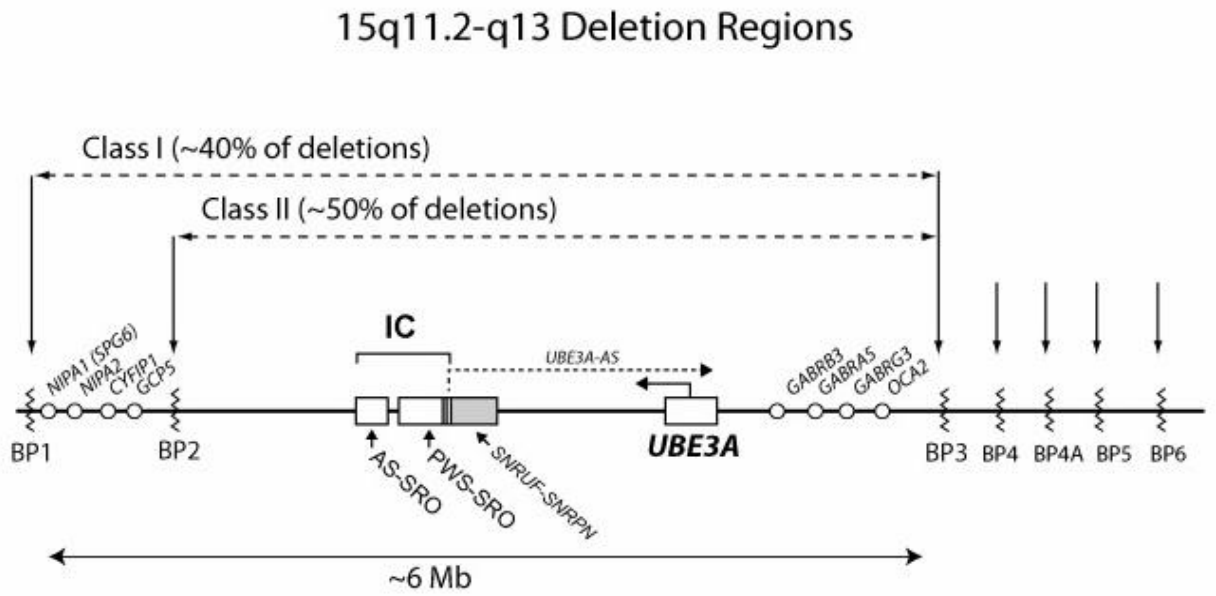
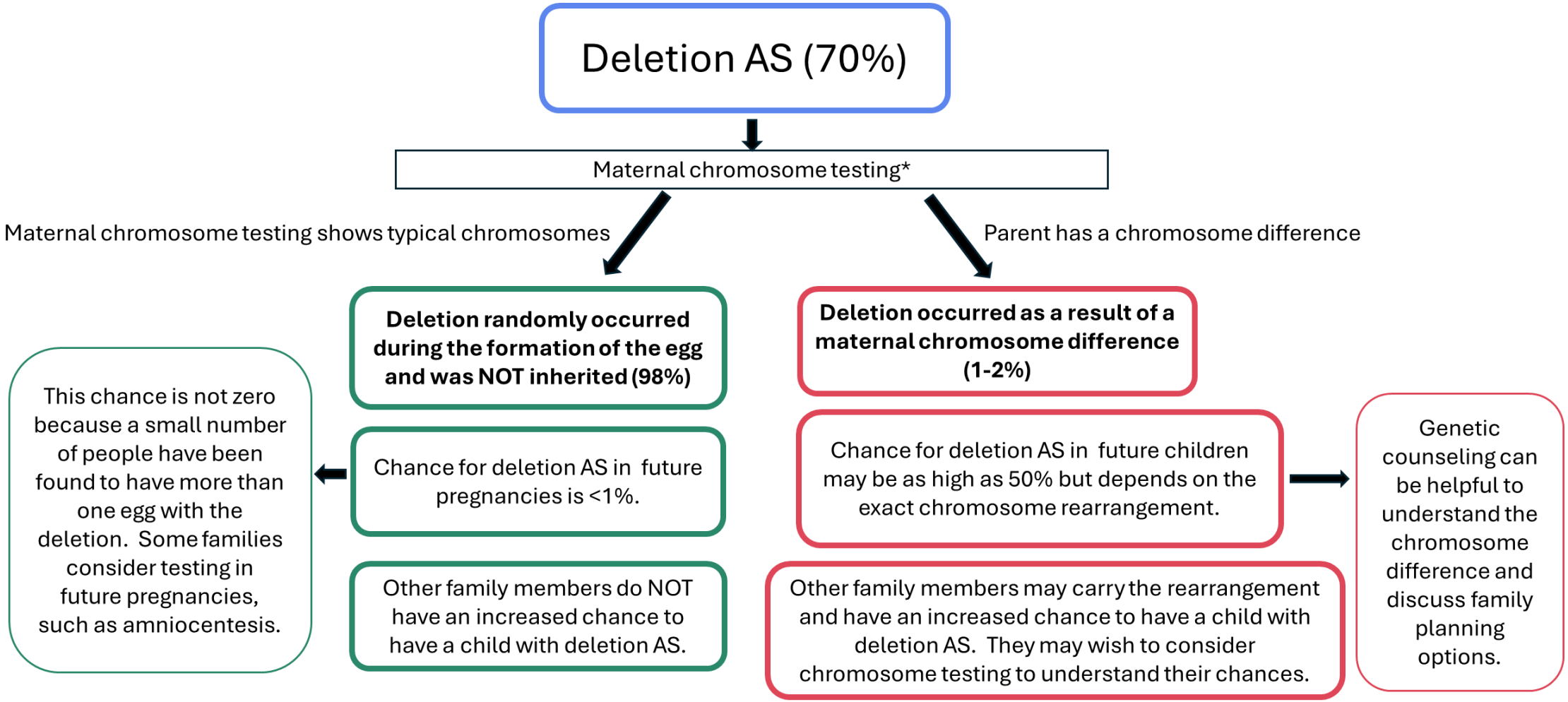
Deletion

- Generally considered the most severe phenotype
  - Highest prevalence of seizures
  - Lowest scores on cognitive and language assessments
- Likely a result of the deletion of the 3 GABA receptor genes
  - GABA receptors=ion channels in the synapses that respond to GABA
  - Chief inhibitory receptor in the CNS
  - Loss of function variants in GABA subunits have been linked to epilepsy and developmental delays
- Unclear if there is a difference between phenotype of the different standard deletion classes
- Hypopigmentation for family background
  - Caused by deletion of *OCA2*



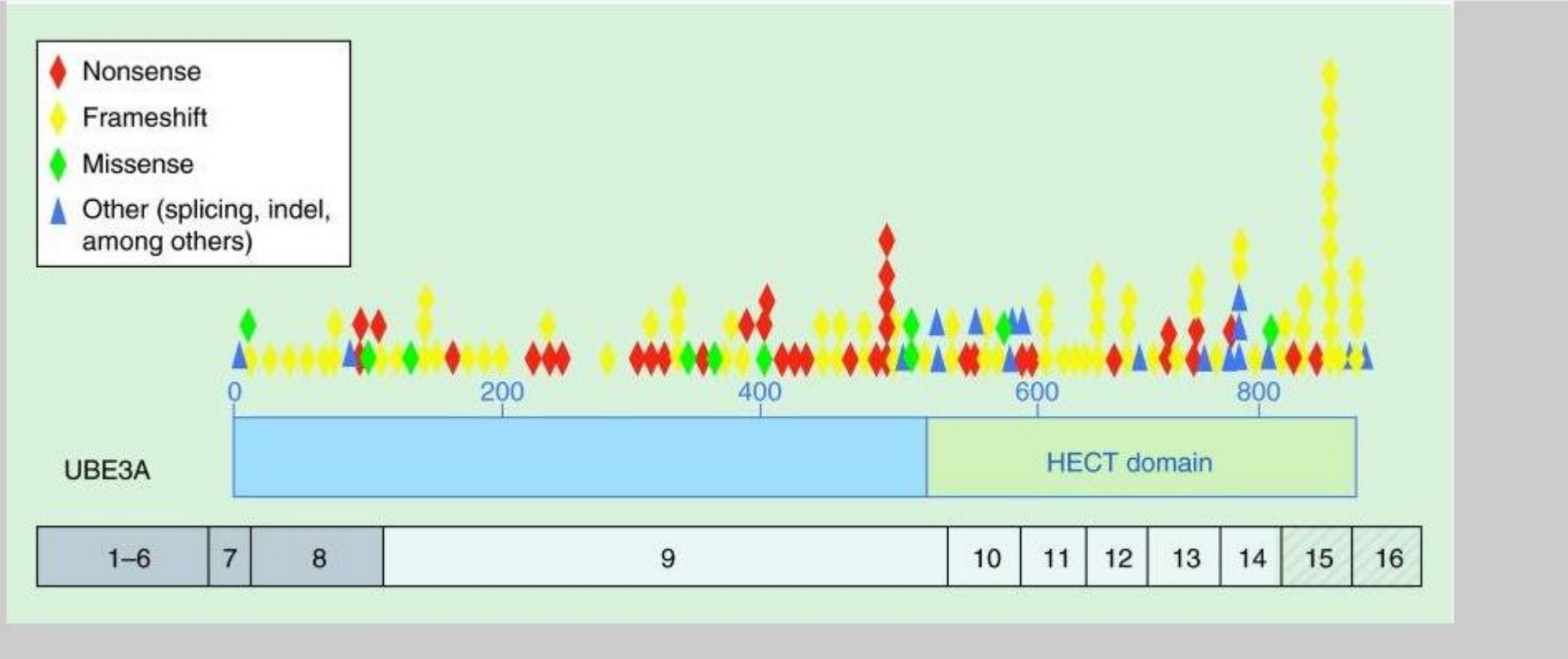
Angelman Syndrome, GeneReviews® [Internet]. Adam MP, Feldman J, Mirzaa GM, et al., editors. Seattle (WA): University of Washington, Seattle; 1993-2024.

Deletion – inheritance and recurrence risk



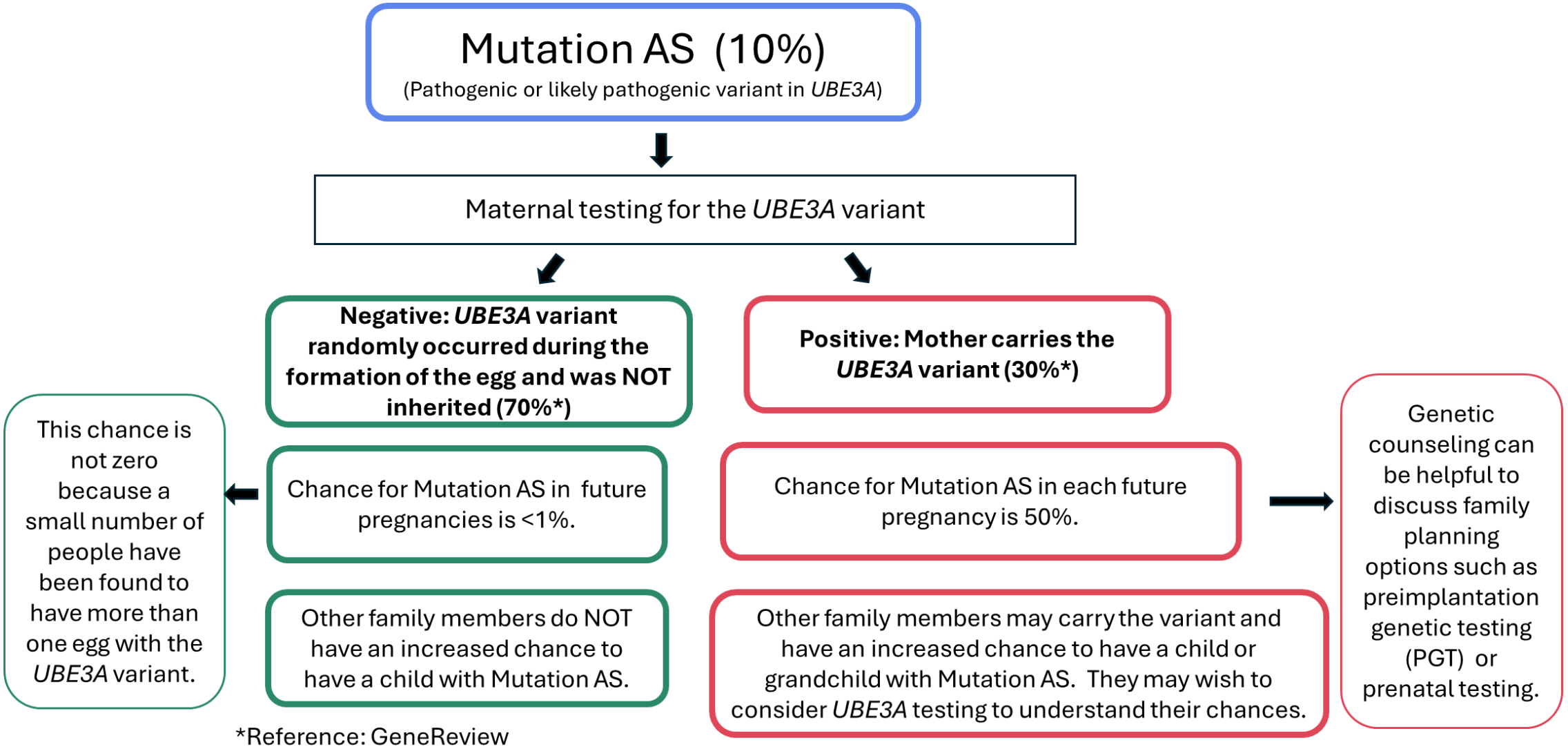
*Exact testing depends upon how the child was diagnosed

Mutation



LaSalle et al. (2015). Epigenetic regulation of UBE3A and roles in human neurodevelopmental disorders. Epigenomics, 7(7), 1213–1228.

Mutation – inheritance and recurrence risk



*Reference: GeneReview

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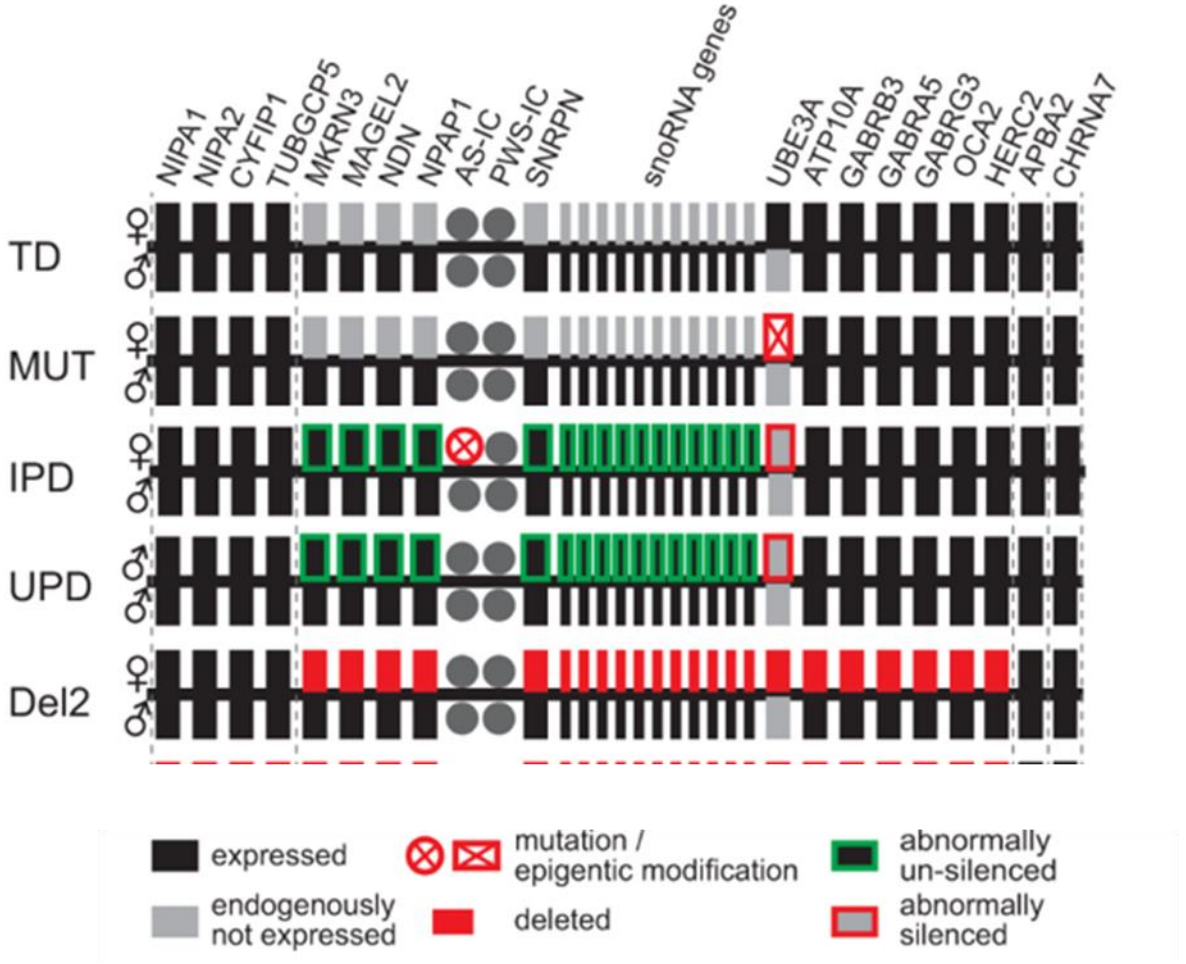
Paternal Uniparental Disomy (UPD)

- Absence of neuronal *UBE3A* expression
- Overexpression of paternally expressed genes, including *MKRN3, MAGEL2, NDN, NPAP1*, *SNRPN*, and the *snoRNA* genes
- Phenotype
  - Lower prevalence of epilepsy and less severe epilepsy
  - Higher risk of obesity



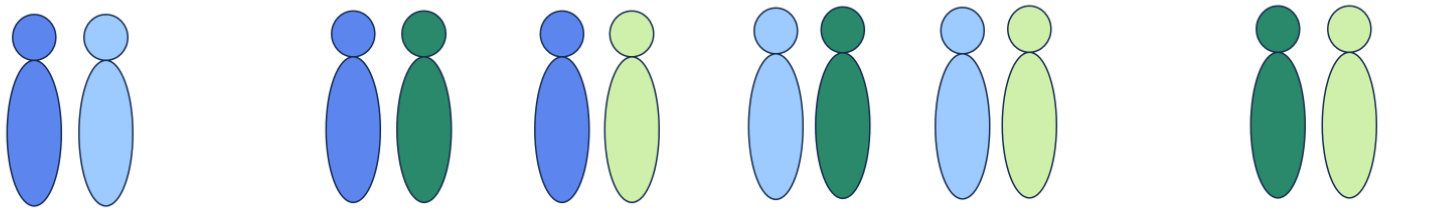
Adapted from Keute, M., Miller, M.T., Krishnan, M.L. *et al.* Angelman syndrome genotypes manifest varying degrees of clinical severity and developmental impairment. *Mol Psychiatry* **26**, 3625–3633 (2021).

Uniparental disomy (UPD)

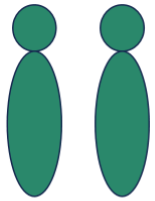
Maternal chromosome 15s

Typical inheritance of chromosome 15s

Paternal chromosome 15s

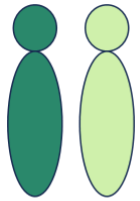


Chromosome 15s when a child has UPD-isodisomy



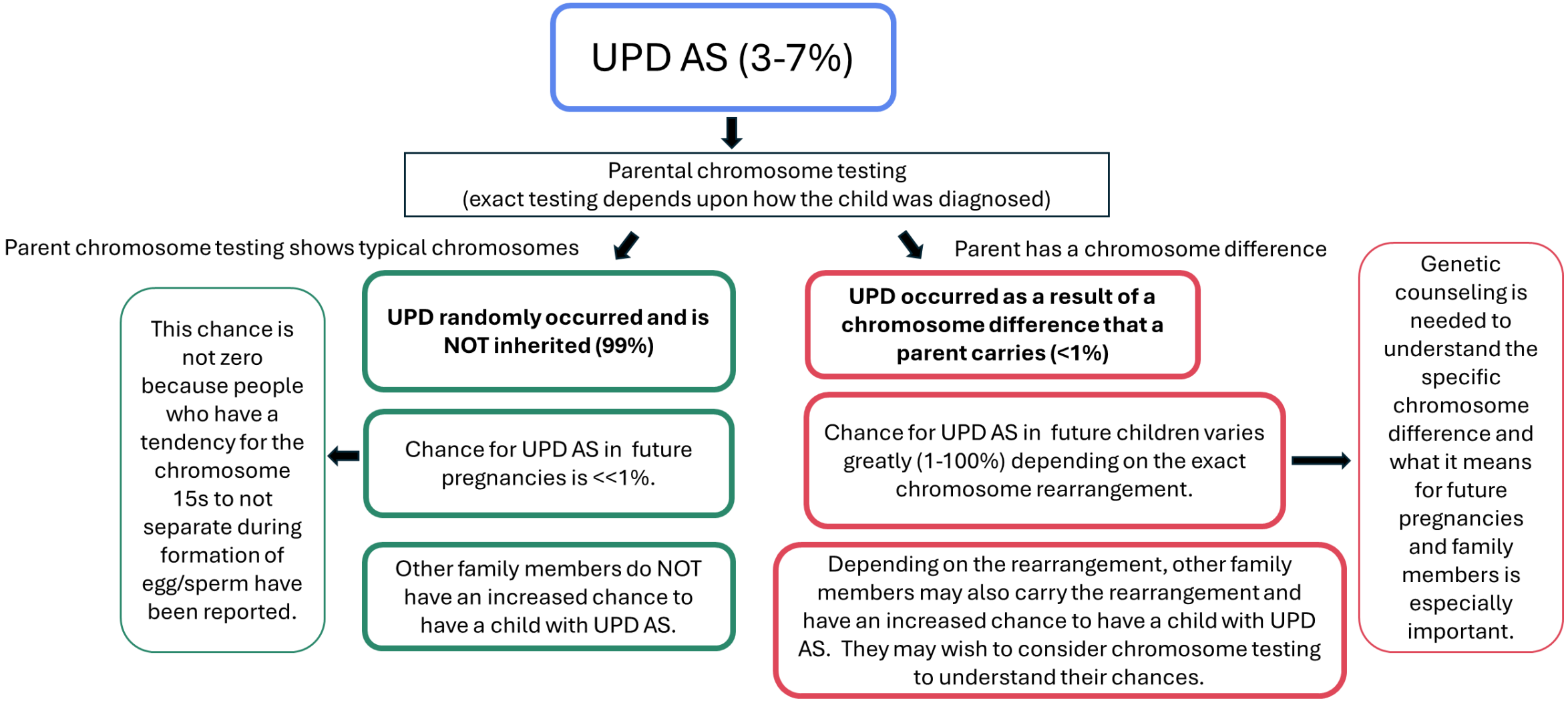
When both chromosome 15s are exactly the same, it is called isodisomy.

Chromosome 15s when a child has UPD- heterodisomy

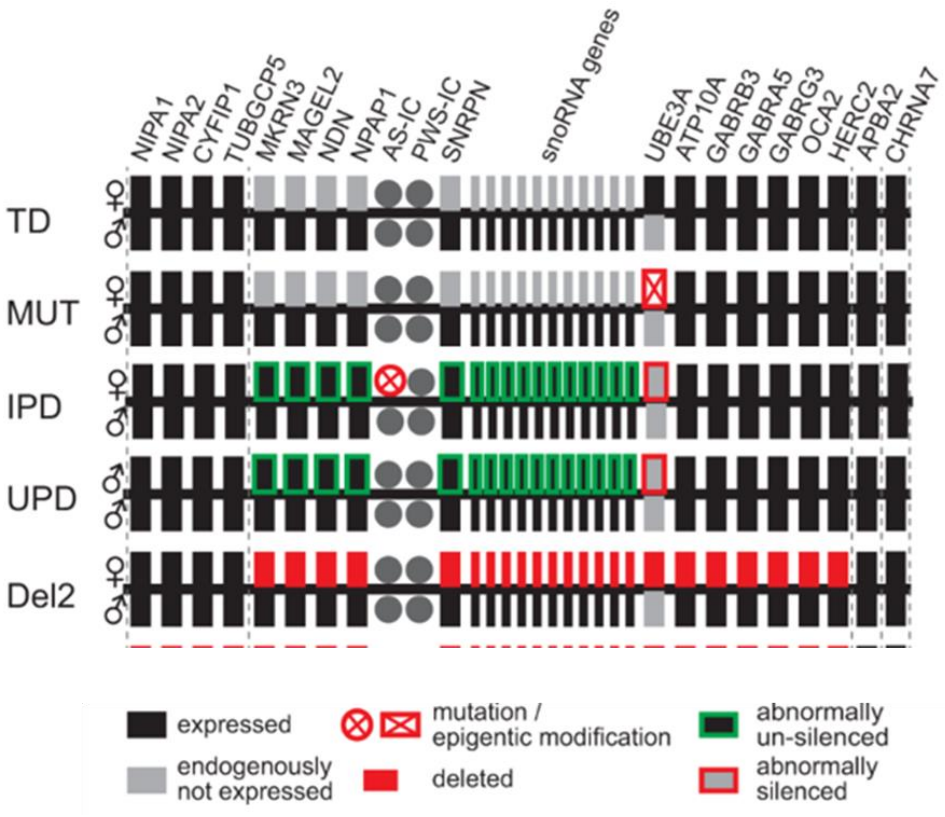


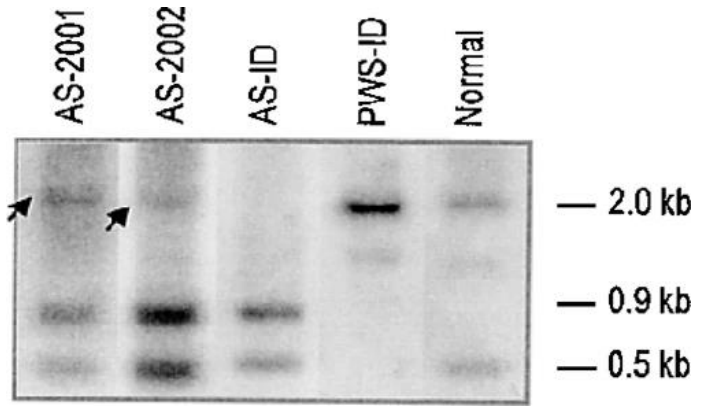
When the chromosome 15s are different, but still both come from the sperm, it is called heterodisomy.

Uniparental disomy (UPD) – inheritance and recurrence risk



Imprinting Center Defect (ICD)

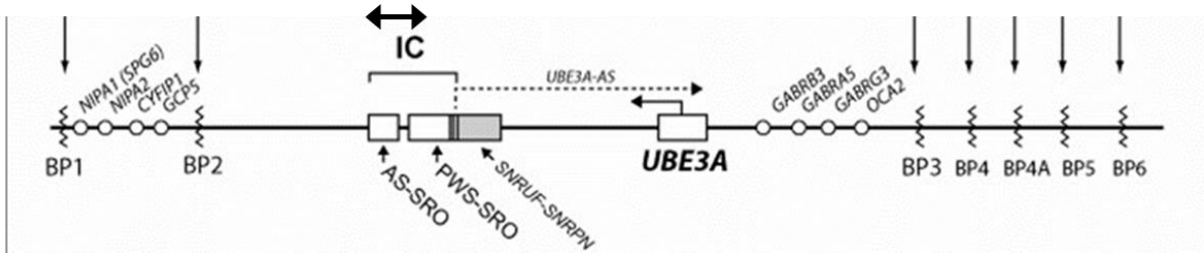




Two separate causes:

- Epigenetic methylation defect that is often mosaic
- Deletion of the imprinting center which may be inherited

Imprinting Center Deletion



Adapted from Angelman Syndrome, GeneReviews® [Internet]. Adam MP, Feldman J, Mirzaa GM, et al., editors. Seattle (WA): University of Washington, Seattle; 1993-2024.

Keute, M., Miller, M.T., Krishnan, M.L. *et al.* Angelman syndrome genotypes manifest varying degrees of clinical severity and developmental impairment. *Mol Psychiatry* **26**, 3625–3633 (2021).

Horsthemke, B., Lich, C., Buiting, K. *et al.* Problems in detecting mosaic DNA methylation in Angelman syndrome. *Eur J Hum Genet* **11**, 913–915 (2003).

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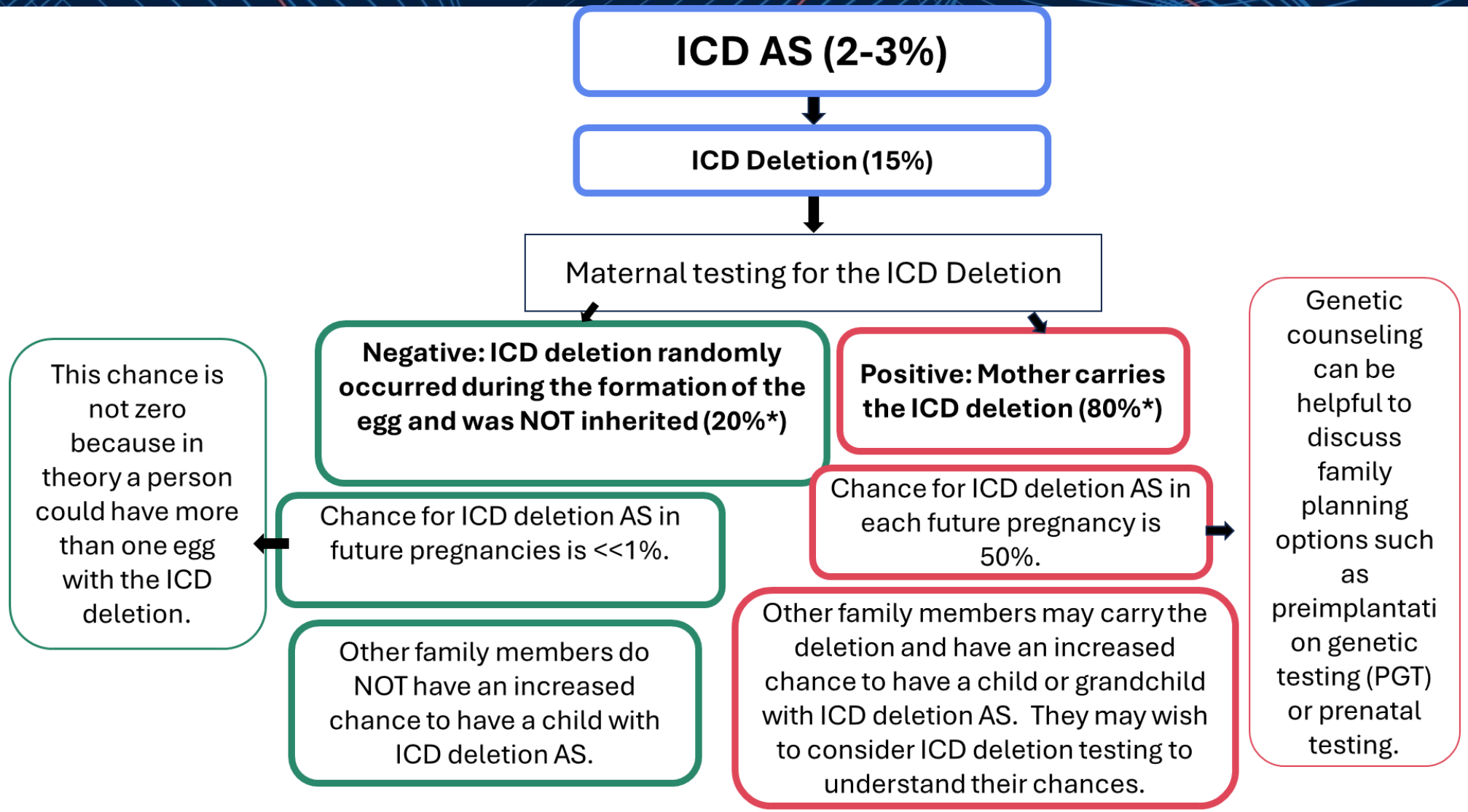
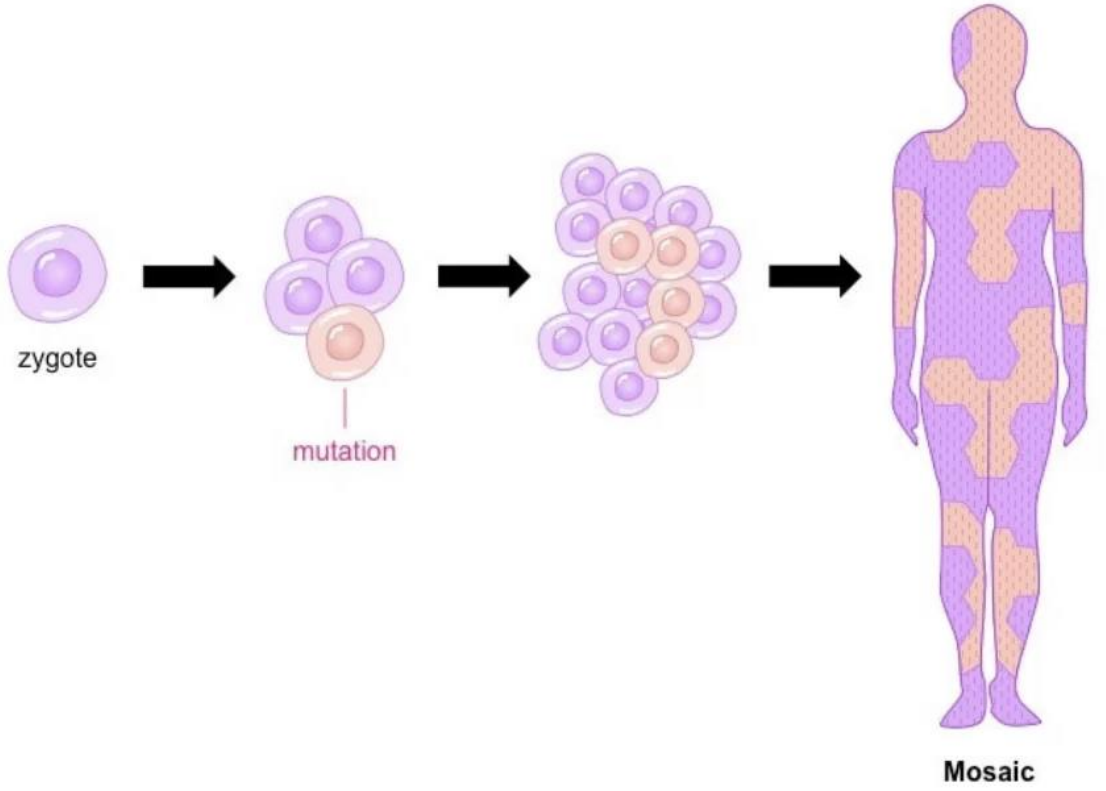
Imprinting Center Defect – inheritance and recurrence risk



*Reference: Horsthemke B, Buiting K: Genomic imprinting and imprinting defects in humans. Adv Genet 61:225–246 (2008).

Mosaicism

- Most commonly caused by epigenetic methylation at the imprinting center that occurs after conception
- Has also been reported in individuals with Deletion, UPD, and Mutation



https://smc1a-epilepsy.org/wp-content/uploads/2023/07/Screen-Shot-2023-05-04-at-1.37.10-PM.png

Future of diagnosis for Angelman syndrome

- Long read sequencing
  - Oxford Nanopore
  - PacBio
- Newborn screening
  - Multiple methylation-based assays in validation
    - Would not detect Mutation AS
  - Included in some sequencing pilots
    - Sequencing pilots have variable ability to detect UPD, Deletion, and ICD
  - No prospective pilot

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Diagnosis of Prader-Willi syndrome and Angelman syndrome by targeted nanopore long-read sequencing

Mamiko Yamada [a], Hironobu Okuno [b], Nobuhiko Okamoto [c], Hisato Suzuki [a], Fuyuki Miya [a], Toshiki Takenouchi [d], Kenjiro Kosaki [a,*]

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[b] Department of Physiology, Keio University School of Medicine, Tokyo, Japan
[c] Department of Medical Genetics, Osaka Women's and Children's Hospital, Osaka, Japan
[d] Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan

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Therapeutic pathway based on the genetics of Angelman syndrome

Niki Armstrong

European Commission

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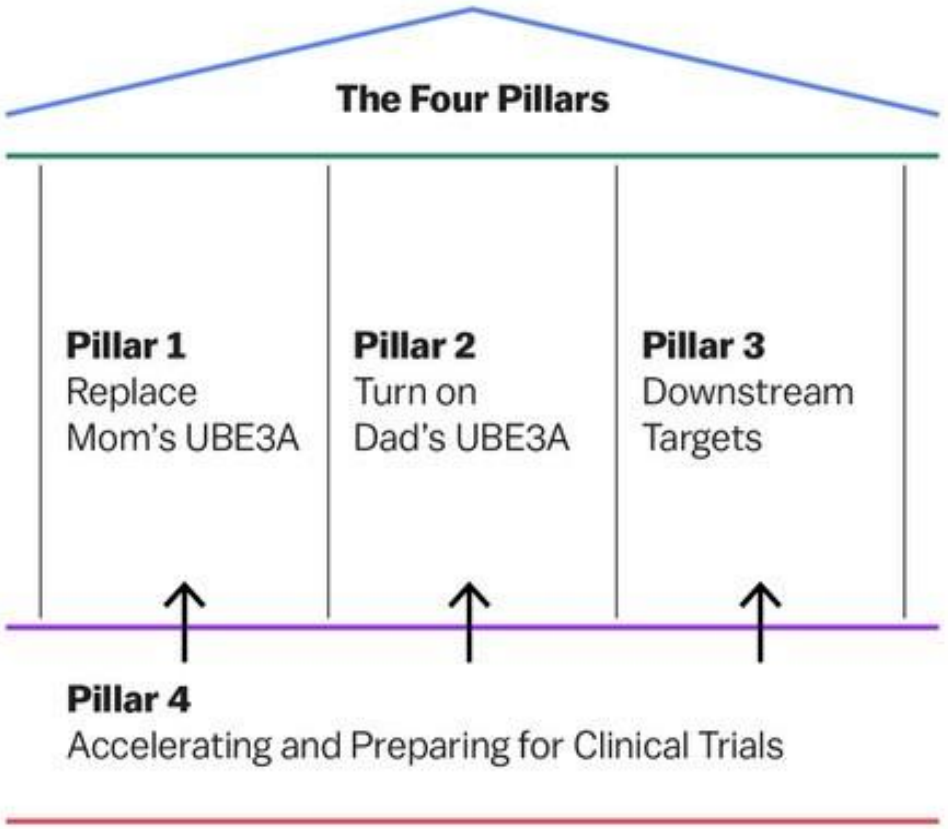
Approaches to transformative therapies in Angelman syndrome: the FAST perspective

fast



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fast

Pillar 1 | Pillar 2

	AAV-GT				HSC-LVV-GT		ASO				CRISPR				ATF
Study	Daley et al. 2011	Nenninger et al. 2022	Wilson et al.	Judson et al. 2021	Adhikari et al. 2021		Meng et al. 2014	Milazzo et al. 2021		Lee et al. 2022	Wolter et al. 2020	Wilson et al. 2020	Jiang et al. 2022		Segal et al. 2023
Approach	*Ube3a* GT (AAV)	STUB-UBE3A (AAV cross correction)	*Ube3a* GT (AAV)	Dual Iso1/Iso2 *Ube3a* GT (AAV)	HSC-GT-*UBE3A* (LVV ex-vivo)		*ASO* paternal activation *Ube3a*	*ASO* paternal activation *Ube3a*		*ASO* paternal activation *Ube3a*	CRISPR paternal activation *Ube3a*	CRISPR paternal activation *Ube3a*	CRISPR paternal activation *Ube3a*		ATF paternal activation
Age	Adult	Adult	Juvenile (2-3- wk)	Birth	Birth	Adol/Adult (6-wk)	Adult (8-16 wk)	Birth	Juvenile (3-wk)	Adult	Birth	Birth	Birth	Juvenile (3-wk)	Adult
LTP	Yes	Yes						+/-	+/-						Yes
Forced swim								Yes							
Seizures				Yes	Yes	Yes	Yes*	Yes	Yes	Yes			Yes	Yes	
Open field	No		Yes (partial)	No	Yes	Yes	No	Yes	No		Yes (partial)	No	Yes	Yes	Yes
Nest building			Yes	Yes			No	No	No			Yes	Yes	Yes	
Rotarod	No	Yes	Yes	Yes (partial)	Yes	Yes	No	Yes (partial)	No		Yes (partial)	Yes (partial)	Yes	Yes	Yes (partial)
Digigait					Yes	Yes									Yes (partial)
Marble burying			Yes	Yes			No	No	No		No	Yes	Yes (partial)	Yes (partial)	
Weight			Yes	No			Yes			Yes	Yes				
Fear conditioning	Yes	Yes (partial)		Yes (partial)			Yes				No				
EEG delta					Yes	Yes									
Morris water maze	Yes														
Novel object recognition					Yes	Yes							Yes	Yes	
Brain weight				No							No				
Hindlimb Clasp	No	Yes	No								Yes				
Y-maze			Yes												
Beam Walking			No		Yes	Yes									
Catwalk			Yes												
UBE3A levels (estimates)	30-50%		50-100%		15-80%	15-80%	35-45%	55-74%	Up to 74%		37-40%	48%	70-80%		28%

Next webinar on Angelman syndrome

- Global Science Summit highlights - updates on research pipeline and clinical trials
  - Prof. Laurent Servais, Pediatric neurologist – CHR La Citadelle – Liège, Belgium & MDUK Oxford Neuromuscular center, Oxford, UK
  - Prof. Nadia Bahi-Buisson, Pediatric neurologist – Hôpital Necker-Enfants Malades – Paris, France

- FAST Global Science Summit – 7 & 8 November 2024, Orlando - USA
  - Free registration: https://cureangelman.org/summit

Questions?

Questions from the survey

What are the most promising new therapies on the horizon for Angelman syndrome, and how might these impact the quality of life for children with AS?

Has there been any recent findings about AS?

What effect is expected from ASO treatment in UPD patients?

What are the results of current trials in Angelman syndrome research?

Thank you for your attention !

- **Feedback survey, before you leave** https://forms.office.com/e/NDmYW0bD1g
- **Get ITHACA's NewsLetter** https://ern-ithaca.eu

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