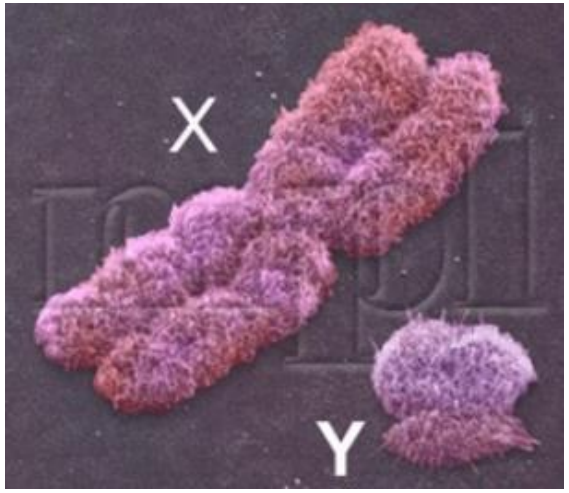

X-Linked Intellectual Disability (XLID) in females

MANAMA Course

March, 16, 2021

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Plan

- **Introduction:** XLID and X-linked inheritance

- **3 Clinical Vignettes of X-linked disorders in females**
  - *HUWE1*
  - *KDM5C*
  - *HDAC8*

- **X-chromosome inactivation** (XCI)
  - Generalities
  - Skewing XCI

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I/Introduction: X-linked Intellectual Disability (XLID)

- Prevalence
  - ✓ **XLID:** 10-12 % in all males with ID (prevalence 1/600-1/1000)
- Interest for the **X chromosome**
  - ✓ Excess of males (ratio: 3/2)
  - ✓ Excess of genes implicating in cognitive function on the X chromosome
- Genetic heterogeneity
  - ✓ Fragile X: 1-2% of all ID
  - ✓ Discovery of various genes: from linkage analysis and candidate genes (**research** laboratory) to X-chromosome or exome (genome) sequencing (**diagnostic** laboratory)

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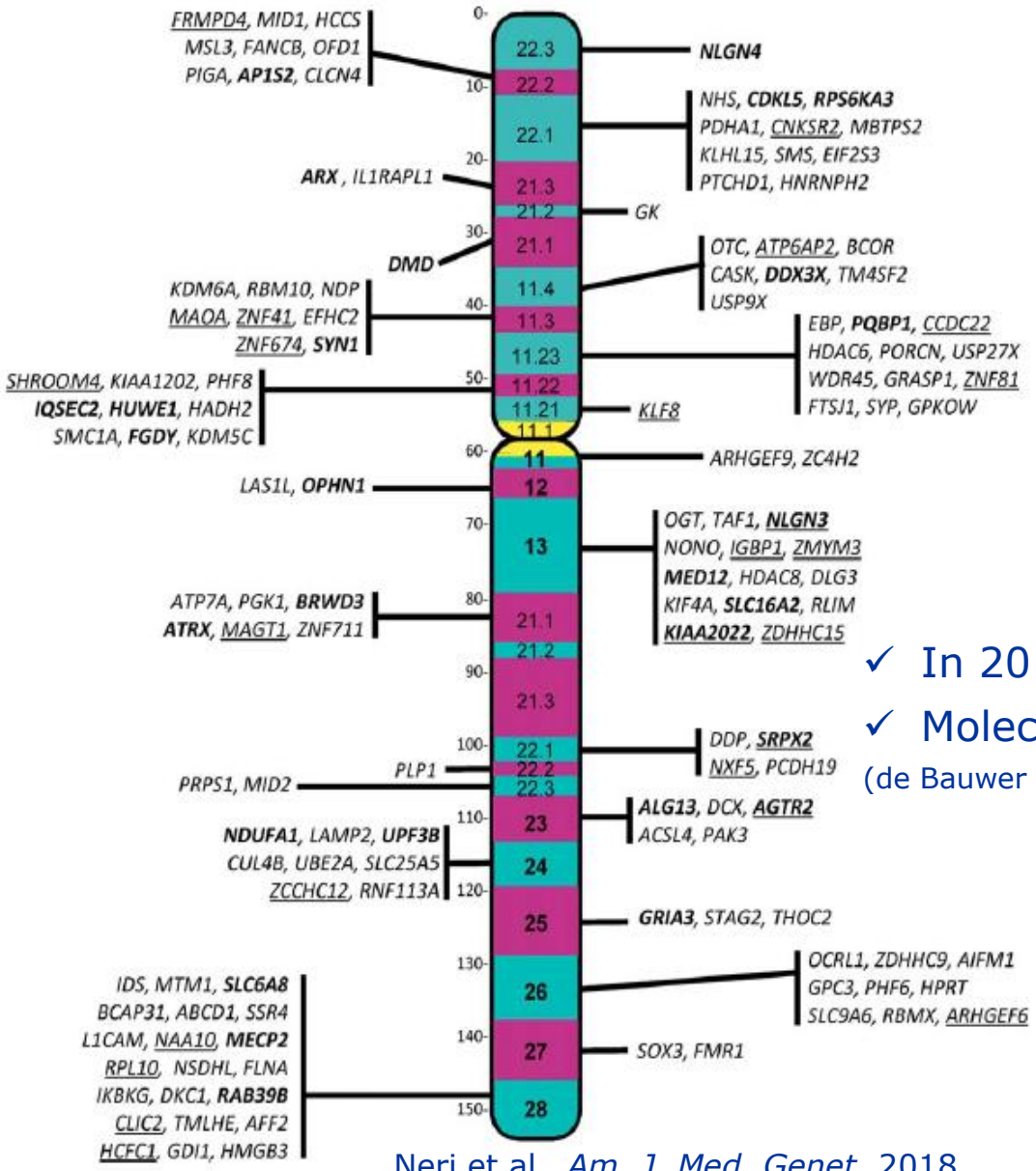
X-linked Intellectual Disability (XLID)



Neri et al., *Am. J. Med. Genet,* 2018

- ✓ In 2018: **141 known XLID genes**
- ✓ Molecular diagnosis: **40-58%**

(de Bauwer *et al.* 2007, Gilissen *et al*. 2014, Hu *et al*. 2016)

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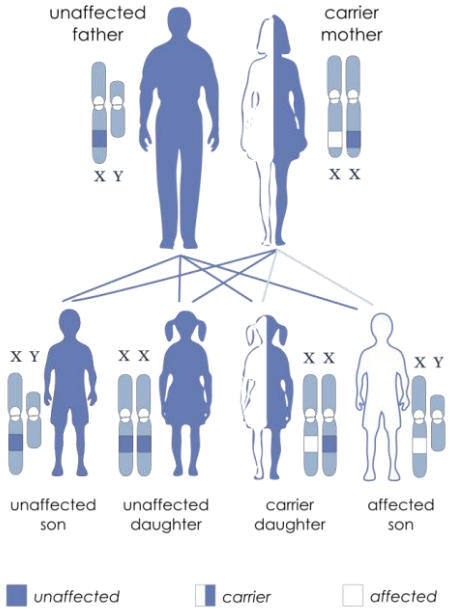
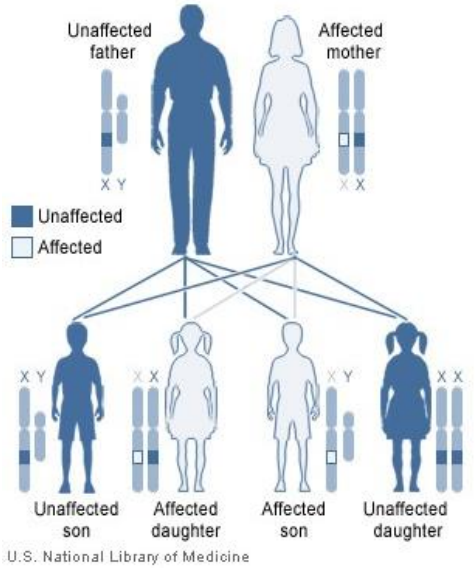
Inheritance of most X-linked traits is not dominant or recessive, **just X-linked**

X-linked recessive



X-linked dominant



- Hemizygous affected males
- Heterozygous carrier females are not or mildly affected (« protected skewing » X chromosome inactivation)

- Usually lethal in males: Rett Syndrome, Incontinentia Pigmenti
- Semi-dominant: Alport Syndrome, Fragile X
- (Dominant with male-sparing: craniofrontonsal syndrome, *EFNB1*; Epilepsy, *PCDH19*)

Mutations in XL genes are identified in severely affected females, as males, with similar or various symptoms and high penetrance!

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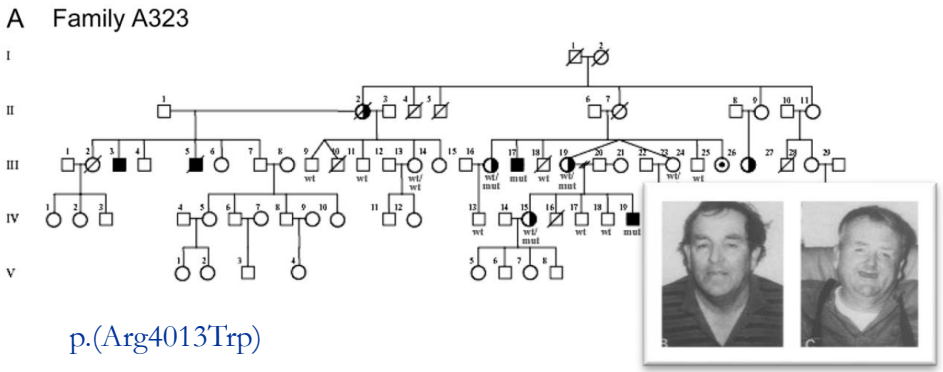
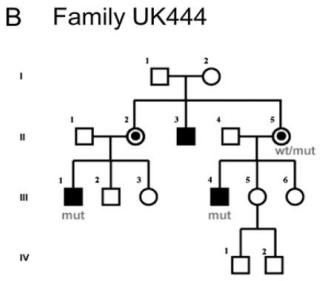
II/ Clinical illustrations: first exemple

Missense variants in *HUWE1*

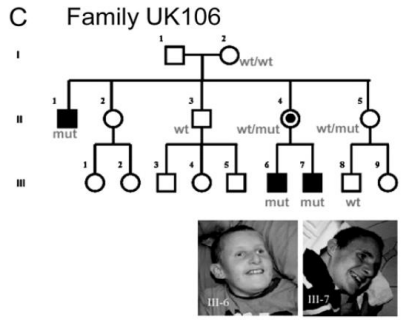
- **Non-syndromic XLID** (2008)



p.(Arg4013Trp)



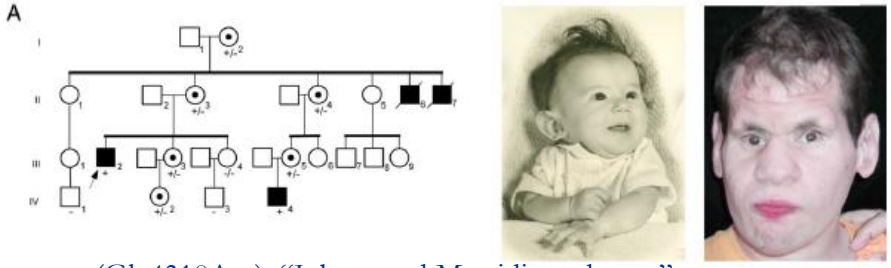
p.(Arg4187Cys)



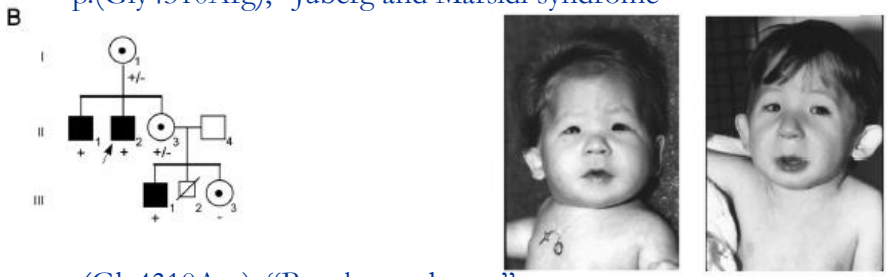
p.(Arg2981His)

Froyen et al, *Am J Hum Genet* 2008

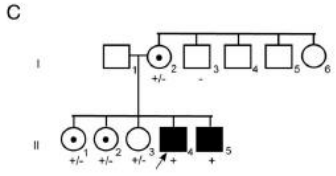
- **Syndromic XLID** (2016)

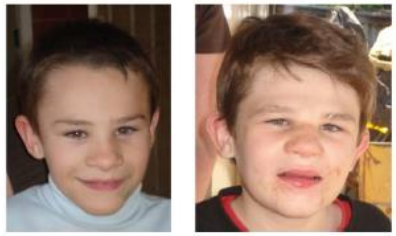


p.(Gly4310Arg), "Juberg and Marsidi syndrome"



p.(Gly4310Arg), "Brooks syndrome"

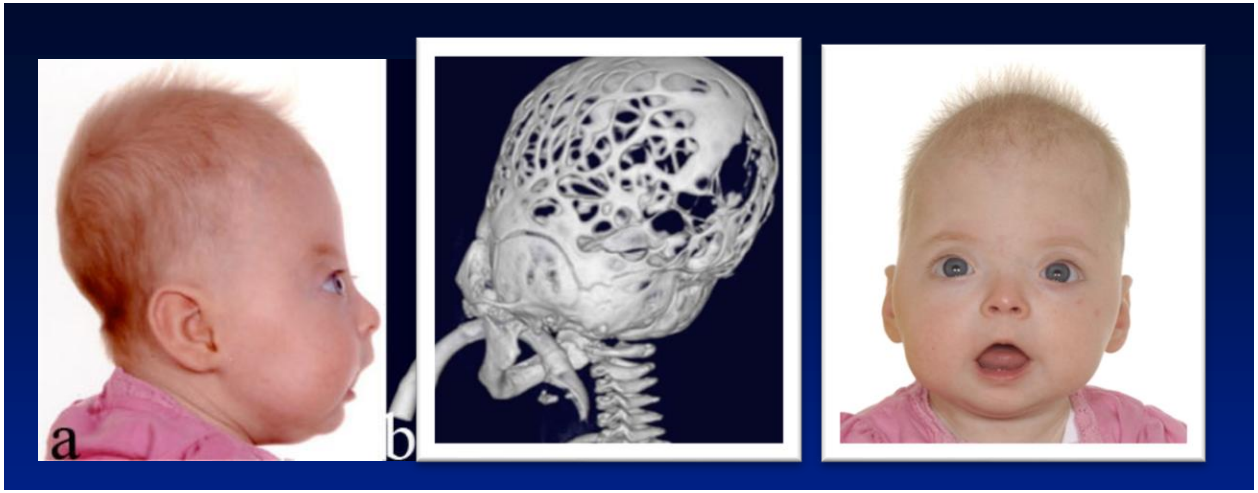




p.(Arg4063Gln)

Frietz et al, *BMJ Open* 2016

- **Taylor** et al, *Nat. Genet* 2015: first **female** patient with learning difficulties and *craniosynostosis*. Skewed XCI (100/0) with preferential expression of the *mutated* allele



***de novo* p.(Arg110Gln)**

+ **Two unrelated males** with *craniosynostosis*, moderate-severe global developmental delay and a missense variant affecting the same amino acid (p.Arg110Trp)

(Taylor et al, *Nat Genet* 2015; Miller et al, *J Med Genet* 2017; Zhu et al, *Genet Med* 2015)

p. Arg110 missense variants could cause a specific phenotype??

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HUWE1 variants

Study of 21 Patients

- Cohort description:
  - ✓ 21 patients with *HUWE1* variants, **14F/7M**
  - ✓ All but two are sporadic patients referred for ID or DD
  - ✓ Age range of 18 months to 31 years
  - ✓ **XCI** in available female patients or obligate carriers
  - ✓ **Expression studies** (cDNA sequencing)

- Clinical features
  - ✓ Moderate to profound **ID** (90%)
    - ✓ Global developmental delay (95%)
    - ✓ Hypotonia (70%)
  - ✓ Absent or **limited speech** (90%)
  - ✓ Postnatal **short stature** (70%)
  - ✓ Seizures (40%)
  - ✓ Skeletal anomalies: small hands and feet (57%), overlapping toes (42%)
  - ✓ **Strabismus** (65%), hypermetropia and/or astigmatism (50%)

Moortgat et al. *Eur J Hum Genet* 2018 Jan 26:64-74

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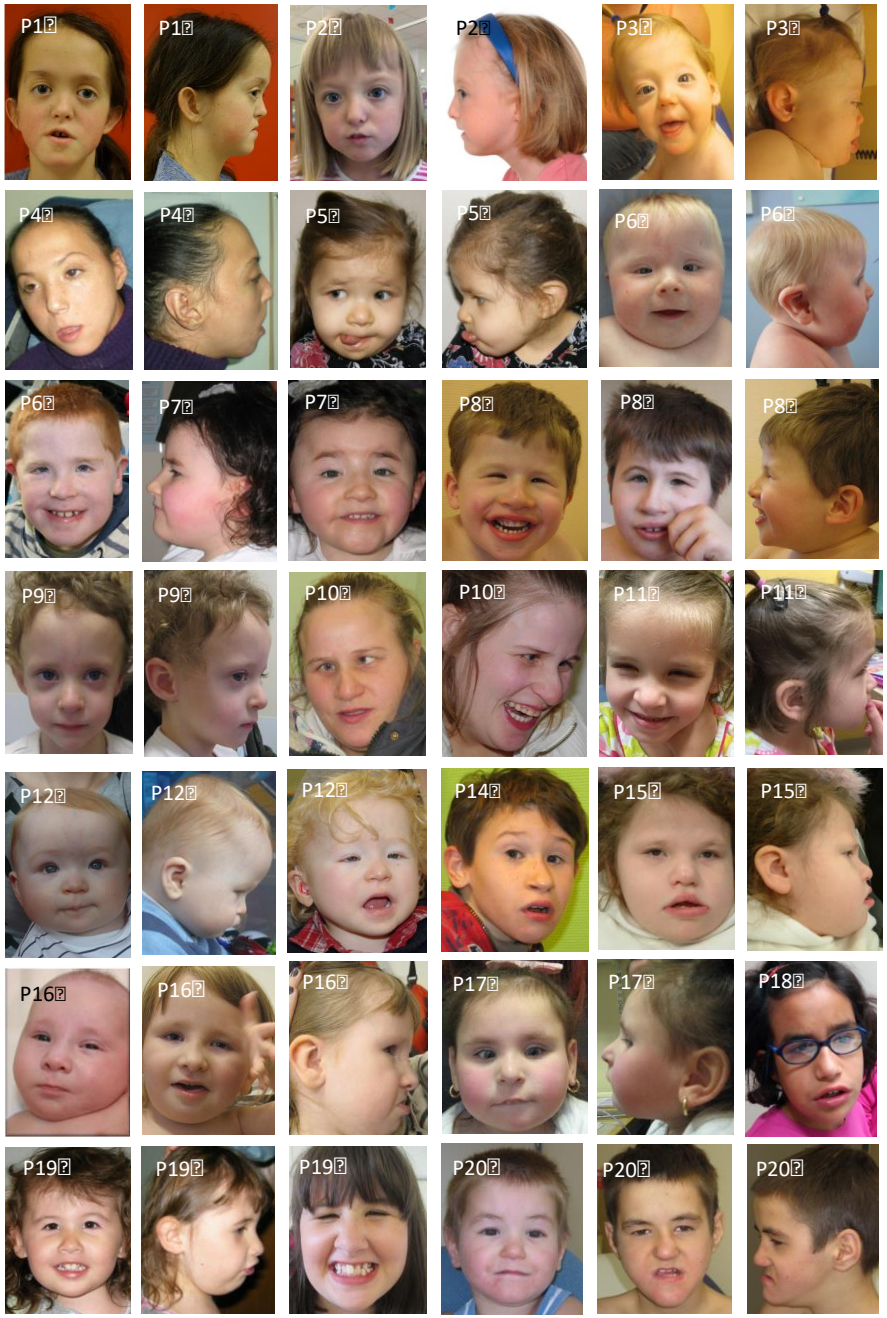
Recurrent facial dysmorphism but *not a recognisable gestalt*



Patient	(n=21)	(%)
Sex	14F/7M	
Inheritance	4mat/17dn	
Growth findings		
Norm birth param.	15/20	75
Height <3rd centile	15/21	71
Weight <3rd centile	11/21	52
Microcephaly	11/21	52
Facies		
Hypotelorism	6/21	28
Hypertelorism	7/21	33
Small nose	8/21	38
Broad nasal tip	19/21	90
Short philtrum	10/21	47
Full lower lip	8/21	38
Thin upper lip	16/21	76
Long face	11/21	52
High forehead	13/21	61
Eyes		
Deep set eyes	15/21	71
Epicanthic folds	14/21	66
Blepharophimosis	12/21	57
Strabismus	13/20	65
Retinopathy	3/16	18
Refraction error	9/18	50
Ears		
Low set	7/19	36
Posterior rotated	8/19	42
Hearing loss	4/18	22
Skeletal anomalies		
Small hands	12/21	57
Overlapping toes	9/21	42
Flexion contract.	5/17	29
Neurological		
Global motor delay	19/20	95
Walking	13/18	72
Delayed speech	17/19	89
Mild ID	3/19	16
Mod-profound ID	16/19	84
Hypotonia	13/19	68
Seizures	8/17	47
Brain MRI anom.	7/16	43
Stereotypies	8/17	47

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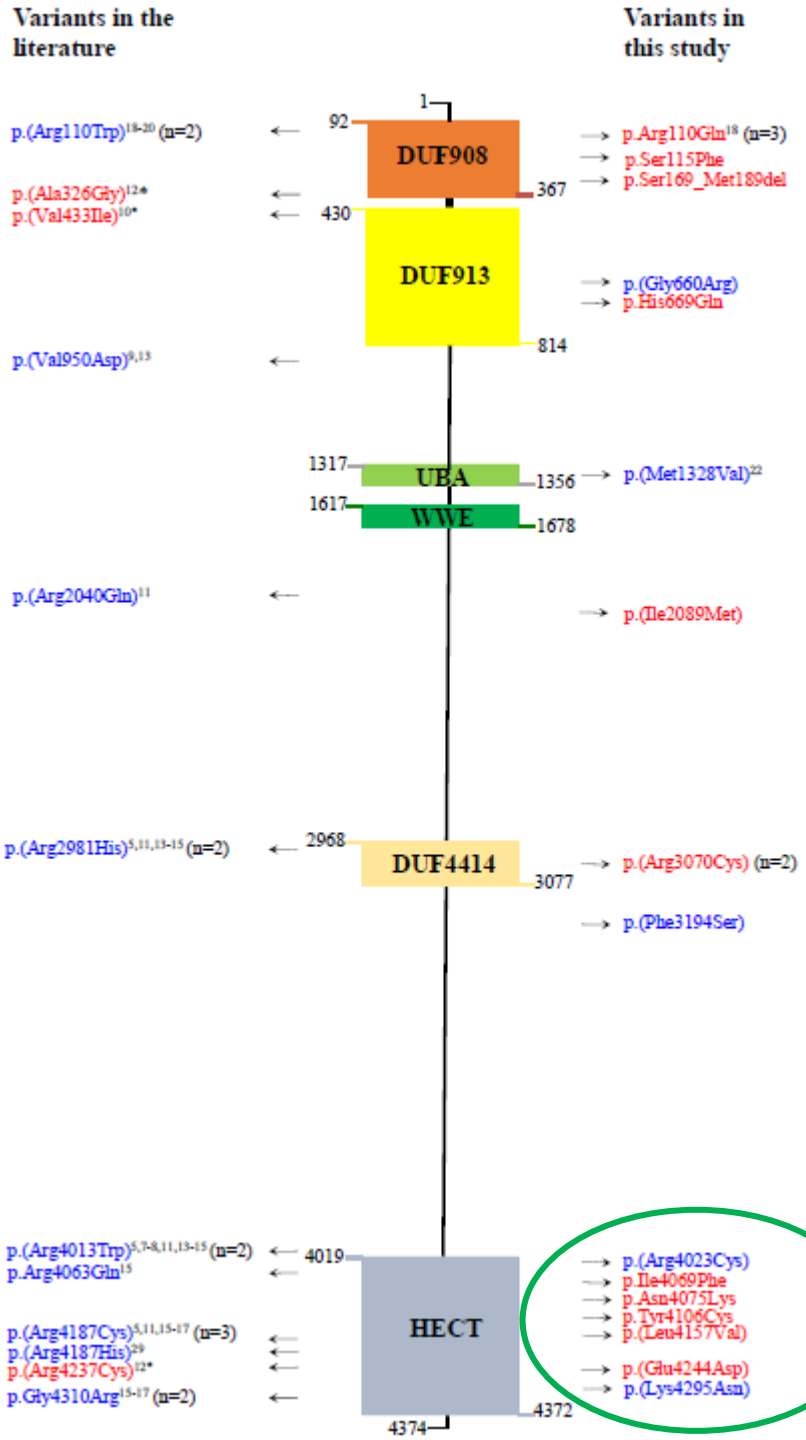
Molecular results

- 16 different variants
- All are **missense** except one splice-site variant leading to in-frame deletion
- Variants are located in **five** out of six different functional **domains** of the protein
- No robust genotype-phenotype correlations (except for p.Arg110Gln…)



Red: female
Blue: male

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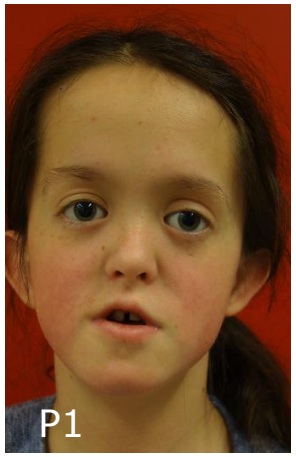
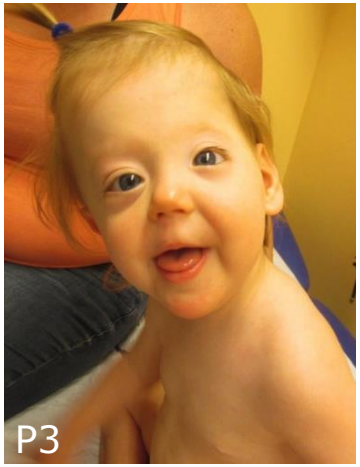
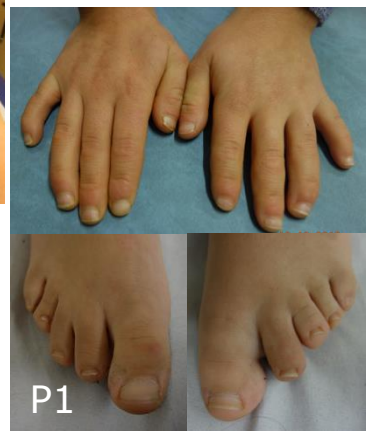
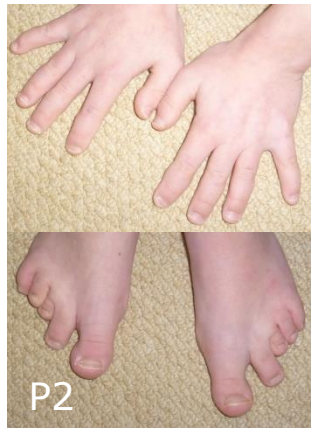
p.Arg110 variants cause a specific phenotype?

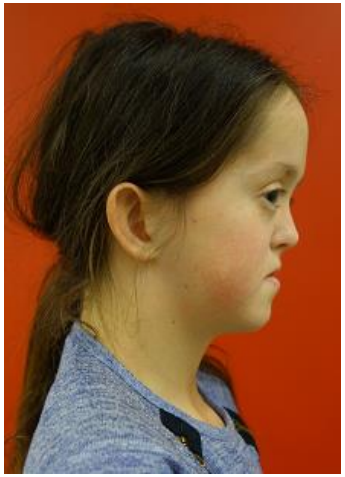
- 3 females with **p.Arg110Gln** variant have a particular phenotype:
  - with mild ID
  - facial dysmorphism
  - skeletal anomalies and craniosynostosis

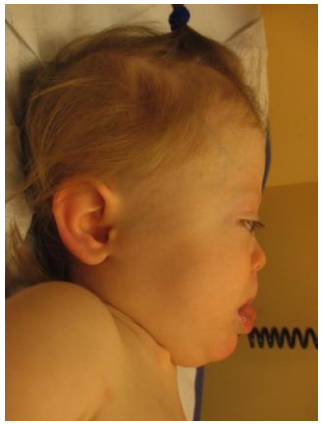












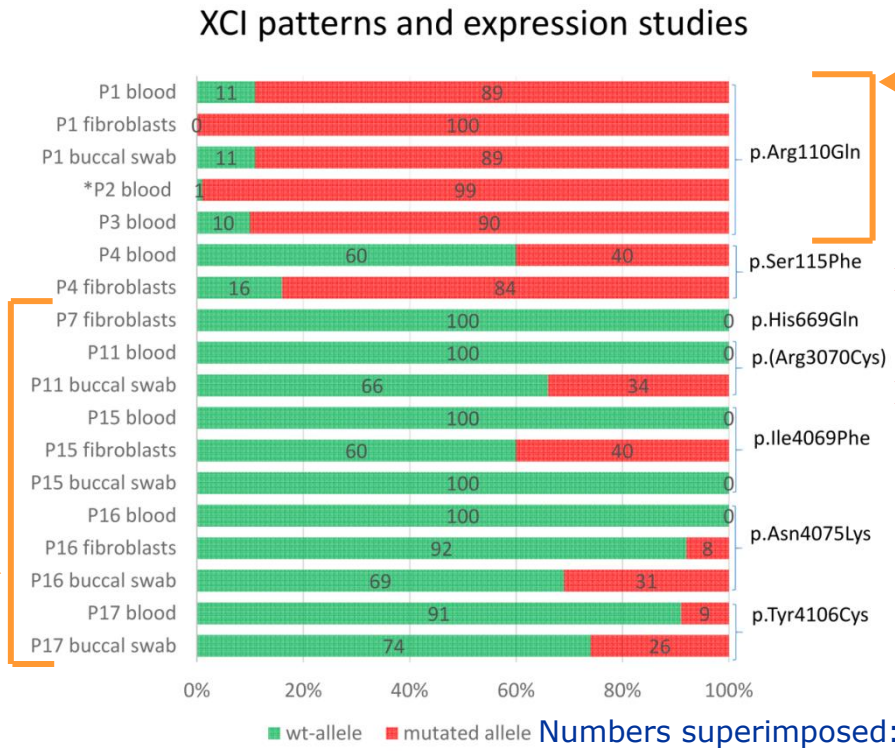
Taylor et al, *Nat. Genet* 2015

X-inactivation studies and RNA expression analysis

- **13/14** female patients had a **skewed X-inactivation ratio** (unaffected obligate carriers also presented with skewed XCI)
- XCI in blood (12F), fibroblasts (6F) and buccal swabs (5F). XCI pattern was not always consistent in different tissues
- **cDNA-sequencing** was used to determine which allele was preferentially expressed (8 patients)

In *severely* affected females:

- extremely skewed XCI
- almost exclusive *expression of the **normal** allele*



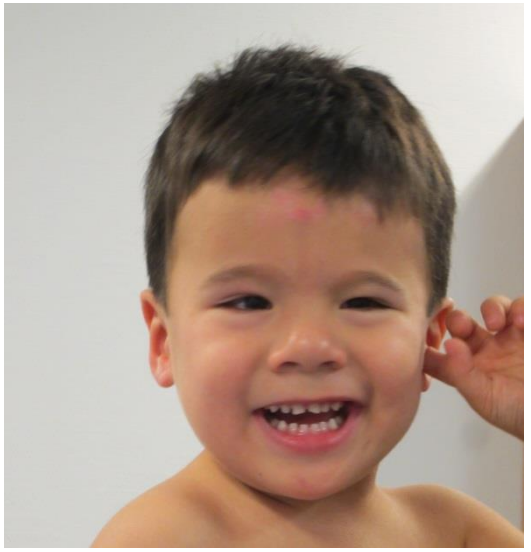
In *p.Arg110Gln*-females:

- extremely skewed XCI
- almost exclusive *expression of the **mutant** allele*

Skewing of X chromosome is not always protective…

II/ Clinical illustrations: second exemple

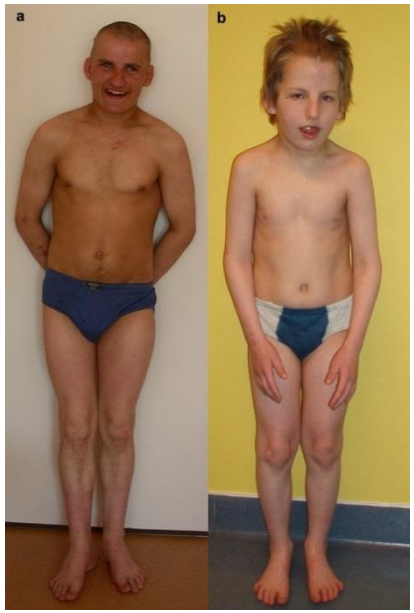
L. 3y

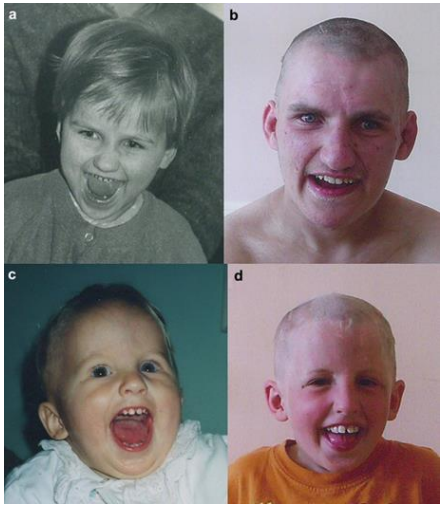


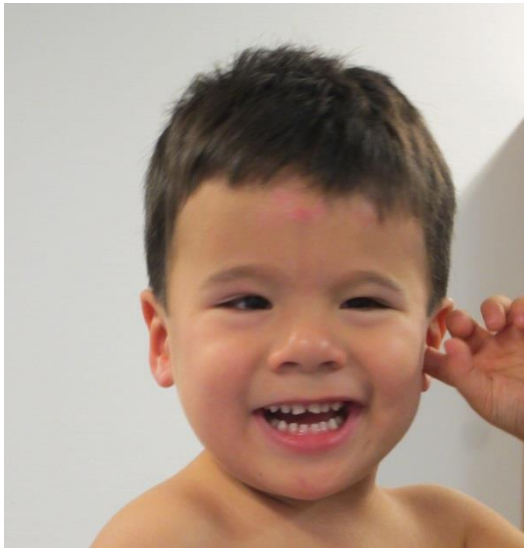
- First child, non consanguinous parents
- Prematurity at 35w +5/7 GA for maternal preeclampsia. W/H/OFC: -1 SD
- Global developmental delay
  - Sat at 18M and walk alone at 2 years 5M
  - Fine motor skills delay
  - Severe speech delay
- Left strabismus
- Limb hypertonia/hyperreflexia and stiffness of ankles
- Brain MRI: periventricular leukomalacia→ Diagnostic of « cerebral palsy »

c.62G>A, p. (Trp21*) *de novo* in the *KDM5C* gene

« Claes-Jensen syndrome »





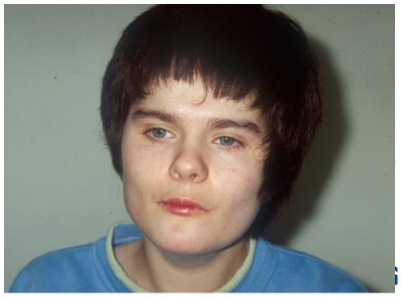


Ouna et al. *Eur J Med Genet* (2012) 178-184

KDM5C/JARID1C (Claes-Jensen type, XLID)

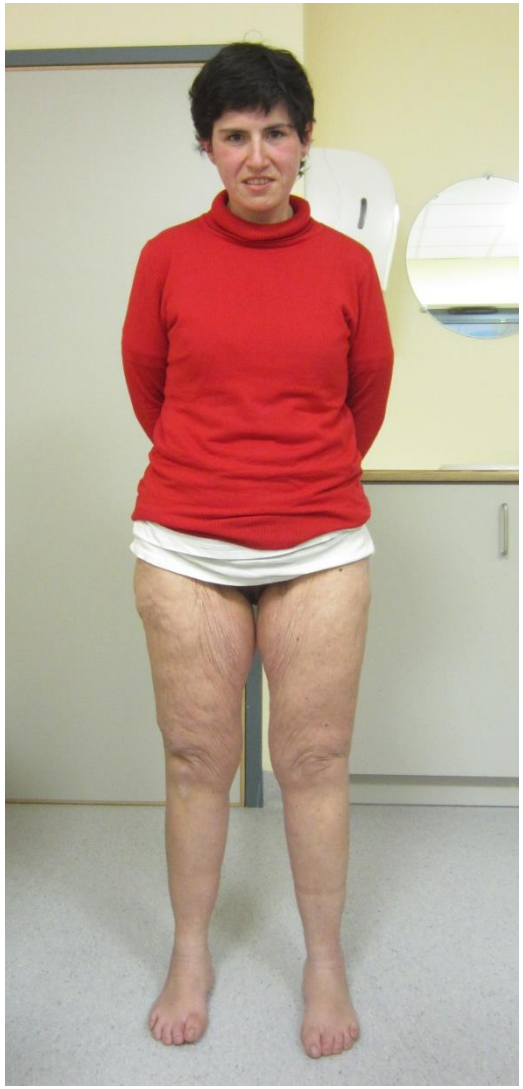
- Located on **Xp11.22**
- Encoding a transcription factor with **histone demethylase** activity
- Loss of function mutations responsible of **1-3% XLID** (fragile X negative and normal CGH)
- Severe **clinical features in male patients** :
  - Intellectual disability: mild (25%), moderate (12%) to severe (63%)
  - Hyperreflexia and spasticity (78%)
  - Short stature (55%)
  - Epilepsy (30%)
  - Behavioral trouble (agressivity , ASD) (35%)
  - High arched palate, malar hypoplasia

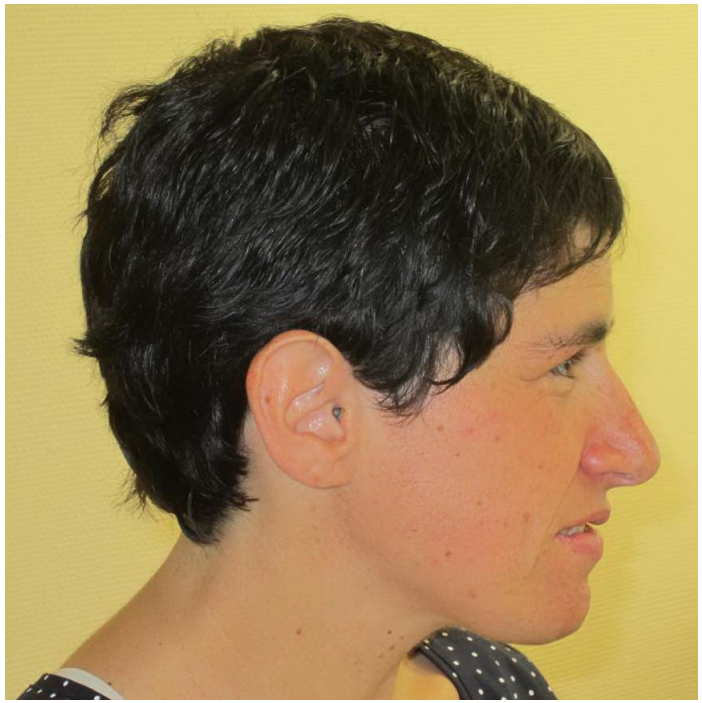
- **Carrier females** usually **asymptomatic** or mildly affected
- A severely affected female with ID/autism, sialorrhea, short stature: microdeletion 0,4 Mb in Xp11.22 encompassing *KDM5C and IQSEC2 ,* skewed XCI (and deletion on the *inactive* allele)
(Fieremans et al. *Eur J Med Genet.* 2015)

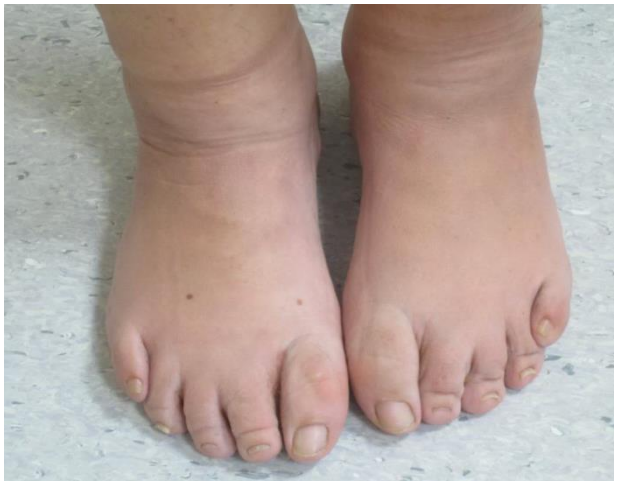


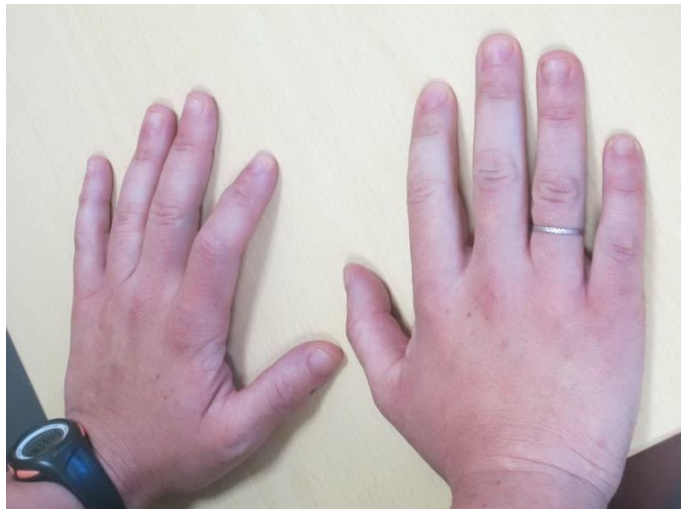
M.L, 31 years

- Unremarkable familial history
- Speech delay
- Moderate intellectual disability
- Behavior trouble
- Obesity (without hyperphagia, gastroplasty). H: 153 cm. OFC:3th centile
- Hypersialorrhea
- No dysmorphism









- Short feet and brachydactyly of toes with short nails and overlapping 5th toe

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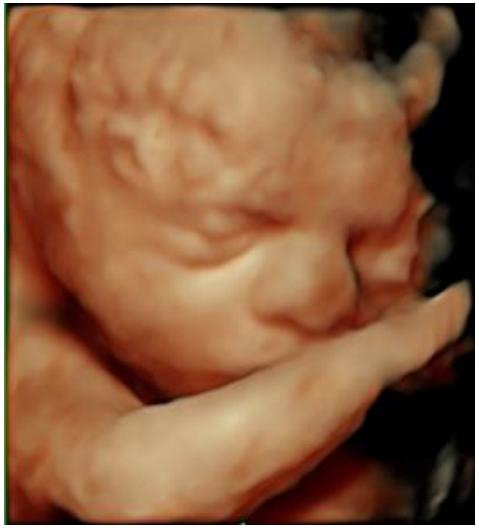
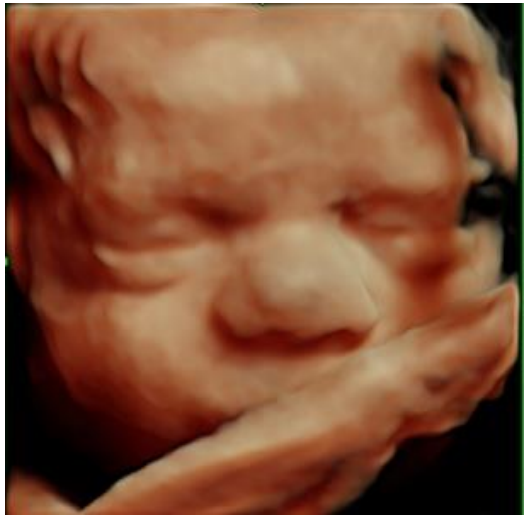
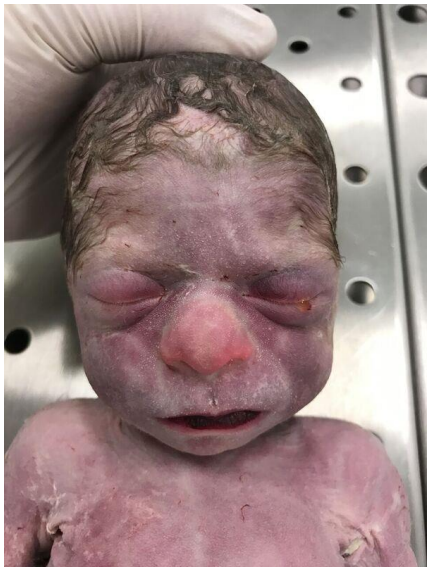
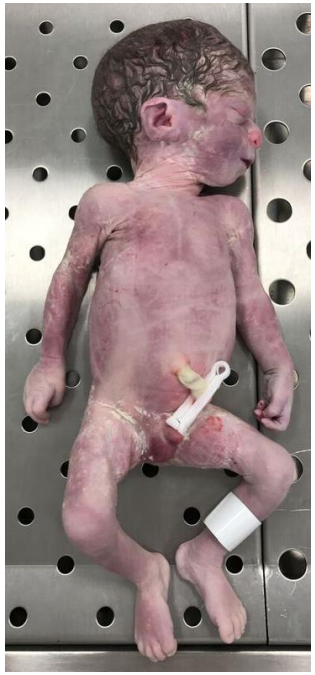
- pregnancy at 29 weeks of gestational age
- *Fœtal US:* sagittal craniosynostosis, pachygyria and facial dysmorphism (short neck, hypertelorism)
- MTP

Genetic studies

- **In the mother:**

c.2383_2384delAG, p.Arg795SerFs*5, *de novo*
in *KDM5C*

« Claes-Jensen syndrome »

Extremely skewed inactivation of X chromosome (ration: 91/9)

- **In the foetus**: hemizygous *KDM5C* mutation

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KDM5C: collaborative study in **10 affected females**

Clinical features in females

- ID: 100% (moderate 7/10)
- Speech delay: 90% (severe, 50%)
- Behavioral troubles: 80% (low frustration tolerance, stereotypies, agressivity)
- Ophtalmologic anomalies: 50% (hypermetropia, myopia)
- Short stature: 40% (-2 SD to -3 SD)
- Overweight/obesity: 40%
- Skeletal abnormalities: 60% (pes panus, short feet)
- Other: seizures (2/10), spasticity (1/10), hyperpilosity (3/10)

Carmignac et al. *Clin Genet*. 2020 Jul;98(1):43-55

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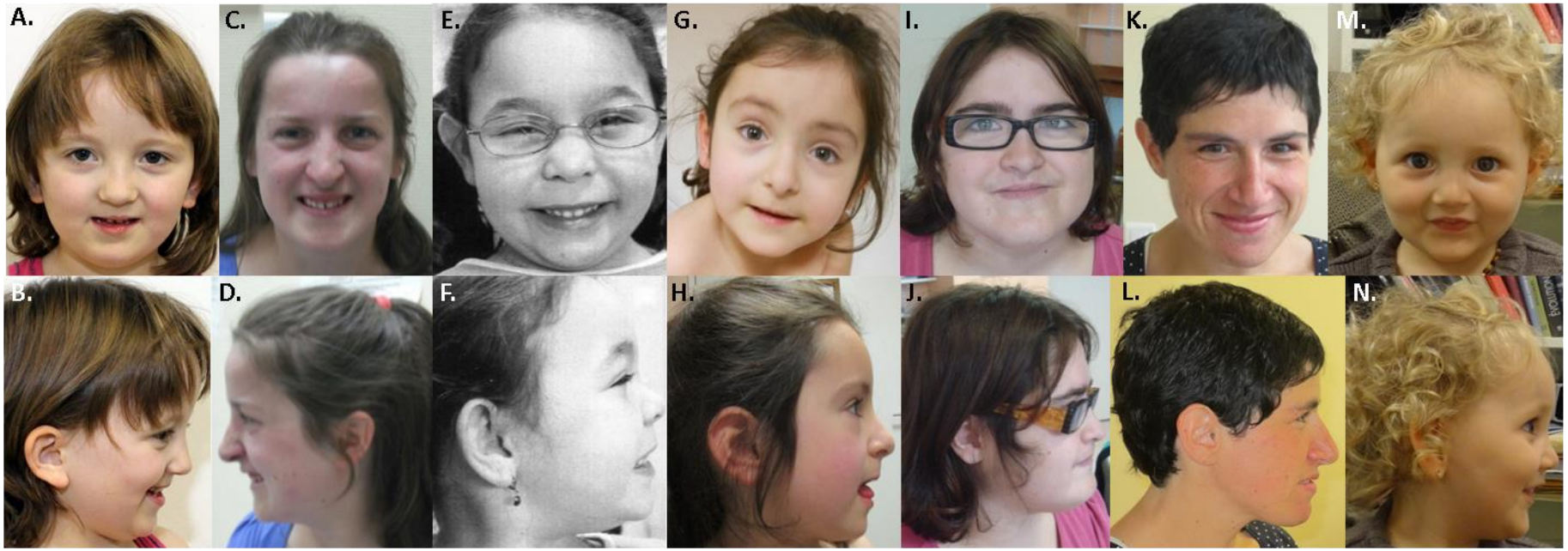

Figure 1: Facial pictures of 6 index cases. A-B: Case 1 at X years. C-D: Case 2 at X years. E-F: Case 3 at X years. G-H: Case 4 at 3 years. I-J: Case 8 at 21 years. K-L: Case 9 at 31 years. M-N: Case 10 at 2 years.

- Round face (8/10)
- Broad nasal bridge (4/10)
- Low columella (6/10)
- Short philtrum (6/10)
- Thin lips (8/10)

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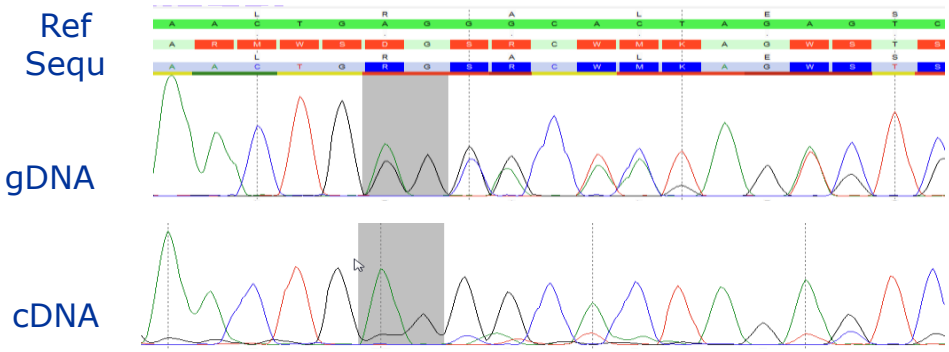
Molecular results

- *5 missense, 3 frameshift and 2 splice site mutations*
- 5 *de novo* mutations (2 inherited and 3 NA)
- **X inactivation studies** performed **in 8/10** females patients
- **5/8** had a **skewed X-inactivation ratio** (3/8 extremely skewed, *>90/10*)
- **Expression study** (cDNA sequencing) in patient with p.Arg795Glyfs*5 mutation and severely skewed XCI: preferential expression of the wild type allele



Skewing of X chromosome is not always protective…

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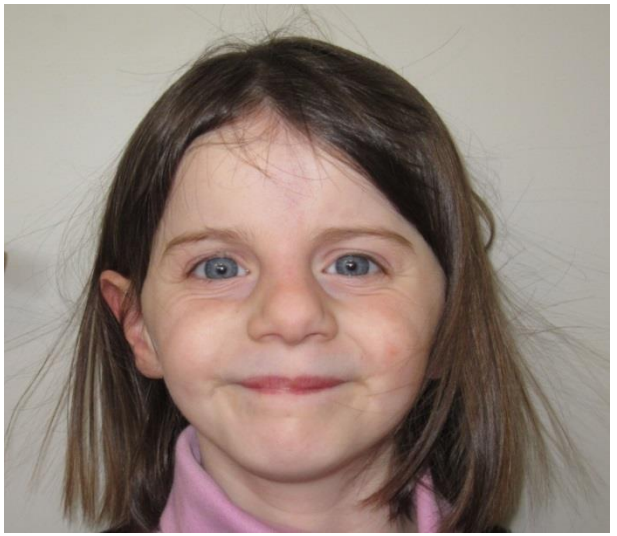
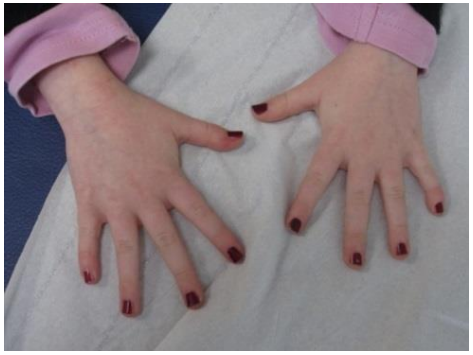
II/ Clinical illustrations: third exemple

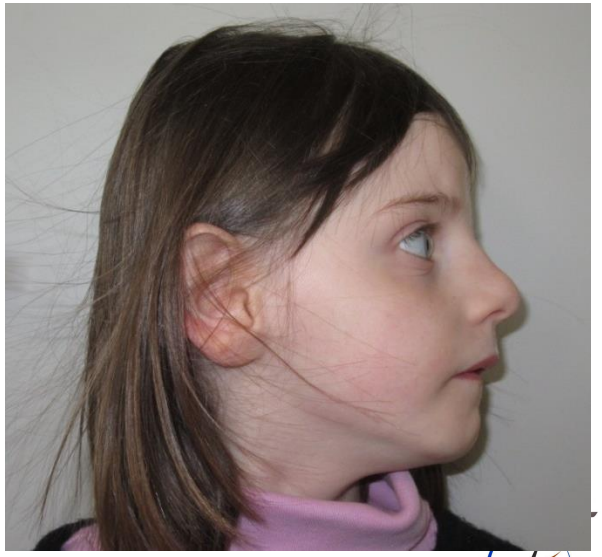
L. 8 y



- 2nd child of healthy and non consanguinous parents
- **IUGR**
- At birth: W:-2SD; H:-3 SD, OFC:-2 SD
- ASD operated
- **Feedings difficulties** and GOR
- **Growth retardation** (W:-4 SD, H:-2,5 SD)

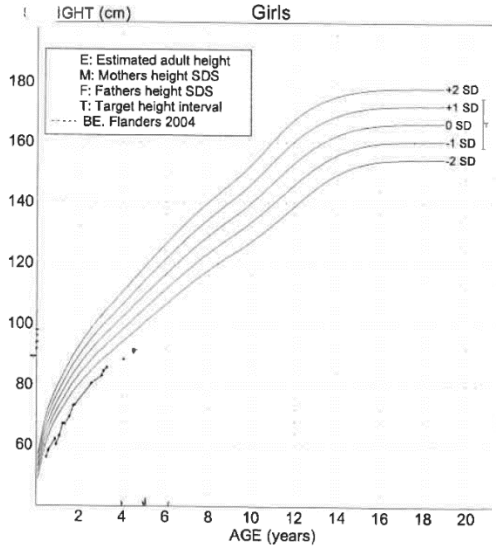
  microcephaly (-3,5 SD)
- Global developmental delay (speech delay ++)
- Moderate ID (IQ:42)

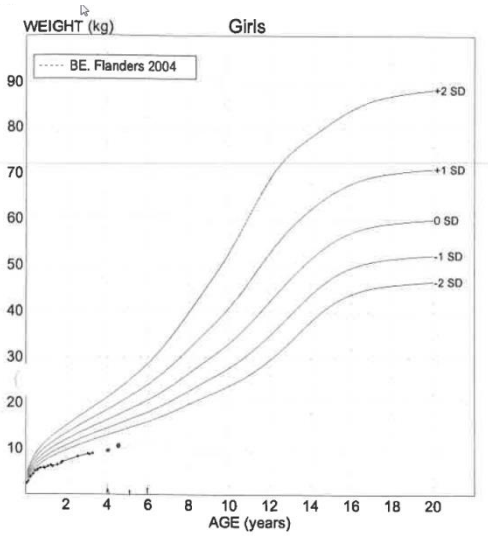




Paraclinic investigations

- Brain MRI: no malformation
- EEG: normal
- Endocrinological investigations normal (IgF1, TSH/T4, coeliac disease)
- Metabolic screening: negative





Genetic investigations

- Micro-array (Agilent 180K): negative
- UPD 7 and methylation 11p15: negative
- Encephopathy panel (150 genes): negative
- Sanger Sequencing of *ANKRD11, ARID1B*: negative
- Trio Targeted Exome Sequencing (« mendeliome »)

c.628+1G>T, *de novo*

in the *HDAC8* gene

« Cornelia de Lange-like syndrome »

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HDAC8

- Located on **Xq13.1**
- Encodes for a SMC3 **histone deacetylase** involved in cohesin recycling
- one of the 5 genes accounting for « Cornelia de Lange-like syndrome » (*NIPBL*, 60%; *HDAC8*, 4%)
- 40 different variants (no hot-spot)
- +/- 70 patients reported in the literature
- Males more severely affected
- **70% are females**, less CdLS, 90% **extremely skewed X** inactivation (ratio >95/5, in favor of the normal allele)
  - In our patient XCI: 100/0 (blood, urine and buccal swab). Expression of normal X chromosome

Skewing of X chromosome is not always protective…

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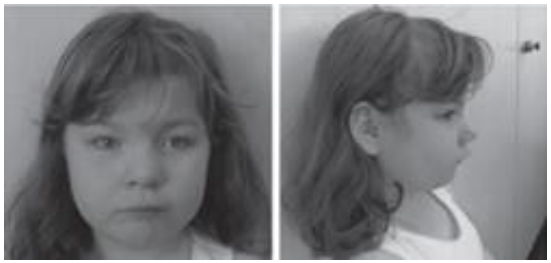
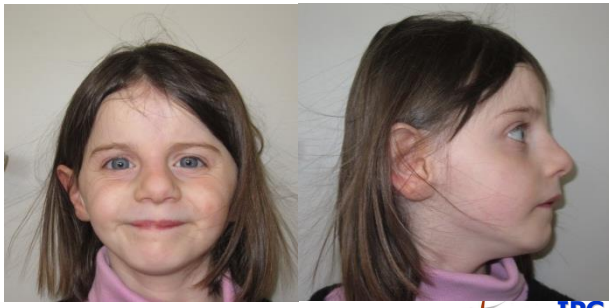
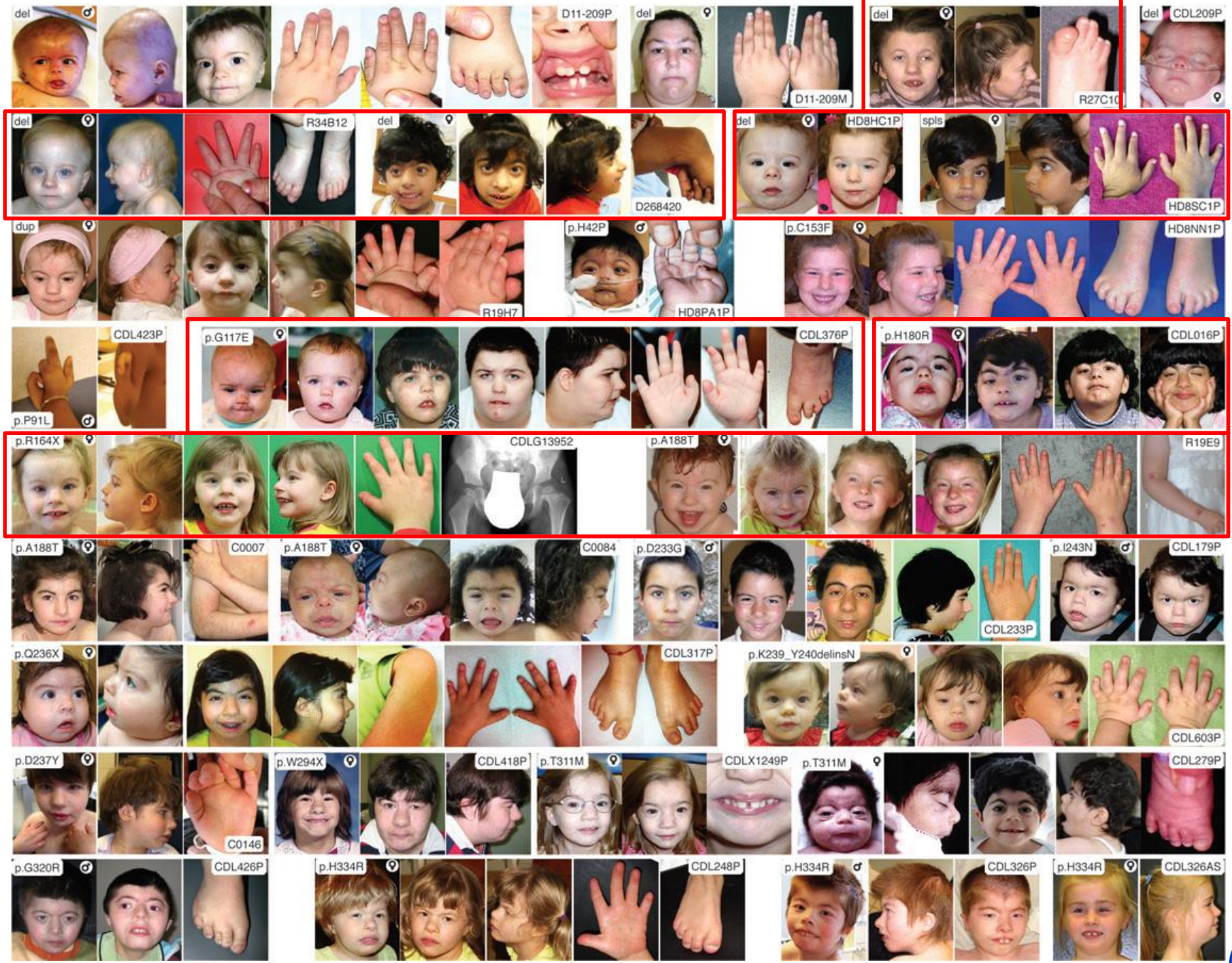
HDAC8: « Cornelia de Lange-like X linked syndrome» and overlapping phenotypes

- **Growth retardation** (70-100%)
- **DD/ID (100%),** mild (47%), moderate (33%), severe (20%)
- Other common clinical features: microcephaly (30-90%), feeding difficulties and GER (50-80%), hearing problems (62%), small hands (62-95%), cardiac anomalies (50%), myopia/astigmatism (50%), epilepsy (12%)
- **Facial dysmorphism**: brachycephaly (70%), synophrys and arched eybrows (90%), anteverted nares (75%) and long smooth philtrum, microretrognathia (60%)
- **Uncommun with CdL**: delayed closure of anterior fontanelle (50%), hypertelorism and/or telecanthus (25-65%), broad nasal tip (66%), hooding of eyelids, dental anomalies (50%), friendly personality (20-50%)
- *No limb deficiency*







Kaiser et al. *Hum Mol Genet* 2014: 35 affected patients with *HDAC8* mutation

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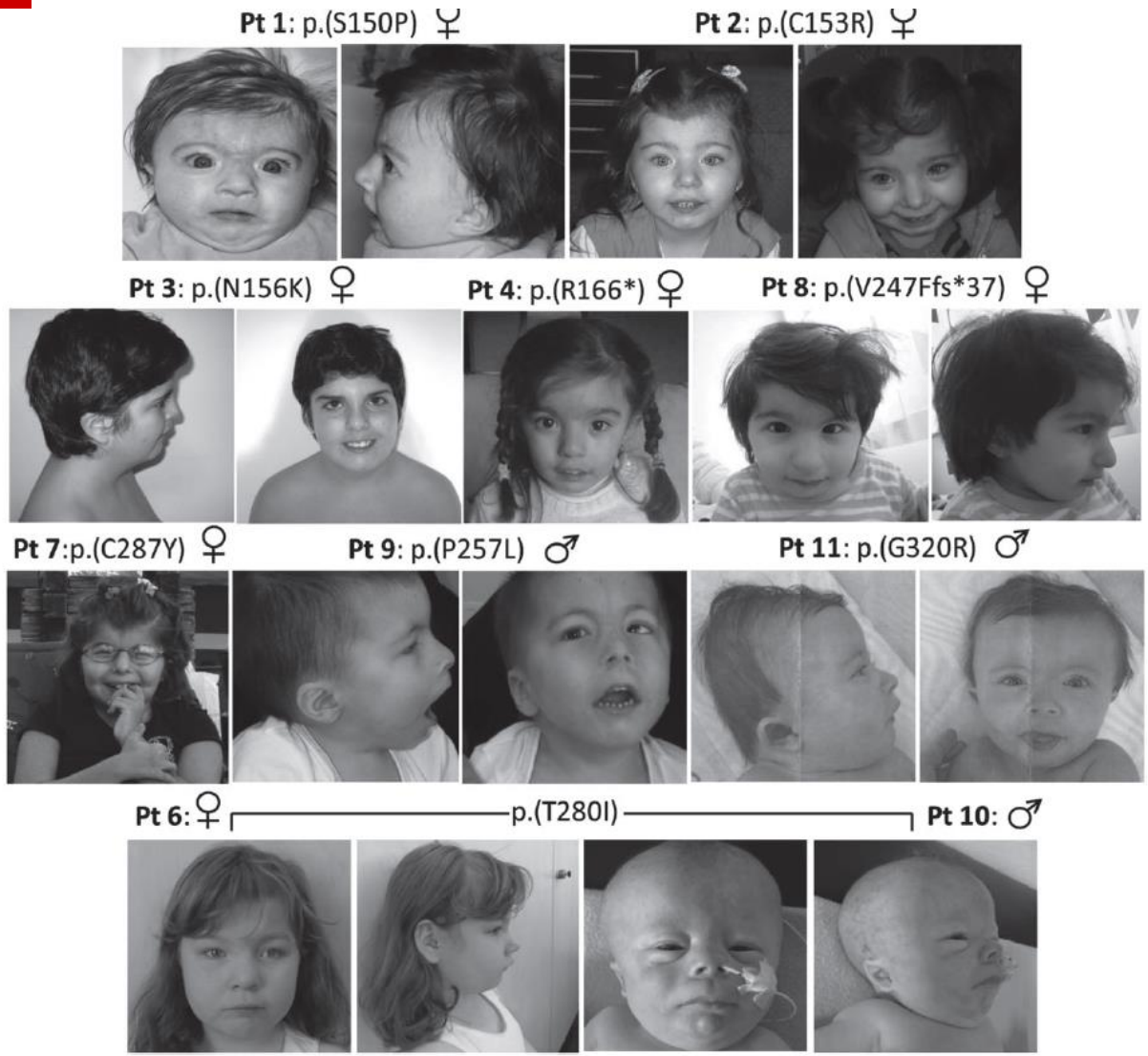
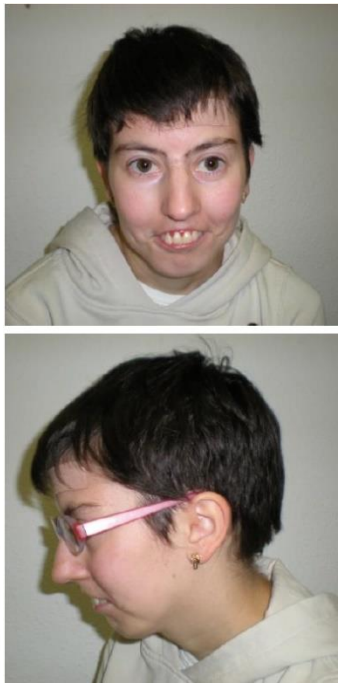
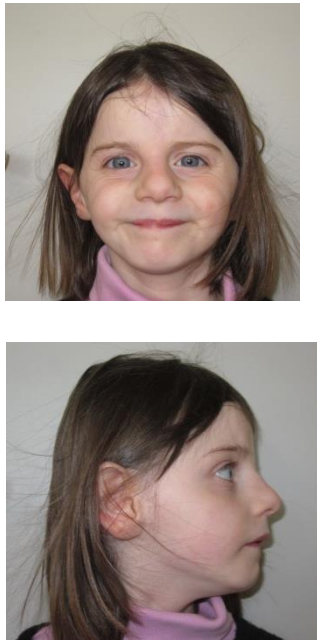


Fig. 1. Facial appearance of the patients harboring variants in *HDAC8*. For each patient, the gender and the variant are indicated. Written informed consent was obtained from each individual participant for the publication of the pictures.

Parenti et al. *Clin Genet* 2015



Fieremans et al.
Hum Mut 2016



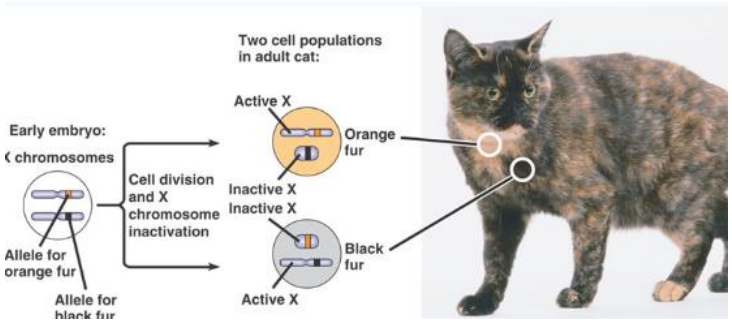
Our patient

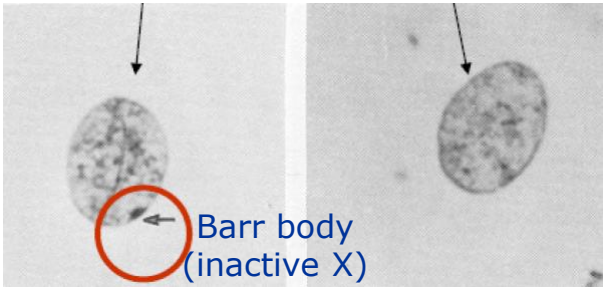
Skewed inactivation pattern in female patients with ID???

Few reminders….

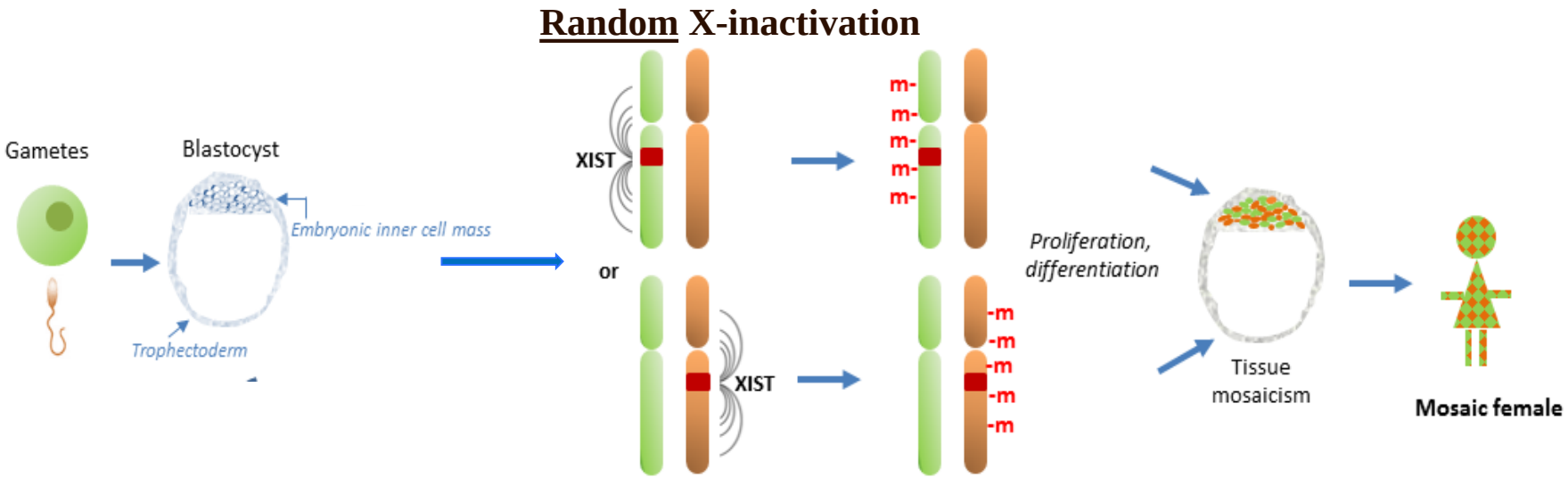
X inactivation process (1/2)

- « Hypothesis of **Lyon** » in 1961: one of the two copies of the X chromosome present in females is *inactivated*
- Dosage compensation mechanism



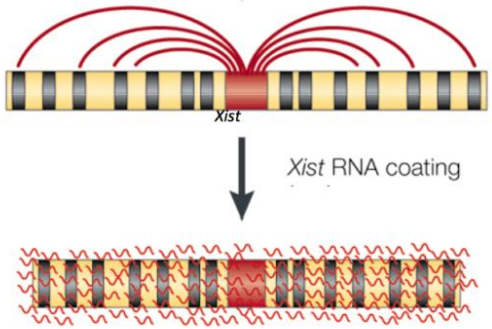


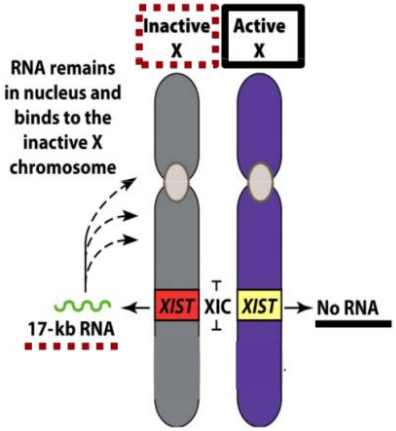
- **Early** in the development (blastocyst stage, 7-9 dpf)
- **Random** choice but then **stably** transmitted

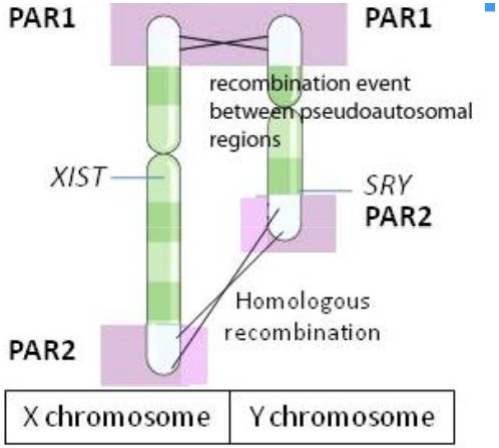


X inactivation process (2/2)

- X inactivation starts at the **XIC** (X inactivation center) containing *XIST* (X-Inactivation Specific Transcript) only expressed from the inactive X
- XIST RNA coats the inactive X-chromosome
- High methylation of DNA and histone hypoacetylation for the stability **of the inactivation**







- **15% escape** X-inactivation (Carrel and Willard 2005)
- **10%** genes show **variable patterns** of inactivation and are expressed to different extents from inactive X chromosome

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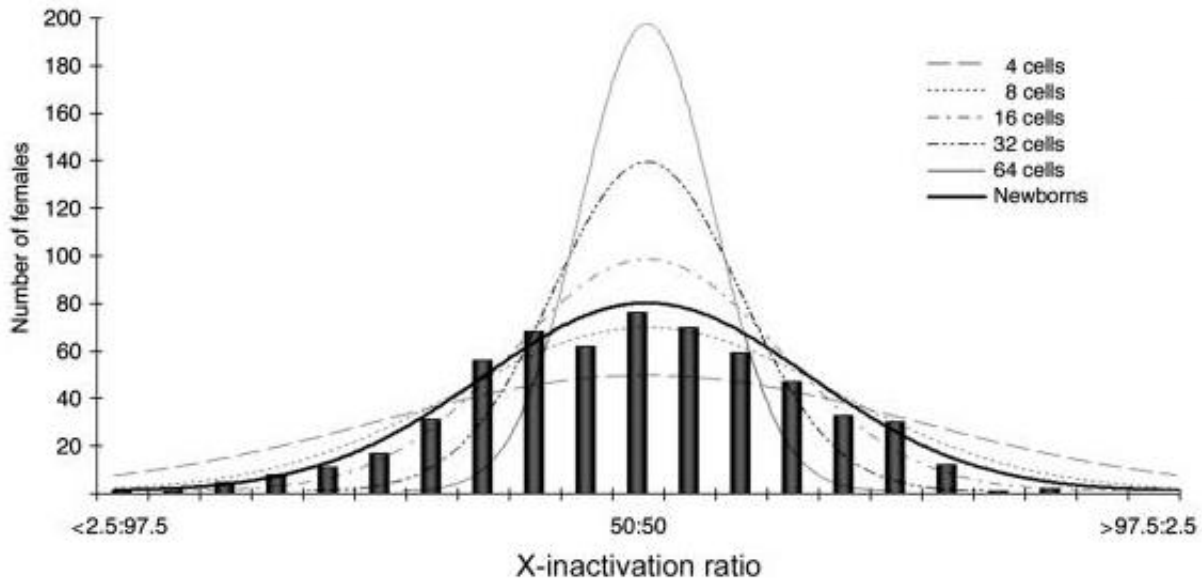
X inactivation ratio



- **Random**: 50/50
- **Skewed**: >80/20 (extremely skewed: >90/10)
- In general population, extreme skewing is present in 3,6% of females
- X inactivation is usually measured in blood

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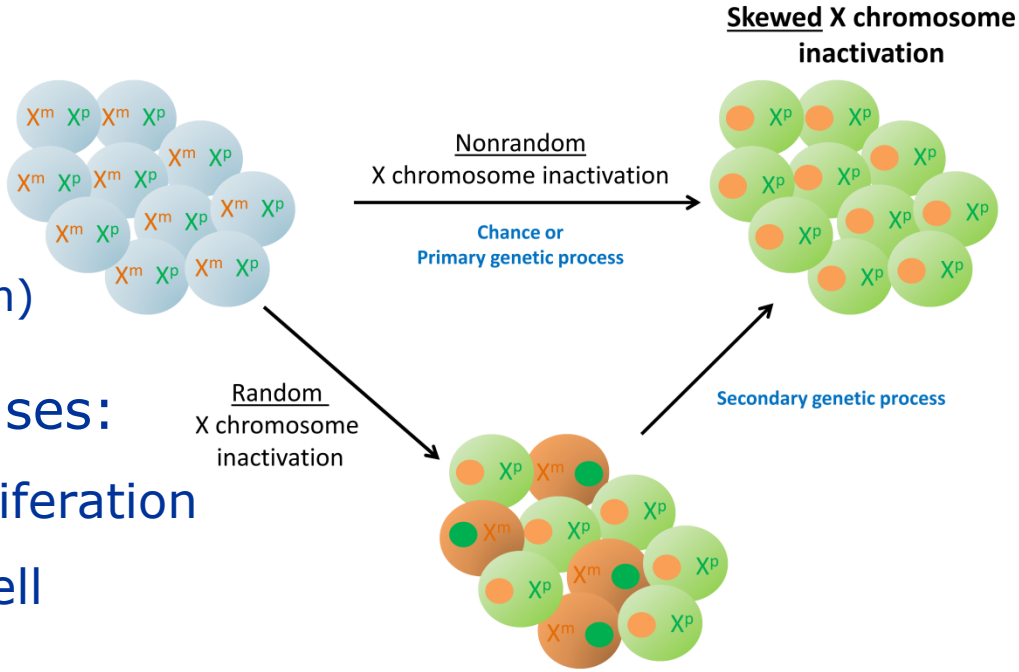
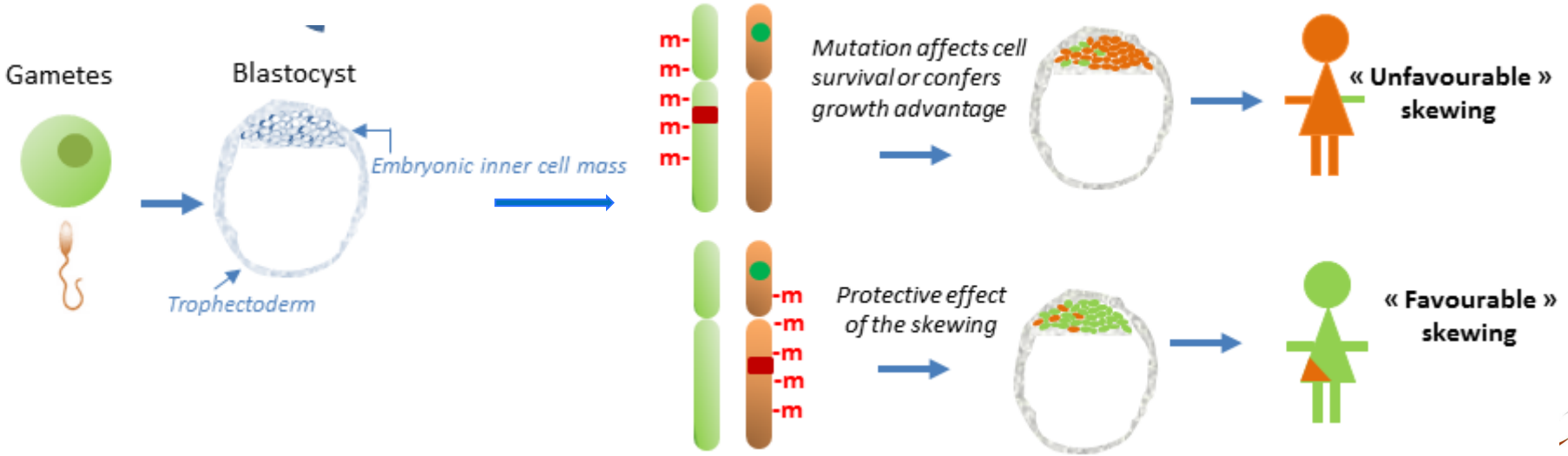
Skewed XCI:

- ✓ By **chance**
- ✓ **Primary** cause (*Xist* mutation)
- ✓ **Secondary** (acquired) causes: genetic factors affect cell proliferation and cause post-inactivation cell selection



Skewed XCI in tissue affected by mutation



In *HUWE1*, *HDAC8* and *KDM5C*:

- Female patients are **affected**
- Female patients present a **skewed XCI**
- They express the **normal X chromosome**
  - The mutations are responsible of the skewing
  - Skewing observed in the blood *may not reflect* the situation in the brain→ Skewing ingluenced by tissue specificity

But…KDM5C escapes X-inactivation!

- Mutations in females should be detrimental, irrespective of whether they are located on the active or inactive X…

- *However*, in the literature, **13/15 mutated KDM5C females** showed **skewed XCI**:
  - Escape is probably partial and incomplete
  - Selective pressure early in the embryogenesis despite the escaping of X-inactivation?
  - In mouse, KDM5C escapes X-inactivation but the degree of escaping is highly variable across different tissues
  - Skewing is also described in other escaping genes: *DDX3X, SMC1A (Fieremans et al. 2016), KDM6A (Lederer et al. 2012)*

- The mutation in the female patient we describe (p.Arg795SerFs*5) is responsible for the phenotype and probably contributes to the skewing of X inactivation

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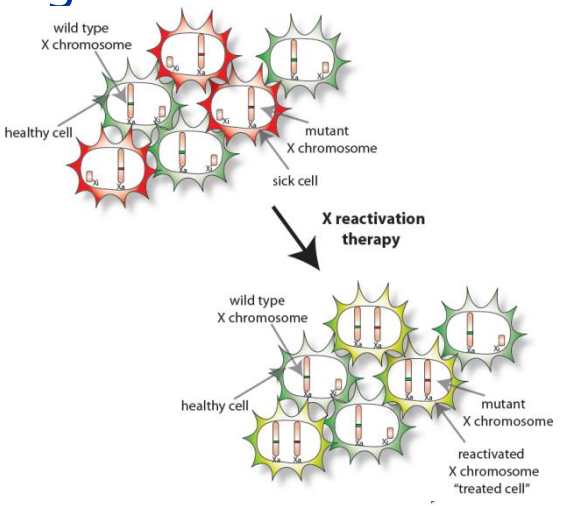
Conclusion and Perspectives

- **XCI** is not only a fascinating biological process but also an important **modifier of X-linked disease in females**
- "X-linked disease is only relevant for males" is subject of change: as in cohorts of *HUWE1* and *KDM5C* mutated females, there are now many reports linking XCI-skewing to the phenotypes in females
- **De novo variants in genes** located on the X chromosome are an important cause for ID and for skewing
- **Therapeutic approach?** Research to develop **targeted X-reactivation methods**



Thank you for your attention

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