Les CNV du chromosome X

Damien Sanlaville

Service de Génétique, CHU de Lyon

Equipe GENDEV, CRNL, INSERM, CNRS, UCBL1

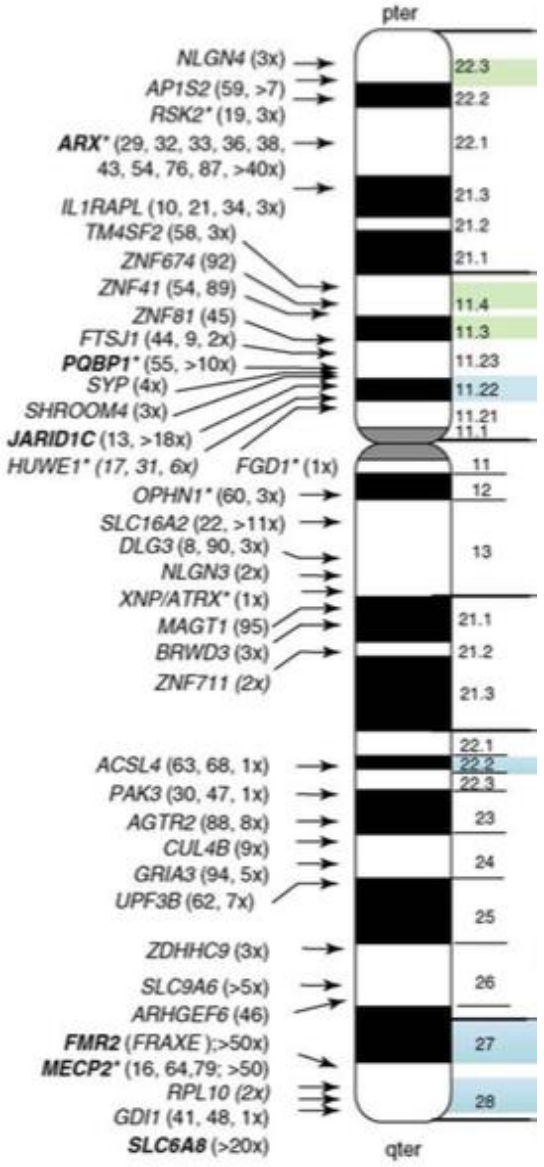
Introduction

- Chromosome X
  - 156 Mb
  - 1805 gènes connus

- 2 chromosomes X chez une fille,
- 1 chez les garçons

- Plus de 100 gènes impliqués dans la DILX

- Chez les femmes, un des deux chromosomes X est inactivé de façon aléatoire mais certaines régions échappent à l'inactivation



Gecz TIG 2009

1) Difficultés **d'analyse** des profils ACPA

Principes et exemples

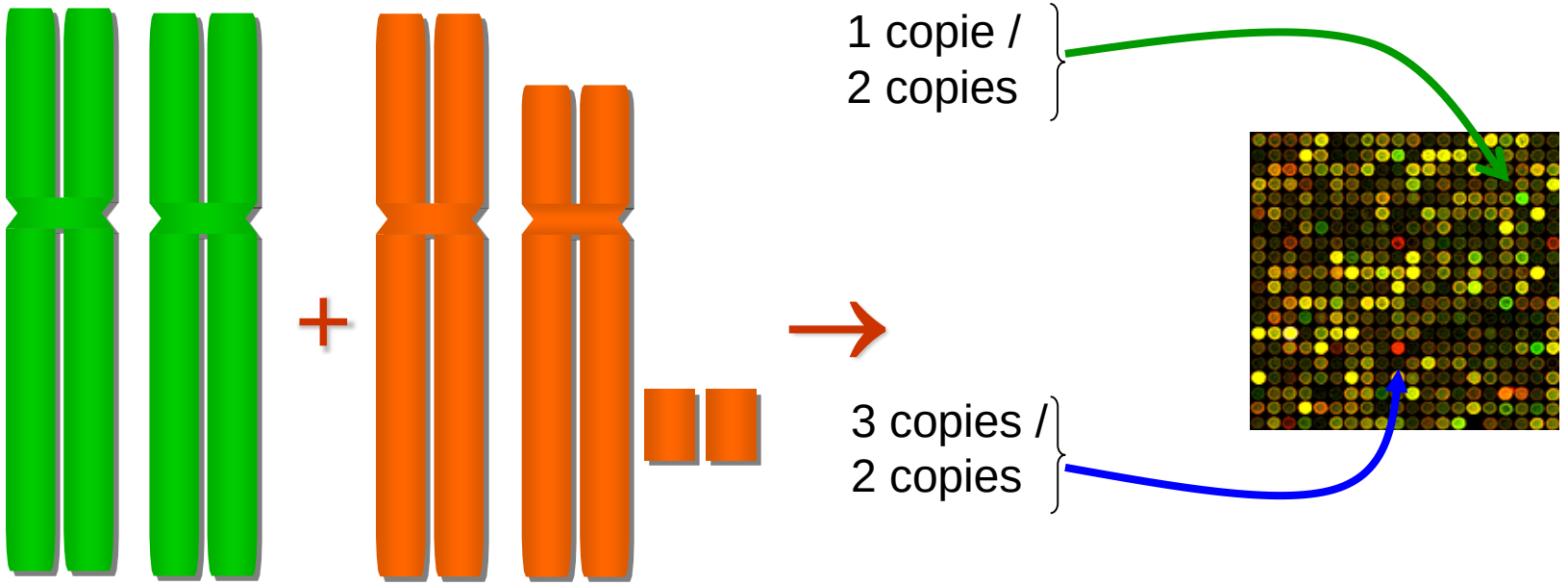
Principe de l'ACPA

Pas de culture, pas de chromosome

ADN
témoin

ADN
patient

Hybridation
Sur des oligonucléotides



D'après P. Gosset

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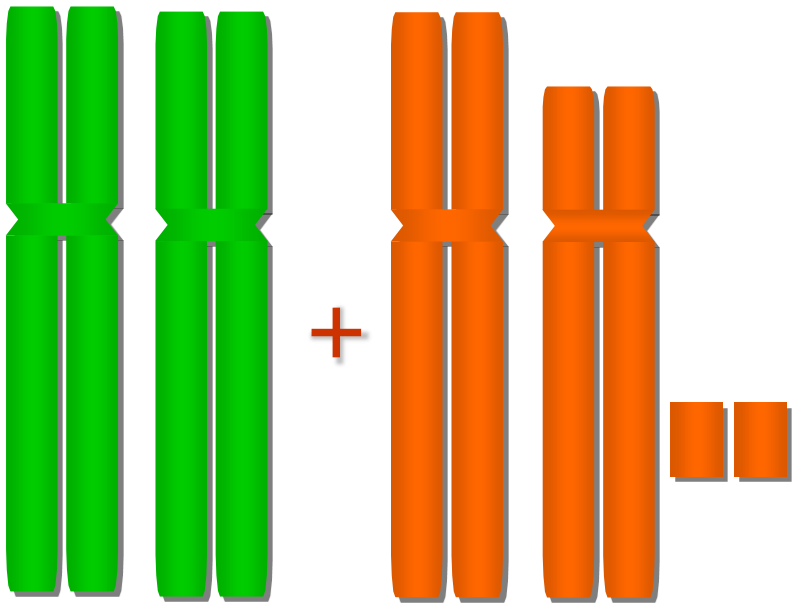
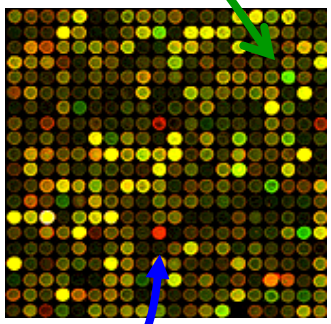


Hybridation
Sur des oligonucléotides

1 copie /
2 copies

Log2 -1



3 copies /
2 copies

Log2 +0,58

Variations du nombre de copie
CNV

D'après P. Gosset

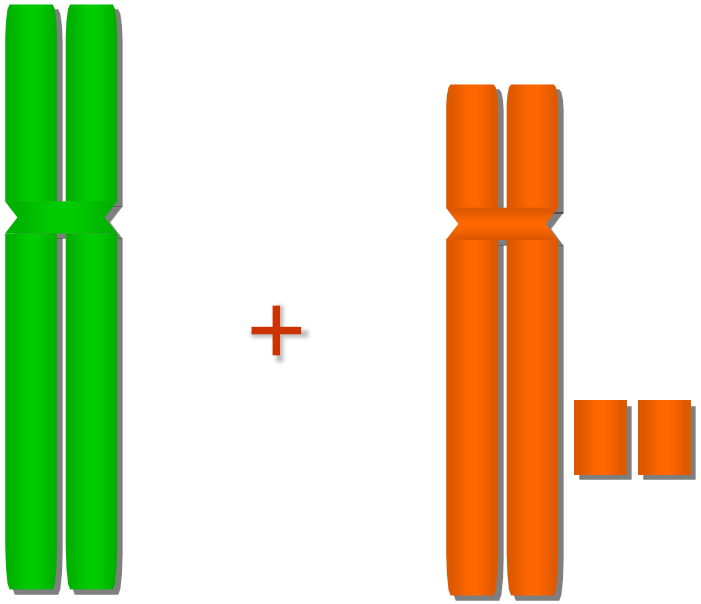
Principe de l'ACPA

Sur le chromosome X : calcul différent

Garçon contre Garçon

ADN témoin

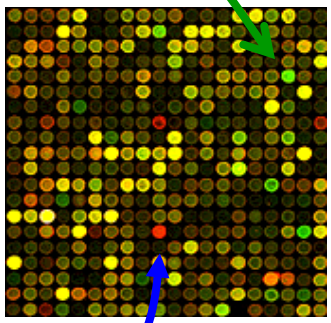
ADN patient



Hybridation
Sur des oligonucléotides

0 copie /
1 copie

Log2 -infini



2 copies /
1 copie

Log2 +1

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Hybridation
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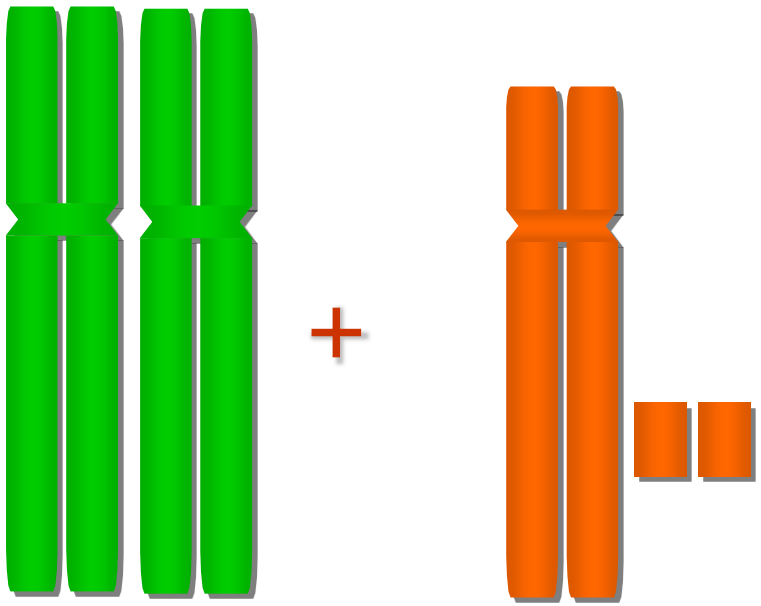
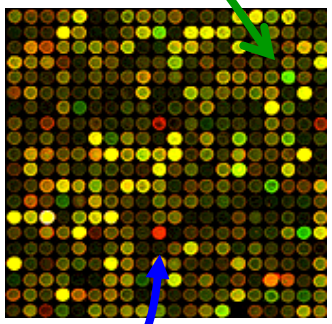


0 copie /
2 copies

Log2 -infini



2 copies /
2 copies

Log2 0

Variations du nombre de copie
CNV

D'après P. Gosset

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Fille contre Garçon

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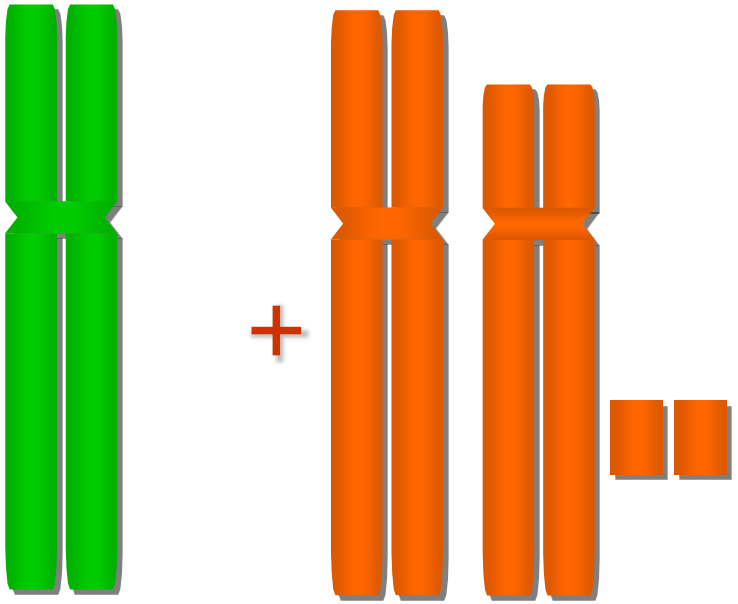
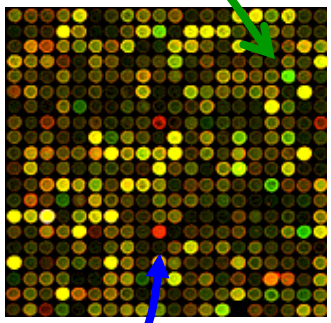
ADN patient



Hybridation
Sur des oligonucléotides

1 copie /
1 copie

Log2 0



3 copies /
1 copie

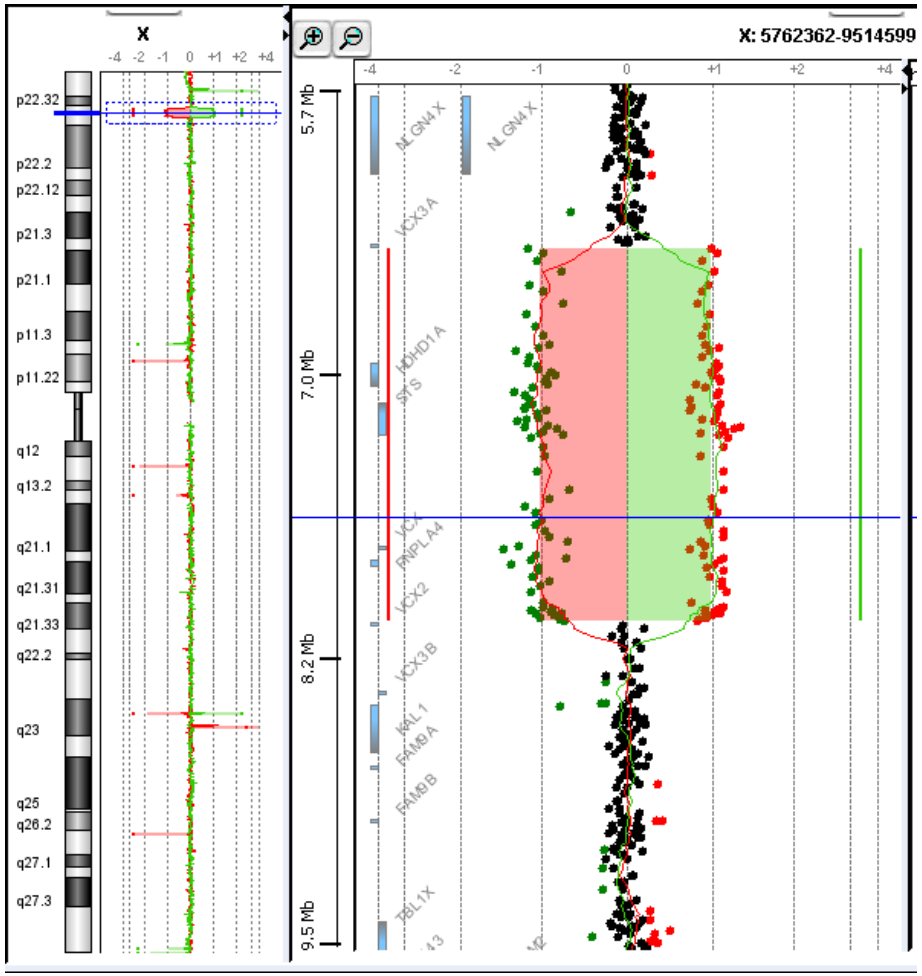
Log2 +1

Variations du nombre de copie
CNV

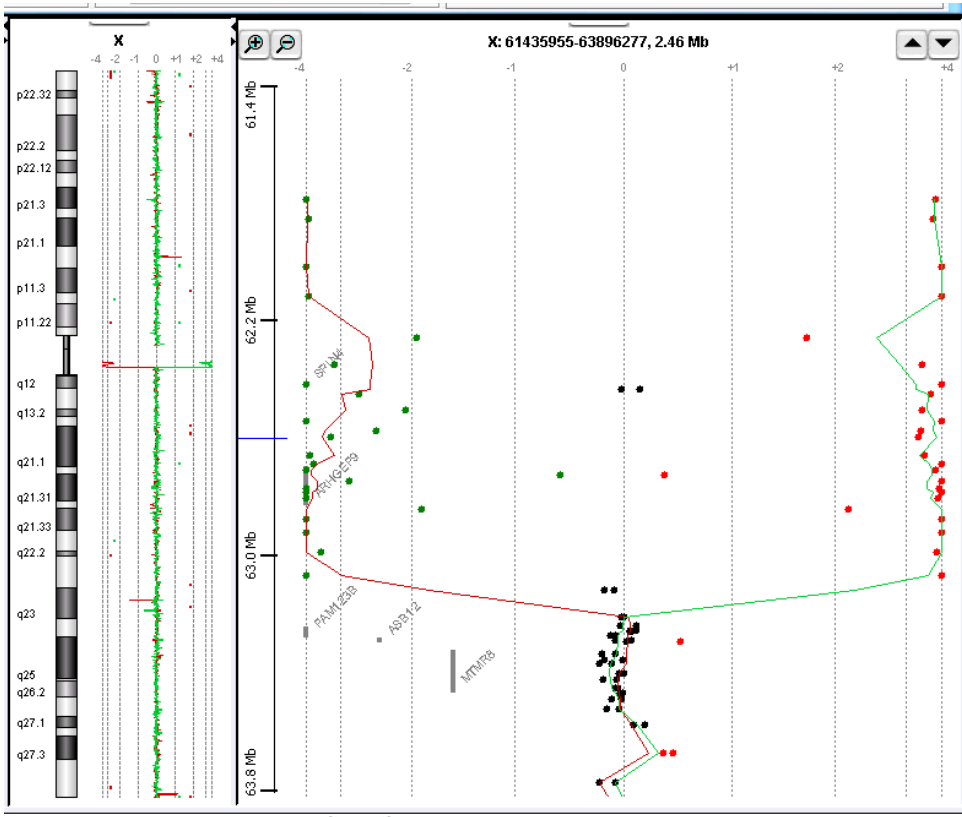
D'après P. Gosset

Deletion (sex match)

Femme (-1)



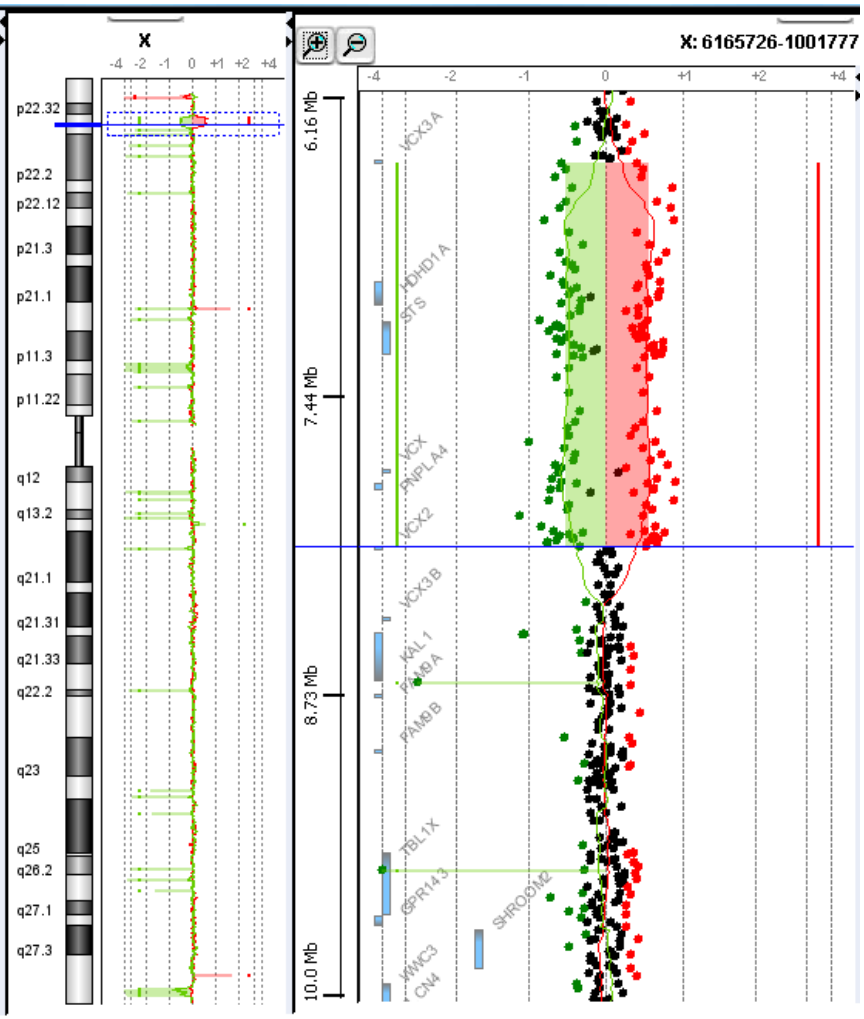
Homme (-infini)



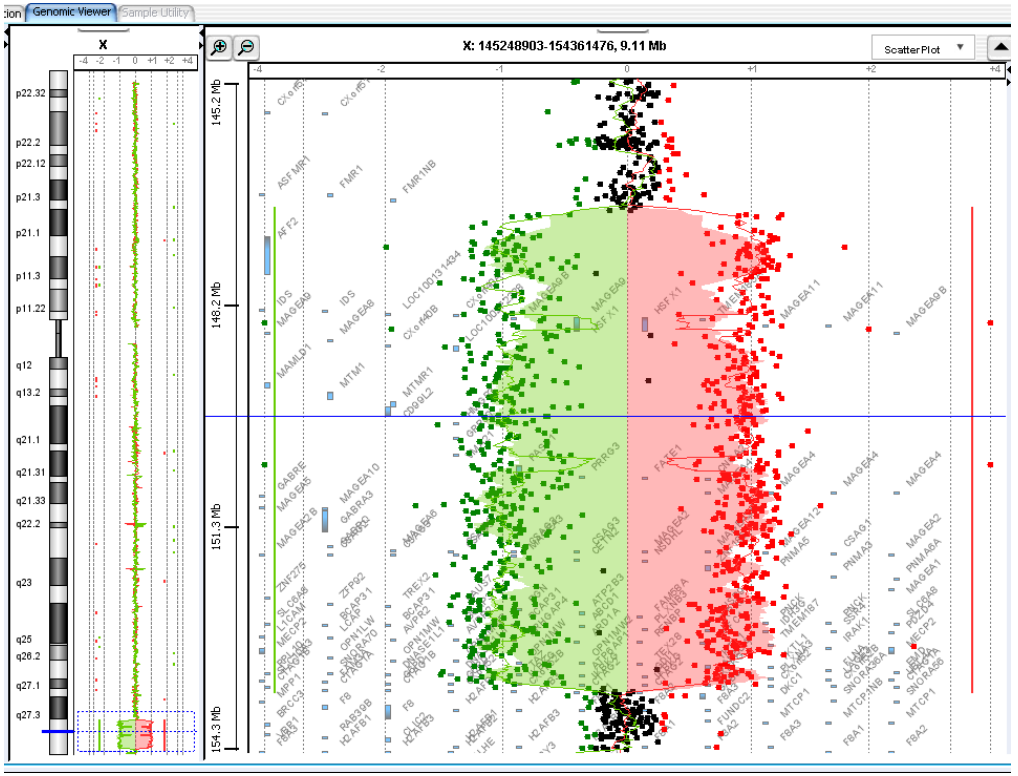
Red profile: Patient in Cyl5 – Control 1 in Cy3
Green profile: Control 2 in Cy5 – Patient in Cy3

Duplication (sex match)

Femme (+0.58)

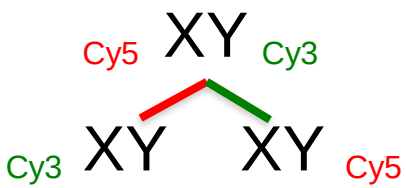


Homme (-1)

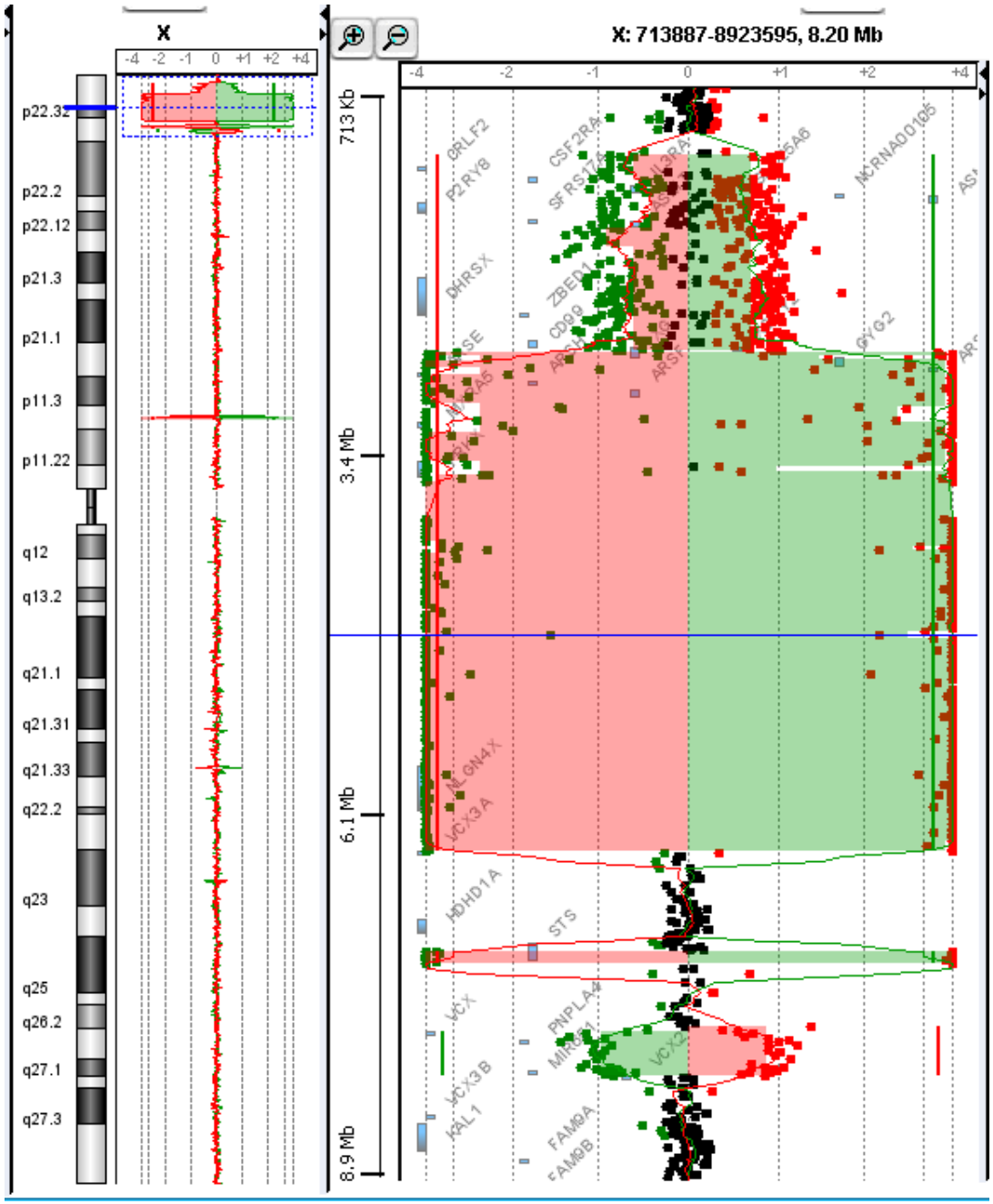


Red profile: Patient in Cyl5 – Control 1 in Cy3
Green profile: Control 2 in Cy5 – Patient in Cy3

Trio

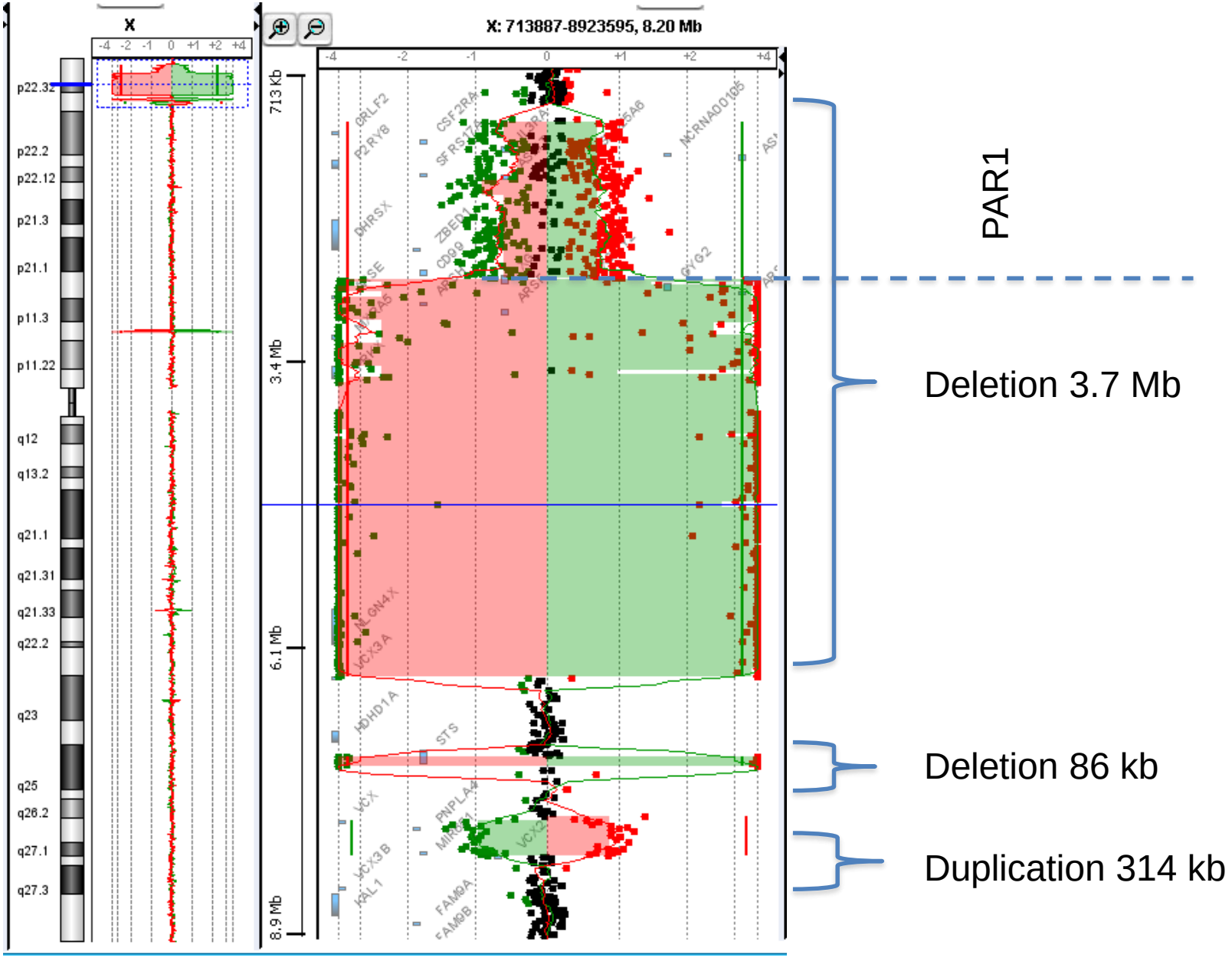


Combien de CNV ?



Red curve : Patient in Al5 – Control 1 in Al3
Green curve: Control 2 in Al5 – Patient in Al3

Ne pas oublier la région PAR



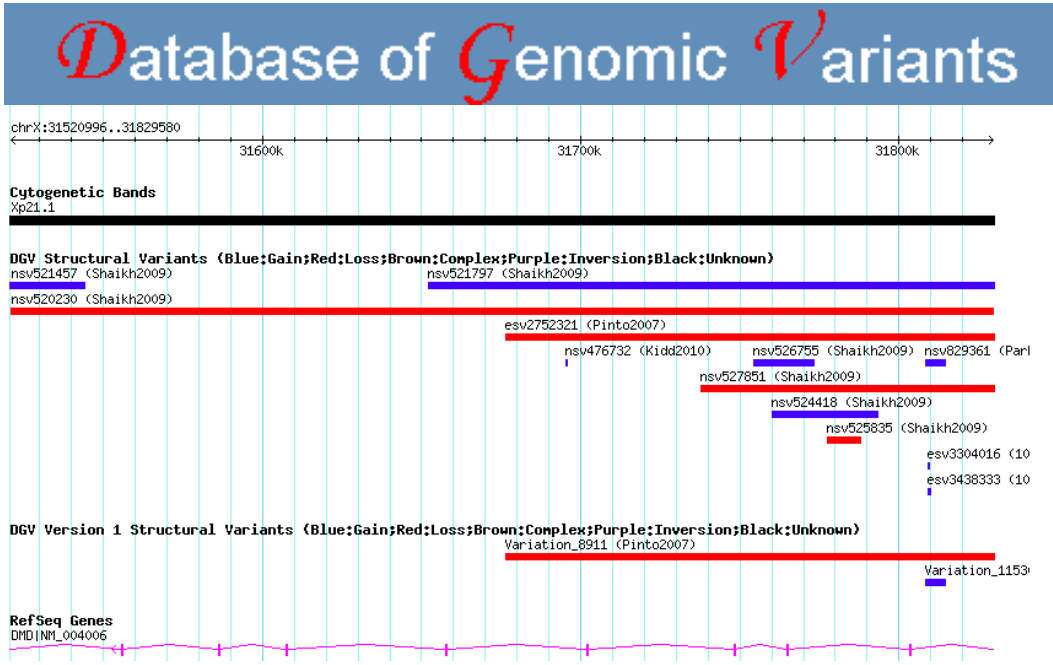
Piège 1

- Femme avec caryotype XY (mutation *RA* mutation, *duplication NROB1* (*DAX1*))
  - Une duplication aura un log2 ratio à 0

- Homme avec un caryotype XX (translocation impliquant SRY)

Piège2

- Dans les bases de données le sexe des patients est rarement renseigné



Reference	Shaikh_et_al_2009
Pubmed ID	19592680
Accession Number(s)	nsv520230

Frequency	**Sample Size**	2026
	Observed Gain	0
	Observed Loss	1
	Observed Complex	0
	Frequency	n/a

Homme/ Femme ?

Message

Une bonne analyse des profils

Permet

De faire une bonne interprétation clinique

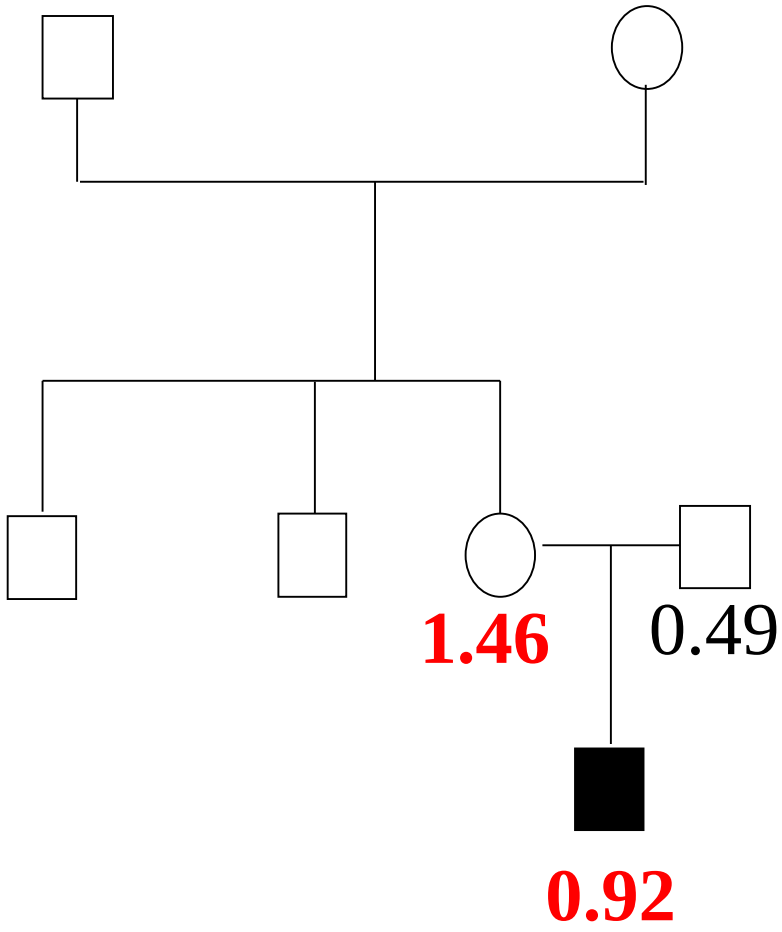
2) **Interpretation** des CNVs

- L'interprétation dépend de
  - Perte ou gain
  - Taille
  - Contenu en gène OMIM
  - Corrélation entre la clinique et les autres cas publiés
  - Hérédité : *de novo* ou hérité
  - …

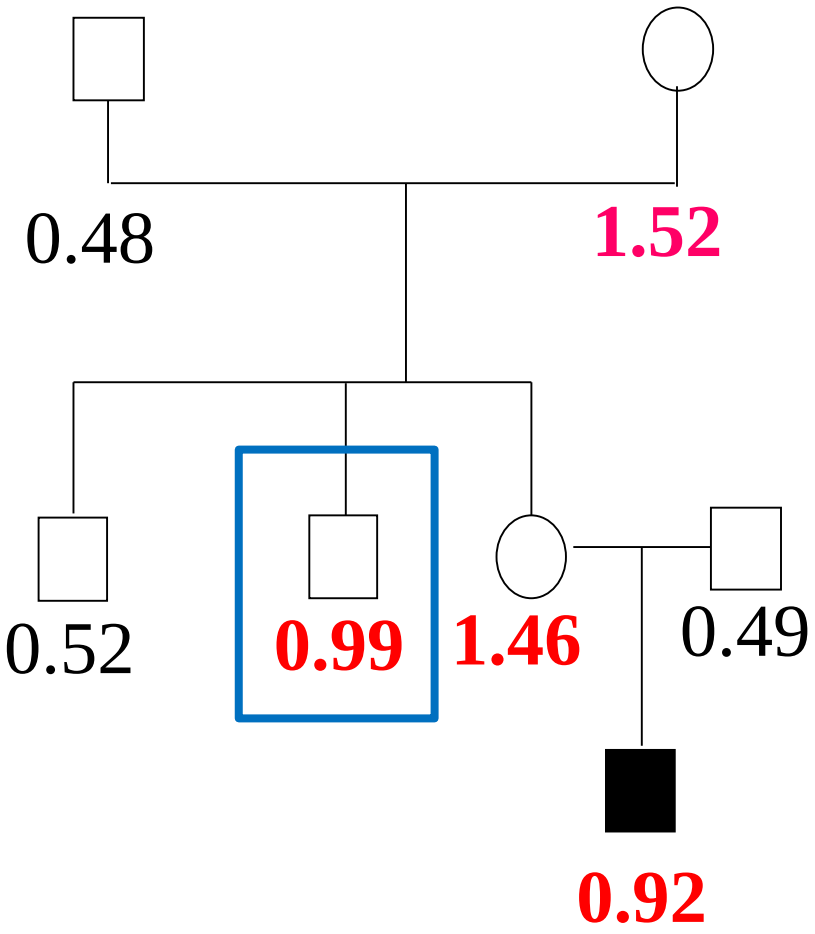
Transmission

- En cas de VOUS : étude des parents
- Les CNVs hérités d'un parent sain sont généralement considérés comme bénins
- Mais pour le chromosome X…
- Généralement les CNVs sur le chromosomes X chez un garçon sont hérités de la mère

Importance de l'arbre généalogique



CNV hérité de la mère



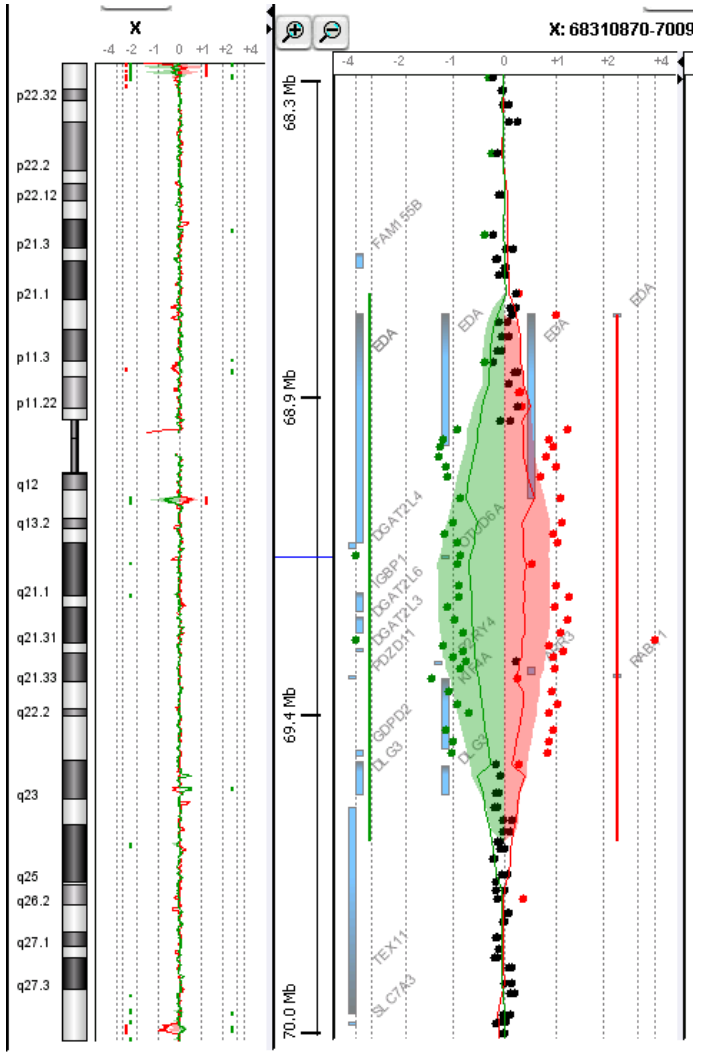
Cas favorable

Probablement bénin

596,6 kb Xq13.1 duplication

- 2008
- Garçon
- RCIU
- Microcéphalie
- Cardiopathie
- Malformations rénale et cérébrale
- Déficience intellectuelle

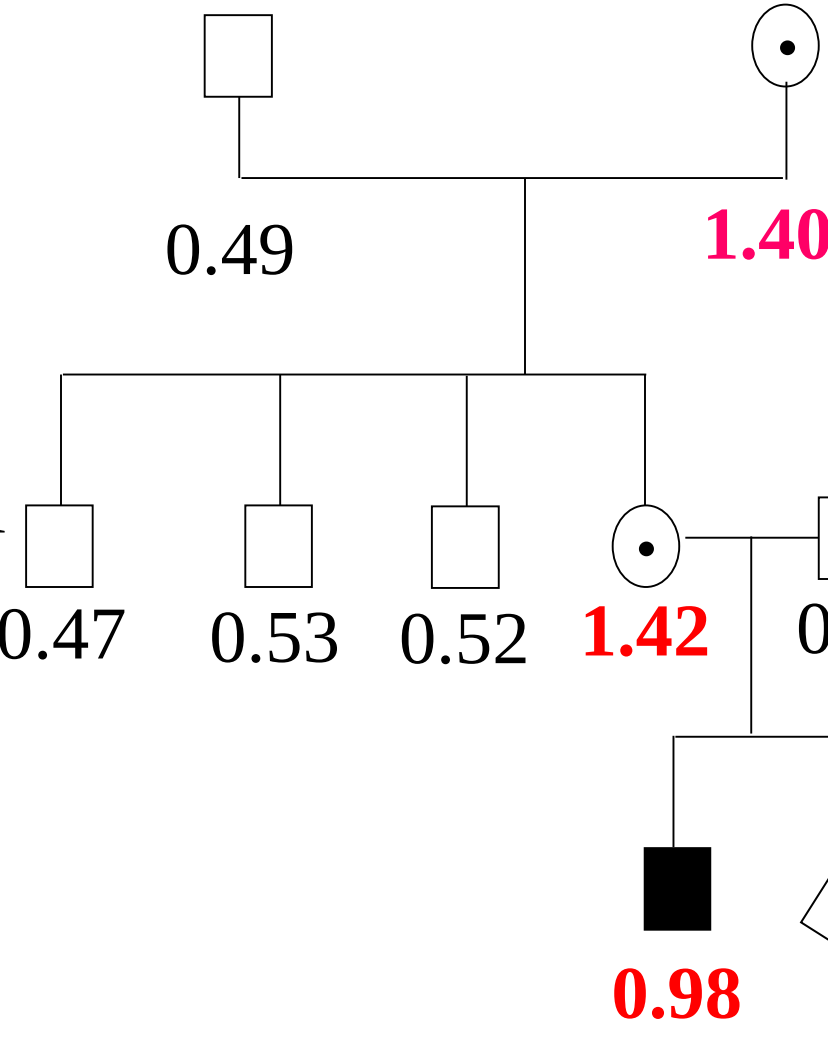
- aCGH : VOUS
- Dup jamais décrite



Dye swap experiment

Patient du Dr Rossi

Famille non informative



Conclusion ?
Pathogène ?

Les parents demandent un DPN …

M. Rossi, CHU Lyon

3) Utilité de l'étude de l'inactivation du chromosome X

- Les femmes ont deux chromosomes X

- Un des deux chromosomes X est inactivé de façon aléatoire

- **2 points importants**
  - **Un défaut d'inactivation conduit à une disomie fonctionnelle du chromosome X**
  - L'inactivation biaisé d'un chromosome X peut être vu chez une femme sans phénotype

Disomie fonctionnelle (DF)

- Disomie fonctionnelle : expression en double dose de gènes exprimés normalement en simple dose
- Chez les hommes :
  - Duplication : DF
  - Grande DF : FCS
- Chez les femmes des remaniements de structure peuvent entrainer un phénotype (t(X;A)).

DF Xp

Functional Disomy of Proximal Xp Causes a Distinct Phenotype Comprising Early Hypotonia, Hypertelorism, Small Hands and Feet, Ear Abnormalities, Myopia and Cognitive Impairment

Matthew Hunter,[1,2] Damien Bruno,[1,2] and David J. Amor[1,2,3]*

AJMG 2009

- Signes clés
  - Hypotonie
  - Retard de dévelopement sévère
  - DI
  - Dysmorphie faciale
  - Epilespsie

DF Xq

Review

Open Access

Distal Xq duplication and functional Xq disomy

Damien Sanlaville*†1,2, Caroline Schluth-Bolard†1,2 and Catherine Turleau†3

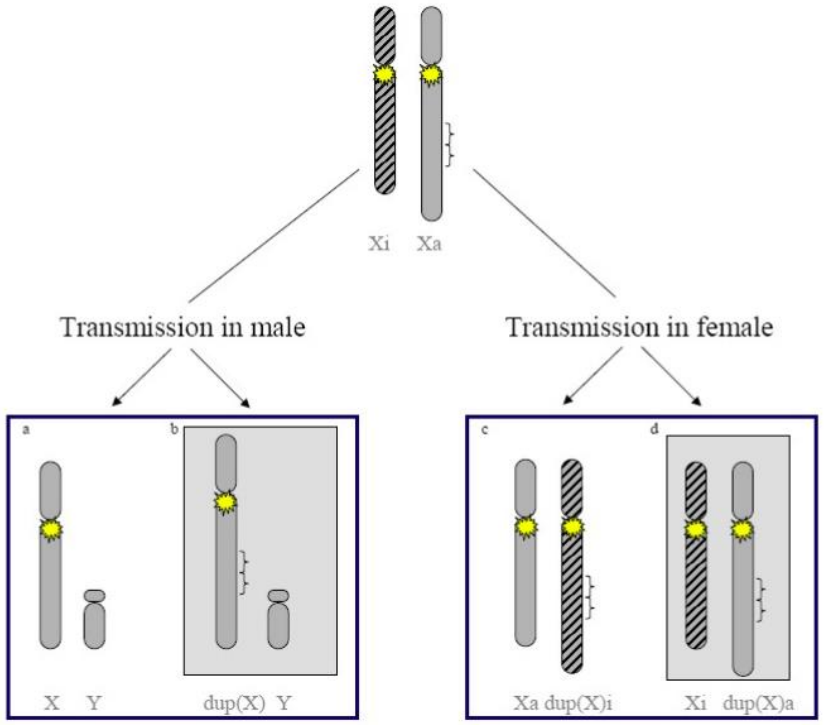


Figure 1
Schematic representation of a Xq duplication transmission. Active X and Y chromosomes are in grey, inactive X is striped in black. A yellow star represents XIC. a) normal XY chromosomes; b) Xq duplication in male leading to Xq functional disomy, associated with an abnormal phenotype; c) Xq duplication in female with inactivation pattern skewed towards the duplicated X, associated with a normal phenotype; d) Xq duplication in female with inactivation pattern skewed towards the normal X leading to Xq functional disomy, associated with an abnormal phenotype.

Table 1: Comparison of clinical symptoms observed in three groups of patients with Xq duplications.

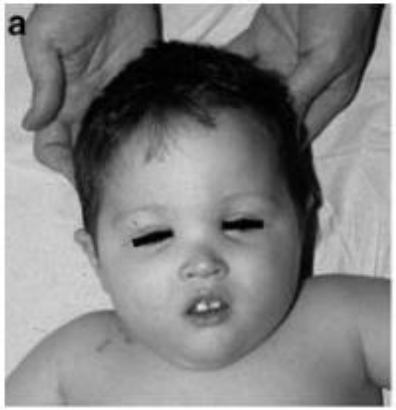
		Xq21q24 DF*	Xq26.3qter DF**	MECP2 duplication ***
Number of cases		12	21	47
Caesarean section		0/1	8/14	nr
Growth				
	Growth retardation	9/10	17/19	2/3
	Microcephaly	4/5	19/19	5/39
Facial dysmorphism				
	Prominent metopic suture	nr	5/15	nr
	Epicanthus fold	nr	5/6	1/8
	Large ears	nr	9/12	4/20
	Small mouth	nr	11/13	6/20
	Abnormal palate/maxillar alveolus	nr	13/14	nr
	Facial hypotonia	nr	3/4	19/28
Neurologic outcome				
	Hypotonia		19/19	29/32
	Developmental delay		19/19	47/47
	Absent or delayed speech		11/14	46/47
	Never walked or limited walking		12/14	21/34
	Spacticity		3/4	17/21
	Seizures		6/16	22/42
Malformations				
	Hypoplastic genitalia/cryptorchidism	11/11	15/19	5/10
Others				
	Severe feeding problems		10/14	15/29
	Gastroesophageal reflux		4/7	13/17
	Constipation		5/5	nr
	Small feet		8/8	nr
	Digital abnormalities		13/19	6/20
	Recurrent infections		15/17	33/40

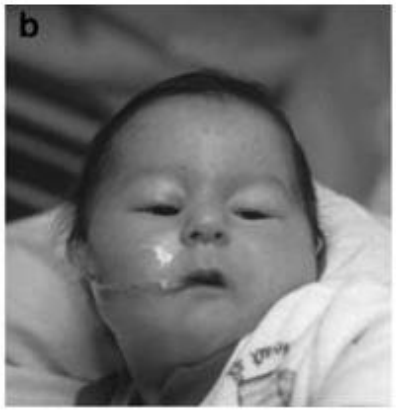
nr : not recorder

* modified from Cheng SF *et al.*[1] and Gabbett *et al.*[19]

** modified from Sanlaville *et al.*[5] and Smyk *et al.*[20]

*** modified from Ariani *et al.*[27], Meins *et al.*[28], Van Esch *et al*[26]., Freiz *et al.*[29], del Gaudio *et al.*[30], Madrigal *et al.*[31], and Smyk *et al.*[20].





3) Utilité de l'étude de l'inactivation du chromosome X

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- Un des deux chromosomes X est inactivé de façon aléatoire

- **2 points importants**
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  - **L'inactivation biaisé d'un chromosome X peut être vu chez une femme sans phénotype**

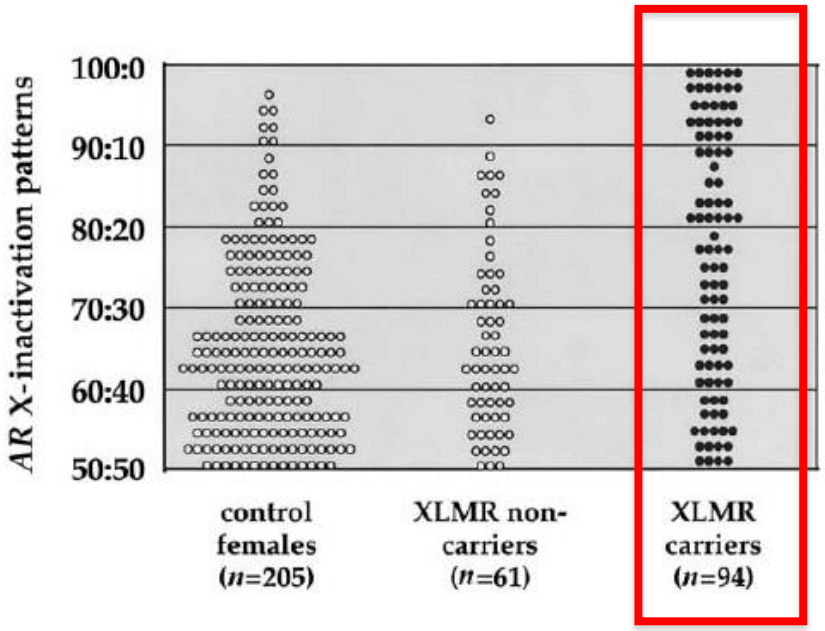
Femmes et biais d’inactivation

Skewed X-Chromosome Inactivation Is a Common Feature of X-Linked Mental Retardation Disorders

AJHG 2002

Robert M. Plenge,[1,*] Roger A. Stevenson,[2] Herbert A. Lubs,[3] Charles E. Schwartz,[2] and Huntington F. Willard[1]

b



Skewed X-Inactivation Patterns in XLMR Carriers

X-Inactivation Pattern	Frequency of Skewed X Inactivation[a] (%)		
	Female Control Subjects	XLMR Noncarriers[b]	XLMR Carriers[c]
≥90:10	3	2	30
≥80:20	9	15	48
≥70:30	30	41	63

X Chromosome–Inactivation Patterns of 1,005 Phenotypically Unaffected Females

AJHG 2006

James M. Amos-Landgraf,* Amy Cottle, Robert M. Plenge,† Mike Friez, Charles E. Schwartz, John Longshore, and Huntington F. Willard

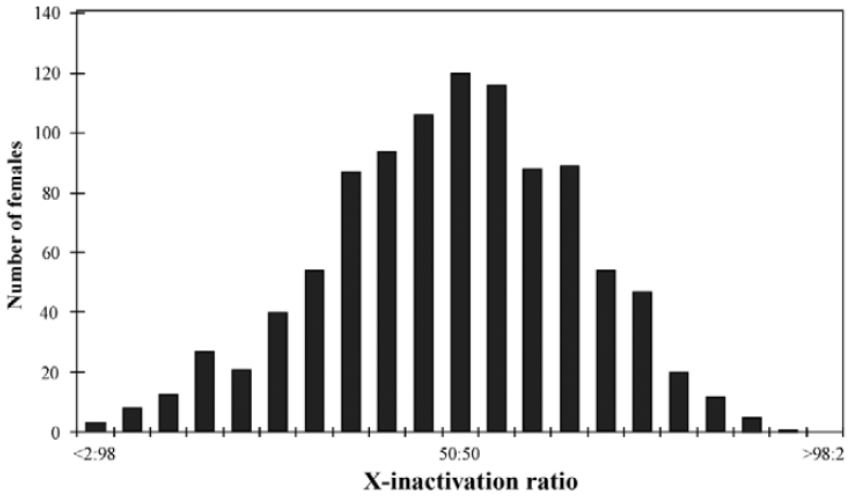


Figure 1. The X-inactivation patterns of 1,005 females were assigned to 21 "bins" with a range of <2:98 to >98:2, with increments of 5%. These are normally distributed, with the mean of the distribution residing at 49:51 and the median at 50:50 (SD of the mean = 17).

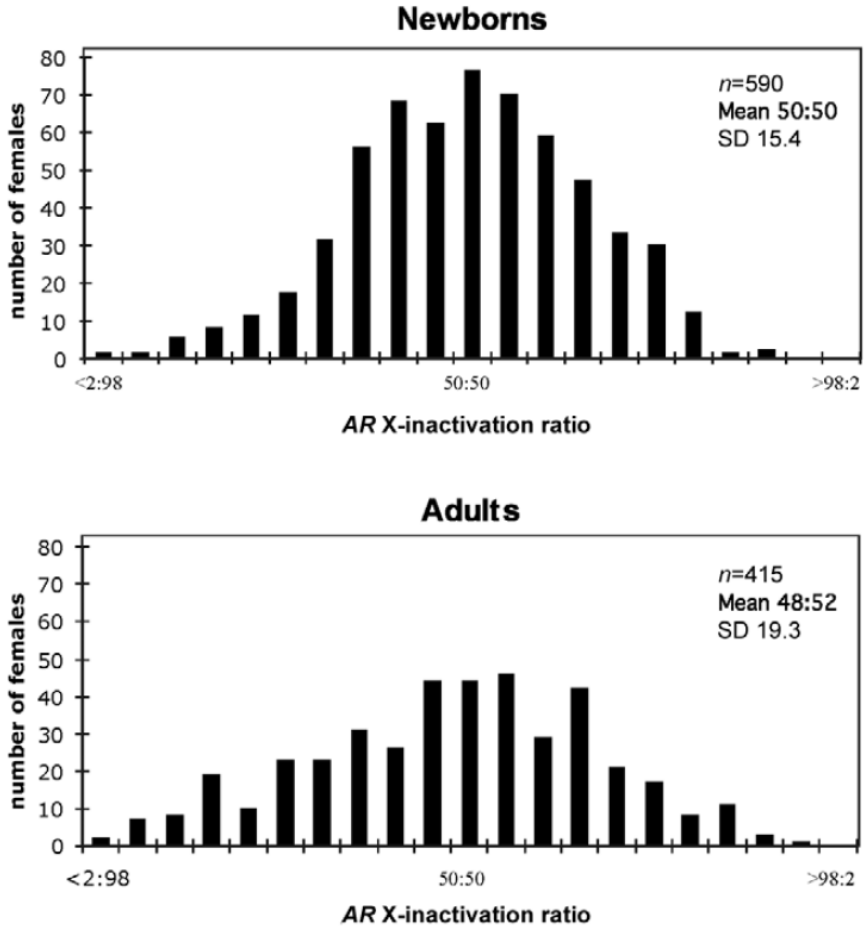


Figure 2. Distributions of X-inactivation ratios of both newborn samples (n = 590) and unaffected adult females (n = 415).

Copy Number Changes on the X Chromosome in Women with and without Highly Skewed X-Chromosome Inactivation

CGR 2012

V. Jobanputra[a] B. Levy[a] A. Kinney[b] S. Brown[c] M. Shirazi[a] C. Yu[d]
J. Kline[b, e, f] D. Warburton[d]

Table 2. Copy number changes[a] for women with and without highly skewed (≥85%) X-chromosome inactivation

N= 90	Skewing ≥85%	Skewing 50 to <75%
Number of women	45	45
Deletions	2	2
Deletions with genes	1	0
Women with deletions	2	1
Women with deletions with genes	1	0
Duplications	2	3
Duplications with genes	2	0
Women with duplications	2	3
Women with duplications with genes	2	0
Total CNCs	4	5
Total CNCs with genes	3	0
Women with one or more CNCs	3	4
Women with one or more CNCs with genes	2	0

[a] Defined as changes ≥100 kb with ≤6 kb mean distance between markers and ≤50% overlap with normal copy number variants according to http://projects.tcag.ca/variation.

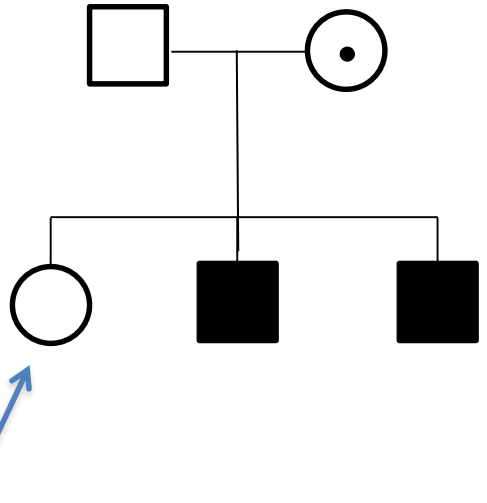
Conclusion: HSXI in a blood sample is rarely due to X-chromosome copy number changes detectable by microarray.

« Grand » CNV > 5-10 Mb
Biais d'inactivation X inactivation

« petit » CNV < 5 Mb ?
biaisé ou non

Un exemple

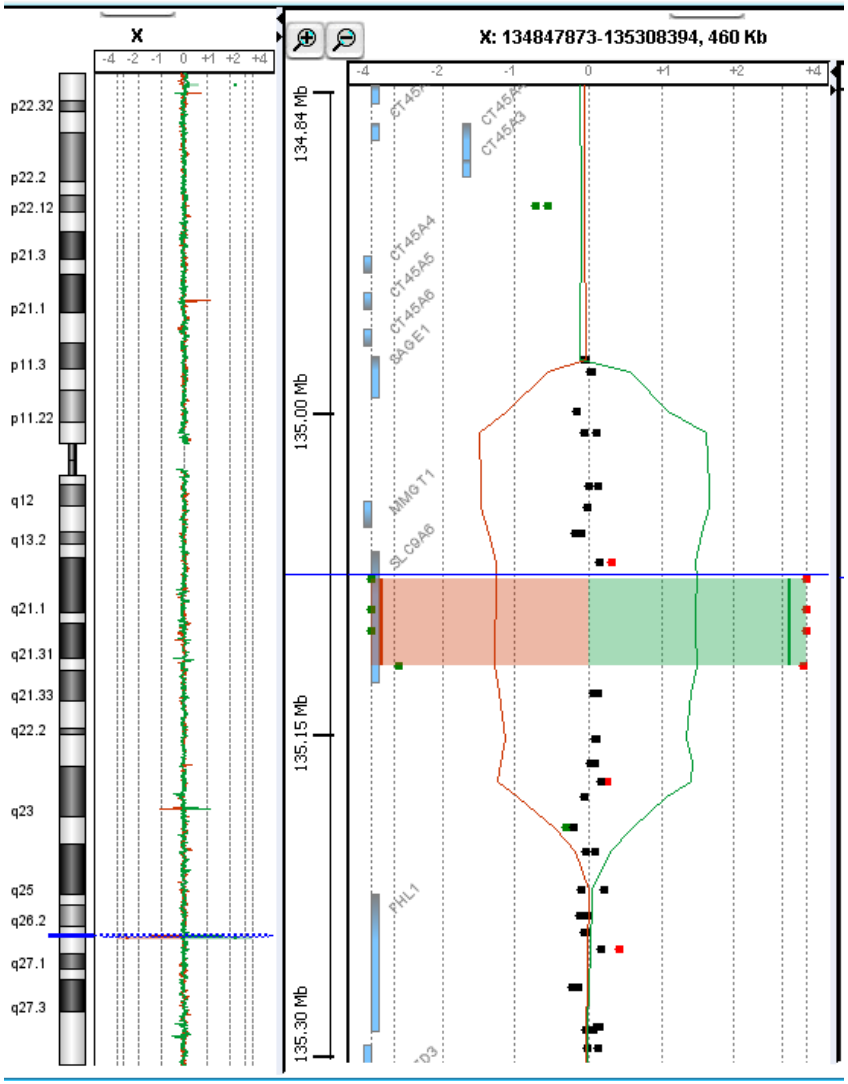
- Deux frères nés en 1990 et 1994
  - Grossesse normal
  - Nés à terme
  - Développement normal jusqu'à 6 mois
  - A 6 mois : microcéphalie (-2,5 SD)
  - Retard de développement majeur
  - Marche à 2 ans
  - spasticité
  - Absence de langage,
  - Epilepsie



Conseil génétique

Dr Till, CHU Lyon

Résultat ACPA

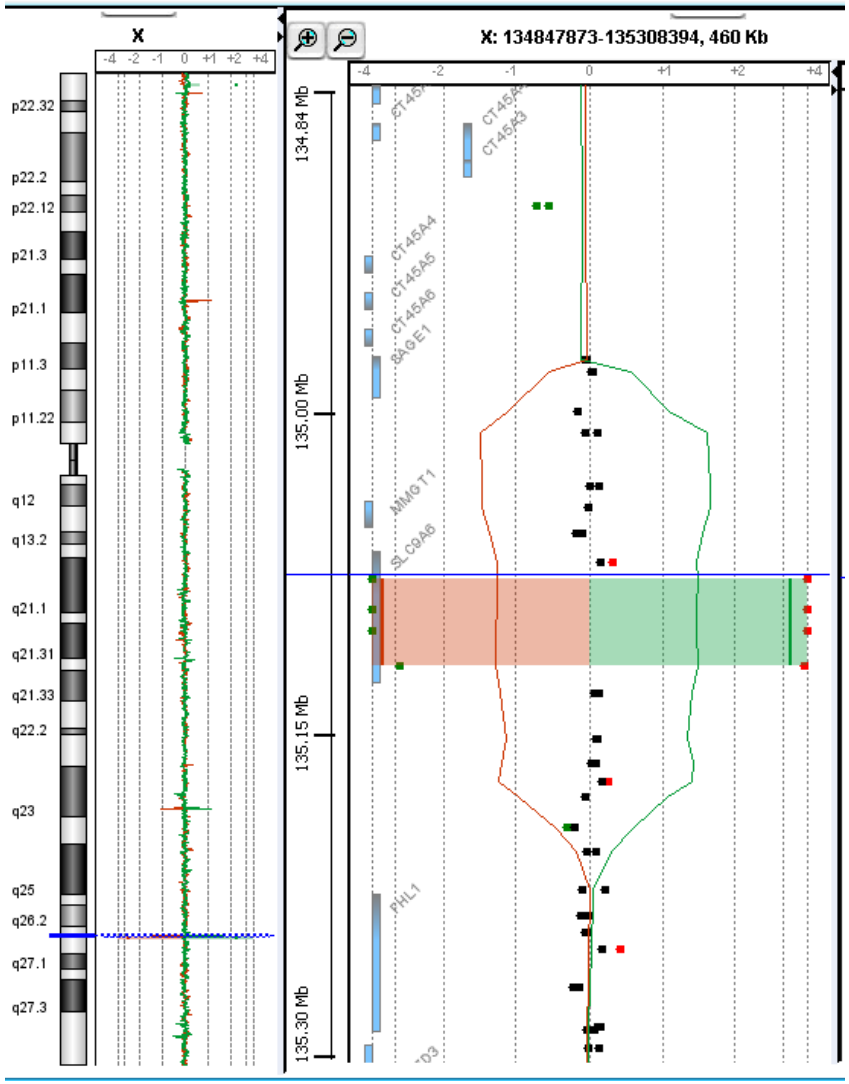


Dye swap

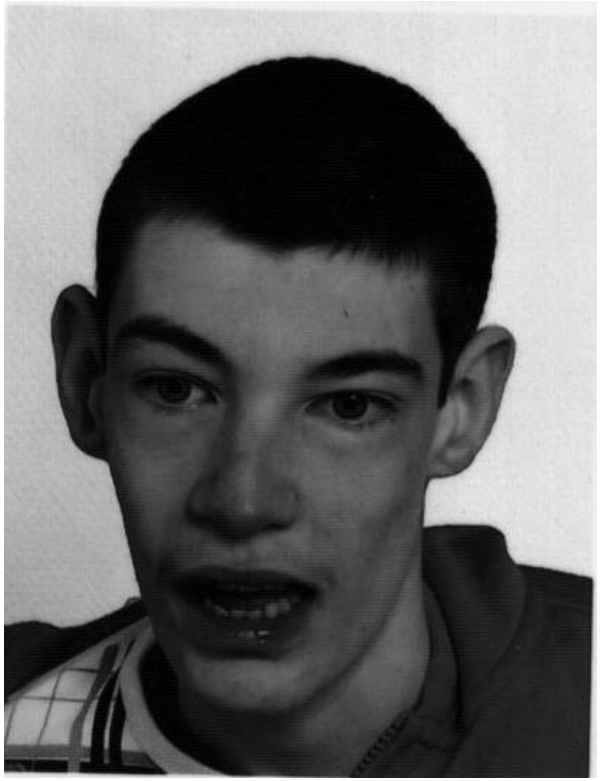
Délétion de 40 kb sur le chromosome X comprenant en partie le gène of *SLC9A6*

Délétion de 40.57 kb : 135,080,988 to 135,121,564 pb (hg19)

aCGH result

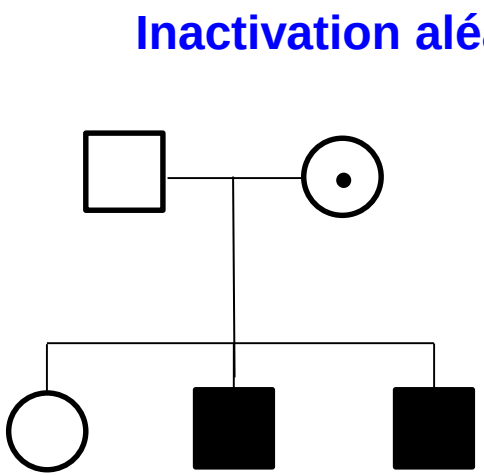


Diagnosis de Christianson syndrome



Etude de l'inactivation du chromosome X

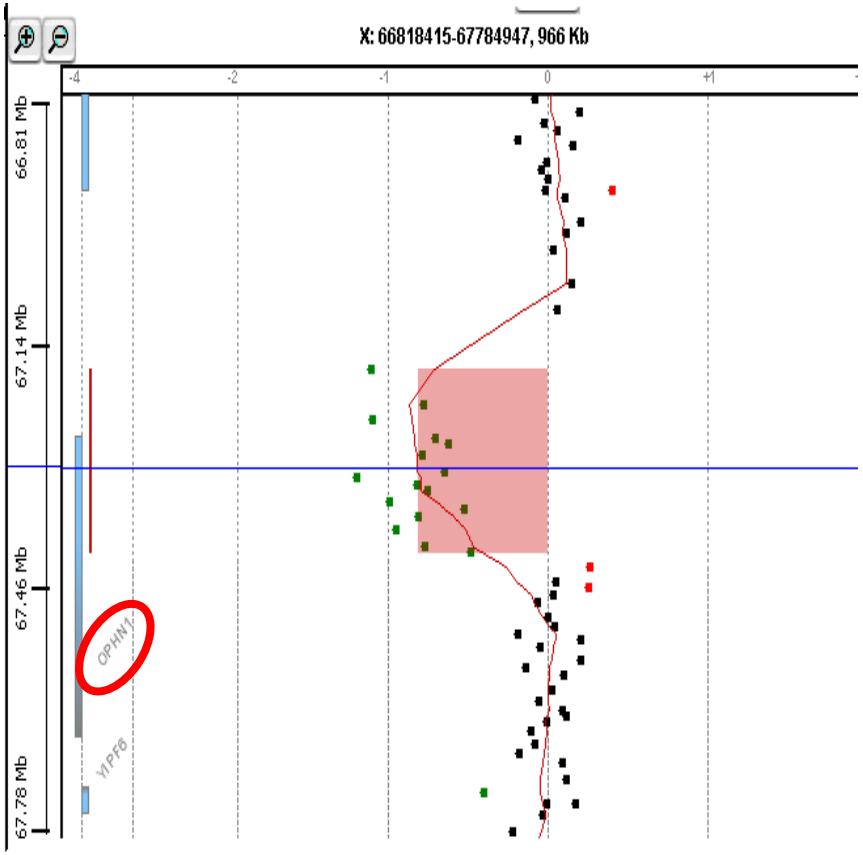
- Inactivation aléatoire chez la mère et pourtant elle est
  - En BS
  - conductrice



- Heureusement la fille n'est pas conductrice

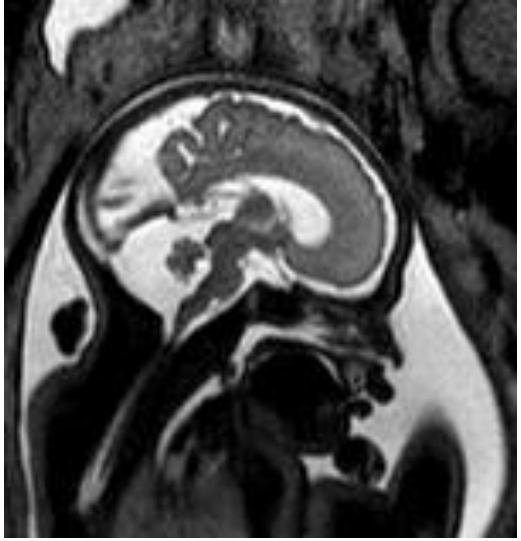
Autre situation en prénatal

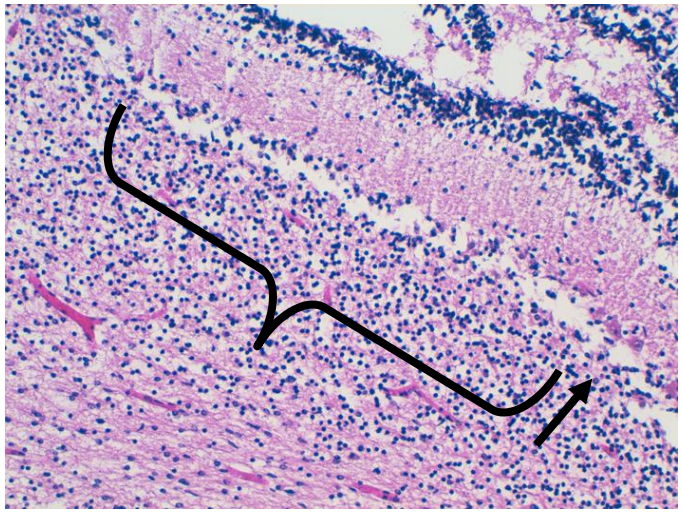
- 3° trimestre : macrosomie, dilatation biventriculaire, hypoplasie cérébelleuse
- Caryotype fœtal : 46,XX
- Décision IMG à 35 SA Parents
- Autopsie fœtale :
  - Hyperplasie thymique
  - Hypoplasie du vermis
  - Diminution des cellules de Purkinje
  - Hétérotopie

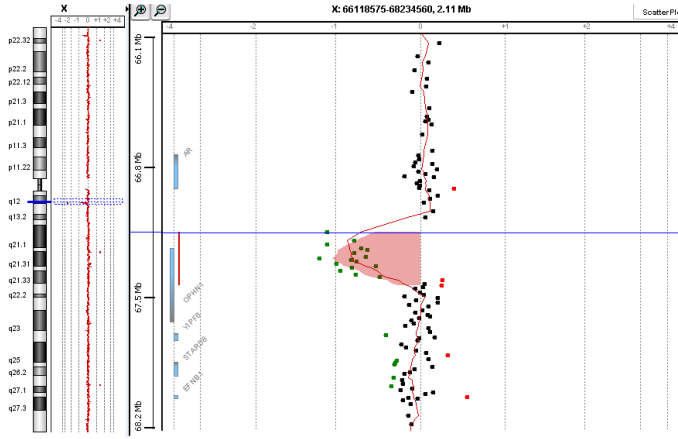


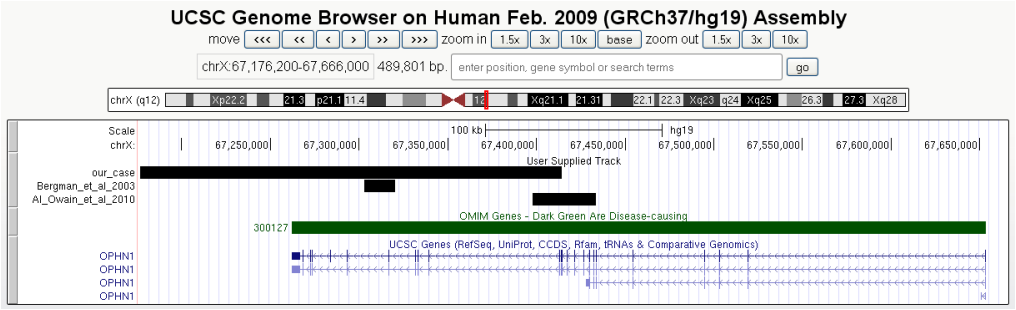
ACPA : 237 kb délétion on Xq12, comprenant le gène *OPHN1*

Dr A. Vasiljevic, CBPE, HCL









Délétion ***de novo***,

Etude Microsatellite : délétion sur le chromosome X d'origine paternelle

Biais d'inactivation complet

Délétion sur le chromosome X paternel, mais inactivation du chromosome X maternel

Biais d'inactivation défavorable chez une fille

Rocas et al, EJMG 2013

Message

Biais d'inactivation chez une mère

N'est pas obligatoirement associé au caractère pathogène d'un CNV mais l'inverse est vrai !

4) Régions PAR

- Des régions du chromosome X échappent au phénomène d'inactivation
  - PAR 1 (Xp): 2.5 Mb, comprend plusieurs gènes dont le gène *SHOX*
  - PAR2 (Xq): 400 kb (*IL9R*)
  - Environ 100 gènes le long du chromosome X

Funct Integr Genomics (2013) 13:285–293
DOI 10.1007/s10142-013-0323-6

ORIGINAL PAPER

Copy number variation-based polymorphism in a new pseudoautosomal region 3 (PAR3) of a human X-chromosome-transposed region (XTR) in the Y chromosome

Avinash M. Veerappa · Prakash Padakannaya · Nallur B. Ramachandra

Table 2 Shared features of pseudoautosomal regions 1 and 2 with XTR (PAR3)

Shared features	PAR1	PAR2	XTR (PAR3)
Sequence homology	>98 %	>98 %	>98 %
Size	2.6 Mb	320 kb	~2.5 Mb
Has allelic homologues on both X and Y	Yes	Yes	Yes
Formed due to duplication	No	Yes	Yes
Gene escape inactivation	Yes	Yes	Yes
Recombination	Yes	Yes	Yes
Recombination frequency	Obligatory per meiosis	1 in 40 times	1 in 40 times
Genes	24	5	3

Il n'est pas clair sir les CNV de PAR3 sont pathogènes

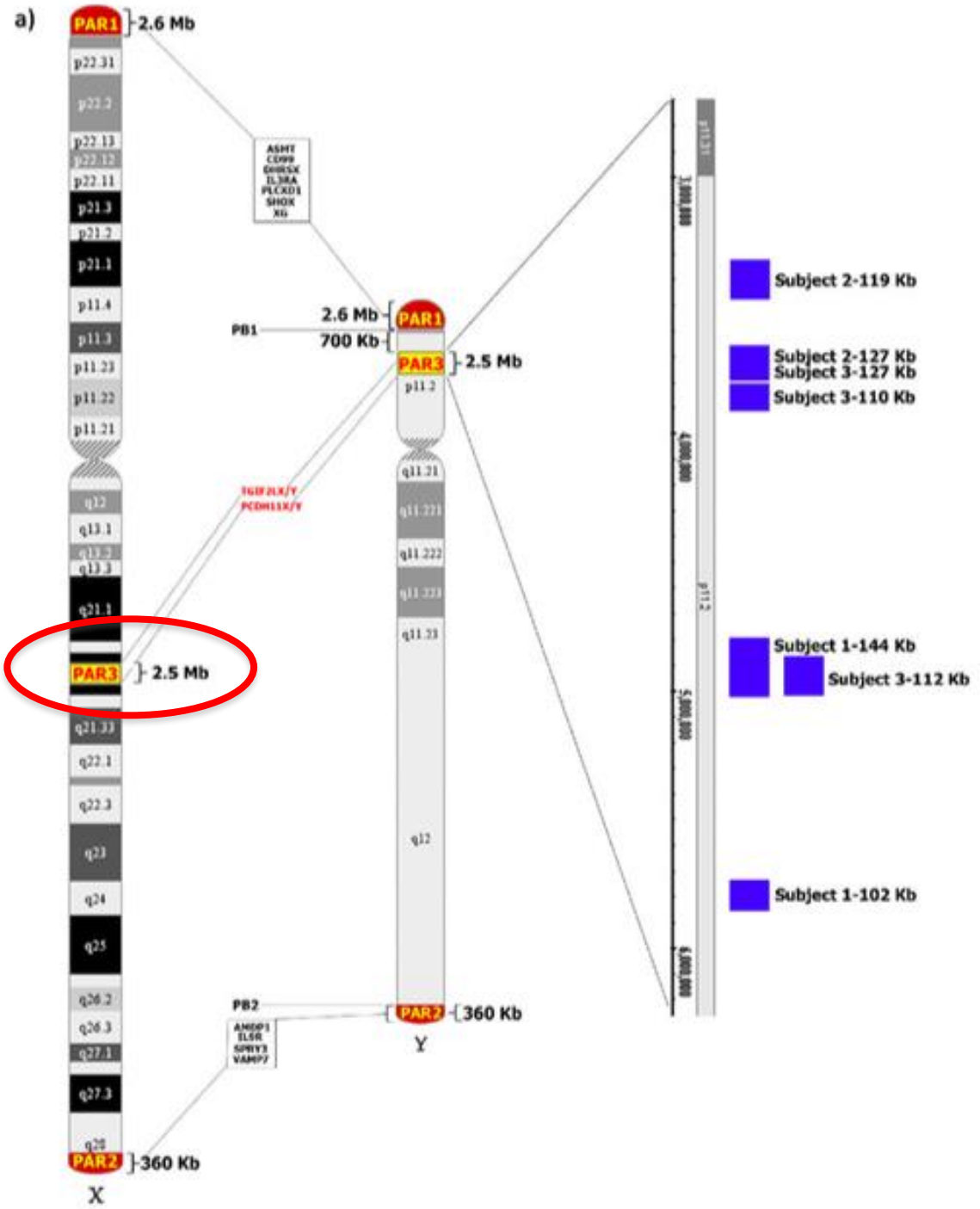


Fig. 1 Proposed PAR3 blocks on both the X and Y chromosomes indicating the size, genes, location, and the pseudoautosomal boundaries together with the already identified PAR1 and PAR2 features. Schematic representation of Yp11.2 blocks of three female subjects recombined with Xq21.3 regions of the X chromosome as revealed by whole-genome scan is also presented

5) CNV récurrents sur le chromosome X

Interprétation médicale dépend

Du sexe

De la localisation : PAR ou non PAR

CNVs récurrents…

- Description de quelques CNVs récurrents sur le chromosome X : NAHR mais pas toujours

- Identification de nombreux CNVs, essentiellement des gains (duplications) chez les garçons, non récurrents

Wagenstaller et al.,

Gijsbers et al.

Whibley et al.

Giorda et al.,
Froyen et al.

Froyen et al.

Bedeschi et al.

Koolen et al.

Mimault et al.,

Koolen et al.

Koolen et al.

Solomon et al.,
Rio et al.

Van Esch et al.,

Segmental Duplications Associated with XLID

Greenwood Genetic Center, updated Nov. 2012

Wagenstaller et al.,

Gijsbers et al.

Whibley et al.

Giorda et al.,
Froyen et al.

Froyen et al.

Bedeschi et al.

Koolen et al.

Mimault et al.,

Koolen et al.

Koolen et al.

Solomon et al.,
Rio et al.

Van Esch et al.,

Xp22.31 Wagenstaller at al. (Am J Hum Genet 81:738, 2007)
VCX3A, HDHD1A, STS, VCX, PNPLA4, and VCX2

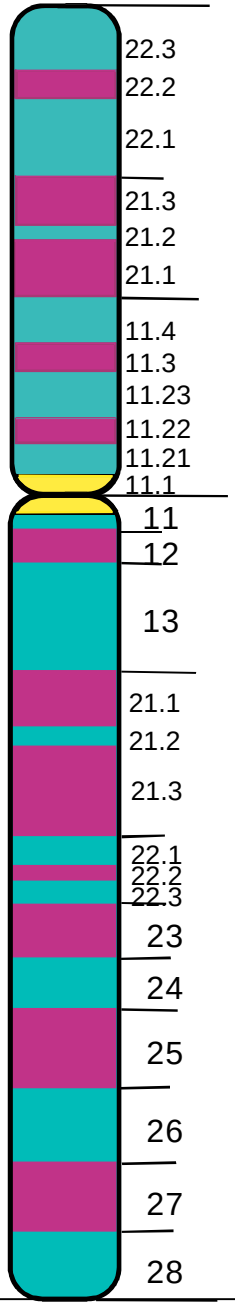
Xp11.22 Giorda et al. (Am J Hum Genet 85:394–400,2009)
PORCN, PQPB1,BMP15,SHROOM4

Xp11.22. Froyen et al. (Am J Hum Genet 82:432, 2008)
RIBC1, HSD17B10 and HUWE1.

Xq21q22. (Mimault et al.: Am J Hum Genet 65:360, 1999)
PLP1

Xq28. Van Esch et al. (Am J Hum Genet 77:442, 2005)
MECP2

Segmental Duplications Associated with XLID



- Wagenstaller et al.,
- Gijsbers et al.
- Whibley et al.
- Froyen et al.
- Froyen et al.
- Bedeschi et al.
- Koolen et al.
- Mimault et al.,
- Koolen et al.
- Koolen et al.
- Solomon et al.,
- Rio et al.
- Van Esch et al.,

Segmental Duplications Associated with XLID

Xp22.31 Wagenstaller (Am J Hum Genet 81:738, 2007)
VCX3A, HDHD1A, STS, VCX, PNPLA4, and VCX2

Xp22.2-p21.3. Honda et al. J Hum Genet 55:590, 2010
REPS2, NHS, and ***ILRAPL1***

Xp22.11p22.13 Gisjbers Clin Genet 2010
AP1S2, ***CDKL5****, SCML1, PDAA1, RPS6KAS, SMX, and* ***ARX***

Xp11.22 Giorda et al. (Am J Hum Genet 85:394–400,2009)
PORCN, PQPB1,BMP15,SHROOM4

Xp11.22. Froyen et al. (Am J Hum Genet 82:432, 2008)
RIBC1, HSD17B10 and HUWE1.

Xq12-q13.1. (Bedeschi et al. Am J Med Genet 146A:1718, 2008)
OPHN1

Xq13.2q21.1. Koolen et al. (Hum Mutat 30:283, 2009).
MED12*, NLGN3, SLC16A2, KIAA2022,* ***ATRX****, and BRWD3*

Xq21q22. (Mimault et al.: Am J Hum Genet 65:360, 1999)
PLP1

Xq27.2-q27.3. Solomon et al.: J Med Genet 41:669, 2004
SOX3

Xq27.3q28 (Rio et al., Eur J Hum Genet 18:285, 2010).
FMR1*, AFF2, IDS MTM.*

Xq28. Van Esch et al. (Am J Hum Genet 77:442, 2005)
MECP2

Microduplications Xp22.31

VCX3A, *HDHD1A,* ***STS***, *VCX, PNPLA4, et VCX2*

Interpretation of clinical relevance of X-chromosome copy number variations identified in a large cohort of individuals with cognitive disorders and/or congenital anomalies

EJMG 2012

Marjolein H. Willemsen*, Nicole de Leeuw, Arjan P.M. de Brouwer, Rolph Pfundt, Jayne Y. Hehir-Kwa, Helger G. Yntema, Willy M. Nillesen, Bert B.A. de Vries, Hans van Bokhoven, Tjitske Kleefstra

- 4407 patients
- 57 CNV sur le chromosome X non vus sur le caryotype (1,3 %)
- Taille de 10 kb à 8,5 Mb

-	Pathogène	VOUS	Polymorphe	Total
Duplication garçon	6	12	5	23
Duplication fille	1	0	11	12
Délétion garçon	7	5	0	12
Délétion fille	1	1	8	10
	15	18	24	57

- 0,3 % CNV pathogènes localisés sur le chromosome X
- 10 % *de novo*. Si hérité : 94 % de la mère
- Biais inactivation chez 21 des 34 mères conductrices : 4/21 (19 %) avec un biais

16 CNVs de la région Xp22.31 (*STS/VCXA3*) / 57 (28 %)

5 pertes de *STS/VCXA3* chez des garçons : VOUS

M	X-linked ichthyosis, concentration and emotional problems	Loss	Xp22.31	6.51–7.66	1.15 Mb/2 genes	NT	NT	*STS* *VCX3A*	X-linked ichthyosis (OMIM id 308100). The CNV explains part of the phenotype, but an association with the psychological problems is uncertain	[32,64–66] Also many cases reported in Decipher. Reported associations with ID are conflicting

Ichtyose plus troubles de l'attention, DI, épilepsie, autisme, spasticité, malformations

4 gains de *STS/VCXA3* chez des filles : Polymorphe

F	ID, Costeff syndrome (genetically confirmed)	Gain	Xp22.31	6.5–8.1	1.55 Mb/6 genes	NT	NT	1) *STS* 2) *VCX3A*	1) X-linked ichthyosis (OMIM id 308100).	[32,64–66] Also many cases reported in Decipher. Reported associations with ID are conflicting 2) Deletion of *VCX3A* was thought to be related to ID because of the gene's absence in XLI patients with ID and its presence in XLI patients without ID [64]. [32,64–66]

Retard de langage, ataxie cérébelleuse, hypotonie, retard de développement

7 pertes de *STS/VCXA3* chez des filles : Polymorphe

F	ID, epilepsy, alopecia, hearing loss	Loss	Xp22.31	6.5–8.1	1.55 Mb/6 genes	Mat	In patient: UI	*STS, VCX3A*	See above	See above

Epilepsie, ataxie cérébelleuse, DI, scoliose et anomalie des cheveux, malformations

6 CNVS comprenant *NLGN4 X*

Aucun cas de duplication de *MECP2*

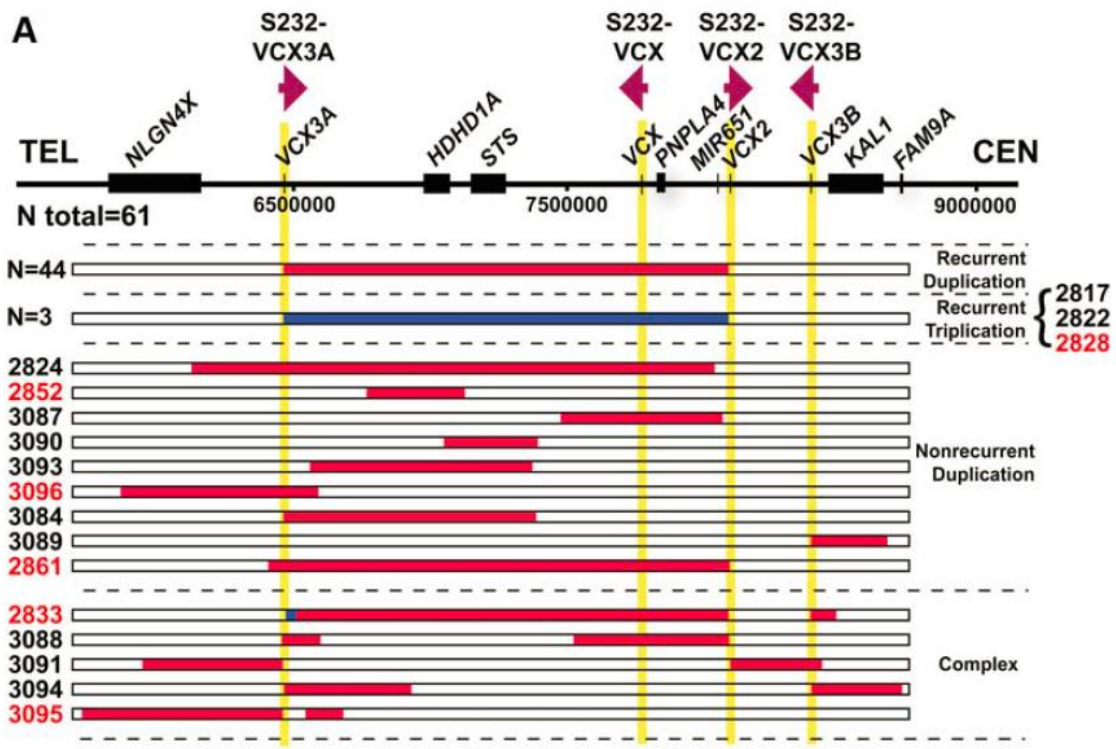
Remaniements Xp22.31

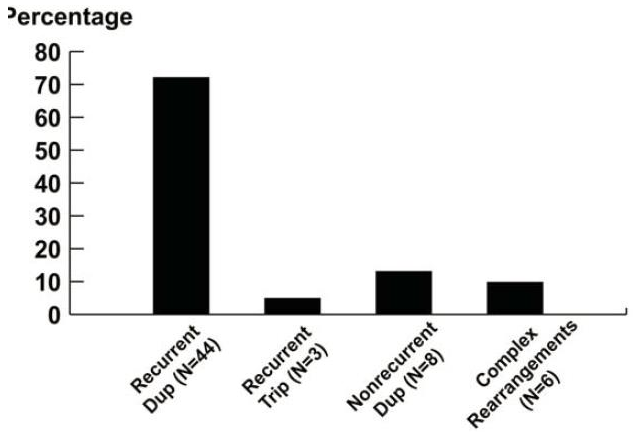
- Patients (H/F) avec MCA/DI : 0,37 % duplication Xp22.31 / 0,15 % dans une population contrôle (Liu et *al,.* 2010). Idem Li *et al.,* 2010

Copy number gain at Xp22.31 includes complex duplication rearrangements and recurrent triplications

HMG 2011

Pengfei Liu[1], Ayelet Erez[1], Sandesh C. Sreenath Nagamani[1], Weimin Bi[1],





Etude de 61 cas

Table 3. Summary of clinical phenotypes for the subjects with the recurrent Xp22.31 duplication or triplication and the comparison with the summarized data from the literature

	Recurrent duplications ($n = 12$)		Recurrent Triplications ($n = 3$)		All duplications from the literature ($n = 35$)[20]
	Male ($n = 11$)	Female ($n = 3$)	Male ($n = 2$)	Female ($n = 1$)	
Gastro-esophageal reflux	3 (27%)	–	1 (50%)	–	3 (9%)
Delay					
Gross motor	6 (55%)	2 (67%)	2 (100%)	1	24 (69%)
Speech	7 (64%)	2 (67%)	2 (100%)	1	
Developmental regression	1 (9%)	1 (33%)	–	–	–
Seizures	1 (9%)	1 (33%)	–	–	4 (11%)
Hypotonia	3 (27%)	1 (33%)	1 (50%)	1	7 (20%)
Behavior problems					
Autism spectrum	7 (64%)	–	–	–	13 (37%)
ADHD	–	–	2 (100%)	–	
Macrocephaly >95%	4 (36%)	–	–	1	–
Microcephaly <5%	–	1 (33%)	–	–	4 (11%)
Short stature	1 (9%)	1 (33%)	1 (50%)	–	2 (6%)
MRI/CT brain abnormalities	2 (18%)	1 (33%)	–	–	–
EEG abnormalities	2 (18%)	1 (33%)	–	–	–

'–' indicates the feature is not present in the corresponding category or this information is not available. In the subjects with duplications, there are two siblings and one set of twins. Note that the data from the last column are phenotypes collected from different types of Xp22.31 gains (not restricted to recurrent duplications).

With these considerations, it remains uncertain whether the recurrent Xp22.31 duplications alone are associated with abnormal phenotypes. Further clinical study is warranted for more individuals with the Xp22.31 recurrent or simple nonrecurrent duplications, triplications and other complex rearrangements in order to reach conclusions whether these changes are pathogenic or benign CNVs.

Facteur de risque

Et les délétions

Remaniements Xp22.31

- Lesca *et al.,* 2005; Mochel *et al.,* 2008; Cueves-Covarrubias *et al.,* 2008 : délétion *VCX3A* : n'explique pas la DI.

Avis de l'ISCA sur CNV (*STS*)

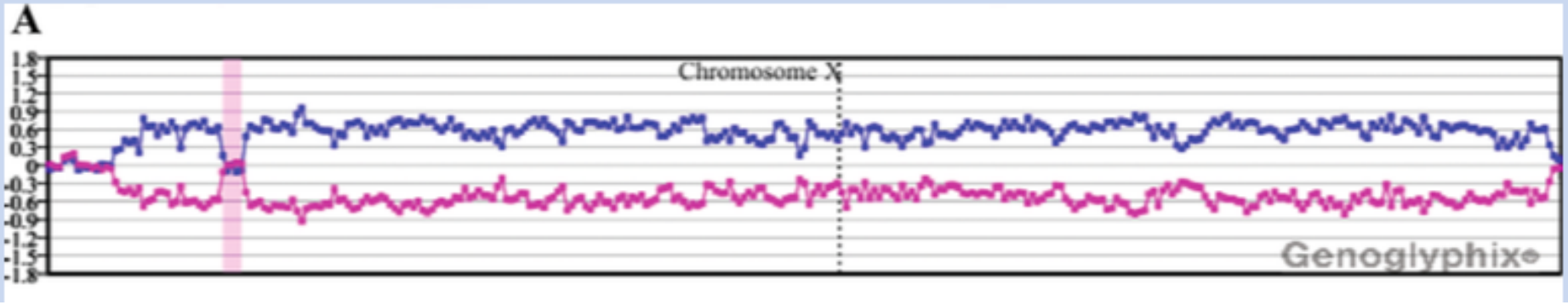
http://www.ncbi.nlm.nih.gov/projects/dbvar/ISCA/index.shtml

- Perte de fonction :
  - Ichtyose chez les garçons
  - Femme atteinte : homozygote ?
  - Possible Clinical Interpretation(s): **Pathogenic** (males); Pathogenic/carrier status (females)
- Gain de fonction :
  - Peu de données en faveur de la pathogénéicité
  - Possible Clinical Interpretation(s): **Benign** (males and females)

- Furrow et al. 2011 : 72 garçons dup *STS/VCX* : Polymorphisme

Duplication of the STS Region in Males Is a Benign Copy-Number Variant

Aubry Furrow,[1] Aaron Theisen,[1] Lea Velsher,[2] Erawati V. Bawle,[3] Sujatha Sastry,[3] Nancy J. Mendelsohn,[4] Kristi Jarvis,[4] Lisa G. Shaffer,[1]* and David Chitayat[5,6]



72 males studied

Based on our results and previously published data, duplication of the STS region in males is likely a benign finding and does not appear to contribute to the abnormal phenotypes found in our patient population submitted for diagnostic microarray analysis. Nevertheless, contributions to a digenic two-hit model or reduced-penetrance dosage effects of other genes in the genomic region encompassed by the CNV gain cannot be ruled out by the present data.

Duplication / Triplication Xq21q22

PLP1

Syndrome de Pelizaeus-Merzbacher

Syndrome de Pelizaeus-Merzbacher

- Forme infantile précoce de leucodystrophie cérébrale progressive
- Démyélinisation de la substance blanche cérébrale
- Clinique
  - Tremblement de la tête
  - Nystagmus
  - Paraplégie spasmodique
  - Déficience intellectuelle
  - Mouvements athétosiques

Three or more copies of the proteolipid protein gene *PLP1* cause severe Pelizaeus–Merzbacher disease

Brain 2005

Nicole I. Wolf,[1,6] Erik A. Sistermans,[7] Maria Cundall,[1] Grace M. Hobson,[8,9]

Table 1 *Clinical characteristics of patients 1–5*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at first symptom	Birth (nystagmus; stridor)	First weeks of life (nystagmus)	3 weeks (nystagmus)	Birth (nystagmus)	Birth (nystagmus; hypotonia)
Age at death	9 months	7 months	Alive (4.7 years)	Alive (14 months)	Alive (15 months)
Nystagmus	+	+	+	+	+
Seizures	+ +	+ +	–	+	+ +
Stridor	+	+	–	+	–
Head control	None	None	Poor	None	None
Muscular hypotonia	+	+	+	+	+
Spasticity	–	–	Mild	Mild	–
Nerve conduction studies	Normal	Not performed	Normal	Not performed	Unreliable

5 copies *PLP1*

4 copies *PLP1*

3 copies *PLP1*

PLP1 (Proteolipid Protein) sensible au dosage génique

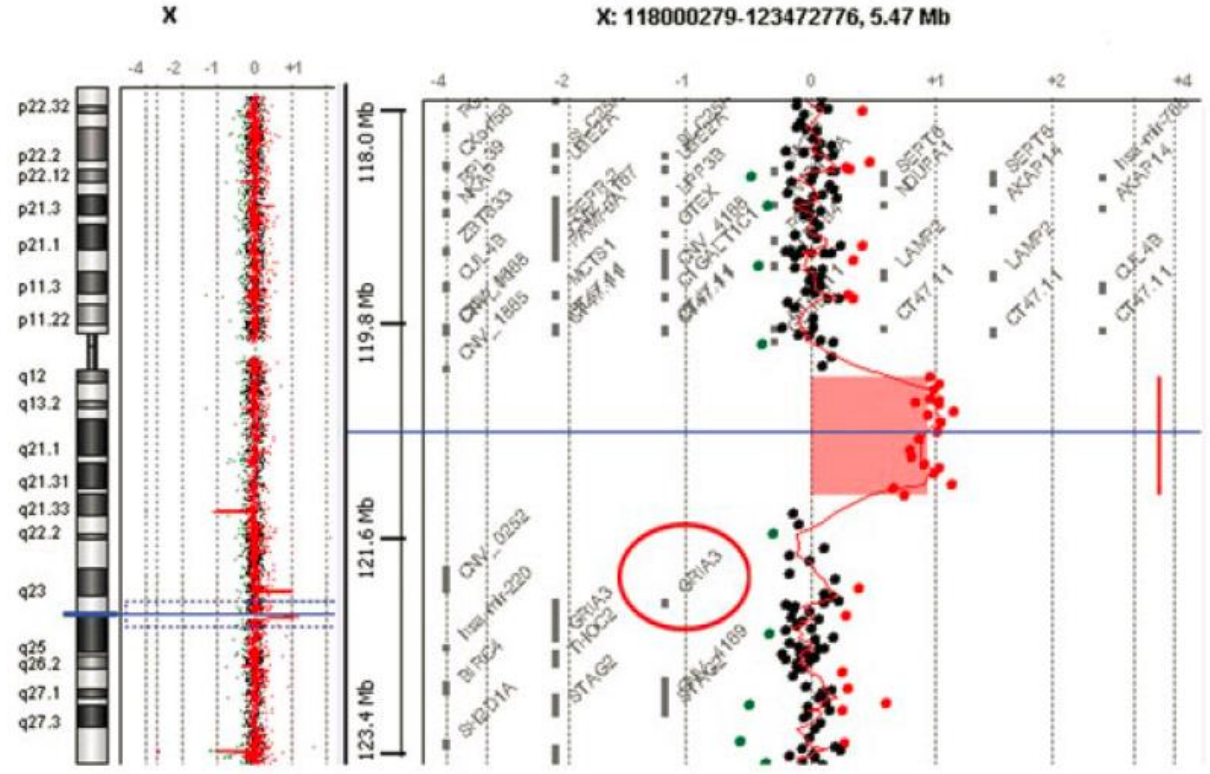
6) Difficultés d'interprétation de certains CNV sur le chromosome X et de conseil

- Effet de position ?

Exploring the Potential Role of Disease-Causing Mutation in a Gene Desert: Duplication of Noncoding Elements 5′ of *GRIA3* is Associated with *GRIA3* Silencing and X-Linked Intellectual Disability

Hum Mut 2009

Céline Bonnet,[1] Alice Masurel-Paulet,[2] Asma Ali Khan,[1] Mylène Béri-Dexheimer,[1] Patrick Callier,[3] Francine Mugneret,[3] Christophe Philippe,[1] Christel Thauvin-Robinet,[2] Laurence Faivre,[2] and Philippe Jonveaux[1]*



Homme
Epilepsie
DI sévère
Dysmorphie faciale
Hypotonie axiale
Cyphose

Dup 970 kb Xq
Pas de gène

GRIA3 encodes glutamate receptor ionotropic AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)

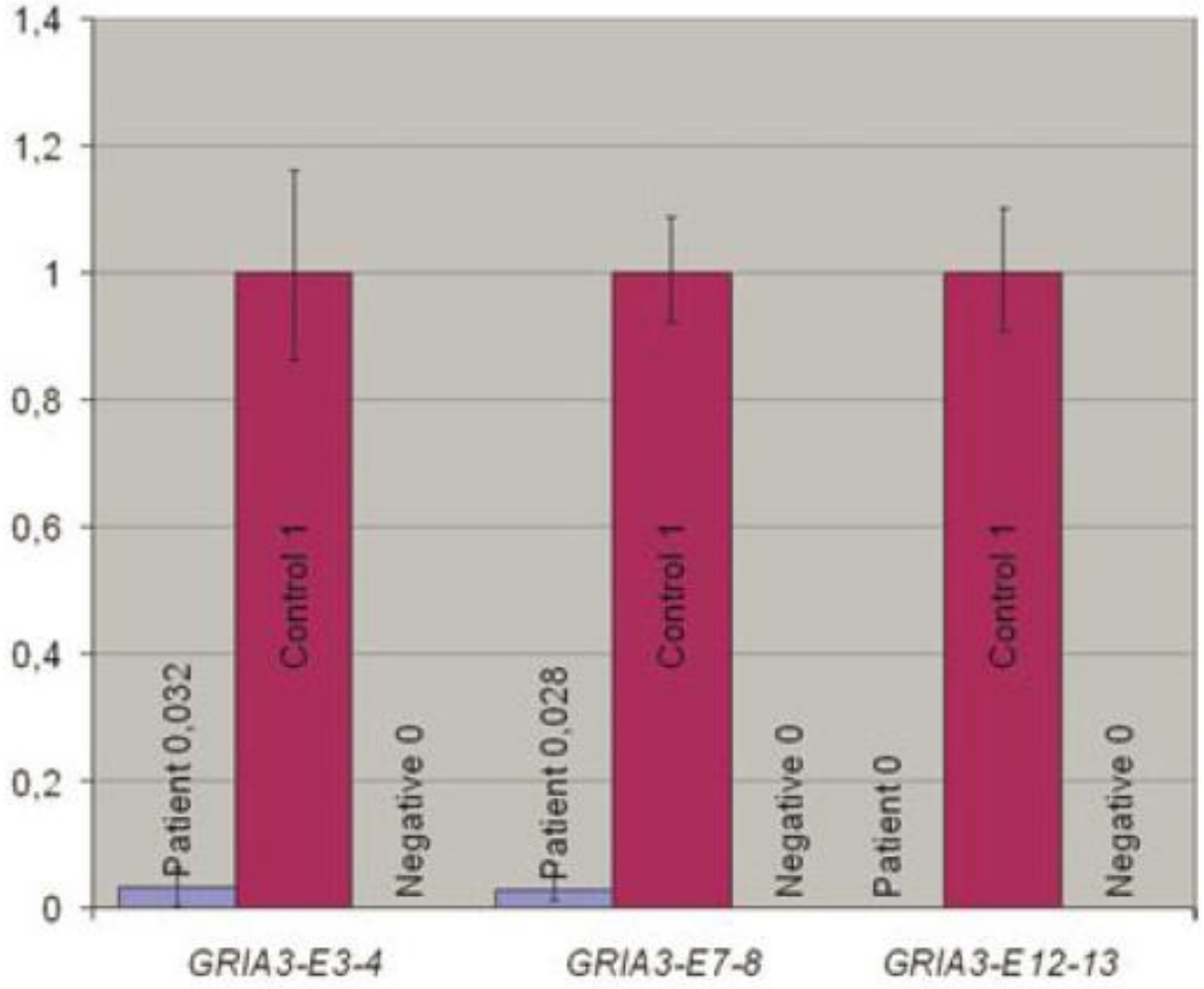


Figure 2. Reverse transcriptase quantitative real-time PCR analysis using three primer sets (exons 3–4, exons 7–8, exons 12–13) shows loss of *GRIA3* expression for the patient relative to a male control.

Absence d'ARNm de *GRIA3*

CNV implique un facteur de transcription ?

6) Difficultés d'interprétation de certains CNV sur le chromosome X et de conseil

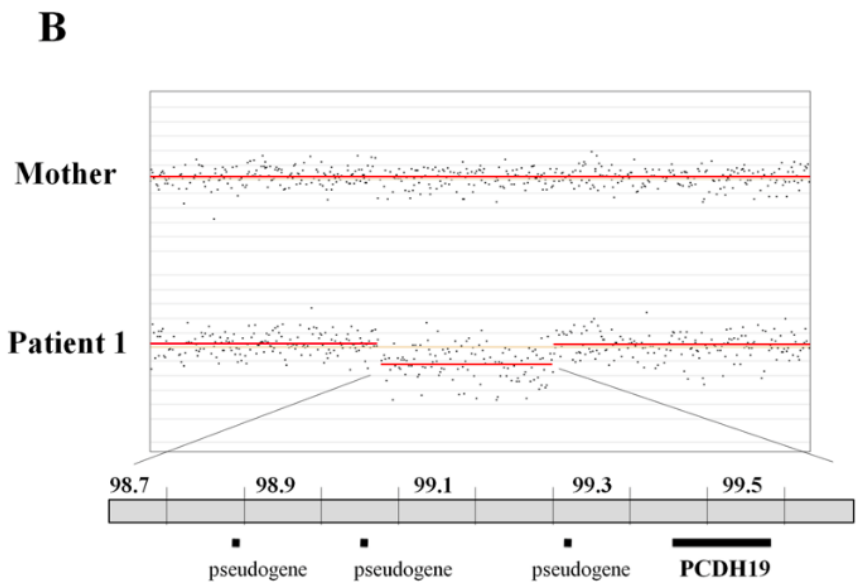
- Effet de position ?

- Attention aux « Dogmes »

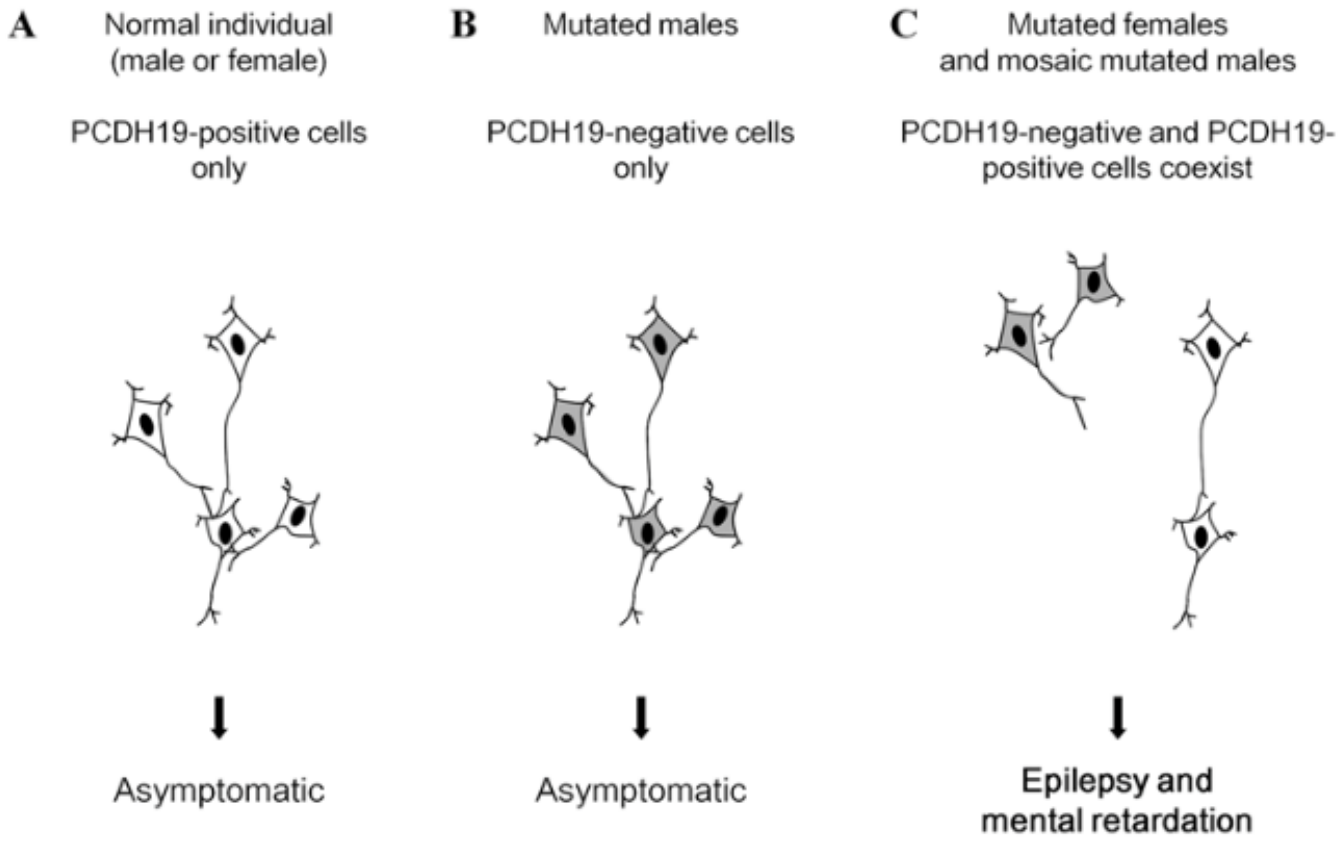
OPEN ACCESS Freely available online PLoS GENETICS

Sporadic Infantile Epileptic Encephalopathy Caused by Mutations in *PCDH19* Resembles Dravet Syndrome but Mainly Affects Females

Christel Depienne[1,2,3]*, Delphine Bouteiller[2], Boris Keren[1], Emmanuel Cheuret[4], Karine Poirier[5], Oriane Trouillard[1], Baya Benyahia[1], Chloé Quelin[5], Wassila Carpentier[6], Sophie Julia[4], Alexandra Afenjar[1,7], Agnès Gautier[8], François Rivier[9], Sophie Meyer[10], Patrick Berquin[11], Marie Hélias[12], Isabelle Py[13], Serge Rivera[14], Nadia Bahi-Buisson[15], Isabelle Gourfinkel-An[2,15,16], Cécile Cazeneuve[1], Merle Ruberg[2,3], Alexis Brice[1,2,3], Rima Nabbout[16,17], Eric LeGuern[1,2,3]

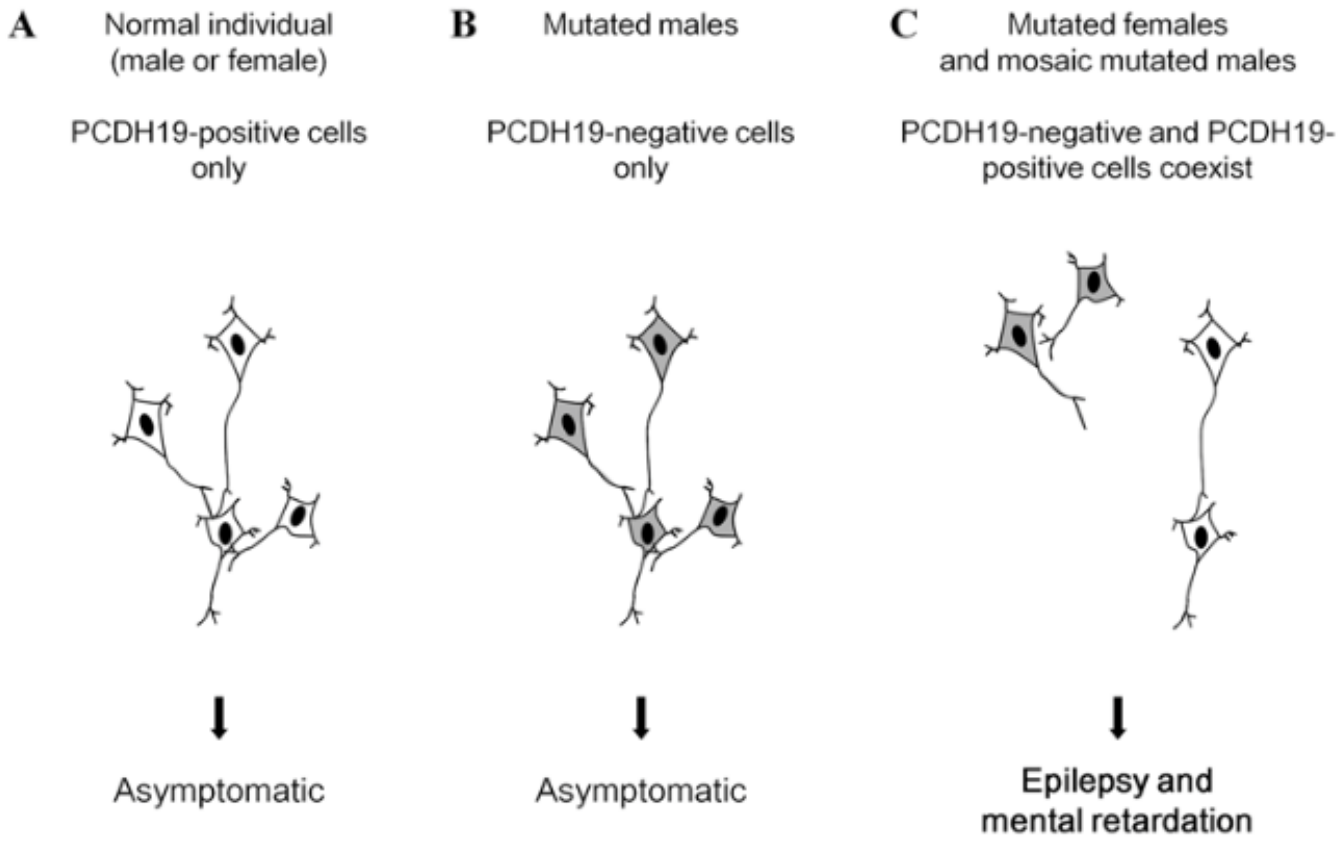


Délétion PCHD19 de novo



Mutation hémizygote chez les garçons : asymptomatique

Mutation hétérozygote chez une femme : DI et épilepsie



Mutation hémizygote chez les garçons : asymptomatique

Mutation hétérozygote chez une femme : DI et épilepsie

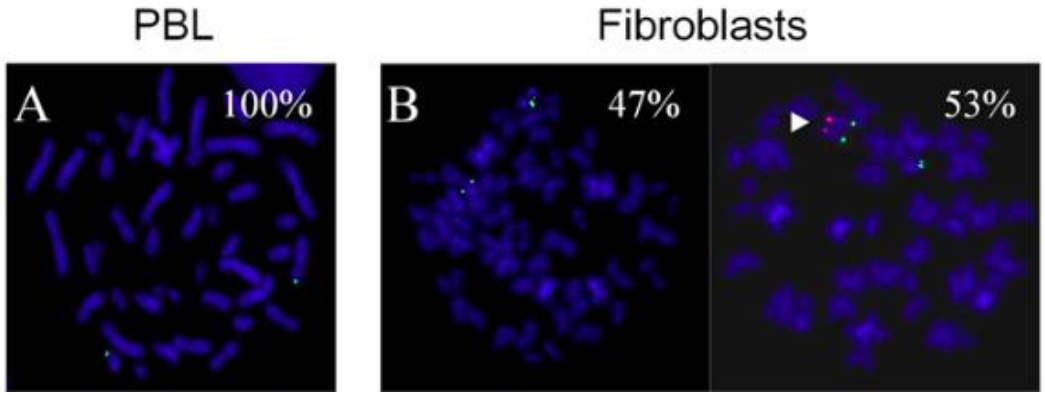
Figure 6. Schematic illustration of the cellular interference mechanism associated with *PCDH19* mutations. A) In normal individuals,

Mécanisme d'interférence cellulaire

Cas d’un garçon symptomatique

Pourquoi ?

Cas d’un garçon symptomatique



Garçon symptomatique :
étude des fibroblastes :
mosaïque !

Dr B. Keren

7) CNV du chromosome CNV et découverte non sollicité

Exemple de *DMD*

Découverte non sollicité

- Patiente adressée pour
  - Encéphalopathie épileptique
  - Nystagmus,
  - Microcéphalie progressive
  - Hypoplasie cérébelleuse à l'IRM
  - *Décès à 14 mois*
  - ACPA : délétion de 227.5 kb sur le chromosome X emportant les exons 3 à 9 du gène *DMD,* prédictif de **BMD**.
- Etude parentale :
  - Le père est porteur asymptomatique mais a des CPK modérément élevées à 1016 IU/l.

8) Puce à façon du chromosome X

Clinical Utility of the X-Chromosome Array

AJMG 2012

Yuri A. Zarate,* Alka Dwivedi, Frank O. Bartel, M. Allison Bellomo, Sara S. Cathey, Neena L. Champaigne, L. Kate Clarkson, Barbara R. DuPont, David B. Everman, Joseph S. Geer, Barbara C. Gordon, Angie W. Lichty, Michael J. Lyons, R. Curtis Rogers, Robert A. Saul, Richard J. Schroer, Steven A. Skinner, and Roger E. Stevenson

TABLE III. Results of Previous Studies That Used Various X-Chromosome Array Platforms

References	Array platform	Indication	Frequency of pathogenic or likely pathogenic CNVs (%)	Comments
Froyen et al. [2007]	1,875 BAC/PAC clones	ID	5/108 (4.6)	3 dup, 2 del
Honda et al. [2010]	1,001 BAC/PAC clones	ID	10/144 (6.9)	13 CNVs, 10 dup, 3 del
Kousoulidou et al. [2007]	558 X-specific 400–600 bp target sequences	XLID	1/20 (5)	1 known dup
Lugtenberg et al. [2006]	1,460 BAC clones	XLID	3/40 (7.5)	3 dup
Madrigal et al. [2007]	1,600 BAC clones	XLID and XL trait	8/54 (14.8)	5 dup, 3 del
Whibley et al. [2010]	385 K Nimblegen® oligonucleotide	XLID	25/251 (10)	18 dup, 7 del
This study	28, 44, and 105 k OGT® oligonucleotide	Multiple	16/59 (27)	16 del

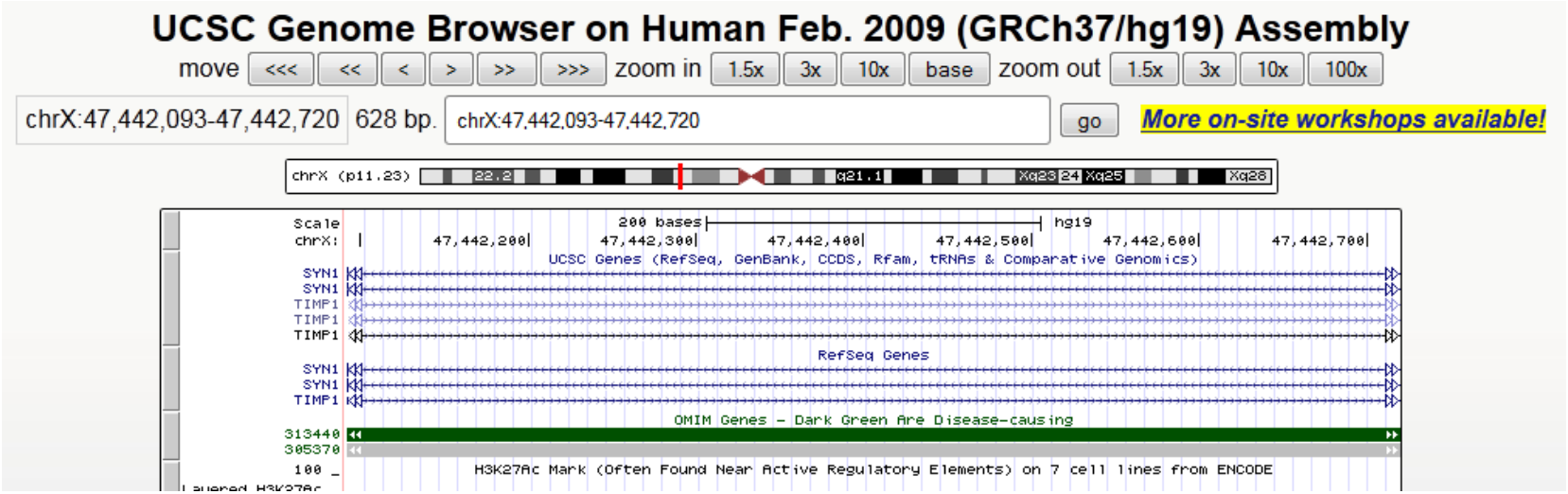
BAC, bacterial artificial chromosome; CNVs, copy number variants; Del, deletion; Dup, duplication; ID, intellectual disability; OGT, Oxford Gene Technology; PAC, P1-derived artificial chromosome; XLID, X-linked intellectual disability.

Custom oligonucleotide array-based CGH: a reliable diagnostic tool for detection of exonic copy-number changes in multiple targeted genes

EJHG 2013

Aurélie Vasson[1], Céline Leroux[1], Lucie Orhant[1], Mathieu Boimard[1], Aurélie Toussaint[1], Chrystel Leroy[1], Virginie Commere[1], Tiffany Ghiotti[1], Nathalie Deburgrave[1], Yoann Saillour[2], Isabelle Atlan[1], Corinne Fouveaut[1], Cherif Beldjord[1,2], Sophie Valleix[1,2], France Leturcq[1,2], Catherine Dodé[1,2], Thierry Bienvenu[1,2], Jamel Chelly[1,2] and Mireille Cossée*[,1,2]

- 12 gènes / 26 sur le chromosome X
- CNV identifiés dans les gènes suivants :
  - *DMD* : 9 dup et 6 del de 2,2 kb à 772 kb
  - *CDKL5* : 2 del de 6,2 kb et 294 kb
  - *DCX* : 1 del et 2 dup : 7,9-740 kb
  - *KAL1* : 3 del : 91-2297 kb
  - *F8* : 1 del de 38,6 kb



Régions introniques: quelles conclusions?
100 petits CNV par array

Fine-Scale Survey of X Chromosome Copy Number Variants and Indels Underlying Intellectual Disability

AJHG 2010

Annabel C. Whibley,[1] Vincent Plagnol,[1,2] Patrick S. Tarpey,[3] Fatima Abidi,[4] Tod Fullston,[5,6]

Table 1. Summary of Rare X Chromosome CNVs

	All CNVs	<1 kb	1–10 kb	10–100 kb	100–500 kb	>500 kb
Deletions	498 (440)	182 (161)	224 (202)	86 (71)	4 (4)	2 (2)
Duplication	454 (421)	150 (146)	174 (165)	84 (69)	31 (26)	15 (15)
Total	952 (861)	332 (307)	398 (367)	170 (140)	35 (30)	17 (17)

Total call numbers are stated, with numbers in parentheses indicating the number of distinct CNV loci after merging calls across samples.

Conclusions

Comment interpréter les CNVs localisés sur le chromosome X

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Original article

Sporadic male patients with intellectual disability: Contribution of X-chromosome copy number variants

M. Isrie [a], G. Froyen [b], K. Devriendt [a], T. de Ravel [a], J.P. Fryns [a], J.R. Vermeesch [a], H. Van Esch [a,*]

[a] Center for Human Genetics, University Hospitals Leuven, KU Leuven, Herestraat 49, 3000 Leuven, Belgium
[b] Human Genome Laboratory, VIB, Center for Human Genetics, KU Leuven, Herestraat 49, 3000 Leuven, Belgium

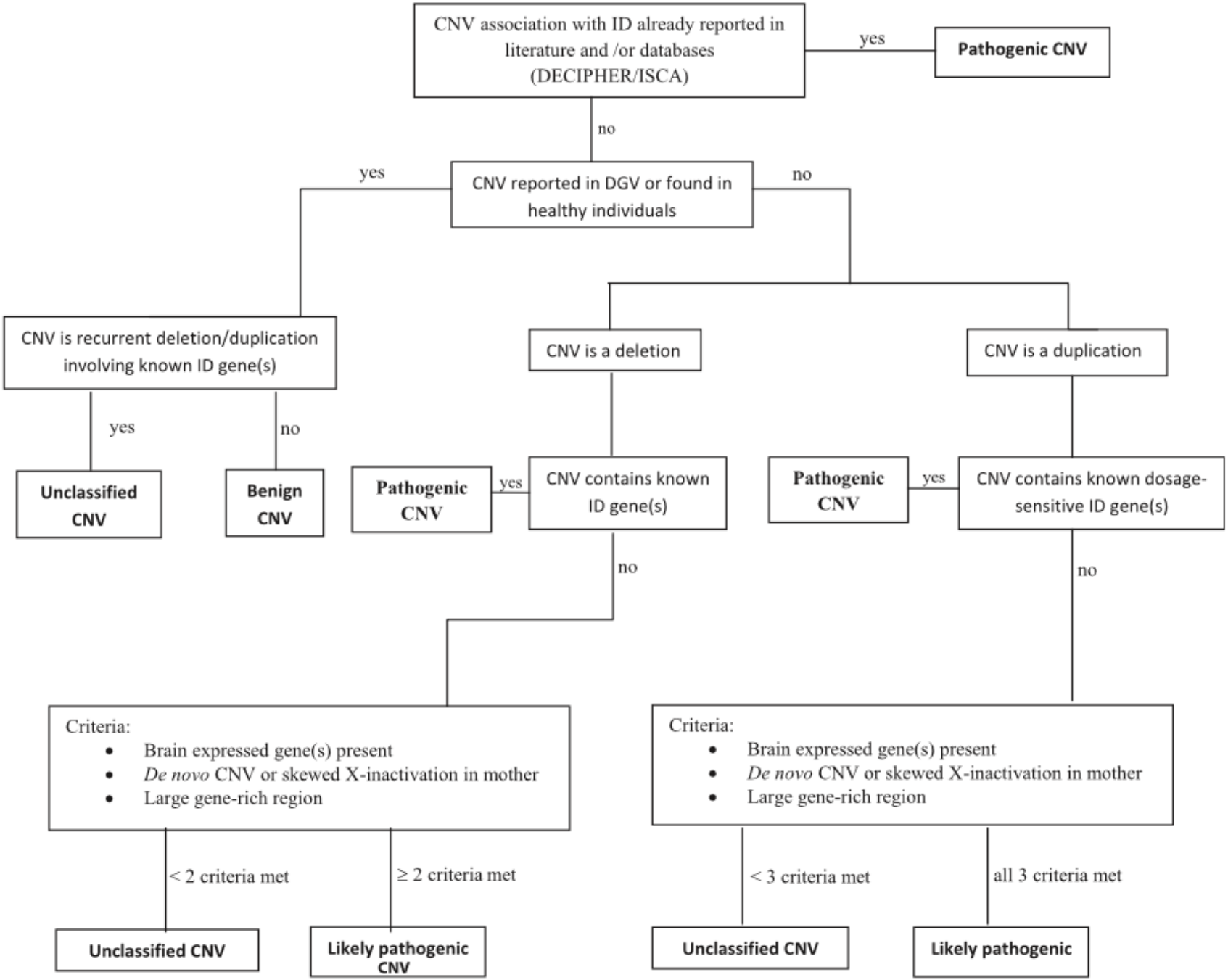


Fig. 1. Proposed decision tree for classification of gene-containing sporadic X-CNVs in males.

CNV chromosome X

Brainstorming
Cytogeneticist
Clinician

Clinical phenotype already described ?

Sex ?

Databases ?
DGV
Decipher
ClinGen
…

?

Gain ?
Loss ?

Size ?

XCI pattern ?

Familial segregation ?

Pahtogenic **VOUS** **Benign**

Take home message

- Bien analyser le profil +++ (sexe patient ?)
- Interprétation clinique dépend du sexe du patient
- Une étude familiale est souvent nécessaire
- Un biais d'inactivation n'est pas synonyme de pathogène
- Combiner les différents éléments avant de conclure

Perspectives

NGS

OPEN

Molecular Psychiatry (2015), 1–16

www.nature.com/mp

ORIGINAL ARTICLE

X-exome sequencing of 405 unresolved families identifies seven novel intellectual disability genes

H Hu[1,41], SA Haas[2,41], J Chelly[3,4], H Van Esch[5], M Raynaud[6,7,8], APM de Brouwer[9], S Weinert[10,11], G Froyen[12,13], SGM Frints[14,15], F Laumonnier[6,7], T Zemojtel[2], MI Love[2], H Richard[2], A-K Emde[2], M Bienek[1], C Jensen[1], M Hambrock[1], U Fischer[1], C Langnick[10], M Feldkamp[10], W Wissink-Lindhout[9], N Lebrun[3,4], L Castelnau[3,4], J Rucci[3,4], R Montjean[3,4], O Dorseuil[3,4], P Billuart[3,4], T Stuhlmann[10,11], M Shaw[16,17], MA Corbett[16,17], A Gardner[16,17], S Willis-Owen[16,18], C Tan[16], KL Friend[19], S Belet[12,13], KEP van Roozendaal[14,15], M Jimenez-Pocquet[8], M-P Moizard[6,7,8], N Ronce[6,7,8], R Sun[2], S O'Keeffe[2], R Chenna[2], A van Bömmel[2], J Göke[2], A Hackett[20], M Field[20], L Christie[20], J Boyle[20], E Haan[16,19], J Nelson[21], G Turner[20], G Baynam[21,22,23,24], G Gillessen-Kaesbach[25], U Müller[26,27], D Steinberger[26,27], B Budny[28], M Badura-Stronka[29], A Latos-Bieleńska[29], LB Ousager[30], P Wieacker[31], G Rodríguez Criado[32], M-L Bondeson[33], G Annerén[33], A Dufke[34], M Cohen[35], L Van Maldergem[36], C Vincent-Delorme[37], B Echenne[38], B Simon-Bouy[39], T Kleefstra[9], M Willemsen[9], J-P Fryns[5], K Devriendt[5], R Ullmann[1,42], M Vingron[2], K Wrogemann[1,40], TF Wienker[1], A Tzschach[1], H van Bokhoven[9], J Gecz[16,17], TJ Jentsch[10,11], W Chen[1,10], H-H Ropers[1] and VM Kalscheuer[1]

X-linked intellectual disability (XLID) is a clinically and genetically heterogeneous disorder. During the past two decades in excess of 100 X-chromosome ID genes have been identified. Yet, a large number of families mapping to the X-chromosome remained unresolved suggesting that more XLID genes or loci are yet to be identified. Here, we have investigated 405 unresolved families with XLID. We employed massively parallel sequencing of all X-chromosome exons in the index males. The majority of these males were previously tested negative for copy number variations and for mutations in a subset of known XLID genes by Sanger sequencing. In total, 745 X-chromosomal genes were screened. After stringent filtering, a total of 1297 non-recurrent exonic variants remained for prioritization. Co-segregation analysis of potential clinically relevant changes revealed that 80 families (20%) carried pathogenic variants in established XLID genes. In 19 families, we detected likely causative protein truncating and missense variants in 7 novel and validated XLID genes (*CLCN4*, *CNKSR2*, *FRMPD4*, *KLHL15*, *LAS1L*, *RLIM* and *USP27X*) and potentially deleterious variants in 2 novel candidate XLID genes (*CDK16* and *TAF1*). We show that the *CLCN4* and *CNKSR2* variants impair protein functions as indicated by electrophysiological studies and altered differentiation of cultured primary neurons from *Clcn4*$^{-/-}$ mice or after mRNA knock-down. The newly identified and candidate XLID proteins belong to pathways and networks with established roles in cognitive function and intellectual disability in particular. We suggest that systematic sequencing of all X-chromosomal genes in a cohort of patients with genetic evidence for X-chromosome locus involvement may resolve up to 58% of Fragile X-negative cases.

Molecular Psychiatry advance online publication, 3 February 2015; doi:10.1038/mp.2014.193

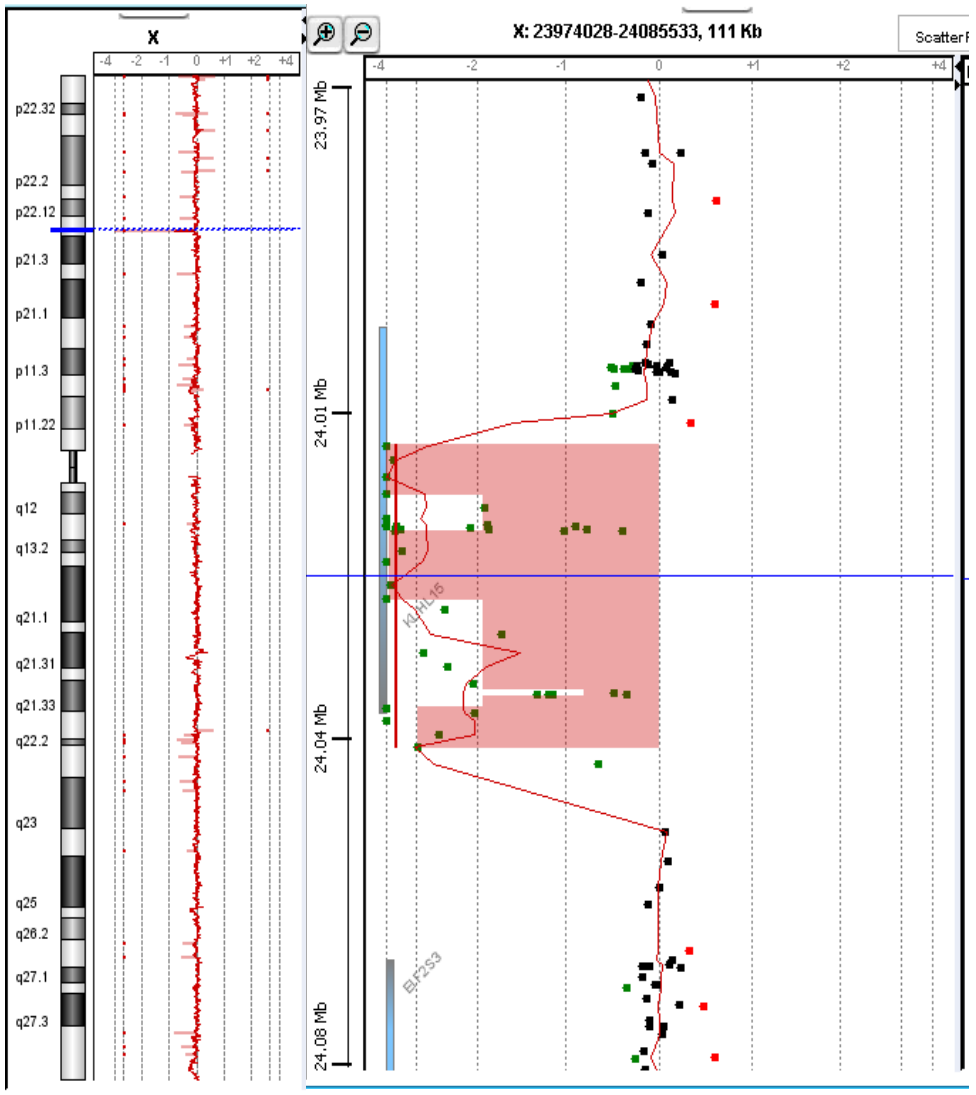
Table 2. Variants identified in novel XLID genes and candidates

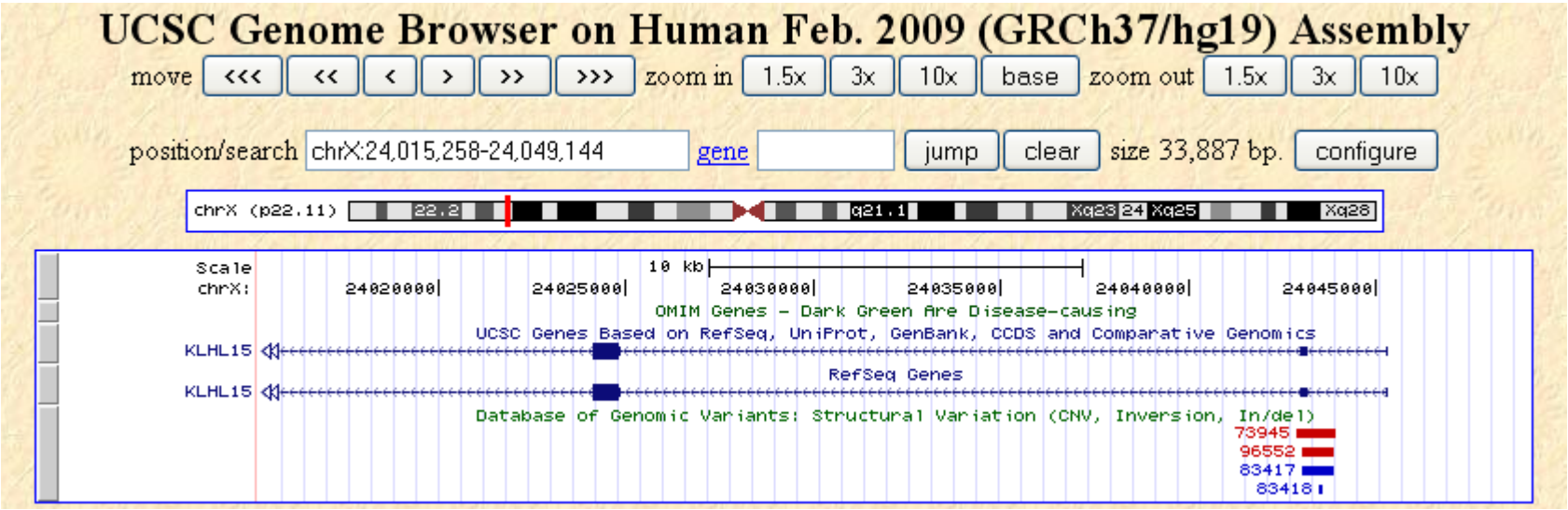
Family ID	*Gene*	*Variant*	*PS score*	*C score*	*Additional information (numbers indicate informative affected/ unaffected males tested for segregation/obligate female carriers)*	*Summary of clinical information for families per gene*
Likely pathogenic variants in novel and validated XLID genes						
MRX49[61]/L19	*CLCN4*	p.Asp15Serfs*18	20	36	4/2/2, F, encodes a proton-chloride antiporter	Non-specific borderline to profound ID
MRX15[62]/T8	*CLCN4*	p.Gly731Arg	8	29	F, cytosolic cystathionine-β-synthase domain, may impair transporter opening	
N70	*CLCN4*	p.Gly78Ser	14	25	1/0/1, F, transmembrane domain	
AU27	*CLCN4*	p.Leu221Val	8	25	2/0/4, F, transmembrane domain	
AU9	*CLCN4*	p.Val536Met	14	27	3/0/7, F, transmembrane domain	
P180	*CNKSR2*	p.Asp152Argfs*8	20	19	3/0/1, F, C, encodes connector enhancer of kinase suppressor of Ras 2, interacts with PSD95, XLID protein DLG3, ID/autism protein SHANK3	ID, attentional problems, hyperactivity, language loss, seizures[99]
P58	*FRMPD4*	p.Cys618Valfs*8	20	38	5/2/2, encodes FERM and PDZ domain containing 4, interacts with PSD95, with ARHGEF7, a guanine nucleotide exchange factor with a role in the regulation of spine morphogenesis, and with actin filaments[104,123]	Mild to severe ID with variable seizures, lack of speech or poor speech, behavioral problems
L87	*FRMPD4*	p.Cys553Arg	4	16	*De novo*	
D60	*KLHL15*	p.Tyr394Ilefs*61	20	33	8/1/4, encodes kelch-like 15, large family with 8 affected in three generations	Mild to moderate ID, mild facial features
MRXS6[66] /AU10	*LAS1L*	p.Ala269Gly	10	18	5/0/19, C, encodes Las1-like, ribosome biogenesis	Wilson-Turner syndrome,[66] mild to moderate ID, obesity, facial features, speech impairment, variable behavioral problems, gynecomastia, small/undescended testes/ hypogonadism, tapering fingers
T50	*LAS1L*	p.Arg415Trp	11	14	3/2/3, C	
MRX61/T11	*RLIM*	p.Pro587Arg	11	13	3/1/3, encodes ring finger protein, LIM domain interacting, E3 ubiquitin-protein ligase, binds to transcription factors that play important roles for the development of neuronal structures and cell types	Non-specific mild to profound ID in two families with variable behavior problems, ID, microcephaly, micrognathia and cryptorchidism in all affected of one family
D72	*RLIM*	p.Arg387Cys	12	12	1/2/8	
AU31	*RLIM*	p.Arg599Cys	14	18	2/3/3	
D177	*USP27X*	p.Ser342Argfs*14	20	10	3/0/2, encodes ubiquitin-specific peptidase 27, interacts with USP22 which deubiquitinates core histones H2A and H2B, USP22 interacts with ARID gene KIF7	Borderline to moderate ID, variable absent or poor speech and behavioral problems
L75	*USP27X*	p.Tyr381His	12	11	1/1/2, this residue is part of a domain (IPR001394) and using HOPE web server (see URLs) the variant is predicted to cause an empty space in the core of the protein or protein complex and to cause loss of hydrophobic interactions	
Potentially deleterious changes in novel candidate genes						
L56	*CDK16*	p.Trp326Valfs*5	20	37	4/1/3, encodes cyclin-dependent kinase 16, also known as PCTK1, PCTAIRE1, and PCT-1	ID, spastic paraplegia
N67	*TAF1*	p.Asn493Asp	13	19	2/0/2, encodes TATA box binding protein (TBP)-associated factor, 250 kDa, subunit of TAFIID which plays a key role transcription initiation. Drosophila homolog phosphorylates histone H2B, variants in *TAF2* cause ARID[78,79]	Mild to severe ID, facial features
D185	*TAF1*	p.Arg1190Cys	14	27	2/4/7	

Abbreviations: C, clinical evidence; F, functional evidence from this study; HGMD, Human Gene Mutation Database; ID, intellectual disability; PS, prioritization score, includes type of variant, evolutionary conservation and predictions from Polyphen2 and SIFT; C score obtained by using Combined Annotation-Dependent Depletion (CADD);[35] XLID, X-linked intellectual disability.

2 brothers with ID : Xp22.11 deletion of 33.88 kb (24,015,258-24,049,144,hg19)

2010

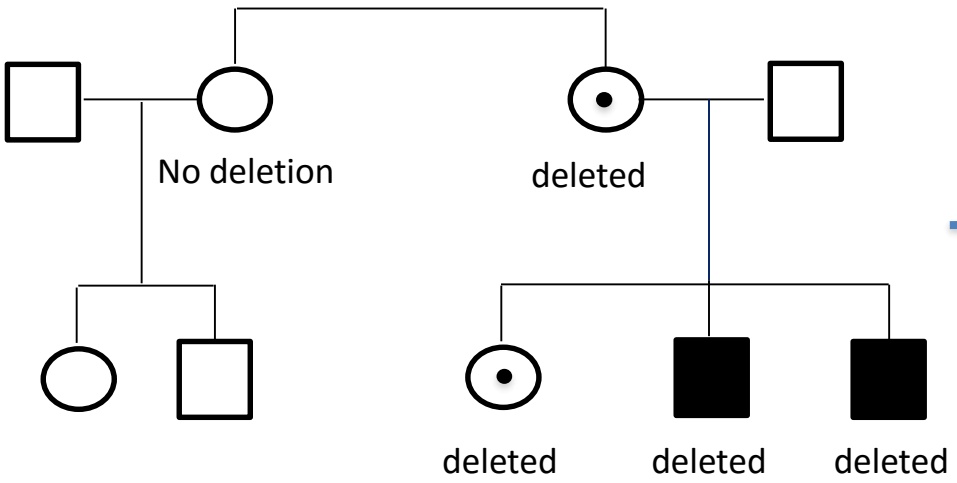




2 exons of *KLHL15*

Family study with qPCR

2010



VOUS

AJMG 2014

Intragenic Rearrangements in X-Linked Intellectual Deficiency: Results of a-CGH in a Series of 54 Patients and Identification of *TRPC5* and *KLHL15* As Potential XLID Genes

Cécile Mignon-Ravix,[1,2] Pierre Cacciagli,[1,2,3] Nancy Choucair,[1,2,4] Cornel Popovici,[3] Chantal Missirian,[3] Mathieu Milh,[1,2,5] André Mégarbané,[1,2,4] Tiffany Busa,[3] Sophie Julia,[6] Nadine Girard,[2,7] Catherine Badens,[1,2,3] Sabine Sigaudy,[3] Nicole Philip,[1,2,3] and Laurent Villard[1,2]*

+ *KHLK15* mutations

Pathogenic

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