Impact des duplications : comment s'en sortir ?

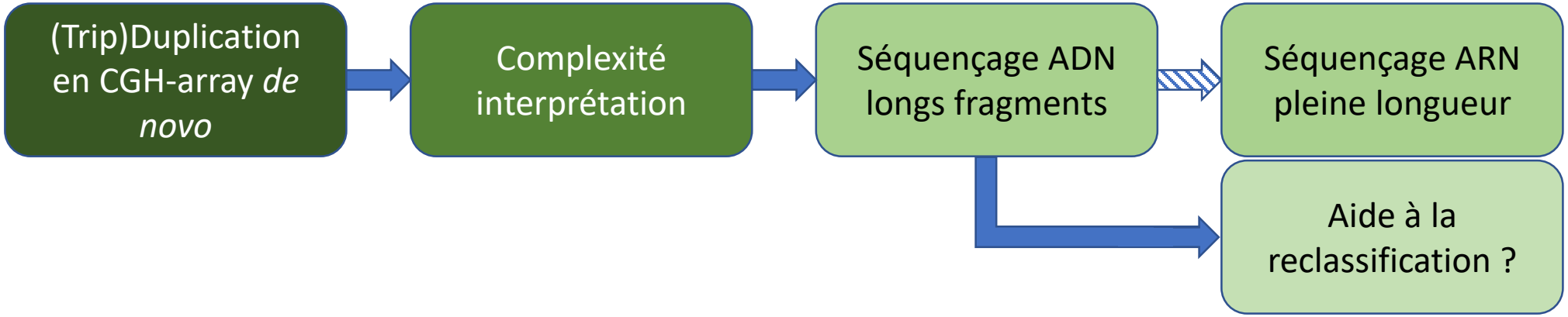
Thomas SMOL

Jade Fauqueux

Journée AChro-Puce

27 novembre 2024

Rationnel et Protocole



- P2i ONT
- Flowcell R10.4.1
- Kit v14

- Génome complet ou adaptive sampling

- Alignement
  - miniMap2 sur GRCh38

- Callers SV/CNV
  - CuteSV
  - sniffles

UPN#02

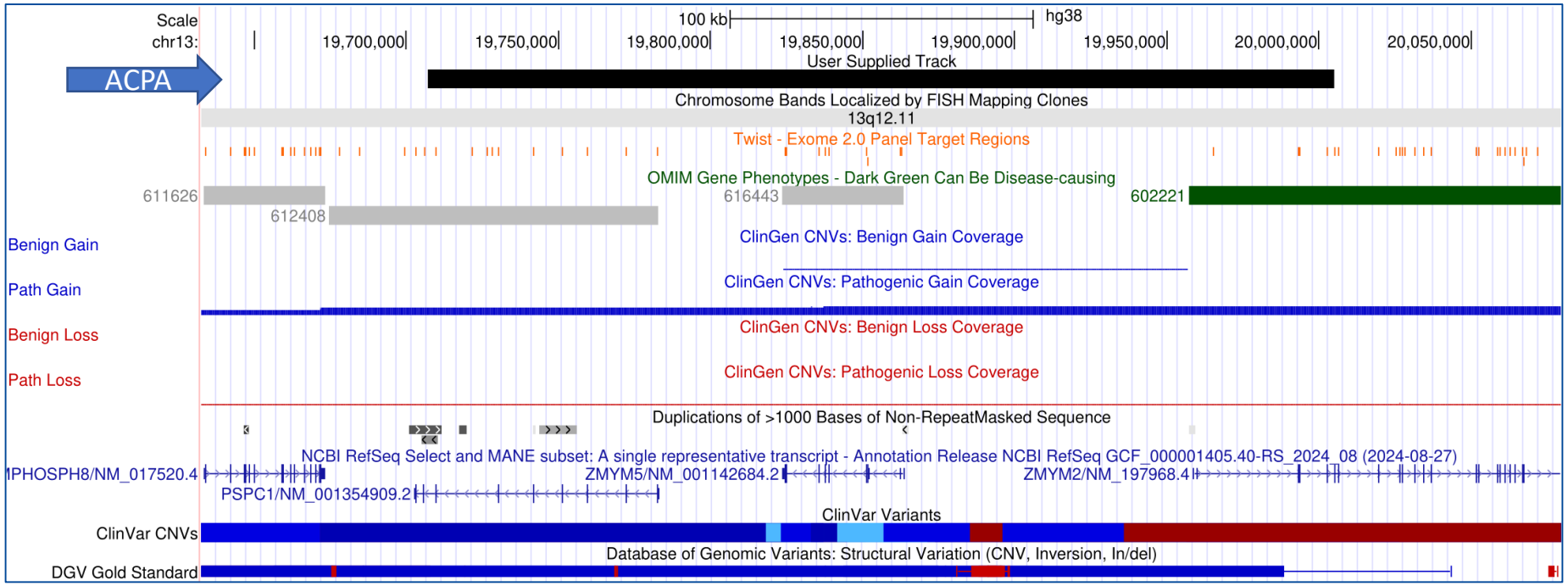
- Patient de 2 ans
  - Retard global acquisitions
  - Difficultés alimentaires
  - Microcéphalie
  - Cryptorchidie

- ACPA

Gain de 2 copies 13q12.11 *de novo*

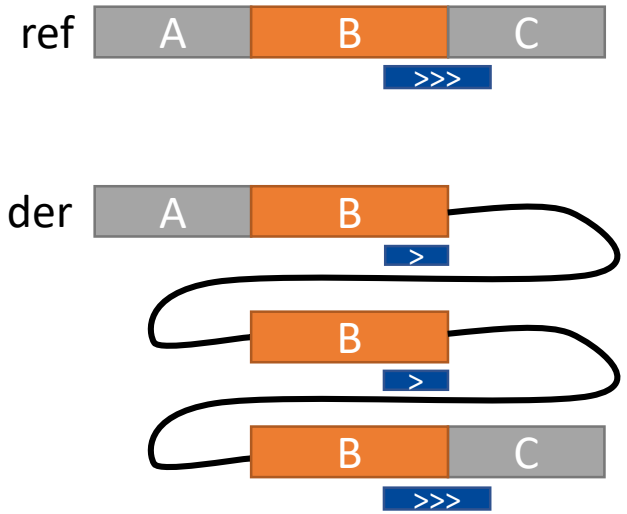
Exons 1 à 6/27 *ZMYM2*

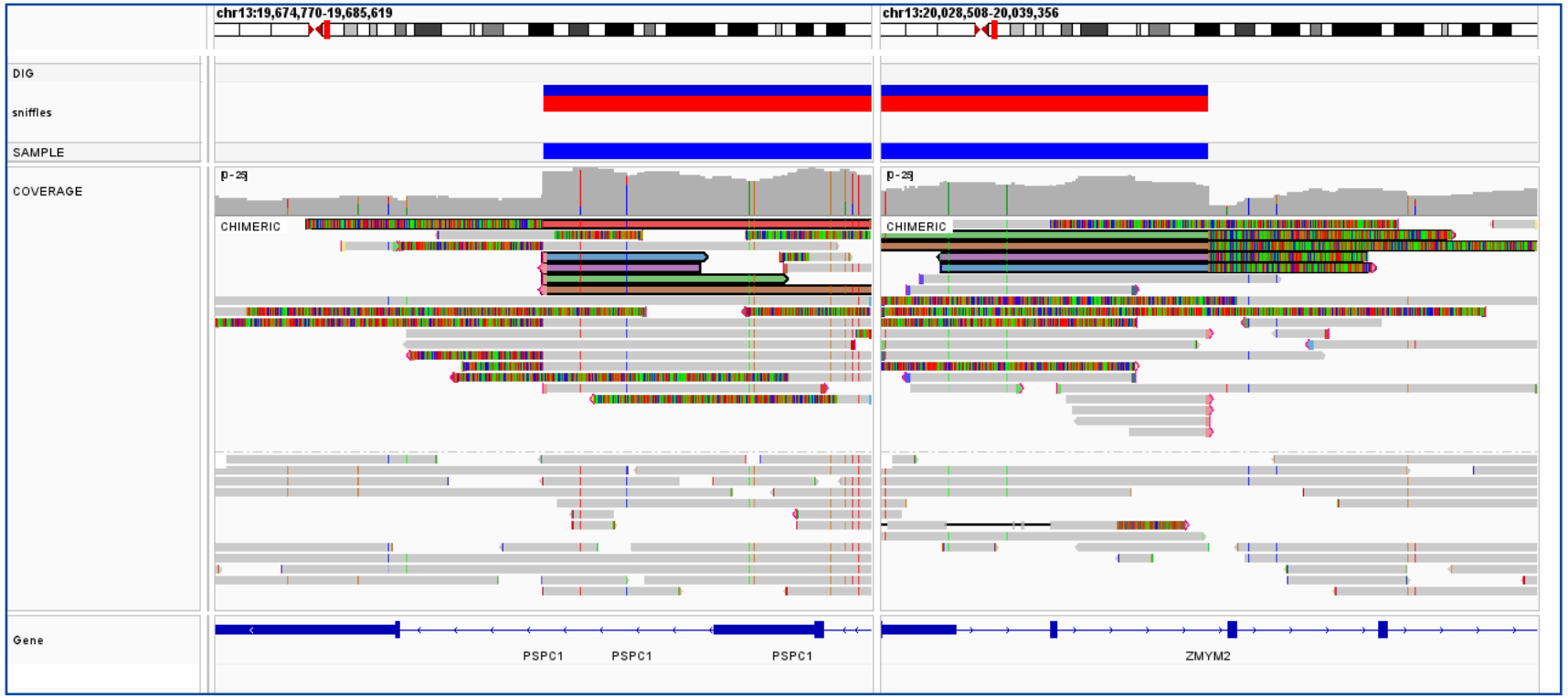
300kb environ



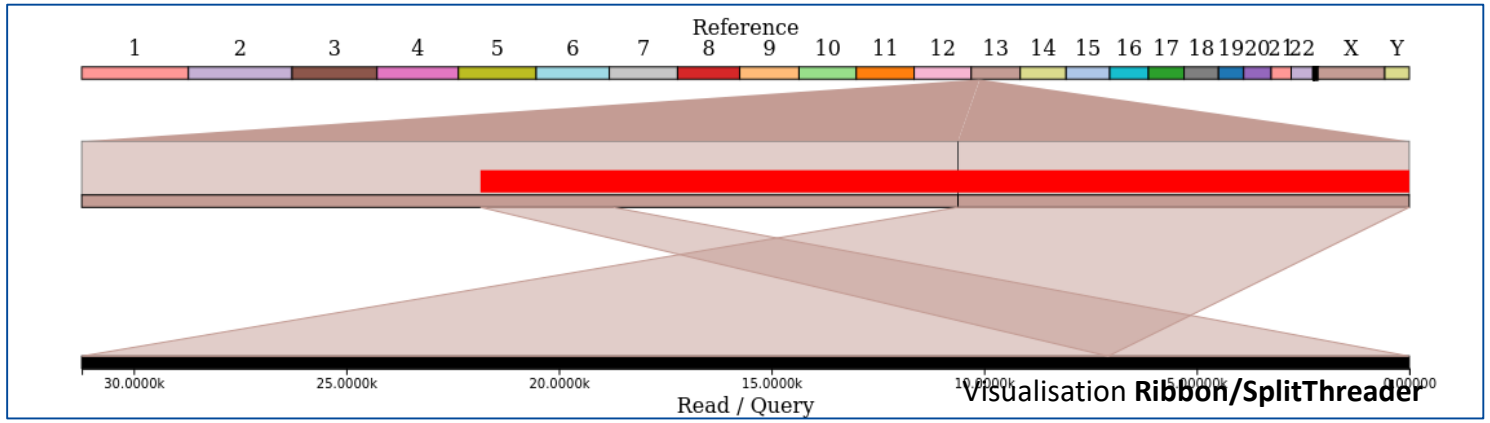
UPN#02 – Explorations longs fragments

- Etude ADN





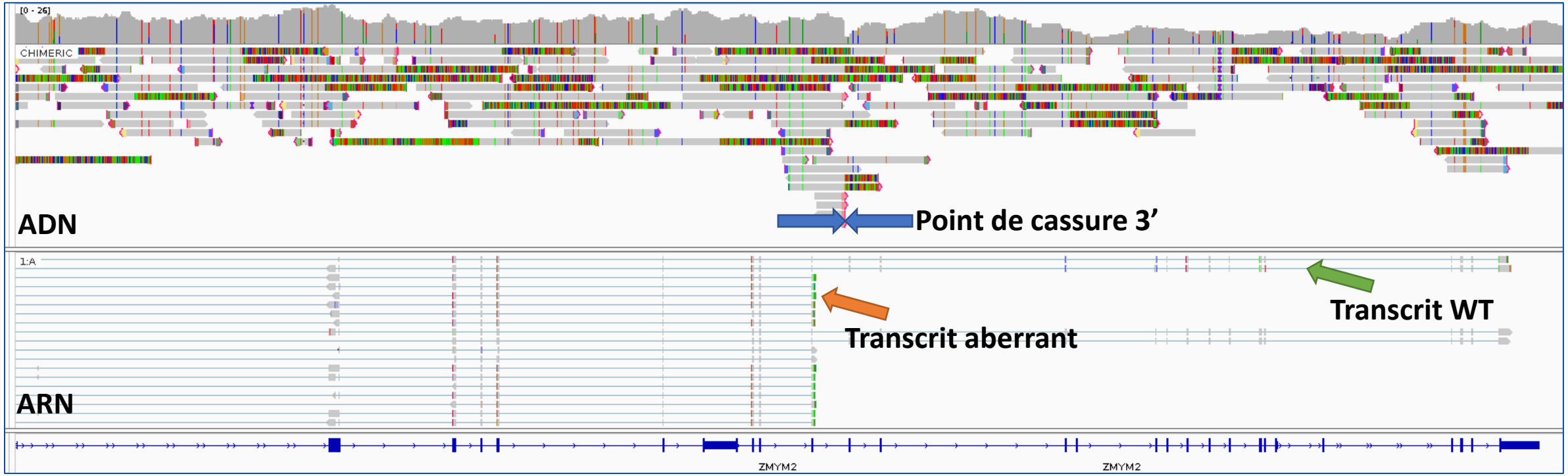
- Triplication en tandem direct



Visualisation **Ribbon/SplitThreader**

UPN#02 – Explorations longs fragments

- Etude ARN



- Equivalent *Frameshift* exon 10

UPN#02 – Mécanisme ?

- MIM #619522 – AD

Neurodevelopmental-craniofacial syndrome with variable renal and cardiac abnormalities – CAKUT Like

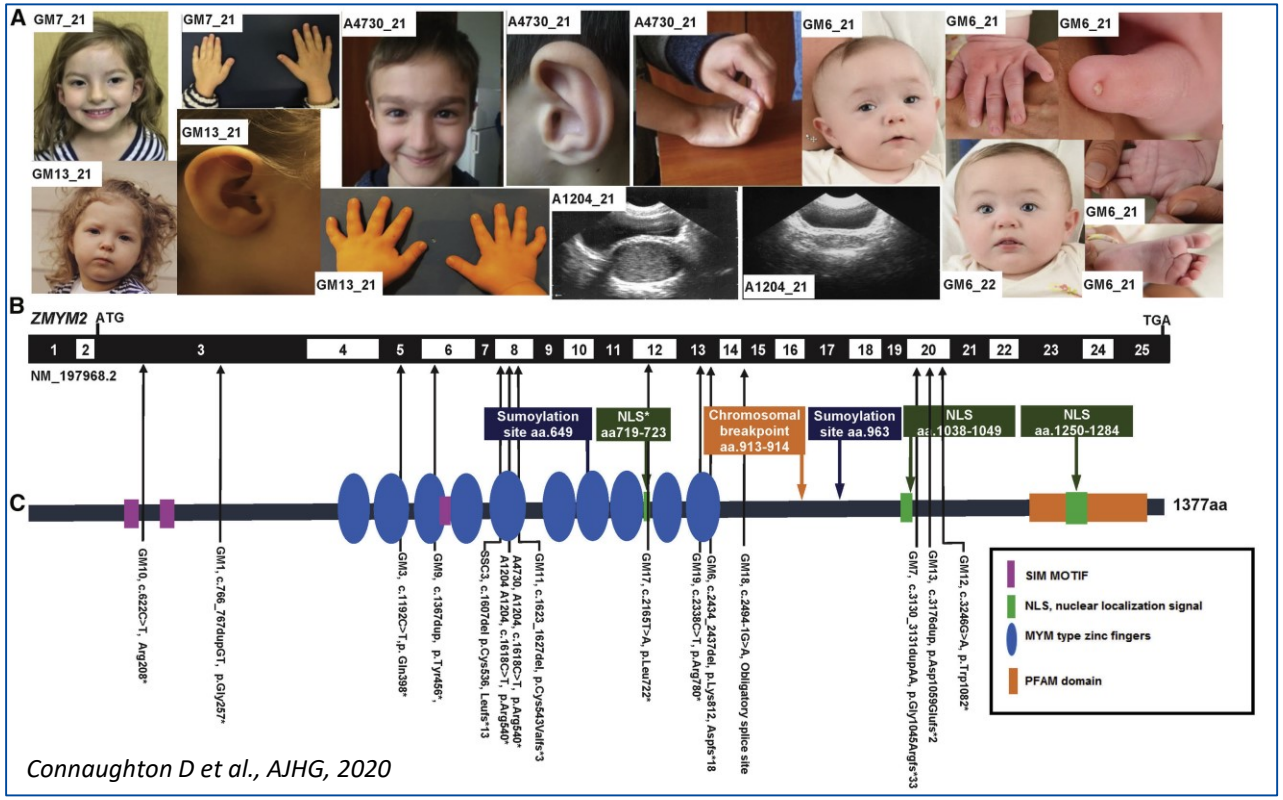
- Mécanismes : LoF et Dnég décrits

Connaughton D et al., AJHG, 2020

ARTICLE

Mutations of the Transcriptional Corepressor *ZMYM2* Cause Syndromic Urinary Tract Malformations

Dervla M. Connaughton,[1,2,46] Rufeng Dai,[1,3,46] Danielle J. Owen,[4] Jonathan Marquez,[5] Nina Mann,[1] Adda L. Graham-Paquin,[6] Makiko Nakayama,[1] Etienne Coyaud,[7,8] Estelle M.N. Laurent,[7,8] Jonathan R. St-Germain,[7] Lot Snijders Blok,[9,10,11] Arianna Vino,[9] Verena Klämbt,[1] Konstantin Deutsch,[1] Chen-Han Wilfred Wu,[1] Caroline M. Kolvenbach,[1] Franziska Kause,[1] Isabel Ottlewski,[1] Ronen Schneider,[1] Thomas M. Kitzler,[1] Amar J. Majmundar,[1] Florian Buerger,[1] Ana C. Onuchic-Whitford,[1,12] Mao Youying,[1] Amy Kolb,[1] Daanya Salmanullah,[1] Evan Chen,[1] Amelie T. van der Ven,[1] Jia Rao,[3] Hadas Ityel,[1] Steve Seltzsam,[1] Johanna M. Rieke,[1] Jing Chen,[1] Asaf Vivante,[1,13] Daw-Yang Hwang,[1] Stefan Kohl,[1] Gabriel C. Dworschak,[1]



Connaughton D et al., AJHG, 2020

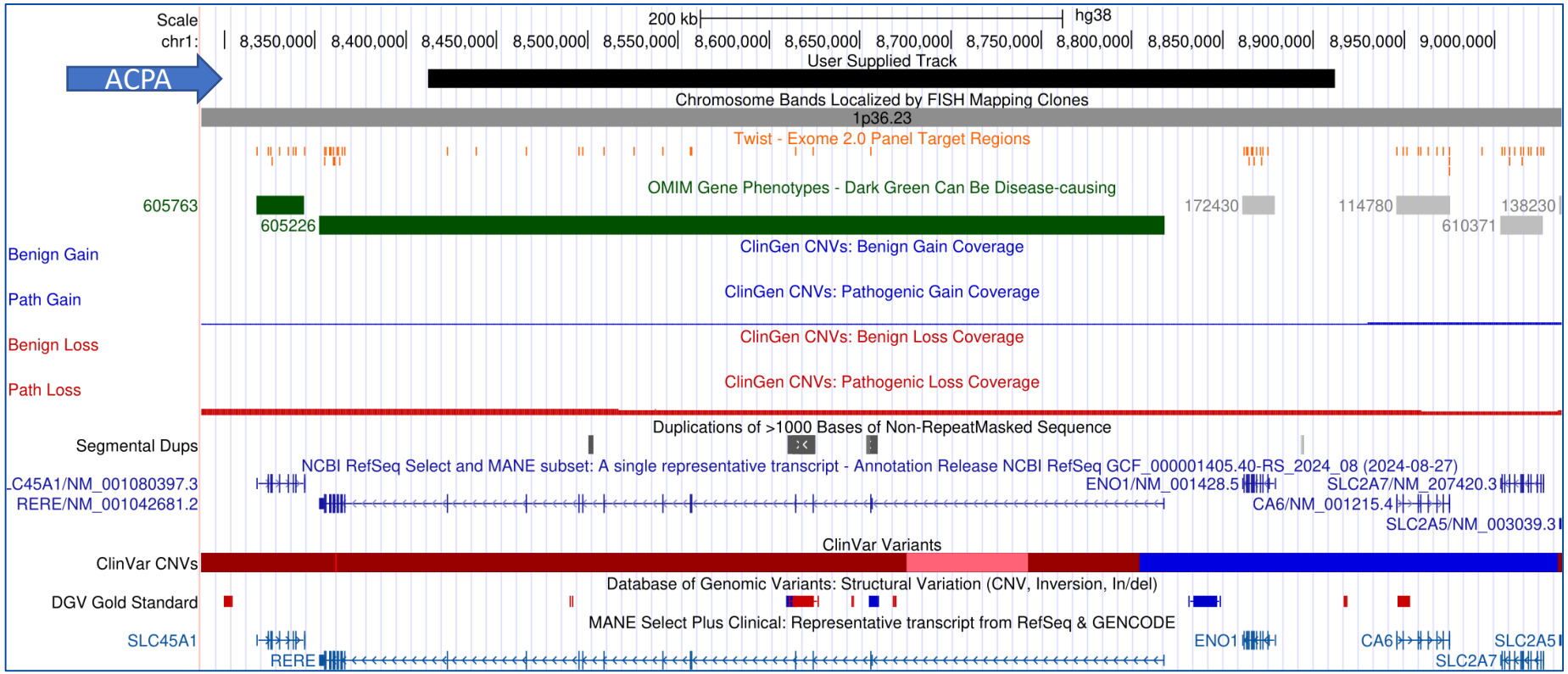
UPN#03

- Patiente de 11 ans
  - Retard global acquisitions
  - Fatigabilité, troubles sphinctériens

- ACPA

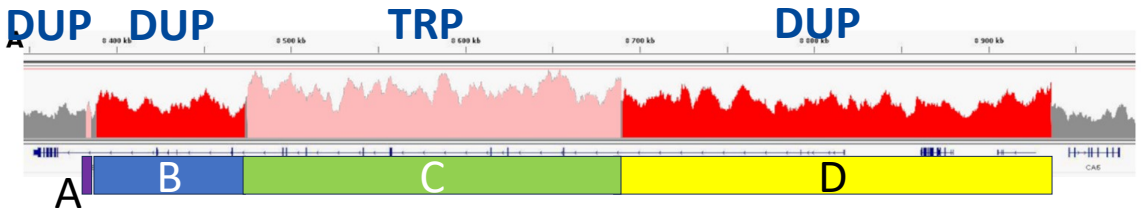
Gain d'1 copie 1p36.23 *de novo*

499kb environ

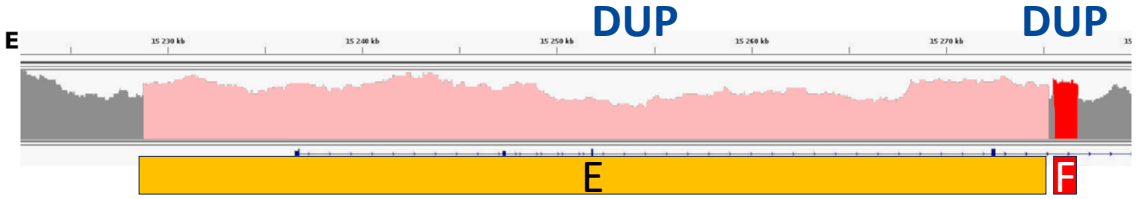


UPN#03 – Explorations longs fragments

Région 1p36.23 : 4 CNV sur env. 500kbp

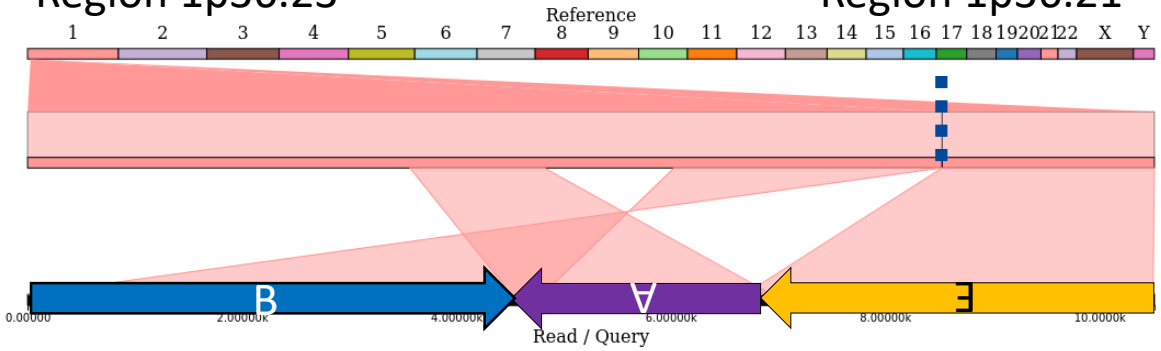


Région 1p36.21 : 2 CNV sur env. 40kbp

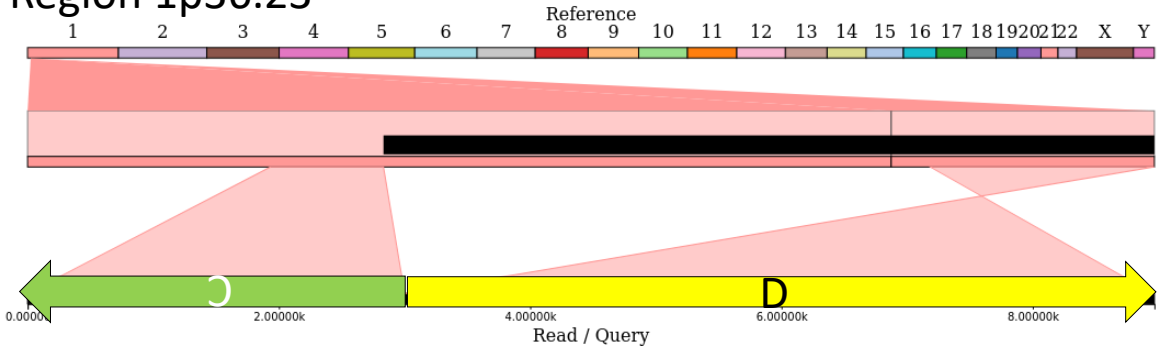


Région 1p36.23

Région 1p36.21

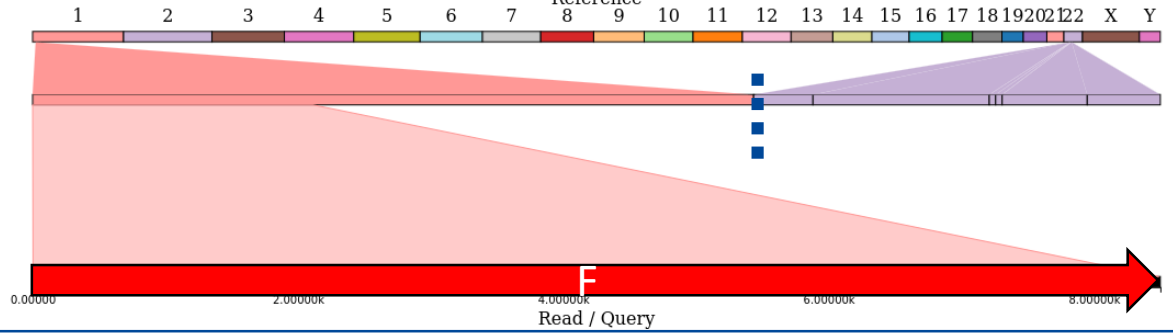


Région 1p36.23



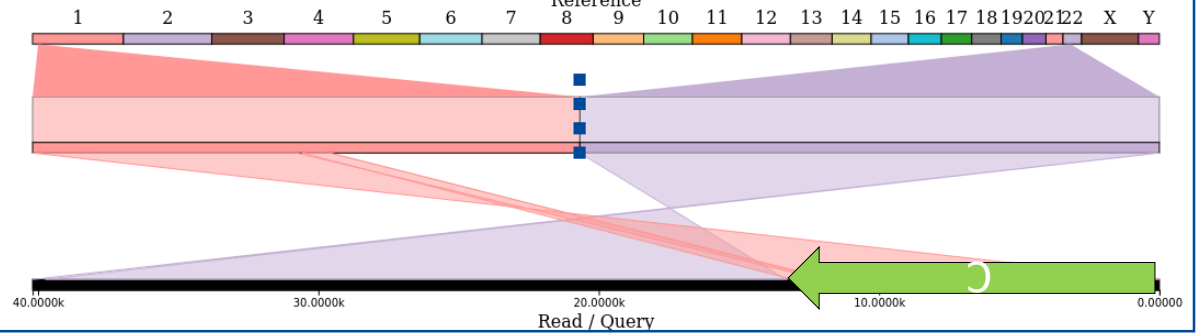
Région 1p36.21

Région 22q11.21

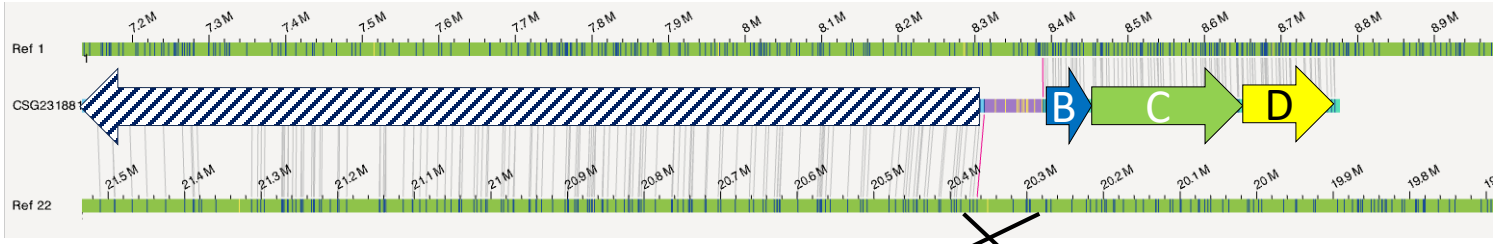


Région 1p36.23

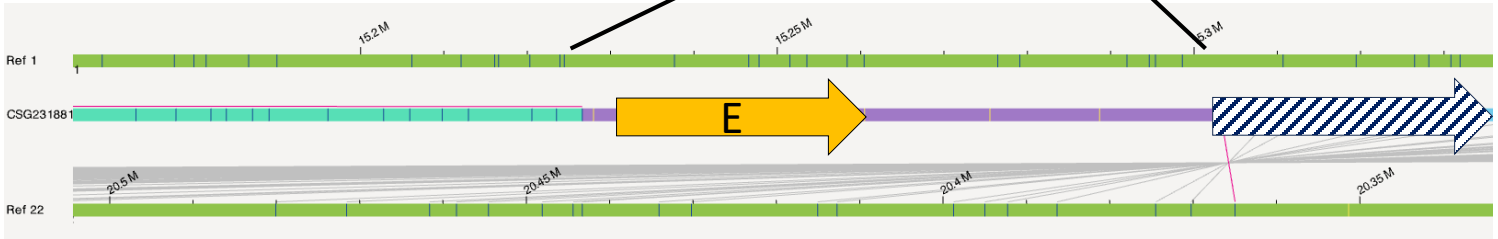
Région 22q11.21



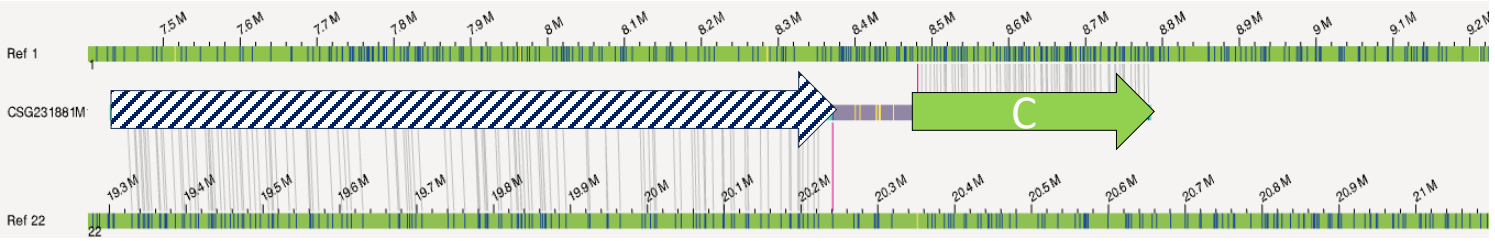
UPN#03 – Cytogénétique comparée



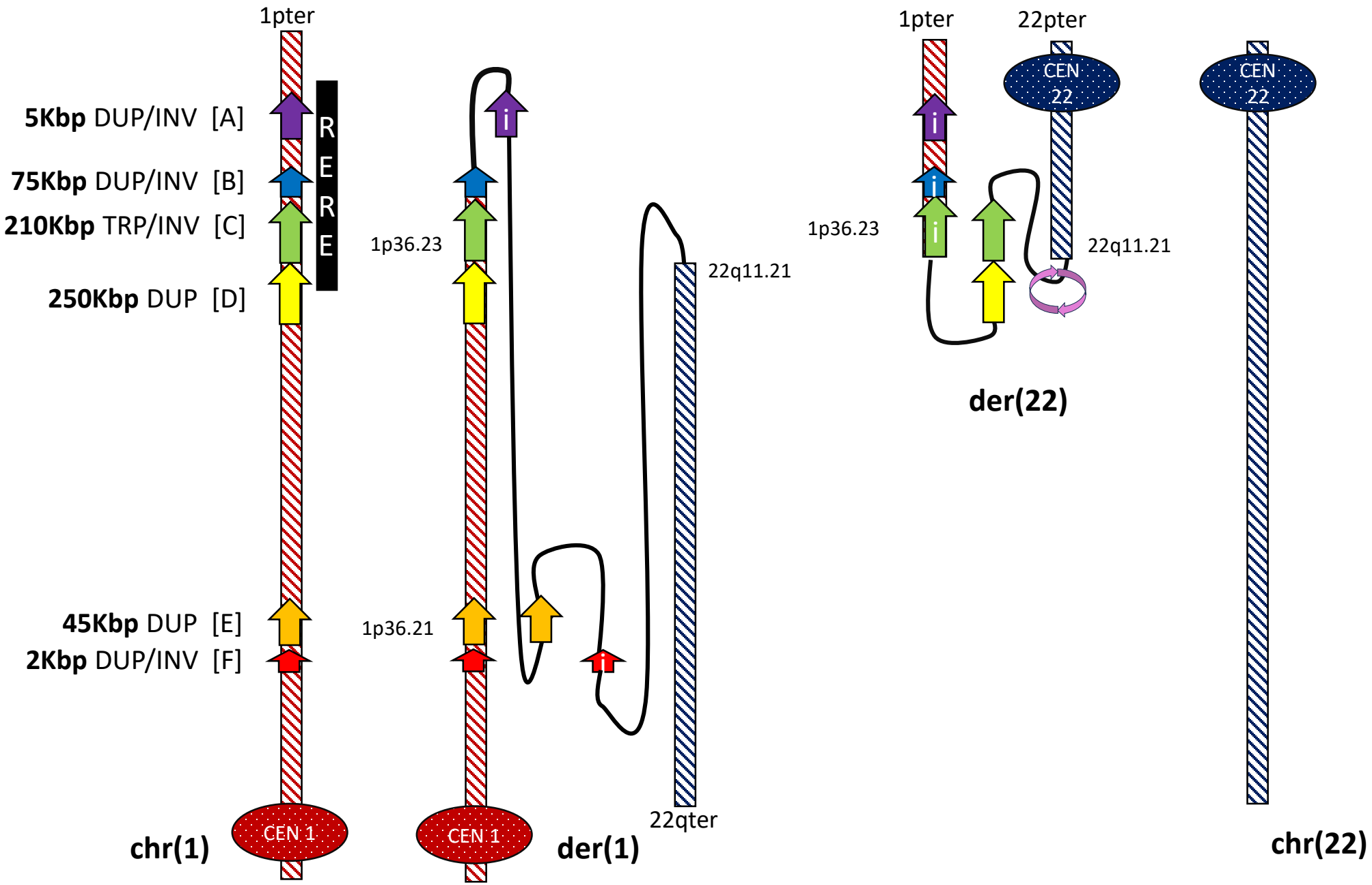
der(1)t(1;22) – zoom 1p36.23



der(1)t(1;22) – zoom 1p36.21

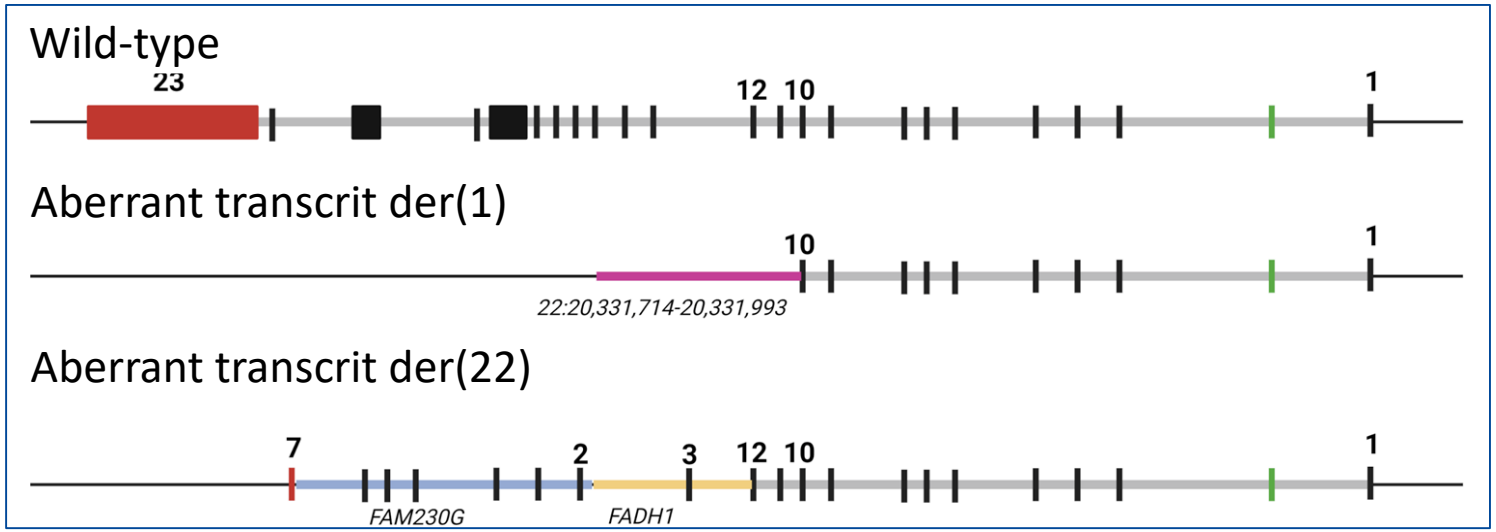


der(22)t(1;22)


1pter
5Kbp DUP/INV [A]
75Kbp DUP/INV [B]
210Kbp TRP/INV [C]
250Kbp DUP [D]
45Kbp DUP [E]
2Kbp DUP/INV [F]
R
E
R
E
CEN 1
chr(1)
1p36.23
1p36.21
i
CEN 1
22qter
22q11.21
der(1)
1pter
22pter
CEN
22
1p36.23
22q11.21
der(22)
CEN
22
chr(22)

UPN#03 – Conséquences ?

- Etude ARN *RERE*
  - 1 transcrit WT
  - 2 transcrits aberrants



***RERE*-Related Disorders**

Synonym: Neurodevelopmental Disorder with or without Anomalies of the Brain, Eye, or Heart (NEDBEH)

Daryl A Scott, MD, PhD[1] and Elliott H Sherr, MD, PhD[2]

Created: March 21, 2019.

arginine-glutamic acid dipeptide repeats gene

- Faux-sens : hypothèse DNég avec phénotype typique
- LoF : haploinsuffisance, présentation plus atténuée

UPN#04 #05

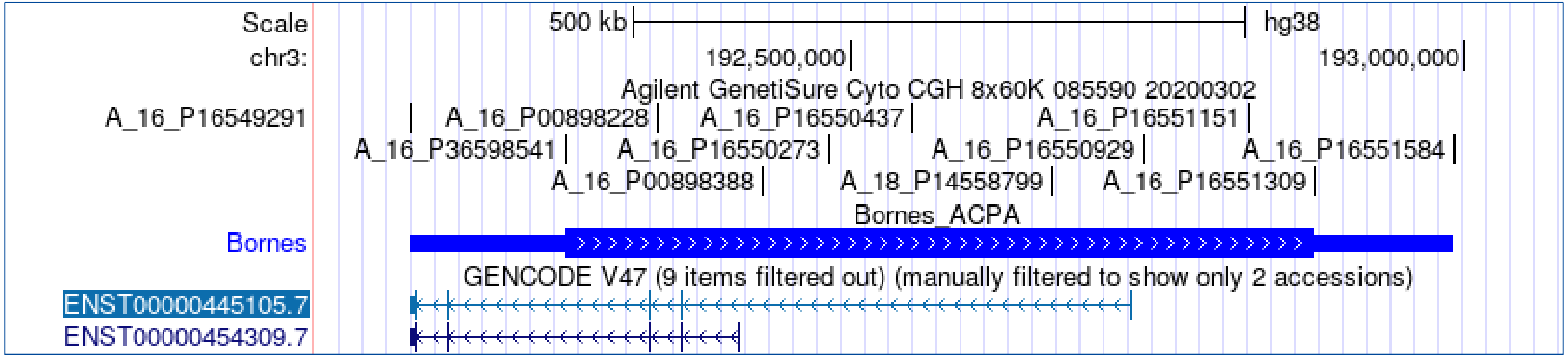
- UPN#04 LILLE
- Deuxième enfant d'une famille non apparentée

- Développement initial normal
- 18 mois: présentation d'épisodes récurrents d'états de mal épileptiques
- Régression psychomotrice

- UPN#05 CAEN
- Enfant d'une famille non apparentée

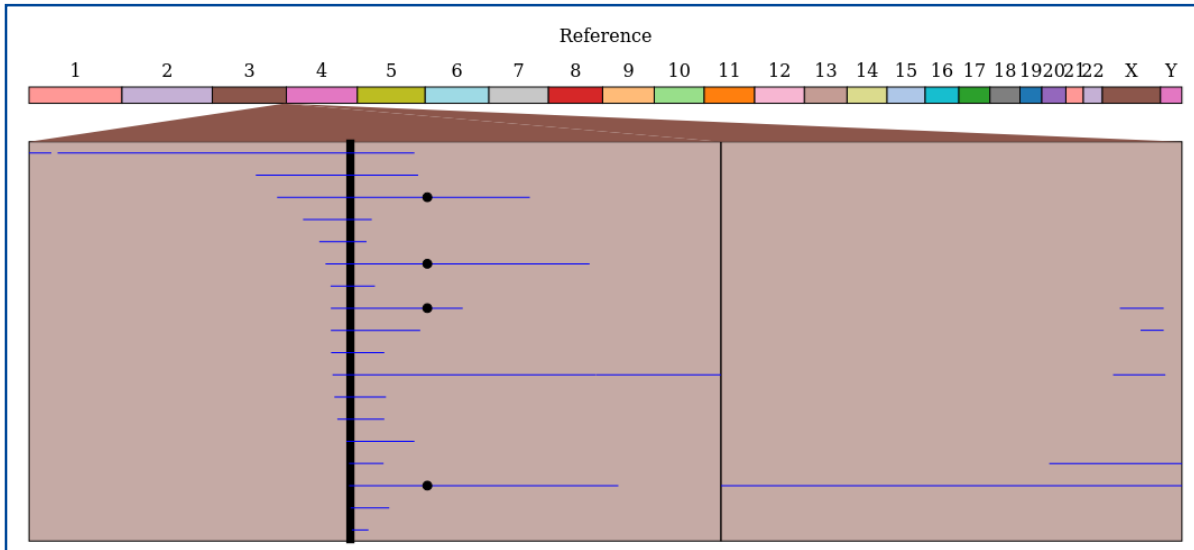
- 5 mois: épisodes d'absence et de clonies
- 6 ans: retard global de développement

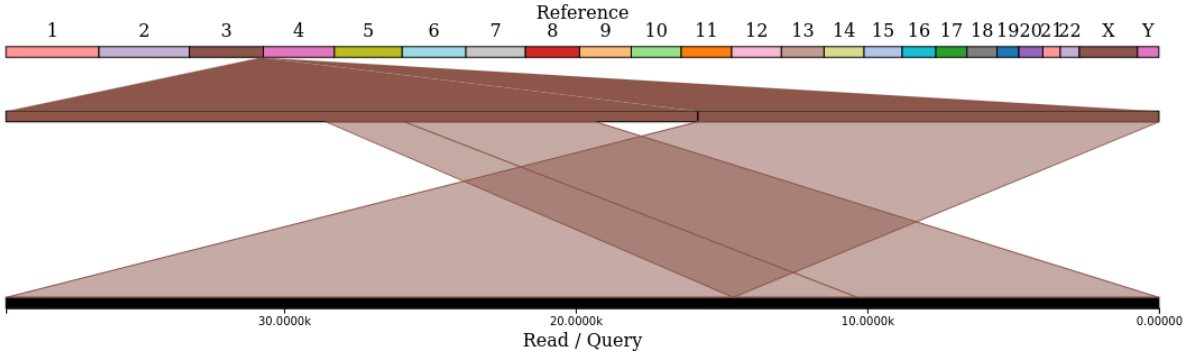
UPN#04 #05 – Remaniement étudié



- ACPA (8x60K) et exome
- Gain d'1 copie **3q28q29** *de novo* ~600kb impliquant *FGF12*
- **Variant faux-sens pathogène :** Encéphalopathie épileptique et développementale (MIM #617166 - AD)

UPN#04 #05 – Explorations longs fragments

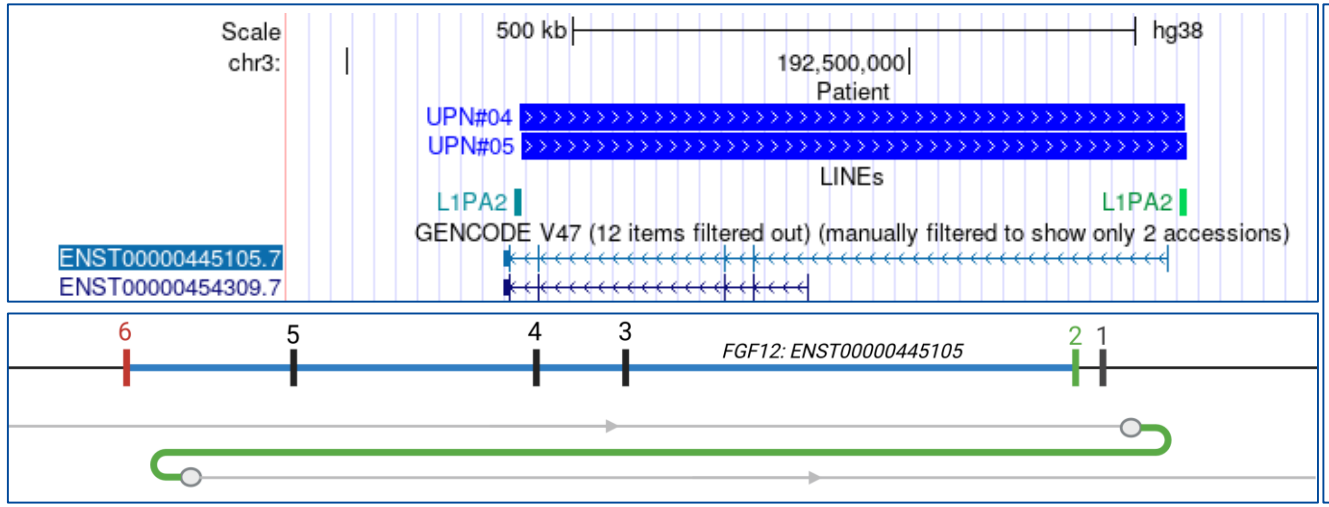


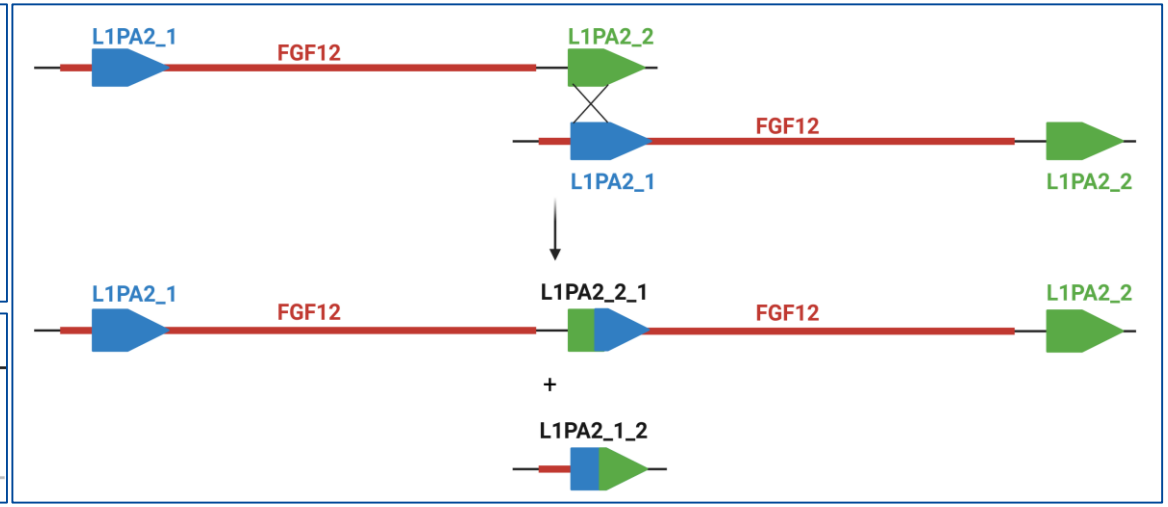


Visualisation **Ribbon/SplitThreader**

- Séquençage Long-Read Nanopore : N50=5100bp, $N50_{readchimeric}$=16 498bp
- Visualisation duplication en tandem
- Hypothèse NAHR – colocalisation points de cassures dans LINE **L1PA2_1 + L1PA2_2**

UPN#04 #05 – Explorations longs fragments





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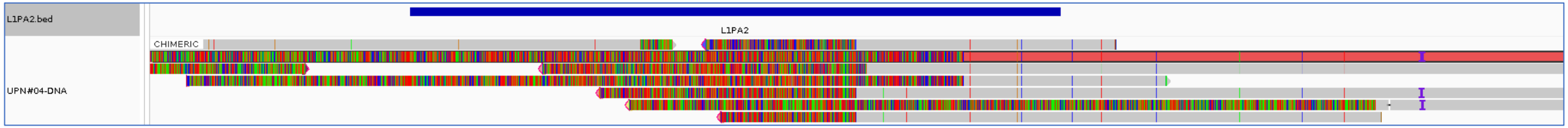
- Visualisation duplication en tandem
- Hypothèse NAHR – colocalisation points de cassures dans LINE **L1PA2_1 + L1PA2_2**

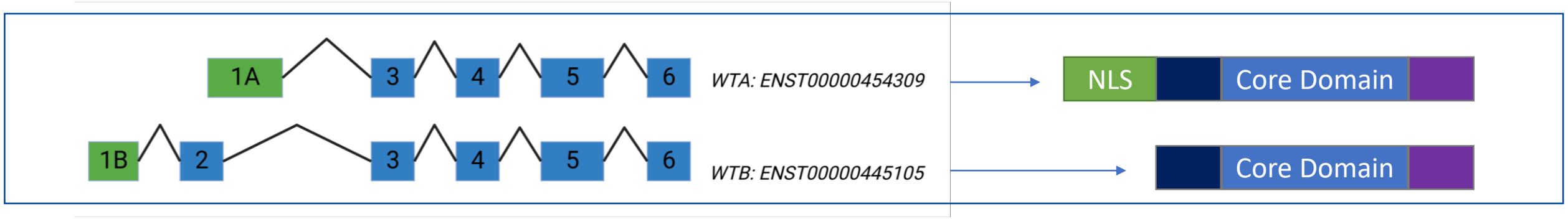
UPN#04 #05 – Explorations longs fragments

L1PA2



- Séquençage Long-Read Nanopore : N50=5100bp, $N50_{readchimeric}$=16 498bp
- Visualisation duplication en tandem
- Hypothèse NAHR – colocalisation points de cassures dans LINE **L1PA2_1 + L1PA2_2**

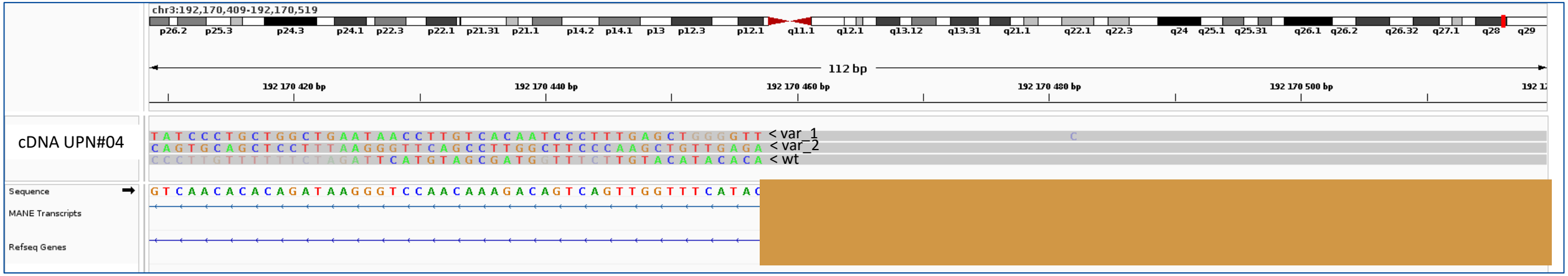
UPN#04 – Etude transcriptionnelle

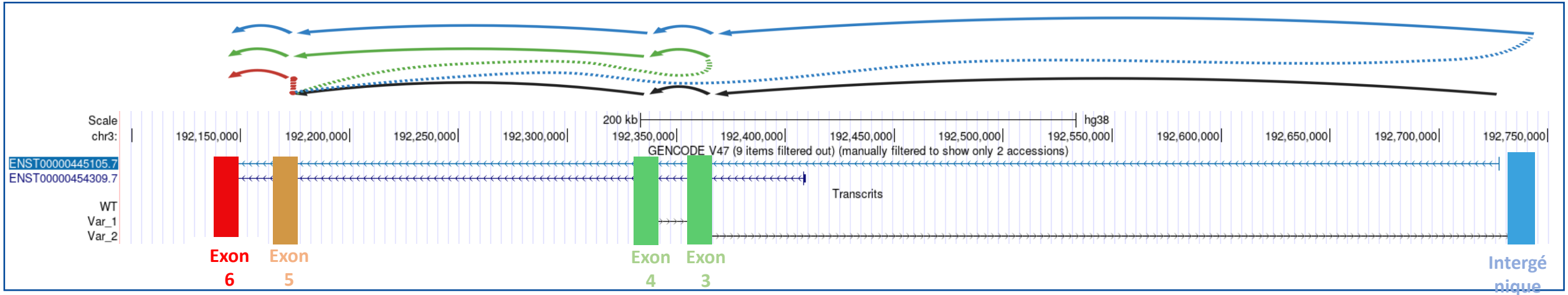


5' upstream sequence			
ENSE00001678043		-	-
Intron 1-2			
ENSE00003497617		-	1
Intron 2-3			
ENSE00003532256	Exon 3	1	1
Intron 3-4			
ENSE00002207494		1	0
Intron 4-5			
ENSE00001005526	Exon 5	0	1
Intron 5-6			
ENSE00001407888		1	-

- Présence de 2 isoformes
  - **WTA** code pour signal NLS
  - Modulation des canaux sodiques-voltages dépendants
- Variant faux-sens : mécanisme GoF connu
- Etude des duplications
  - Mise en évidence de 2 transcrits aberrants

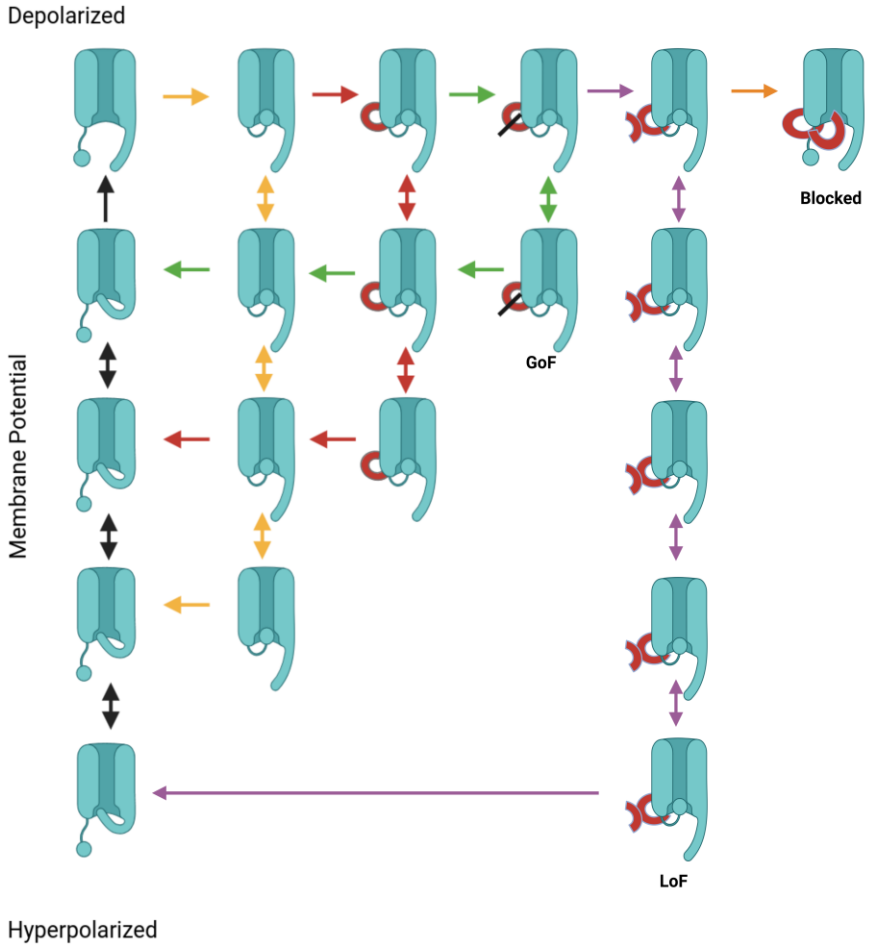
UPN#04 – Etude transcriptionnelle





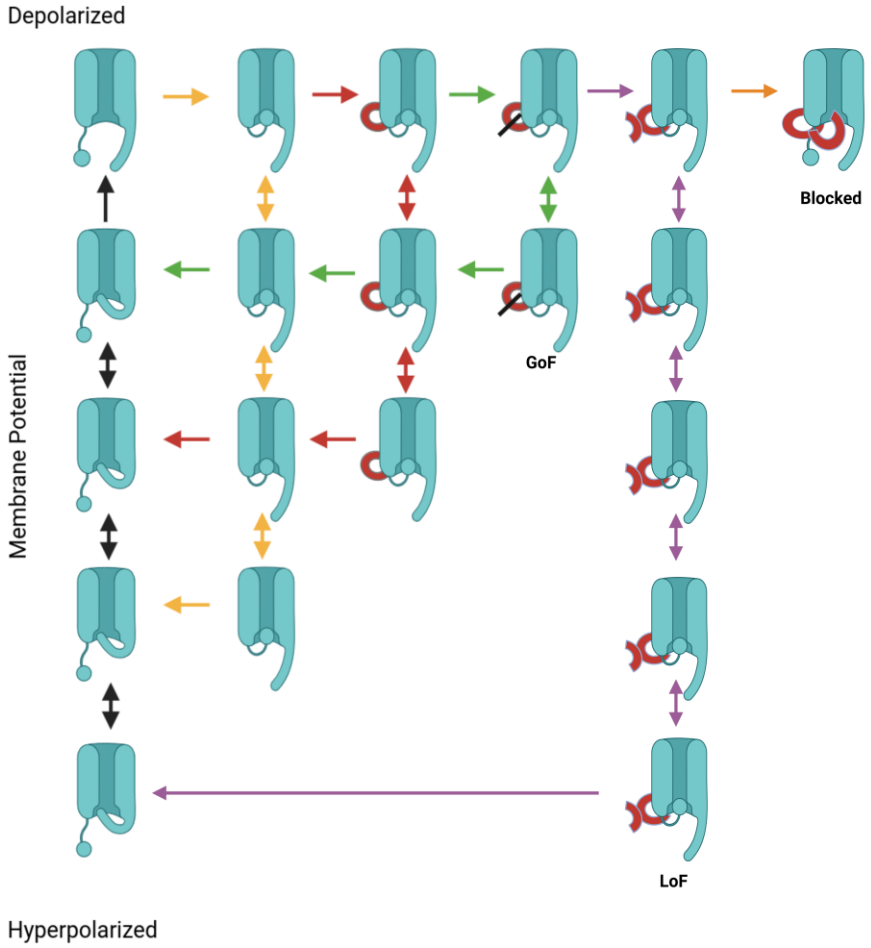
- Présence de 2 transcrits aberrants
  - var_1: chevauchement exon 5 puis exon 3 — Exons en phase, élongation protéique
  - var_2: chevauchement exon 5 puis insertion intergénique — Codon stop prématuré

UPN#04 – Impact sur la fonction des canaux



Jaune: WT
Rouge: Présence de FGF12
Vert: FGF12 avec variant ponctuel pathogène
Violet: Duplication de FGF12
Orange: Isoforme WTA

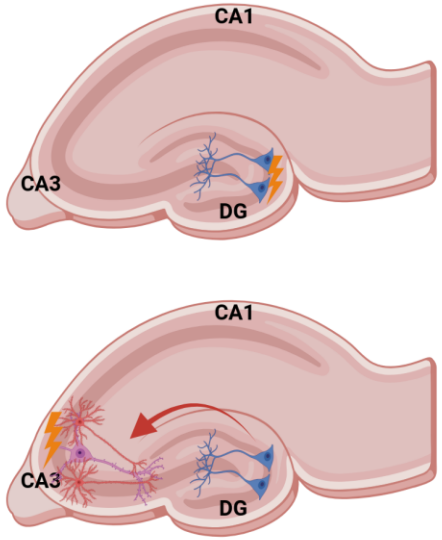
UPN#04 – Impact sur la fonction des canaux



Jaune: WT
Rouge: Présence de FGF12
Vert: FGF12 avec variant ponctuel pathogène
Violet: Duplication de FGF12
Orange: Isoforme WTA

Variant Ponctuel → Gain de Fonction des canaux →

Duplication → Perte de fonction du canal dans le Gyrus Denté: Gain de fonction dans les neurones inhibiteurs →



Modulating effects of FGF12 variants on $Na_V1.2$ and $Na_V1.6$ being associated with developmental and epileptic encephalopathy and Autism spectrum disorder: A case series

Simone Seiffert [1], Manuela Pendziwiat [2], Tatjana Bierhals [3], Himanshu Goel [4], Niklas Schwarz [1], Amelie van der Ven [3], Christian Malte Boßelmann [1], Johannes Lemke [5], Steffen Syrbe [6], Marjolein Hanna Willemsen [7], Ulrike Barbara Stefanie Hedrich [1], Ingo Helbig [8], Yvonne Weber [9]

Impact des duplications : comment s’en sortir ?

Au final… 3 gains de copies

- Approches combinées +++
  - ADN long fragments : caractérisation ensemble du remaniement et identification mécanisme sous jacent
  - ARN (long fragments) : conséquences transcriptionnelles

- UPN#02 〉〉 Triplication en TD 〉〉 **LoF** ou DNég
- UPN#03 〉〉 Chromoanagénésie 〉〉 LoF
- UPN#04 & #05 〉〉 Duplication en TD 〉〉 GoF

Remerciements

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Marine Tessarech
Perrine Brunelle
Caroline Thuillier
Delphine Ceraso
Pauline Grave
Alexis Leurent
Heidi Tampere
Julien Merlin

CHU Lille, Neuropédiatrie

Laurence Chaton
Adeline Traufflers
Pierre Cleuziou

CHU Caen, Cytogénétique

Nicolas Gruchy
Marion Gérard

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Céline Villenet

CHU Lille, Bioinformatique

Emilie Ait-Yahya