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institut de génétique et de biologie moléculaire et cellulaire

phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

In vivo chromosomal engineering in rodents to analyse structural variants through Crismere

Translational Medicine and Neurogenetics Department

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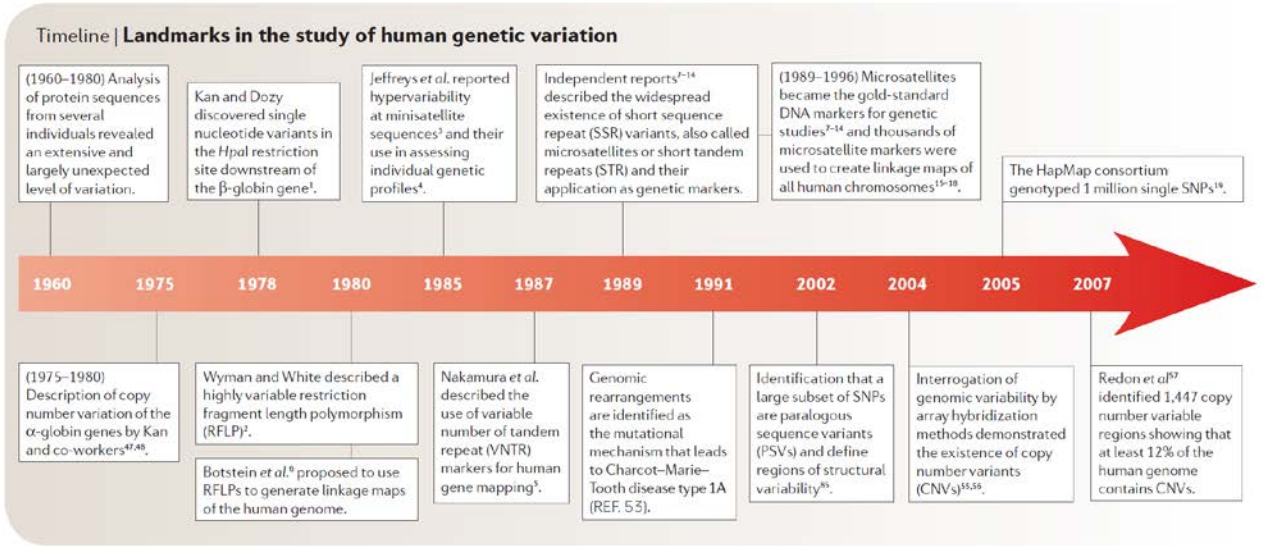
Outlook

- Short Introduction on copy number variants in human genetic variations
- Chromosomal engineering revolution in the CrispR/Cas9 age: « Crismere »
- Ex: copy number disease with intellectual disabilities (Down syndrome and 16p11.2 deletion syndromes) to better understand the genetic behing behavior and cognition defects, and to carry preclinical evaluation
- Conclusion and Future perspectives

Human genetic variation

- *SNP*
  - *90% of all sequence variation*
  - *on average about every 100 to 300 bases*
  - *Functional, or non-synonymous, SNP*
  - *Neutral, or synonymous SNPs are still useful as genetic markers in GWAS*
- SV
  - deletions, inversions, insertions and duplications
  - a typical human has 2,100 to 2,500 structural variations: approximately 1,000 large deletions,160 copy-number variants, 915 Alu insertions, 128 L1 insertions, 51 SVA insertions, 4 NUMTs, and 10 inversions (1000 genome projects)
- CNV
  - Deletion or duplication, invertion of large regions of DNA
  - 0.4% of the genomes of unrelated humans differ with respect to copy number
- *Epigenetics*
  - *Changes in the chemical tags that attach to DNA or to Chromatin components*



OPINION

Copy number variants and genetic traits: closer to the resolution of phenotypic to genotypic variability

Jacques S. Beckmann, Xavier Estivill and Stylianos E. Antonarakis

Improving the technology for chromosomal engineering

Chromosome engineering in mice

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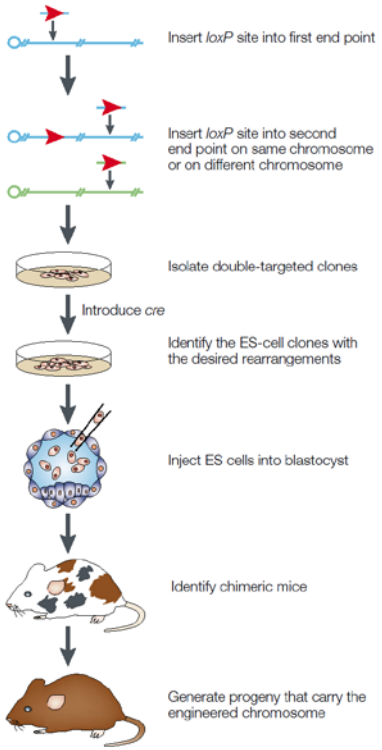
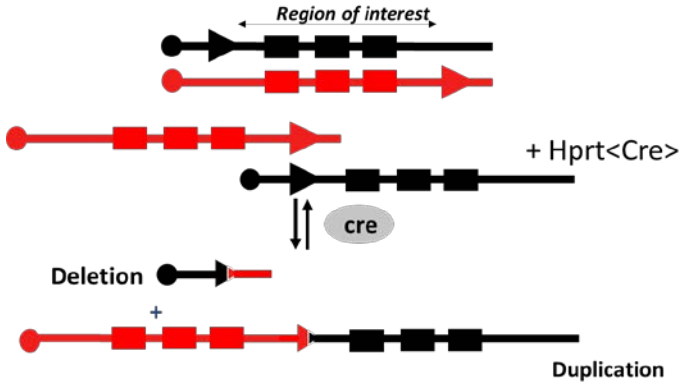
NATURE · VOL 378 · 14 DECEMBER 1995

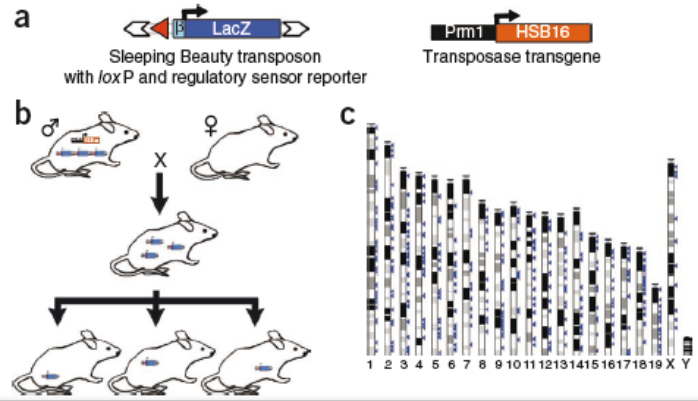


Targeted Meiotic recombination
(TAMERE loxP/Cre; Herault et al. 1998)



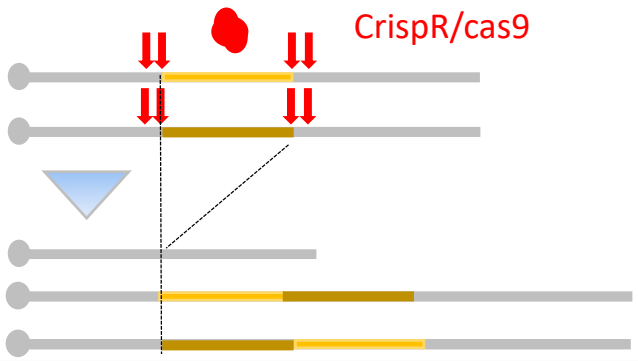
3-5 years

String method to insert loxP sites
with SB transposon (Ruff et al, 2011)



3 years

CRISpr MEdiated Rearrangement
CRISMERE (Birling et al., Sci Rep.,2017)



6 months!!

No additional minigene !
No loxP sites needed!
No more ES cells work!

phenomin
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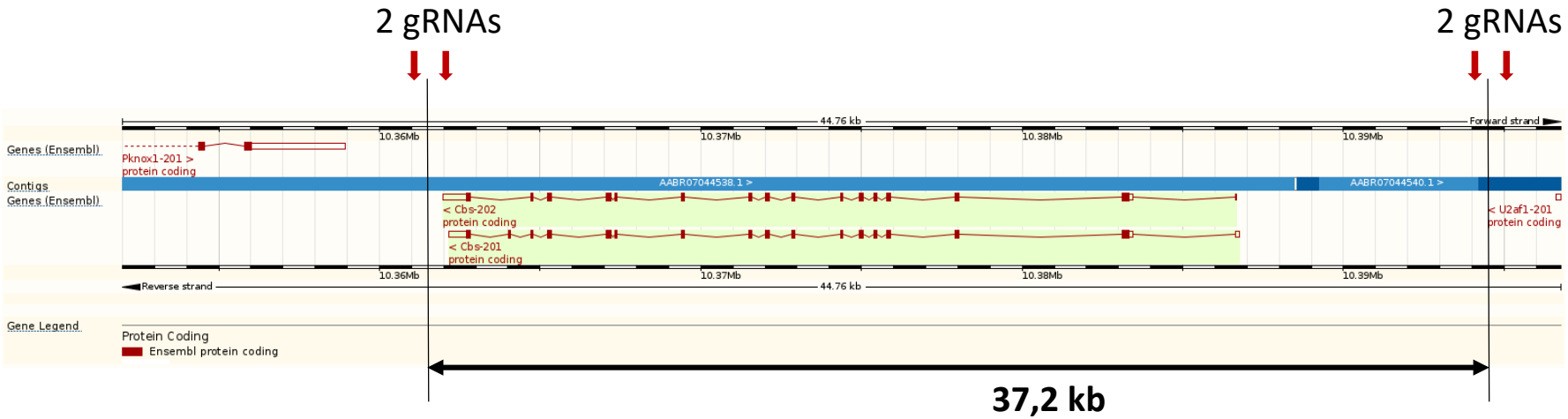
and new species!...

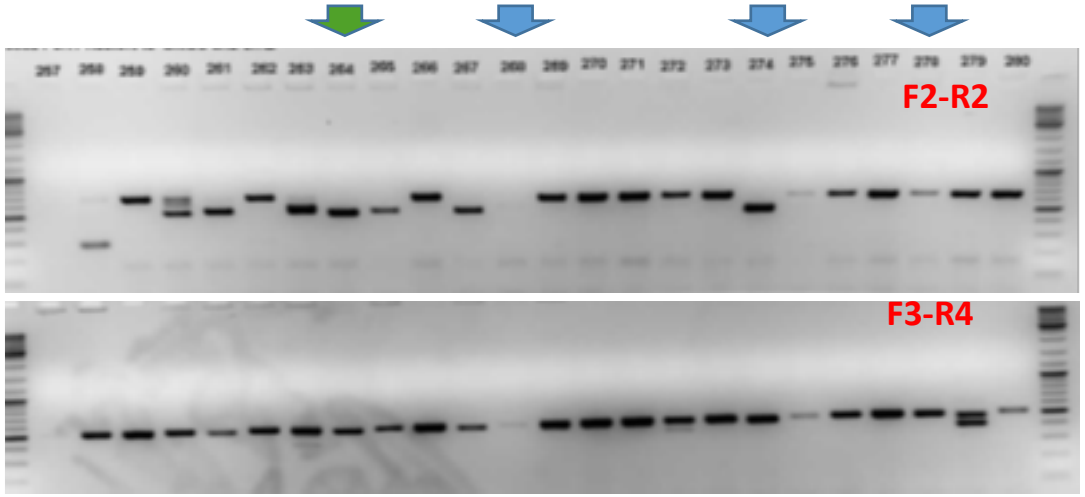
An example

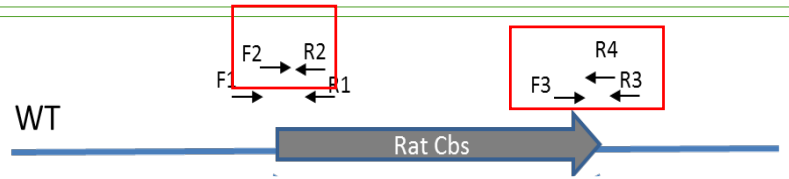
Generation of a model for monosomy/trisomy of rat *Cbs*



Injection conditions (Sprague Dawley fertilized oocytes) :
-50 ng/µl Cas9 WT + 25 ng/µl for the 4 gRNAs
-25 ng/µl Cas9 WT + 12 ng/µl for the 4 gRNAs

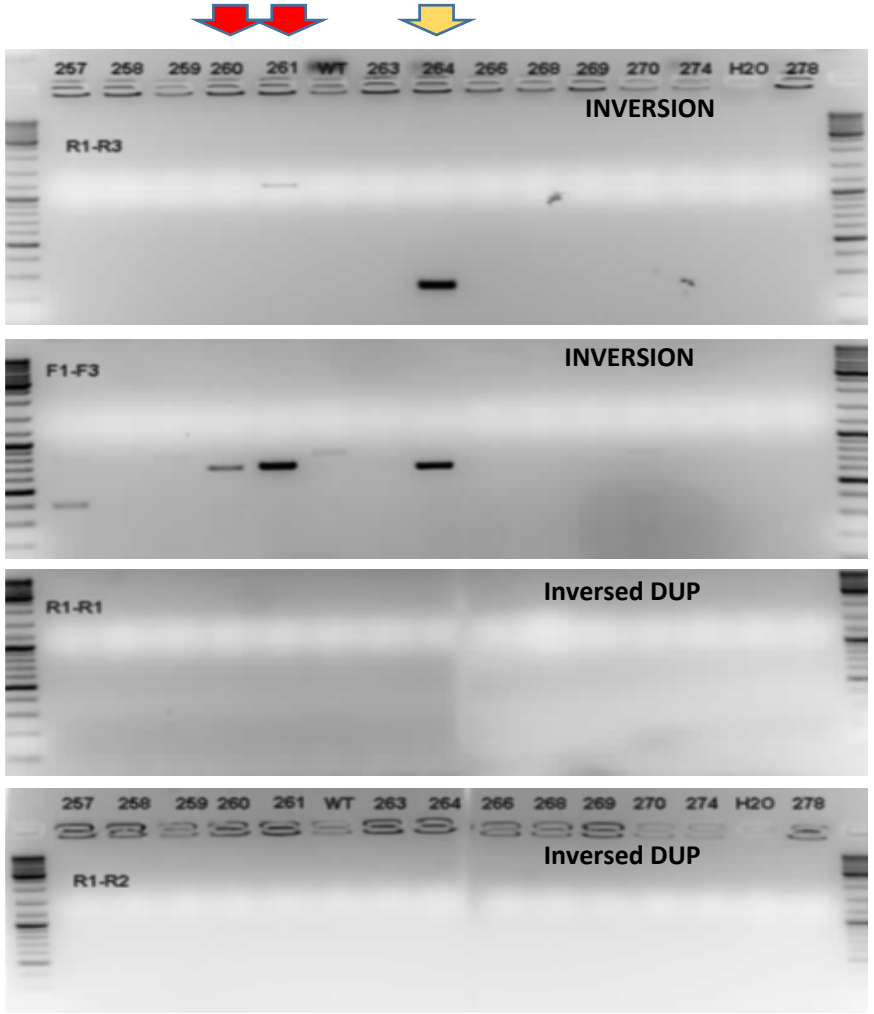
Looking for *Cbs* alleles generated by Crismere

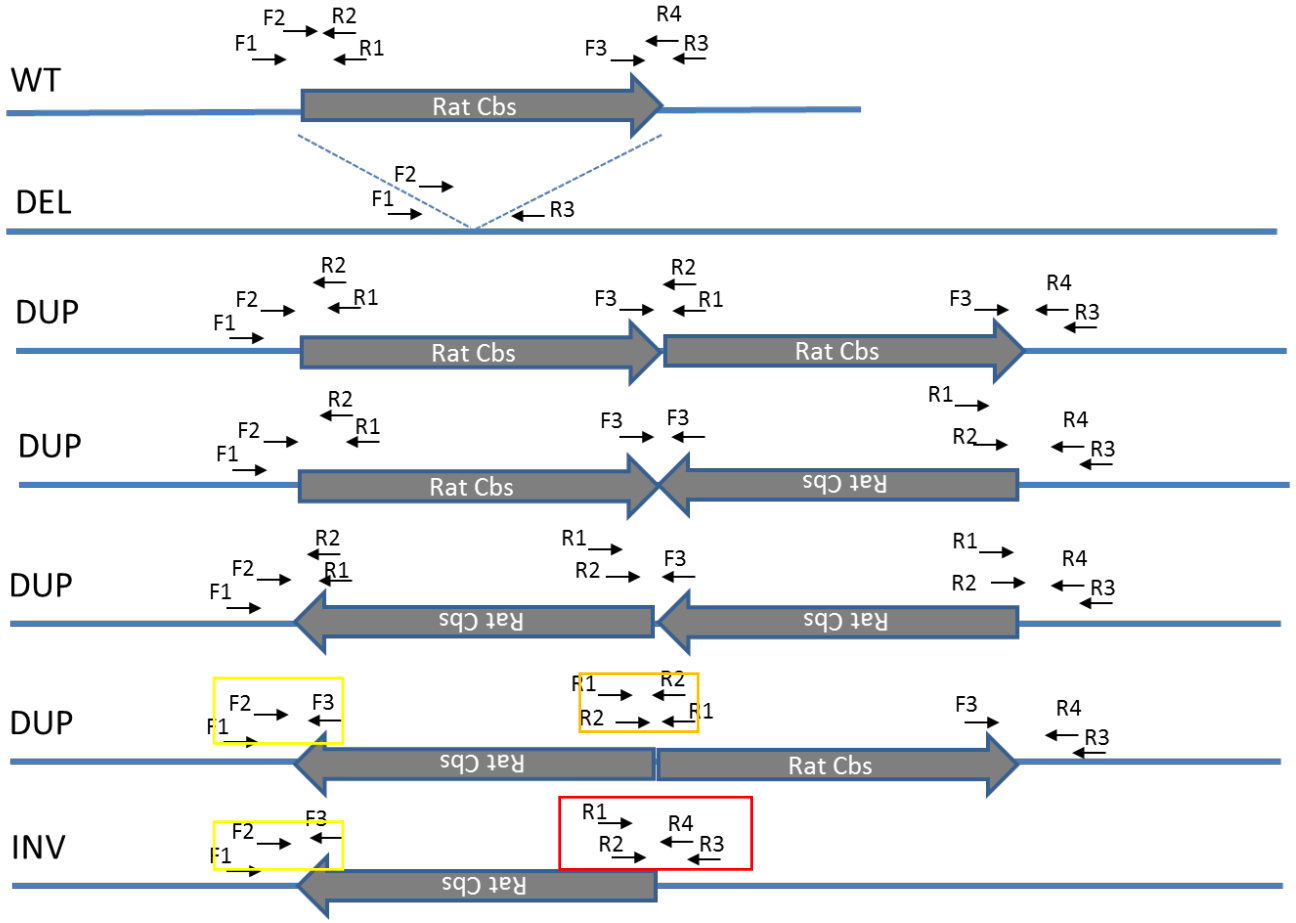




DEL: 257, 263, 268, 274 et 278 (274 and 278 : DEL confirmed by sequencing)
DUP: 264 (DUP confirmed by sequencing)

An even larger set of *Cbs* alleles generated





SCIENTIFIC REPORTS

OPEN

Revealing hidden complexities of genomic rearrangements generated with Cas9

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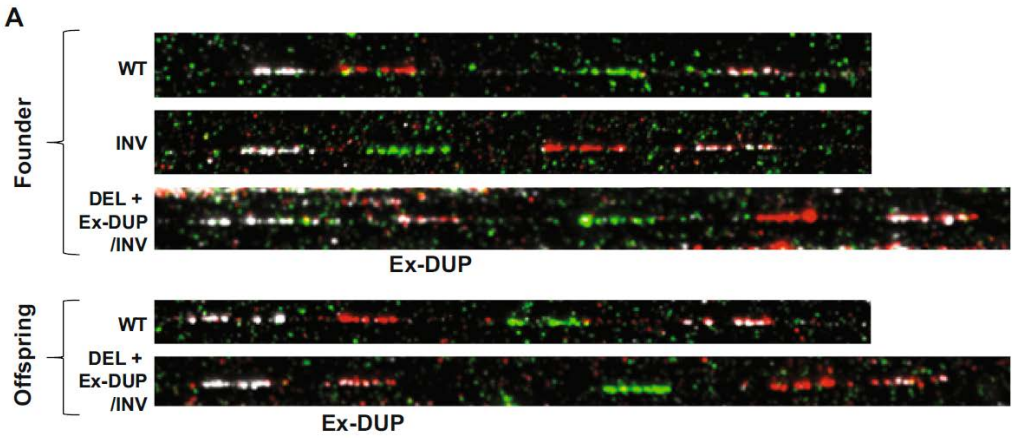
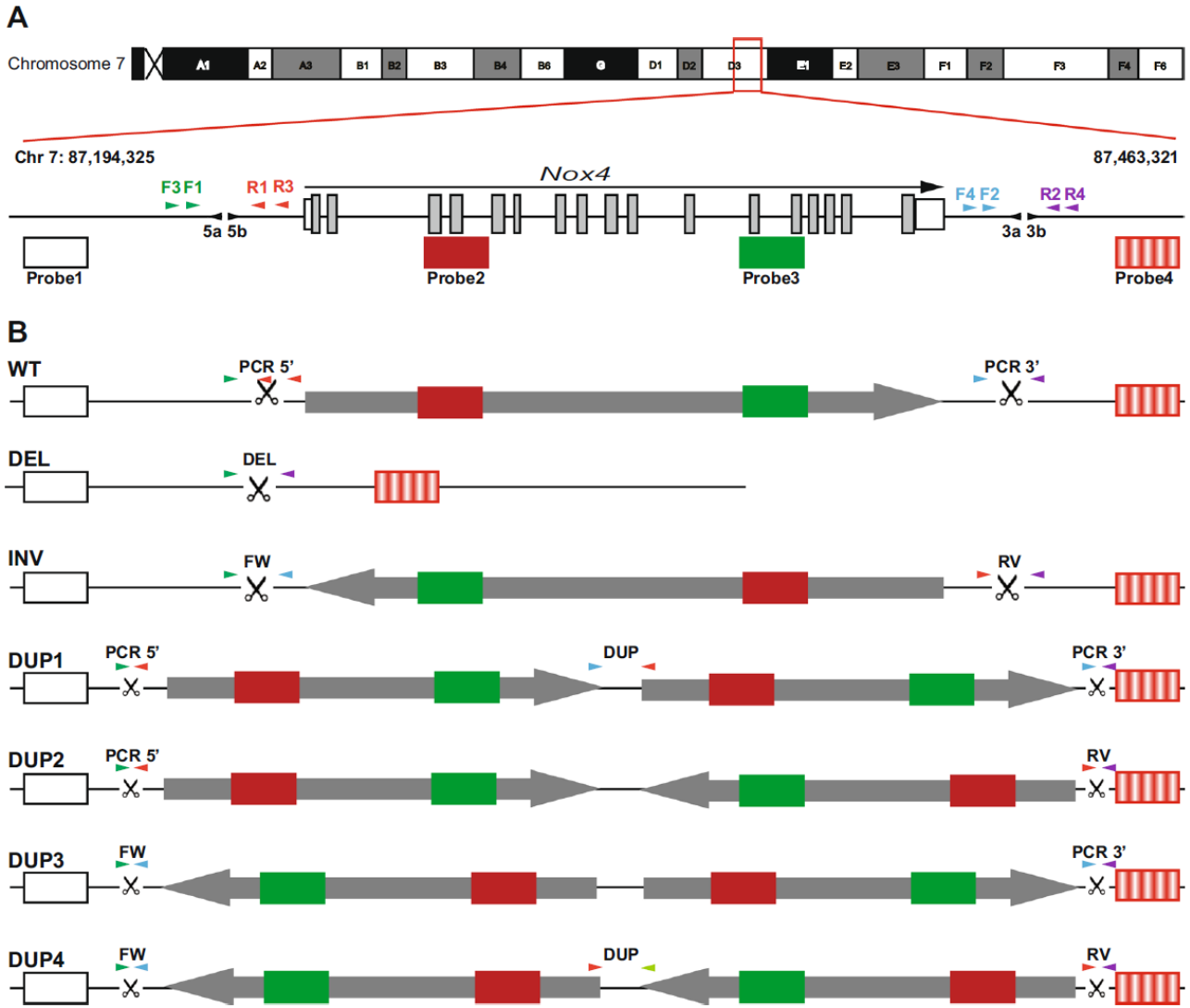
Published online: 09 October 2017

Katharina Boroviak, Beiyuan Fu, Fengtang Yang, Brendan Doe & Allan Bradley

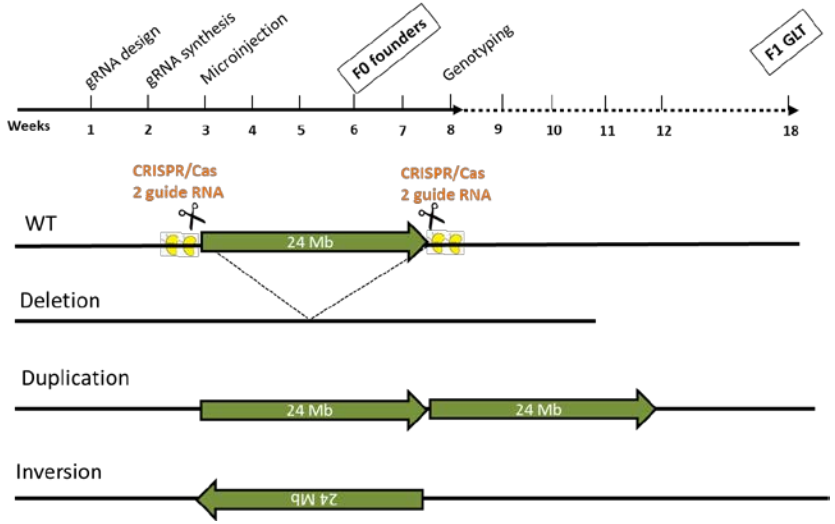
Modelling human diseases caused by large genomic rearrangements has become more accessible since the utilization of CRISPR/Cas9 in mammalian systems. In a previous study, we showed that genomic





Conclusion on generating structural variants

- Before CRISPR : labor-intensive, multiple breeding steps
- With CRISPR: easy production of deletions, inversions and duplications from a few bp to 24,4 Mb



Generation of structural variants is now straightforward

Similar but quicker than in ES cells *(Kraft et al 2015 Cell Reports 10:833-839)*

Larger fragments than *Li et al 2015 J Mol Cell Biol 7(4):284-98*

But the genotyping determination must be done carefully….

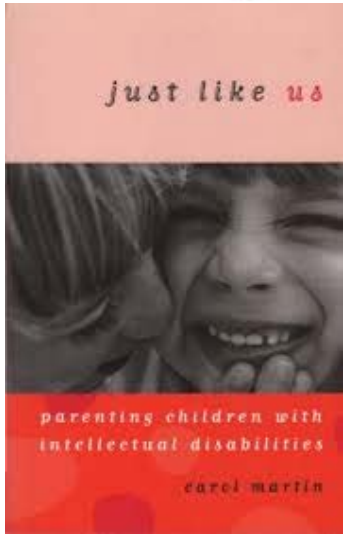
Copy number diseases with Intellectual Disability (ID)

~~Mental retardation~~

- Belong to rare disorders and are defined with IQ<70 (but can be profound (IQ<20) to mild (50<IQ<70))
- Induce limitations in two or more adaptive behaviors such as communication, self-care, social skills, community use, self-direction, health and safety
- Have an estimated prevalence of 2-3 % of the worldwide population with a strong socio-economic impact
- Occur in isolation with or without additional dysmorphism (syndromic vs non-syndromic)
- Display inherited transmission or de novo mutation

- Trisomy (Down syndrome) and partial monosomy,
- 59 copy number variants (>400kb):
  - 16p11 Del with ID and ASD
  - 17q21.31 Del with ID
  - *Auts2* ….

- X-linked (120genes)
- Autosomal Dominant (271 genes)
- Recessive genes (530 genes)



Down Syndrome

- Most common cause of ID and chromosomal aneuploidy (1 / 700),
- Affects about 8 million people around the world (strong effect of maternal age on prevalence; still 1<2000 even with prenatal diagnosis)

-80 clinical features:

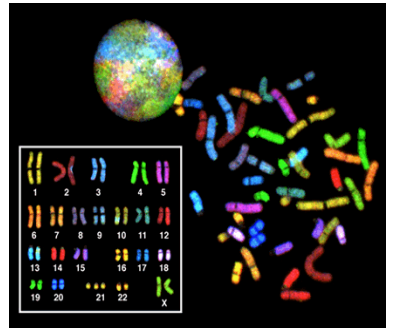
-**Intellectual disabilities** (100%; language, working memory defects and maladaptive behavior…) high variability (medium IQ 40-45, from 30 to 70), **anxiety, hypotonia and motor deficit**,

-**Craniofacial changes** (80%),short stature, skeletomuscular anomalies,

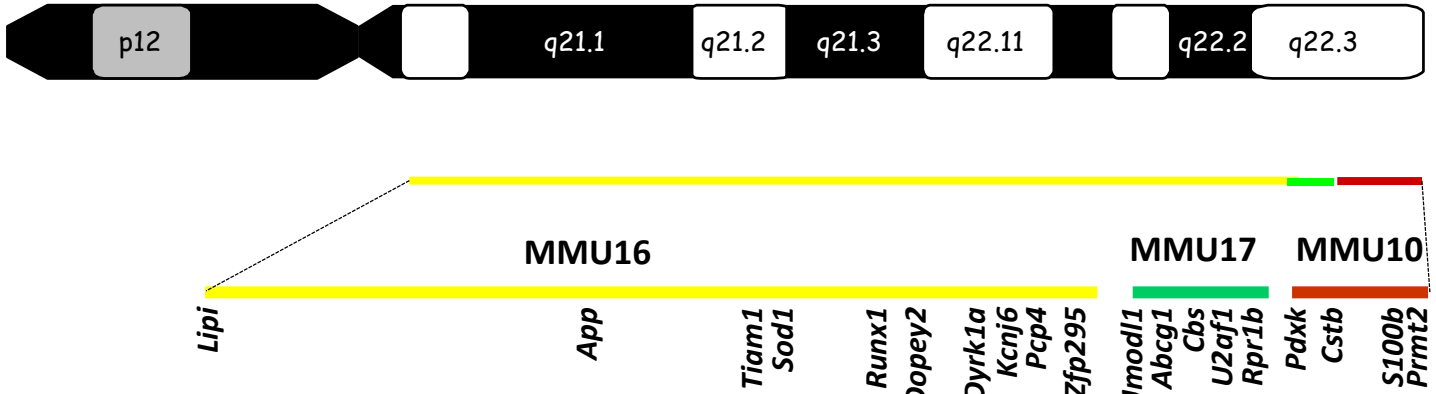
-**Cardiac** (50%) and gastro-intestinal (30%) **defects** …

-**Metabolism**: diabetes, hematological anomalies, hypothryroidism…

-Associated pathology: leukemia (10-20x), autism (7%), epilepsy (2-5%), early onset Alzheimer (10% at age 40 and 100% at age 60 years)…



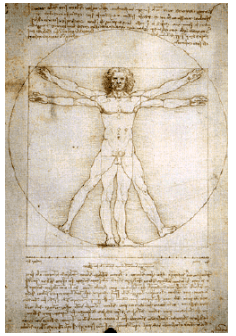
Animal models to better understand Down syndrome



1) to get knowledge on genotype-phenotype relationship

2) To identify target pathways and genes

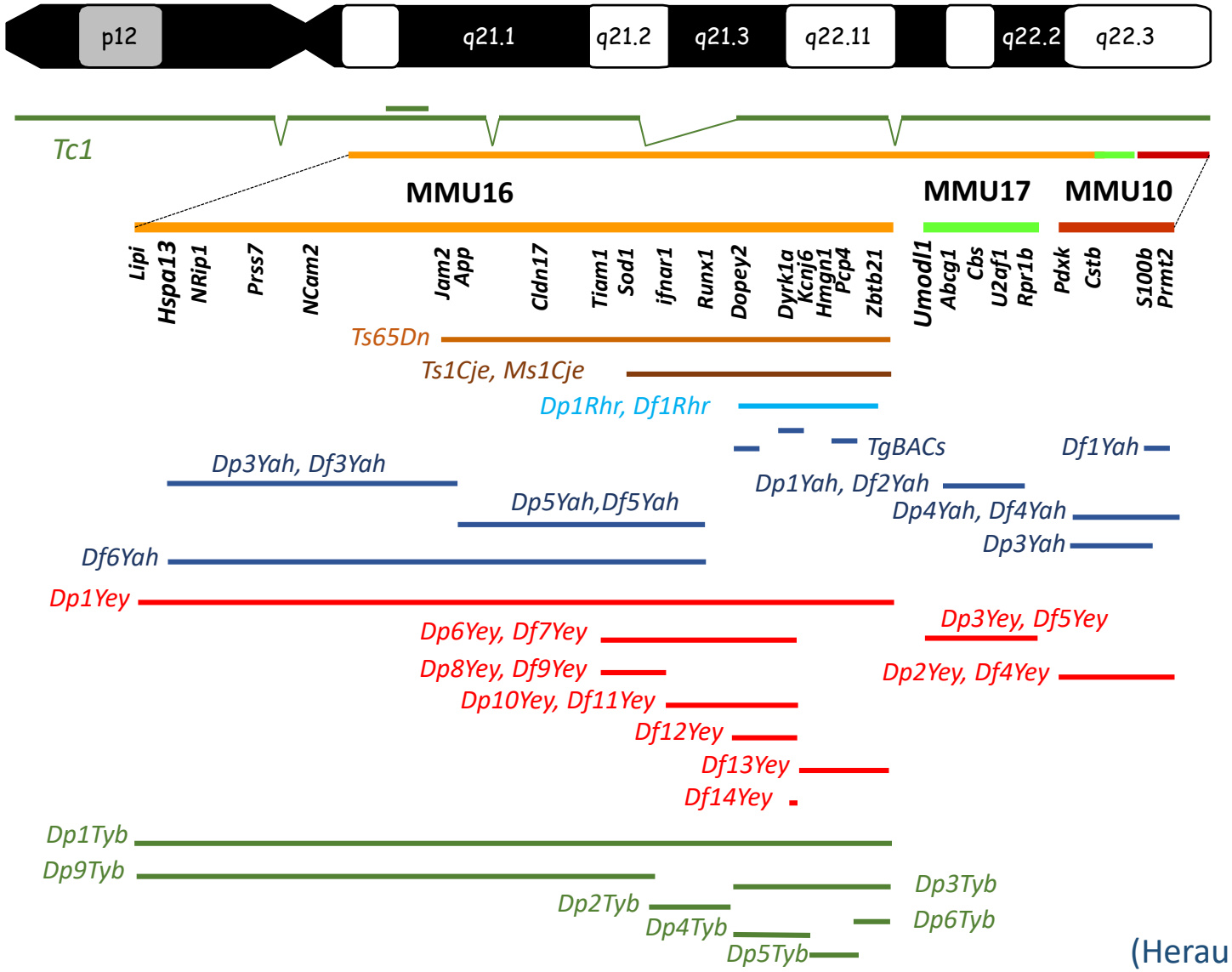
3) To test therapeutic intervention



DS Mouse model (Sept 2017)







(Herault et al., 2017 Dis. Mod. Mech)

3. Validating therapeutic approaches to rescue the cognitive defects in DS mouse models

Neurogenesis, brain development (12)

Oxidative stress, development (5)

Candidate therapeutic approaches for DS (64)

55 analysis done with the Ts65Dn model

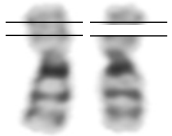
Neurodegeneration (10)
Alzheimer's Disease

Neurotransmission (36)
GABA, memantine, fluoxetine,…

Individual genes (9)
Dyrk1a (8) and *Kcnj6 (1)*

(Herault et al., 2017 Dis. Mod. Mech)

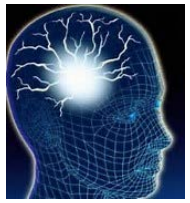
The Human 16p11.2 syndromes



DEL/+

INTELLECTUAL DISABILITIES
(Prevalence 1/1000, 1% ID)
(Jacquemont, Nature 2011; Cooper, Nat Genet 2011)

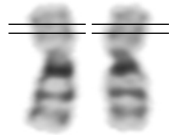
AUTISM *(Weiss, N Eng J Med 2008; Marshall, AJHG 2008; Fernandez, J.Med.Genet 2010; Sanders, Neuron, 2011; Hanson, BiolPys 20115)*



EPILEPSY *(Ghebranious J Med Genet 2007; Shinawi J Med Genet 2010; Zufferey J Med Genet 2012)*

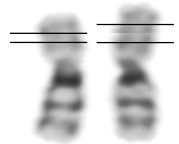
MACROCEPHALY
(Shinawi, J Med Genet 2010)

OBESITY
(Walters, Nature 2010; Zufferey, JMedGen 2012)



NORMAL

Gene dosage effect



DUP/+

INTELLECTUAL DISABILITIES
(Prevalence 1/1000, 1% ID)
(Jacquemont, Nature 2011; Cooper, Nat Genet 2011)

AUTISM *(Weiss, N Eng J Med 2008; Marshall, AJHG 2008; Fernandez, J.Med.Genet 2010; Sanders, Neuron, 2011; Hanson, BiolPys 20115)*

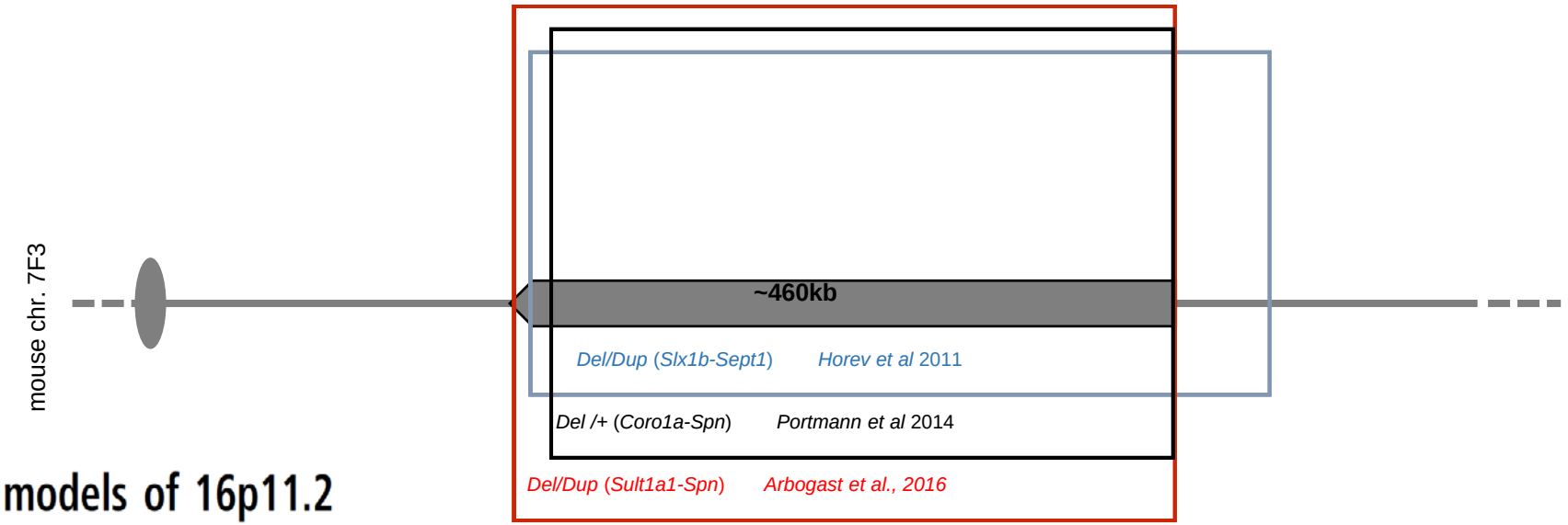
EPILEPSY *(Reinthaler Hum Mol Genet 2014)*

SCHIZOPHRENIA
(McCarthy, Nat Genet 2009)

MICROCEPHALY
(Shinawi, J Med Genet 2010)

UNDERWEIGHT
(Jacquemont, Nature2011)

3 mouse models for the 16p11.2 deletion syndrome



Dosage-dependent phenotypes in models of 16p11.2 lesions found in autism

Guy Horev[a], Jacob Ellegood[b], Jason P. Lerch[b], Young-Eun E. Son[a], Lakshmi Muthuswamy[a,1], Hannes Vogel[c], Abba M. Krieger[d], Andreas Buja[d], R. Mark Henkelman[b], Michael Wigler[a,2], and Alea A. Mills[a,2]

17076–17081 | PNAS | October 11, 2011 | vol. 108 | no. 41

Behavioral Abnormalities and Circuit Defects in the Basal Ganglia of a Mouse Model of 16p11.2 Deletion Syndrome

Thomas Portmann,[1,2] Mu Yang,[3,13,14] Rong Mao,[1,2,13] Georgia Panagiotakos,[1,2,4,13] Jacob Ellegood,[5] Gul Dolen,[6] Patrick L. Bader,[2,7] Brad A. Grueter,[2,8] Carleton Goold,[1,2] Elaine Fisher,[1,2] Katherine Clifford,[1,2] Pavitra Rengarajan,[1,2] David Kalikhman,[3] Darren Loureiro,[3] Nay L. Saw,[9] Zhou Zhengqui,[9] Michael A. Miller,[9] Jason P. Lerch,[5,10] R. Mark Henkelman,[5,10] Mehrdad Shamloo,[2,9,11] Robert C. Malenka,[2,8] Jacqueline N. Crawley,[3,14] and Ricardo E. Dolmetsch[1,12,*]

Cell Reports 7, 1–16, May 22, 2014

PLOS | GENETICS

RESEARCH ARTICLE

Reciprocal Effects on Neurocognitive and Metabolic Phenotypes in Mouse Models of 16p11.2 Deletion and Duplication Syndromes

Thomas Arbogast[1,2,3,4], Abdel-Mouttalib Ouagazzal[1,2,3,4], Claire Chevalier[1,2,3,4], Maksym Kopanitsa[5], Nurudeen Afinowi[5], Eugenia Migliavacca[6,7], Belinda S. Cowling[1,2,3,4], Marie-Christine Birling[8], Marie-France Champy[8], Alexandre Reymond[6], Yann Herault[1,2,3,4,8]*

PLOS Genetics | DOI:10.1371/journal.pgen.1005709 February 12, 2016

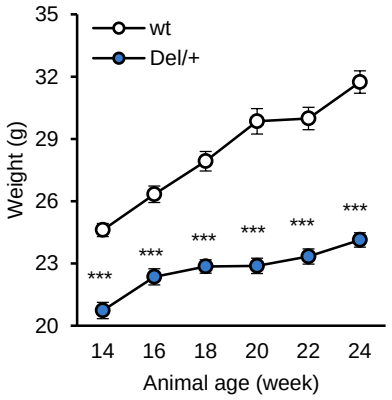
Likeness in the 16p11.2 mouse models

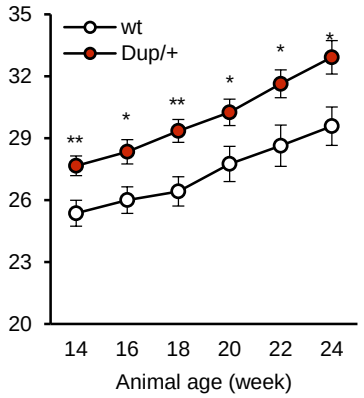
Genetic strain	B6N129Sv		B6N129Mo	B6N		B6NC3B		
Genotype	*Del/+*	*Dup/+*	*Del/+*	*Del/+*	*Dup/+*	*Del/+*	*Dup/+*	
Diurnal activity	H				H	V		
Nocturnal activity	H	H		V	V+H	V+H	V	*
Open field activity	H		H	TC	H+TC			
Repetitive behavior	C		C+Ci	R+J		C	C	*
Recognition memory			1	0.5+3	3	3	3	*
Social interaction								
Social preference								
Reference	[Horev et al 2011]		[Portman et al 2014]	[Arbogast et al., 2016]				

- Similar behavioral defects with robust phenotypes observed in 3 labs

- But Opposite metabolic deficits

B6N





Summary

- Modelling genetic diseases due to copy number variation in rodents is easy and feasible now
- Almost no limit now to rearrange the mouse genome and even to create new chromosome to model copy number disease (control the recombined loci)
- Modeling of CNV disease increases knowledged on
  - genetic interaction,
  - *candidate gene and pathways*
  - preclinical approach
- Mouse is a premier model with all the genetic/phenotyping tools available to date but rat is an alternative model of choice for more sophisticated cognitive functions

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