MGH/HST Athinoula A. Martinos Center for Biomedical Imaging

MASSACHUSETTS GENERAL HOSPITAL

Harvard-MIT Health Sciences & Technology

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Défi Science

Maladies Rares du Développement Cérébral et Déficience Intellectuelle

FILIÈRE NATIONALE DE SANTÉ

Hospices Civils de Lyon

Centre de référence national maladies rares
X fragile et autres Déficiences Intellectuelles liées au chromosome X

Point sur la recherche dans le syndrome de l'X fragile.

Dr Aurore Curie

Service de neuropédiatrie, Hôpital Femmes Mères enfants,
Institut des Sciences Cognitives, CNRS UMR5304,
Lyon, France

Association X fragile Belgique asbl

Colloque scientifique, *Association X Fragile Belgique*, Bruxelles, le 12 octobre 2018.

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ripps

ISC Marc Jeannerod

PLAN

1. Introduction
2. Principes de base des essais thérapeutiques
3. Théorie glutamatergique et premiers essais
4. Effet placebo
5. Importance des critères de jugement
6. Nombreuses pistes thérapeutiques possibles
   - *études pré-cliniques*
   - *études cliniques*
   - *nouvelles stratégies*
7. Conclusion

1. Introduction

Histoire des essais thérapeutiques

- **Avicenne**

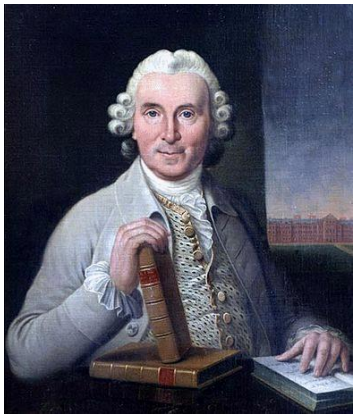
980-1037

Il établit les règles de l'expérimentation des médicaments *(livre des lois médicales)*

- **James Lind**

En 1747: compare effet de différentes substances sur des groupes de marins ayant le scorbut

→ Le groupe ayant reçu oranges et citrons guérissent en 6 jours!



1716 - 1794

Développement d'un nouveau médicament

- **Phase préclinique**

In vitro puis in vivo sur des modèles animaux (rongeurs, chiens, porcs, primates…)

- **Phase I**

Evaluer la tolérance et l'absence d'effets indésirables chez des sujets le plus souvent volontaires sains, indemnisés

- **Phase II ou étude pilote**

Déterminer la dose optimale du médicament et ses éventuels effets indésirables chez des patients (IIa: estime l'efficacité sur nombre limité de patients, IIb: détermine la dose thérapeutique à plus grande échelle)

- **Phase III**

Etude comparative d'efficacité proprement dite. Elle compare le traitement soit à un placebo, soit à un traitement de référence. Coût++

- **Phase IV**

Suivi à long terme lorsque le traitement est autorisé sur le marché, doit permettre de dépister des effets secondaires rares ou des complications tardives

2. Principes de base des essais thérapeutiques

But: éviter la survenue de biais

= la différence observée à la fin de l'essai entre les deux groupes est due à un autre facteur que le traitement étudié

- **l'essai contrôlé randomisé en double insu**
  - Prospectif
  - Comparatif (groupe contrôle)
  - Randomisé (groupes de patients comparables)
  - En double aveugle/insu
  - Sans données manquantes et analysé en intention de traiter

Quantifier l'efficacité

- **Principes des tests statistiques**

- Fluctuations aléatoires d'échantillonnage → Erreur statistique

De 1ère espèce (α) de 2nde espèce (β)

- **Le test statistique**: permet de rechercher s'il existe une réelle différence entre deux groupes

Probabilité p que la différence observée soit due au hasard en l'absence d'effet du traitement

Risque acceptable d'erreur α : 5% classiquement

Moyen de contrôler le risque d'erreur α
Ne prend pas en compte le risque β

- **Principes des tests statistiques**

- Lorsque $p<0,05$

→ alors la différence est dite statistiquement significative

- Lorsque $p>0,05$

→ alors la différence n'est pas statistiquement significative

Mais cela ne signifie pas qu'il y a absence d'effet
L'absence de preuve n'est pas la preuve de l'absence

Manque de puissance par exemple pour mettre en évidence la différence

• Problématique des comparaisons multiples

- Lorsque plusieurs tests statistiques sont réalisés: le risque global d'erreur de 1ère espèce s'accroit

- La répétition à chaque test du risque d'obtenir un résultat significatif par hasard augmente le risque global de conclure à tort à l'efficacité du traitement

Nombre de test (seuil α à 5%)	Risque global d'erreur α global
1	5%
2	10%
10	40%
50	92%
k	$1-(1-\alpha)^k$

Inflation du risque α

• Critère de jugement principal

- Unique
- Doit être soigneusement choisi
- Doit correspondre au critère le plus cliniquement pertinent
- Seul le critère principal permet de conclure sur l'efficacité du traitement

• Critères de jugement secondaires

- Plusieurs possibles
- À titre documentaire
- Puissance de l'essai calculé pour le critère principal
- Intérêt des tests hiérarchisés:

Peut conclure sur plusieurs critères de jugement simultanément

3. Théorie glutamatergique et premiers essais thérapeutiques

Fonction de FMRP

Modulation de la plasticité synaptique et de la maturation dendritique

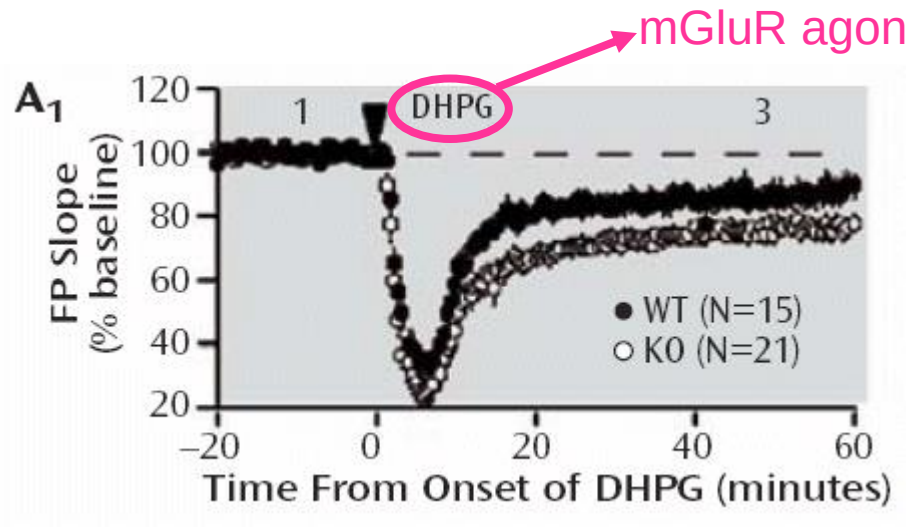
- **Protéine se liant à l'ARN post-synaptique** (et présynaptique/axones)
  - **inhibe la synthèse protéique dans les dendrites**: diminue la traduction protéique de nombreux ARNm
  - interagit avec des ARN non codants (BC1 RNA et microRNAs)
  - **stabilise les ARNm**
- Modifie le **cytosquelette** d'actine
- Contribue à la **plasticité synaptique** (LTP and LTD)
- Participe à la **maturation dendritique**
- est nécessaire à l'apprentissage!

Huber et al., PNAS, 2002
Weiler et al., PNAS 2004
Aschrafi et al., PNAS, 2005
Bagni et al., Nat Review Neurosci. 2005
Grossman et al., J Neurosci 2006

BC1 RNA : Small dendritic noncoding Brain Cytoplasmic RNA

Plasticité synaptique

= modifications durable dans le temps de la force synaptique accompagnées de modifications de taille et de morphologie des épines dendritiques



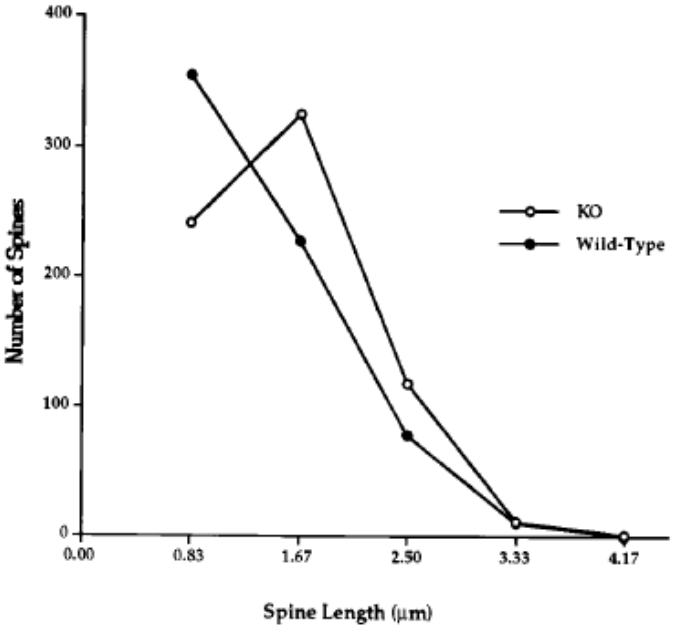
Nécessaire pour l'apprentissage, la mémoire et le développement cognitif

Hippocampus of *Fmr1* KO mice as compared to wildtype (WT) littermate control mice

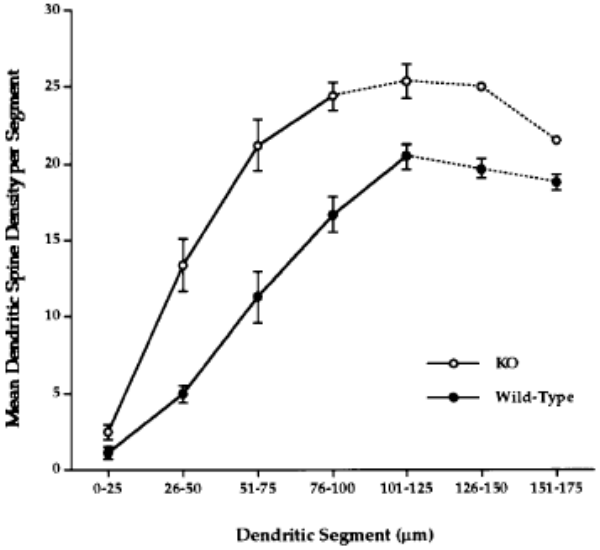
Exaggération de la LTD dépendante de mGLuR dans l'hippocampe de souris KO *Fmr1*

Huber, 2002

Morphologie des épines dendritiques

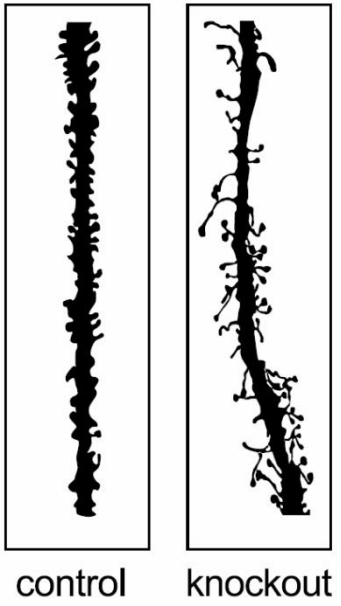


Fmr1 KO mice have fewer short spines and more long spines
(χ^2=46.29, df=3, p<0.0005)



Overall spine density is significantly greater in *Fmr1* Ko mice than in WT controls

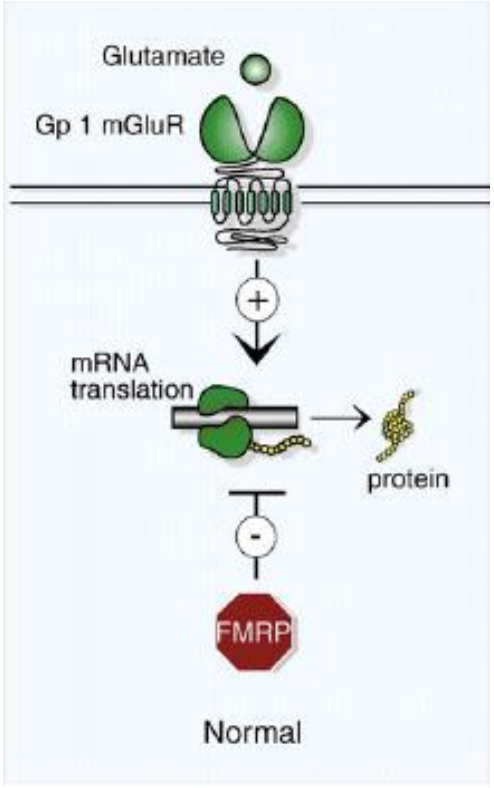
Fmr1 KO mice

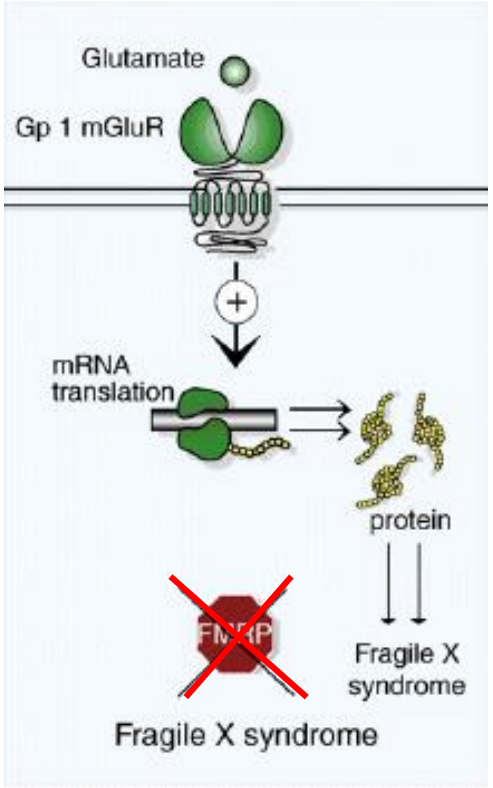


Dendritic spines in *Fmr1* KO: long, thin and immatures

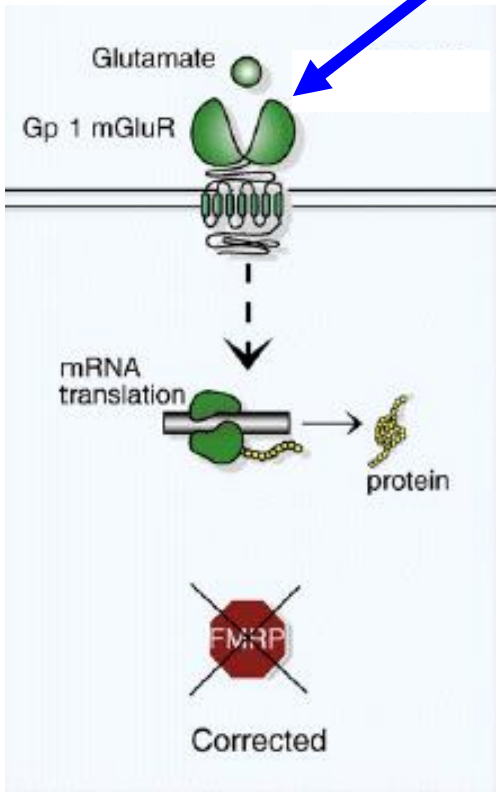
Comery et al, PNAS, 1997

Théorie glutamatergique dans le syndrome de l'X fragile





Antagoniste glutamatergique ou mutation



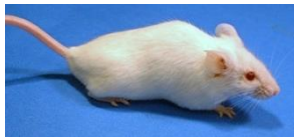
Normal

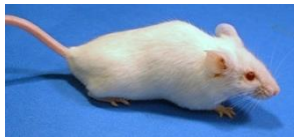
X fragile

Bear et al., 2004
Dölen et al., 2010

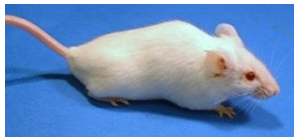
Etudes précliniques Double KO

Double KO FMR1 et mGluR5





FMR1 - /Y mGluR5 - /+



FMR1 : - / y
mGluR5: - /+

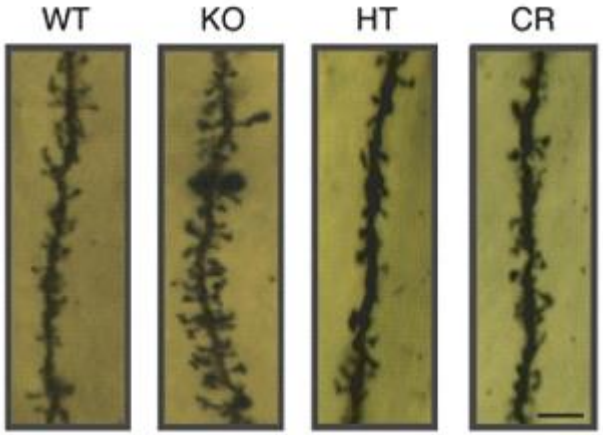
	WT (XYMM)	KO (xYMM)	HT (XYmM)	CR (xYmM)
Fmr-1	+/Y	-/Y	+/Y	-/Y
Grm5	+/+	+/+	+/-	+/-

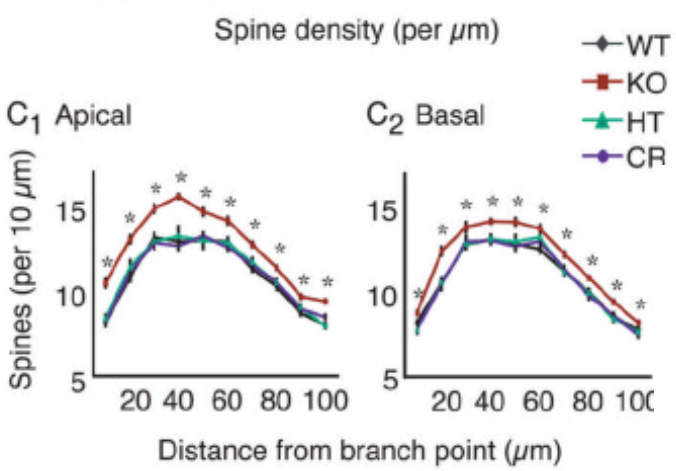
souris: - **WT** (wide type) = normale

- **KO** (knock out) pour gène Fmr1 = X fragile
- **HT** (heterozygote type) pour Rc glutamate de type 5
- **CR** (double hétérozygote) = X fragile avec 50% d'expression des Rc glutamatergiques de type 5

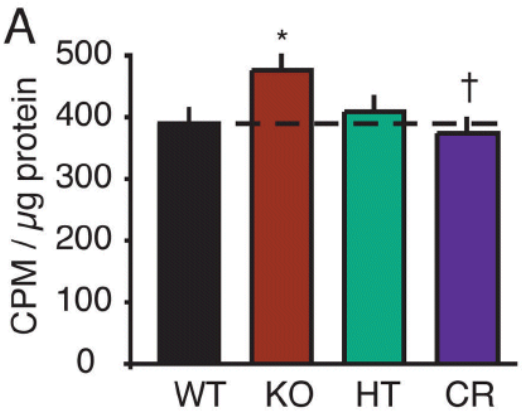
Dölen et al., Neuron, 2007

Etudes précliniques Double KO

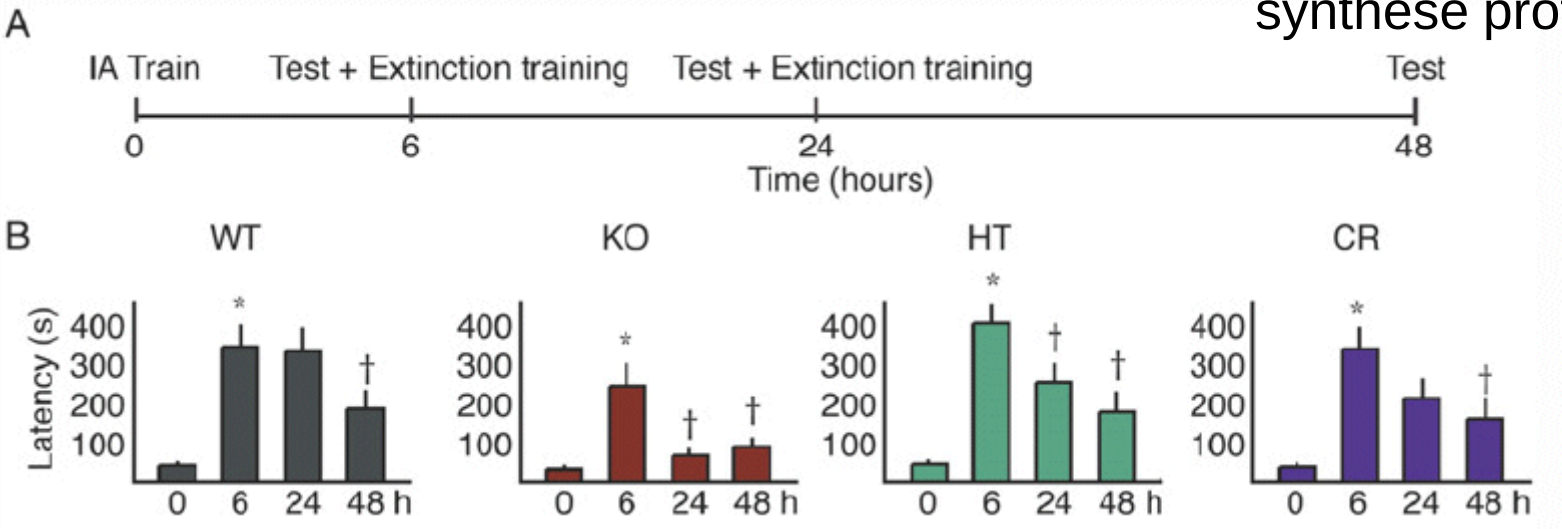




Restauration de la morphologie des épines dendritiques



Restauration de la synthèse protéique



Dölen et al., Neuron, 2007

Neuron 74, 49–56, April 12, 2012

Chronic Pharmacological mGlu5 Inhibition Corrects Fragile X in Adult Mice

Aubin Michalon,[1,4] Michael Sidorov,[3,4] Theresa M. Ballard,[1] Laurence Ozmen,[1] Will Spooren,[1] Joseph G. Wettstein,[1] Georg Jaeschke,[2] Mark F. Bear,[3,*] and Lothar Lindemann[1,*]

[1]Discovery Neuroscience

[2]Discovery Chemistry

Pharmaceuticals Division, F. Hoffmann-La Roche, CH-4070 Basel, Switzerland

[3]Howard Hughes Medical Institute, The Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

- souris adultes KO FMR1 (5 à 6 semaines de vie)
- traitement pendant 2 mois avec antagoniste mGluR5
- “Correction” du phénotype

L'X fragile, un retard mental réparable ?

MÉDECINE | Chez des souris adultes, un traitement par une molécule bloquant des récepteurs au glutamate corrige les troubles associés à ce syndrome. Des essais cliniques sont en cours

SANDRINE CABUT

Jusqu'ici, les retards mentaux étaient considérés comme irréversibles. Peut-être plus pour longtemps. En traitant par une petite molécule des souris adultes atteintes du syndrome de l'X fragile – la cause de déficience intellectuelle héritée la plus fréquente chez l'homme –, des chercheurs suisses et américains ont corrigé des signes cardinaux associés à ce trouble du développement cérébral : hyperactivité, déficit d'apprentissage et de mémorisation, sensibilité aux convulsions. Des altérations morphologiques cérébrales, tel un excès d'épines dendritiques, ont aussi régressé.

« C'est une belle démonstration de la plasticité cérébrale »

PROFESSEUR VINCENT DES PORTES
Centre de référence du syndrome de l'X fragile, à Lyon

Ce syndrome complexe touche un enfant sur 4 000.

ABK/BSIP

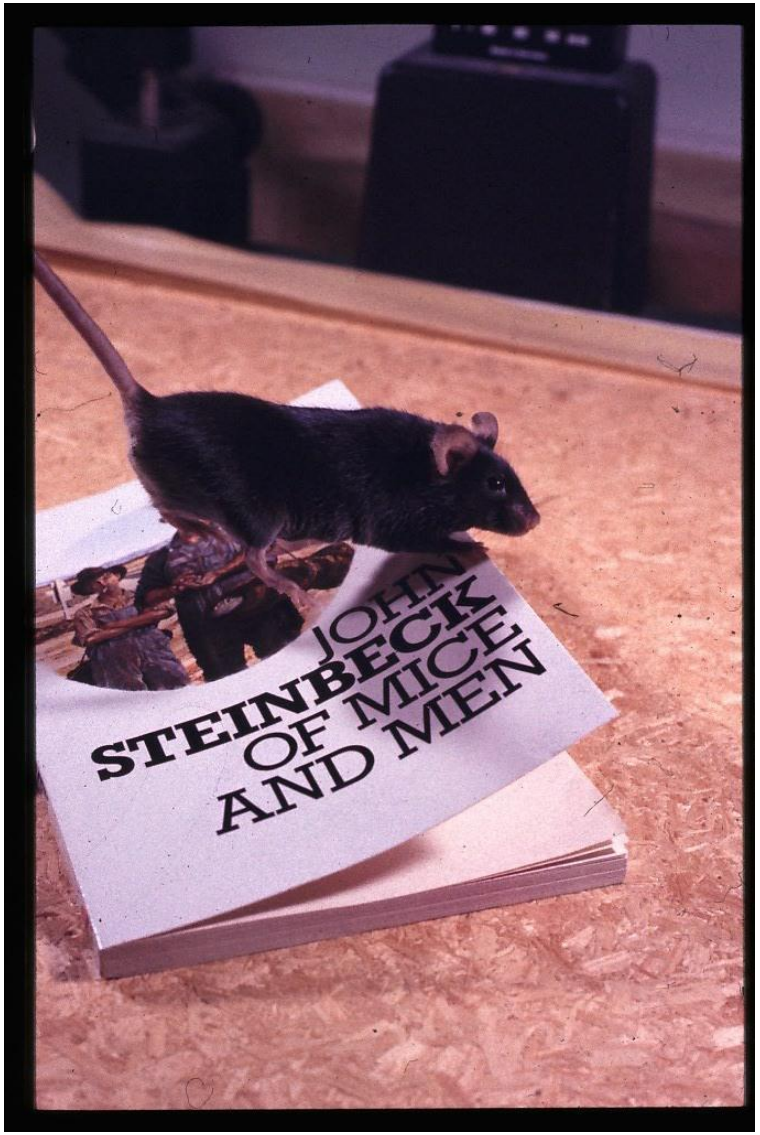
général plus sévère chez les garçons – dotés d'un seul chromosome X.

L'X fragile est dû à une mutation très particulière du gène FMR1 *(fragility mental retardation 1)*, situé sur le

de la conception. Depuis, plusieurs antagonistes des récepteurs mGlu5 ont été développés, qui ont obtenu des résultats intéressants lors d'essais cliniques préliminaires sur de

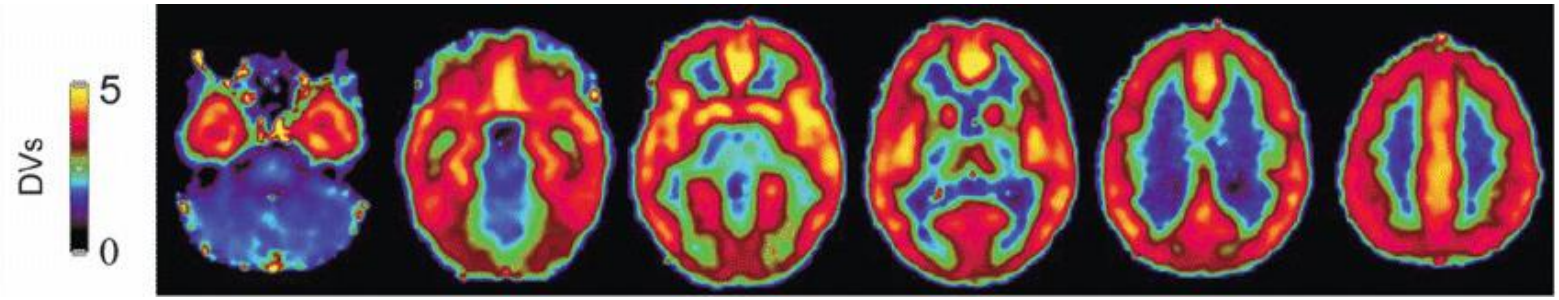
dans des essais thérapeutiques », note le professeur Vincent Des Portes (Centre de référence du syndrome de l'X fragile, Lyon).

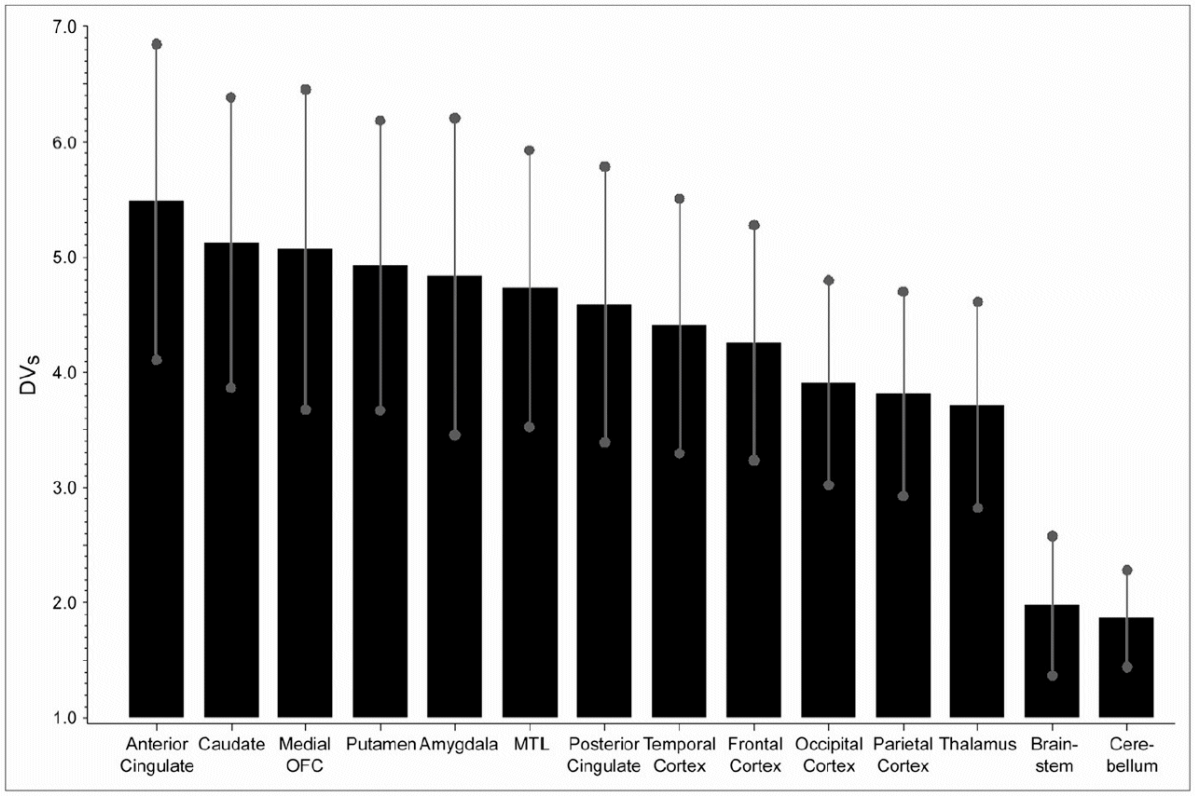
Deux antagonistes de mGlu5



de la souris à l’homme ?

PET study of a mGluR5 antagonist ^{11}C-ABP688





mGluR5 expression

prefrontal cortex
Striatum
Accumbens Nucleus
Amygdalia
Hippocampus

< cerebellum

***Ametamey** et al., 2007*

Human Behaviors involving mGluR1 and 5 receptors

Anxiety, response to fear

Memory of a scaring situation: mGluR5 dependant LTP in amygdala

Rodrigues et al., J Neurosci 2002

Anxiolytic Properties of mGluR5 antagonists

Tatarcynska et al., Br J Pharmacol 2001

Dysfunction of amygdala in fmr1KO mice

Paradee et al., Neurosci 1999

OCD: Obsessive Compulsive disorder

The cortico-striatal LTP is mGluR1 and mGluR5 dependant

Gubellini et al., Neuropharmacology 2003

Tactile Hyperarousal

mGluR1 and 5 are expressed in C fibers of the skin, involved in hyperalgesia

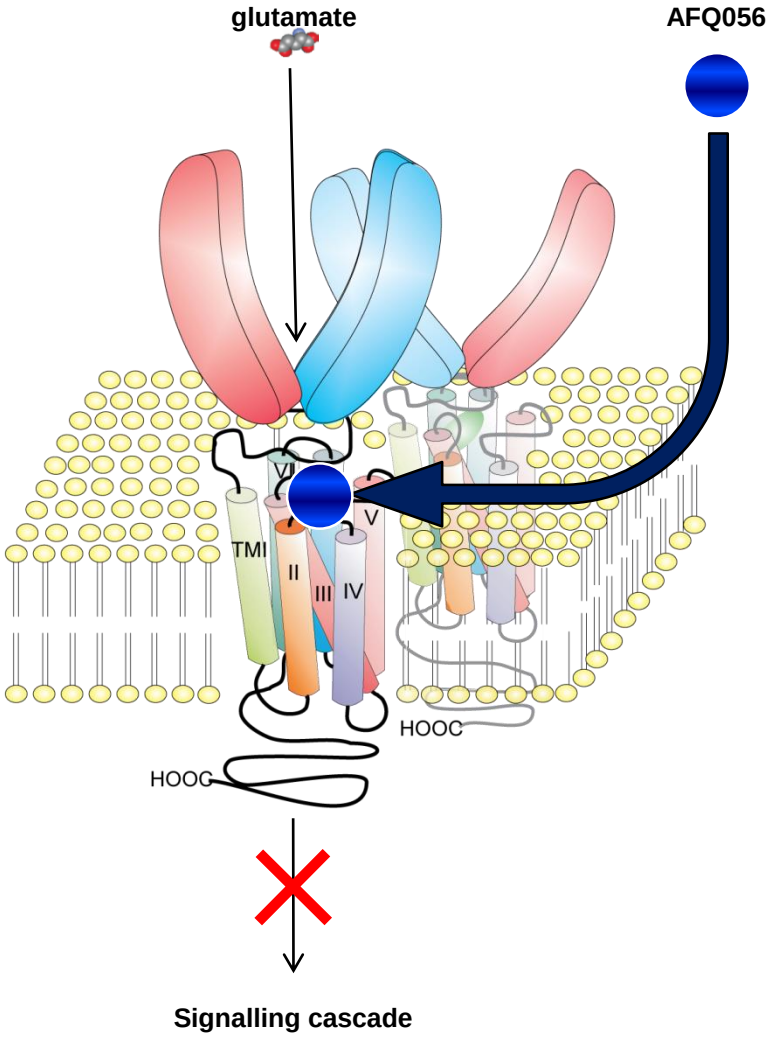
Neugebauer et al., J Neurophysiol 1999; Walker et al., Neuropharmacology 2001

Sleep troubles

The circadian rythm (suprachiasmatic nucleus) ismGluR1 and mGluR5 dependant

Inoue et al., Curr Biol 2002

AFQ056 inhibits the activation of mGluR5 by glutamate, blocking the downstream signalling cascade



- AFQ056 is a novel, selective mGluR5 antagonist developed by Novartis
- AFQ056 is selective for mGluR5
- AFQ056 inhibits the activation of mGluR5 by glutamate, blocking the downstream signalling cascade

Gasparini et al, 2008

AFQ056 (Mavoglurant)
un antagoniste sélectif du mGluR5

Un essai croisé, AFQ056 vs placebo, randomisé, en double aveugle, contrôlé contre placebo

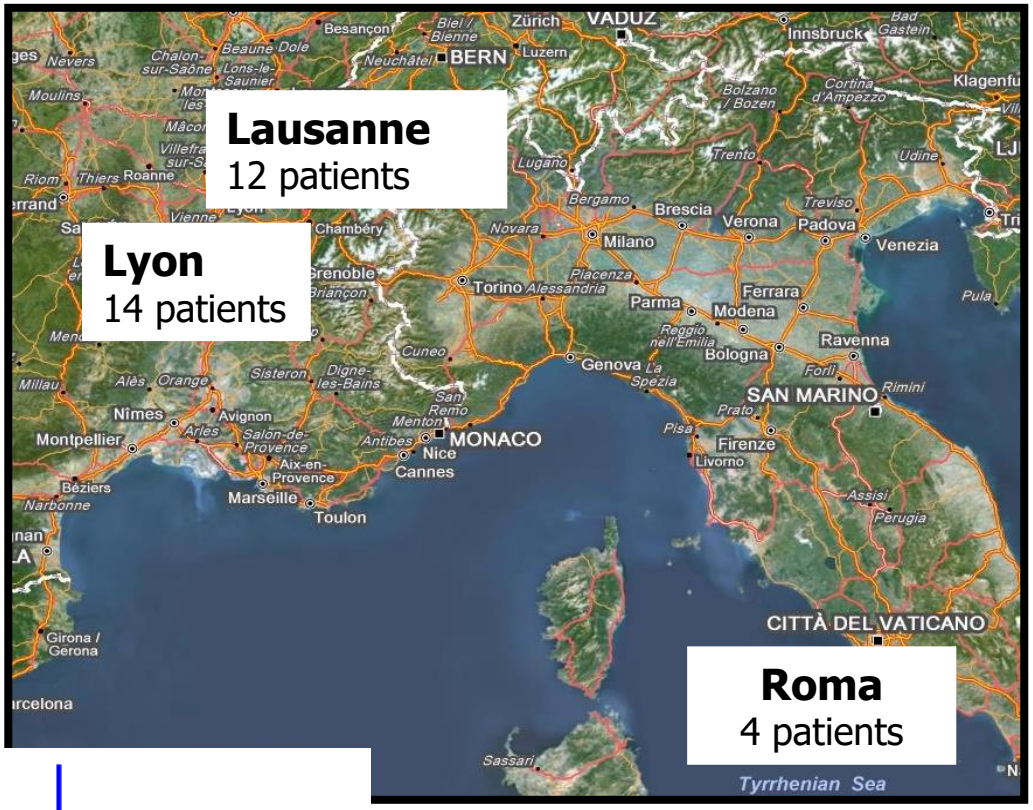
Etude preuve de concept

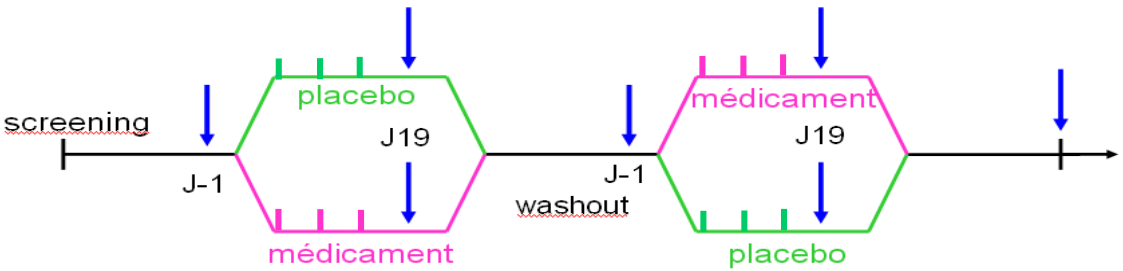
30 patients X fragile inclus

- adultes : 18 - 35 ans.
- 7 ≈ jours complets de visites à l'hôpital et 8 visites à domicile 210 visites à l'hôpital

240 visites à domicile…

De Mai 2008 à Février 2009





AFQ056 – Methods: **outcome measures**

- **Standard evaluations : 9 repeated measures**
  (day 1, 8, 20, 28) of each treatment period + end of study

  PRIMARY OUTCOME MEASURE

  – **Aberrant Behavioral checklist : ABC-C**

  SECONDARY OUTCOME MEASURES

  – **Clinical Global Impression: CGI**
  – Social responsiveness scale (**SRS**)
  – Repetitive Behavior Scale (**RBS**)
  – Verbal skills (Peabody).
  – KITAP: Computerized evaluation of attention, vigilance, distractibility.

- **Exploratory evaluations** : 4 repeated measures
  (day 1, 20) of each treatment block
  – Eye tracking: Evaluation of gaze avoidance and facial processing
  – Pre-Pulse Inhibition: Evaluation of sensory gating

Subscale structure of the original generic ABC scale

Irritability	Lethargy / Social Withdrawal	Stereotypy	Hyperactivity	Inappropriate Speech
2. Injures self	3. Listless, sluggish, inactive	6. Meaningless, recurring movements	1. Excessively active at home, school, work	9. Talks excessively
4. Aggressive to others	5. Seeks isolation	11. Stereotyped, repetitive behavior	7. Boisterous	22. Repetitive speech
8. Screams inappropriately	12. Preoccupied, stares into space	17. Bizarre in behavior	13. Impulsive	33. Talks to self loudly
10. Temper tantrums	16. Withdrawn, prefers solitary activities	27. Moves head back and forth	15. Restless, unable to sit still	46. Repeats words/phrase over & over
14. Irritable & whiny	20. Fixed facial expression	35. Repetitive hand, body, head movements	18. Disobedient	
19. Yells inappropriately	23. Only sits and watches others	45. Waves/shakes extremities repeatedly	21. Disturbs others	
25. Depressed mood	26. Resists physical contact	49. Rocks back and forth	24. Uncooperative	
29. Demands must be met immediately	30. Isolates him/herself from others		28. Does not pay attention to instructions	
34. Cries over minor annoyances	32. Sits/stands in one position for a long time		31. Disrupts group activities	
36. Quick mood changes	37. Unresponsive to structures activities		38. Does not stay in seat during lesson period	
41. Cries/screams inappropriately	40. Is difficult to reach or contact		39. Will not sit still for any length of time	
47. Stamps feet/bang objects/slams doors	42. Prefers to be alone		44. Easily distractable	
50. Deliberately hurts him/herself	43. Doesn't communicate by word/gestures		48. Constantly runs or jumps	
52. Does physical violence to self	53. Inactive, never moves spontaneously		51. Pays no attention when spoken to	
57. Has outburst when doesn't get way	55. Responds negatively to affection		54. Excessively active	
	58. Shows few social reactions		56. Deliberately ignores directions	

Effet de l'AFQ056 sur le comportement des patients

Analyse sur le critère principal: ABC-C

Comparaison de la modification du score pour la ABC-C entre J19 et J1 entre
la période sous AFQ056 versus la période sous placebo

Pas de différence significative

Différence liée au traitement (90% CI) -2.10 (-8.26 to 4.06), p = 0.573

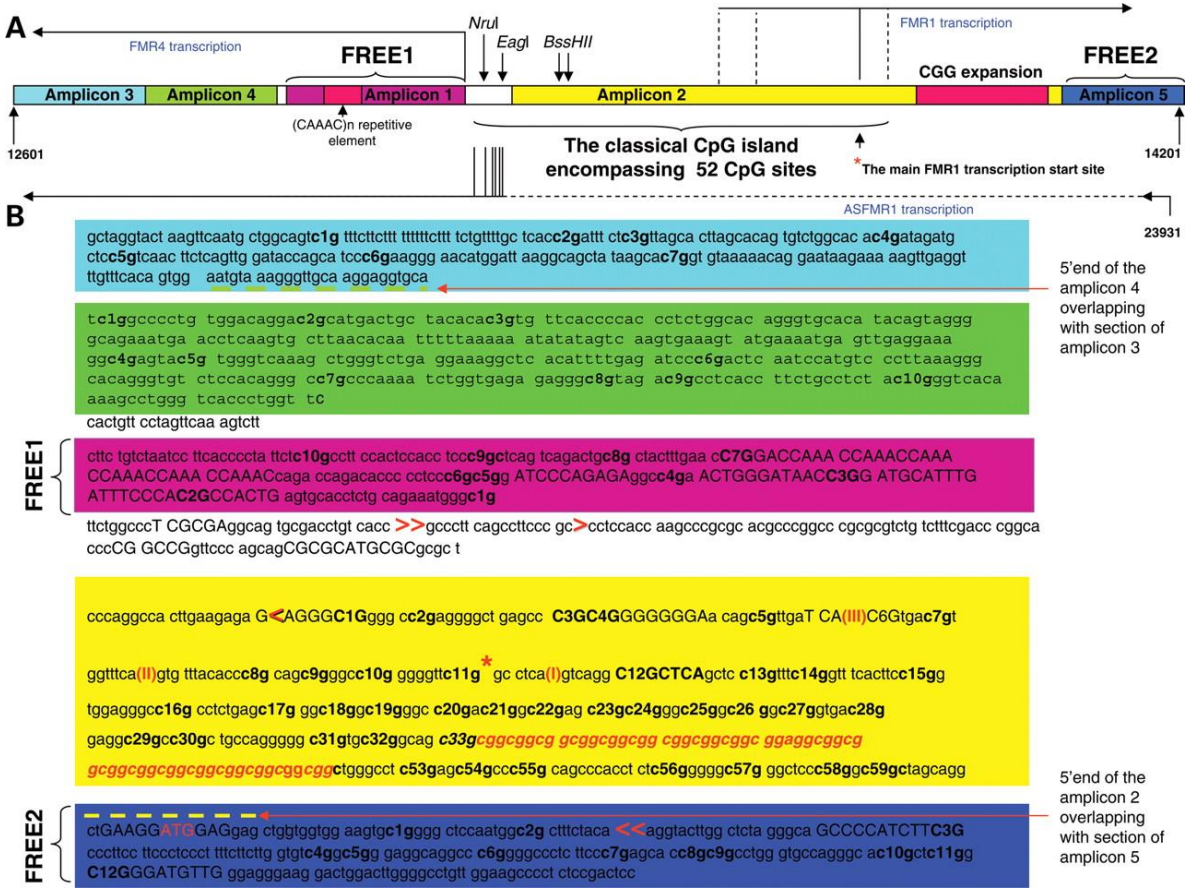
AFQ056 *vs* placebo
critères de jugement secondaires à J 19

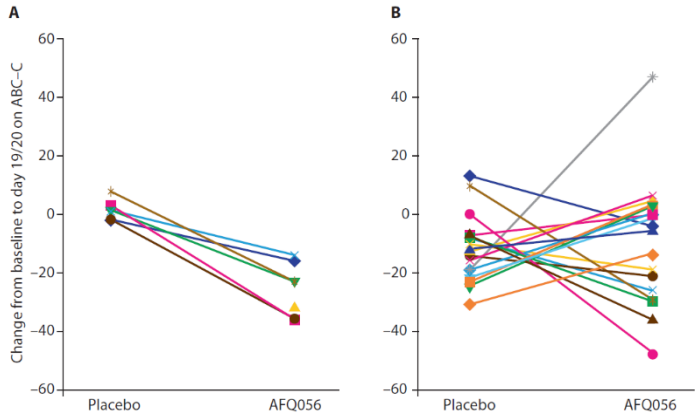
	Difference* (90% CI) AFQ056 – Placebo	*P*
CGI-I	0.01 (–0.38 to 0.41)	0.955
CGI efficacy index	–0.01 (–0.41 to 0.39)	0.974
VABS	0.82 (–5.07 to 6.72)	0.814
RBS-R	–3.81 (–6.91 to –0.70)	0.046
Stereotypic behavior	–1.26 (–2.03 to –0.48)	0.010
Self-injurious behavior	–0.37 (–0.85 to 0.11)	0.201
Compulsive behavior	–0.55 (–1.19 to 0.10)	0.163
Ritualistic behavior	–0.56 (–1.31 to 0.18)	0.211
Sameness behavior	–0.56 (–1.86 to 0.74)	0.470
Restricted interests	–0.66 (–1.12 to –0.19)	0.022
SRS	–1.14 (–7.71 to 5.43)	0.773
VAS	5.18 (–3.89 to 14.25)	0.345

*Difference in least-squares means between AFQ056 and placebo, adjusted for baseline covariate.

Méthylation

Promoter region on the FMR1 gene and the adjacent 5′ and 3′ loci.





Méthylation complète

Méthylation partielle

Godler D E et al. Hum. Mol. Genet. 2010;19:1618-1632.

RESEARCH ARTICLE

Science Translational Medicine
AAAS

FRAGILE X SYNDROME

Epigenetic Modification of the *FMR1* Gene in Fragile X Syndrome Is Associated with Differential Response to the mGluR5 Antagonist AFQ056

Sébastien Jacquemont,[1]* Aurore Curie,[2]* Vincent des Portes,[2] Maria Giulia Torrioli,[3] Elizabeth Berry-Kravis,[4] Randi J. Hagerman,[5] Feliciano J. Ramos,[6] Kim Cornish,[7] Yunsheng He,[8] Charles Paulding,[8] Giovanni Neri,[9] Fei Chen,[1,10] Nouchine Hadjikhani,[10,11] Danielle Martinet,[1] Joanne Meyer,[8] Jacques S. Beckmann,[1] Karine Delange,[2] Amandine Brun,[2] Gerald Bussy,[2] Fabrizio Gasparini,[12] Talita Hilse,[13] Annette Floesser,[13] Janice Branson,[12] Graeme Bilbe,[12] Donald Johns,[14] Baltazar Gomez-Mancilla[14†]

Disclosure:
Investigators have received funding from NOVARTIS to conduct this clinical trial

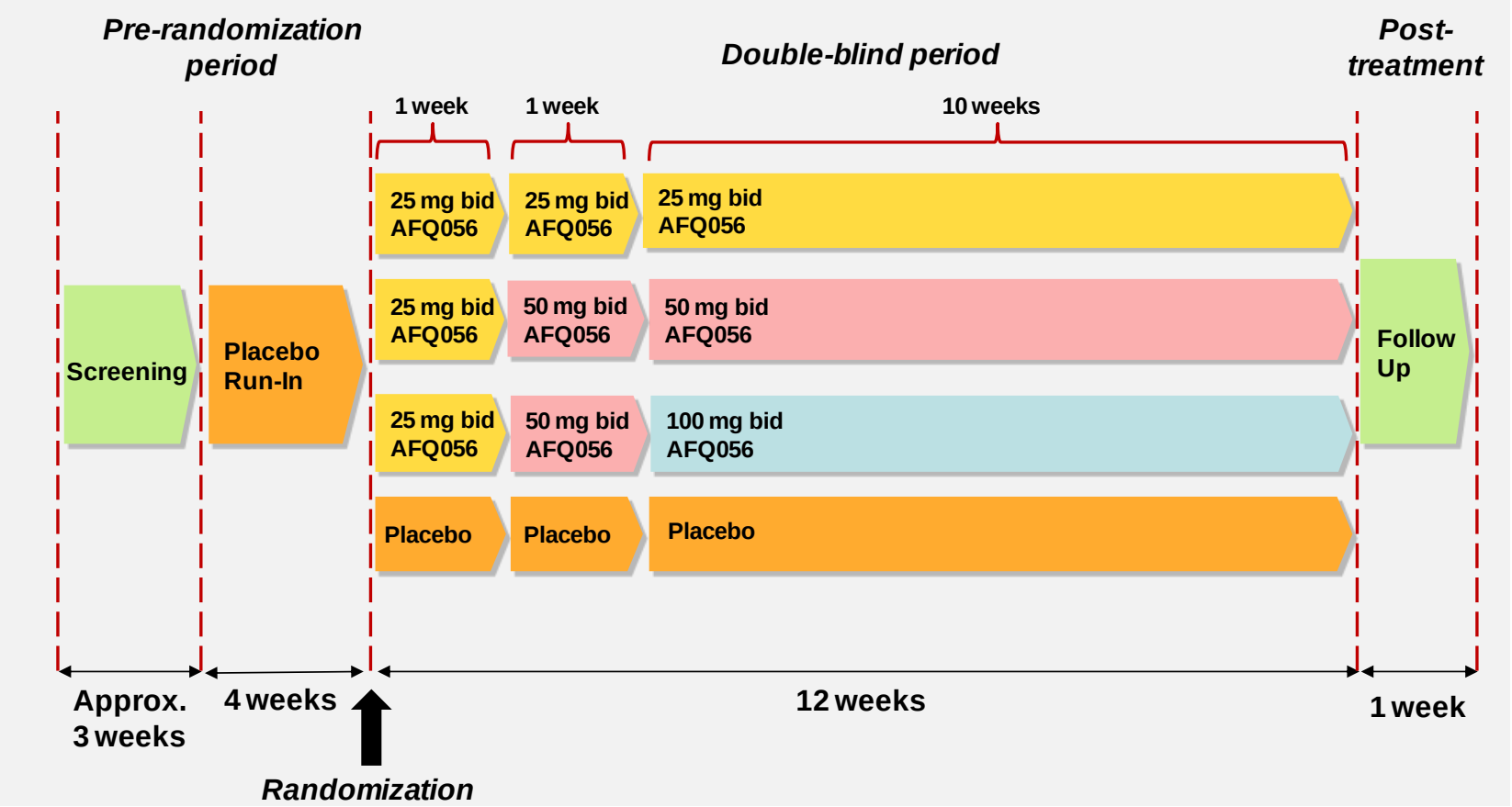
JT - France 3

Etude X fragile

27 janvier 2011

Two phase 2b, multicentre, randomized, double-blind, placebo-controlled, parallel-group studies of mavoglurant

- in 175 adults (>18yrs) (NCT01253629)
- in 139 adolescents (12 to 17yrs) (NCT01357239)

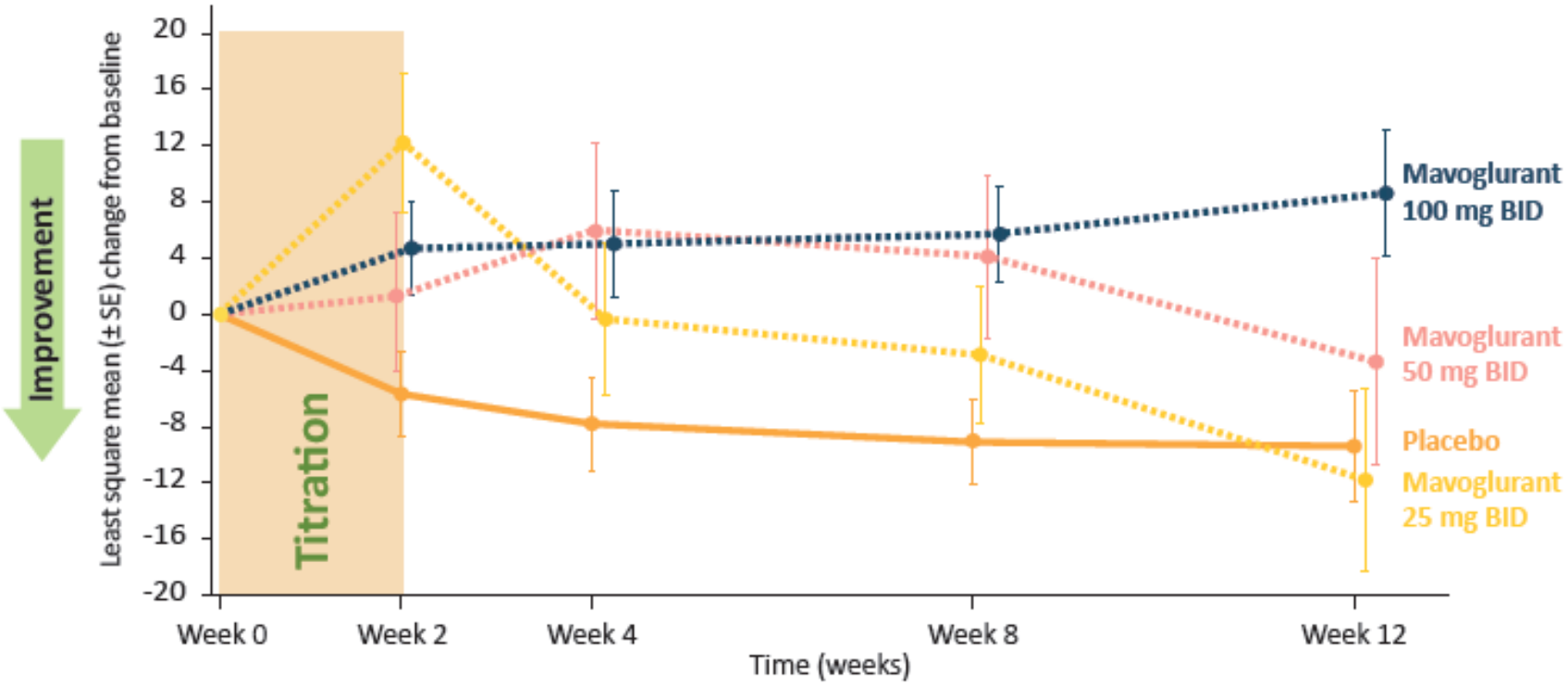


Disclosure:

Investigators have received funding from NOVARTIS to conduct this clinical trial

Résultats

AFQ056 et placebo : pas de difference (ABC)



NOVARTIS

www.ScienceTranslationalMedicine.org 13 January 2016 Vol 8 Issue 321

RESEARCH ARTICLE

Science Translational Medicine AAAS

FRAGILE X SYNDROME

Mavoglurant in fragile X syndrome: Results of two randomized, double-blind, placebo-controlled trials

Elizabeth Berry-Kravis,[1]* Vincent Des Portes,[2,3]* Randi Hagerman,[4] Sébastien Jacquemont,[5,6] Perrine Charles,[7] Jeannie Visootsak,[8] Marc Brinkman,[9] Karin Rerat,[10] Barbara Koumaras,[11] Liansheng Zhu,[12] Gottfried Maria Barth,[13] Thomas Jaecklin,[14] George Apostol,[14] Florian von Raison[14†]

*These authors contributed equally as co-first authors.

Fragile X syndrome (FXS), the most common cause of inherited intellectual disability and autistic spectrum disorder, is typically caused by transcriptional silencing of the X-linked *FMR1* gene. Work in animal models has described altered synaptic plasticity, a result of the up-regulation of metabotropic glutamate receptor 5 (mGluR5)–mediated signaling, as a putative downstream effect. Post hoc analysis of a randomized, placebo-controlled, crossover phase 2 trial suggested that the selective mGluR5 antagonist mavoglurant improved behavioral symptoms in FXS patients with completely methylated *FMR1* genes. We present the results of two phase 2b, multicenter, randomized, double-blind, placebo-controlled, parallel-group studies of mavoglurant in FXS, designed to confirm this result in adults (n = 175, aged 18 to 45 years) and adolescents (n = 139, aged 12 to 17 years). In both trials, participants were stratified by methylation status and randomized to receive mavoglurant (25, 50, or 100 mg twice daily) or placebo over 12 weeks. Neither of the studies achieved the primary efficacy end point of improvement on behavioral symptoms measured by the Aberrant Behavior Checklist—Community Edition using the FXS-specific algorithm (ABC-C_{FX}) after 12 weeks of treatment with mavoglurant. The safety and tolerability profile of mavoglurant was as previously described, with few adverse events. Therefore, under the conditions of our study, we could not confirm the mGluR theory of FXS nor the ability of the methylation state of the *FMR1* promoter to predict mavoglurant efficacy. Preclinical results suggest that future clinical trials might profitably explore initiating treatment in a younger population with longer treatment duration and longer placebo run-ins and identifying new markers to better assess behavioral and cognitive benefits.

Why these trials did not confirm previous results of the PoC Study?

1. **Different study designs**

   parallel groups, sample sizes, trial duration

Different study designs

1. Proof of Concept Study (2009)

- 30 patients
- 3 weeks
- crossover trial
- Effect for a subgroup: 7 patients

2. Two phase IIb studies (2012 – 2014)

- 314 patients (175 + 139)
- 3 months
- Parallel groups
- No effect

Why these trials did not confirm previous results of the PoC Study?

1. **Different study designs**
   parallel groups, sample sizes, trial duration
2. **Outcome measures**
   pitfalls of ABC-C and other scales

Why these trials did not confirm previous results of the PoC Study?

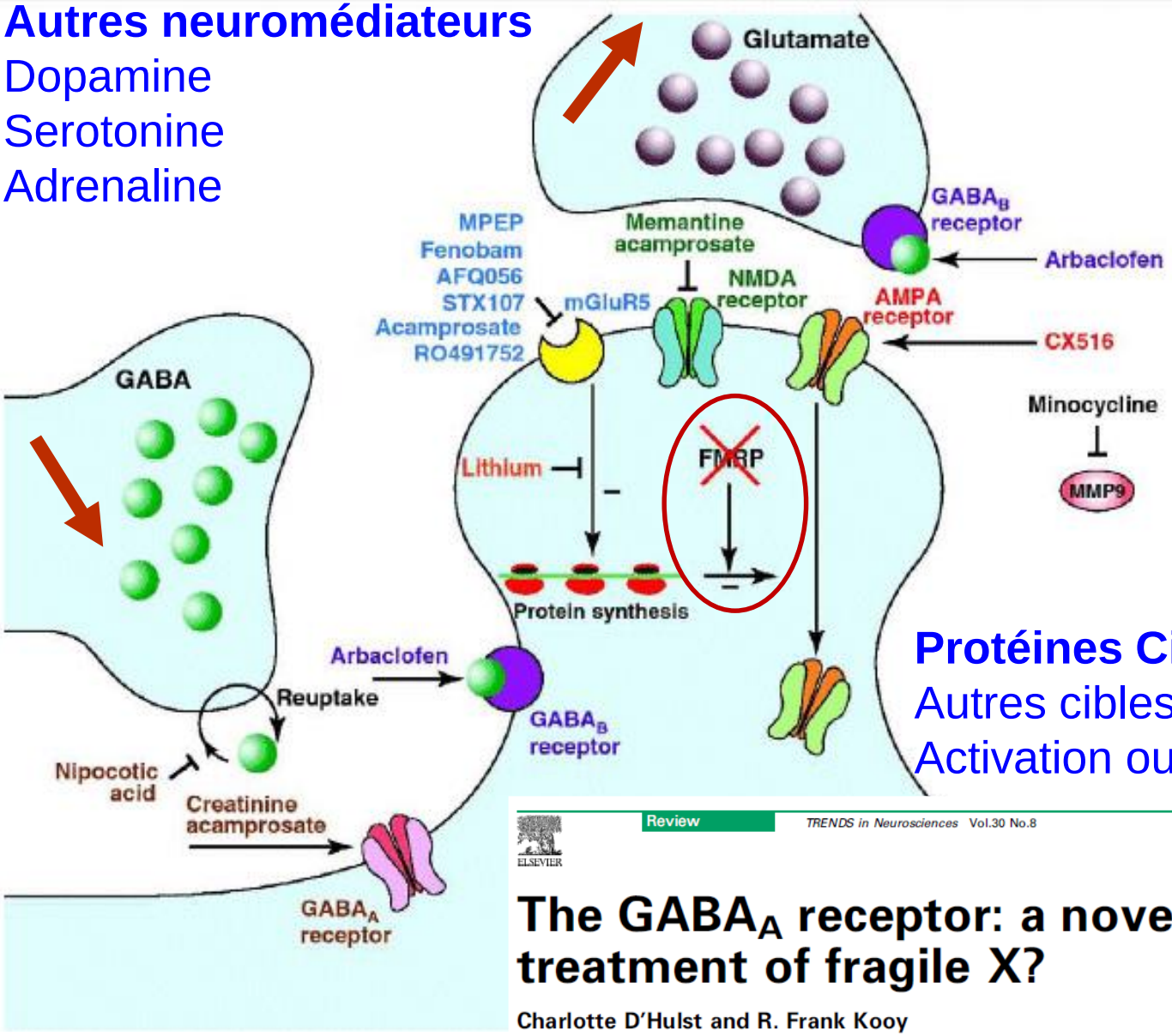
1. **Different study designs**
   parallel groups, sample size, trial duration
2. **Outcome measures**
   pitfalls of ABC-C and other scales
3. **Choice of accurate therapeutic target**
   glutamate vs GABA

Autres neuromédiateurs

Dopamine

Serotonine

Adrenaline



Protéines Cibles de FMRP

Autres cibles inconnues ?

Activation ou répression?

Review TRENDS in Neurosciences Vol.30 No.8

The GABA$_A$ receptor: a novel target for treatment of fragile X?

Charlotte D'Hulst and R. Frank Kooy

Department of Medical Genetics, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium

Limites de la théorie glutamatergique

Berry-Kravis et al. Journal of Neurodevelopmental Disorders (2017) 9:3
DOI 10.1186/s11689-016-9181-6

Journal of
Neurodevelopmental Disorders

RESEARCH Open Access

Arbaclofen in fragile X syndrome: results of phase 3 trials

Elizabeth Berry-Kravis[1*], Randi Hagerman[2], Jeannie Visootsak[3], Dejan Budimirovic[4], Walter E. Kaufmann[5], Maryann Cherubini[6], Peter Zarevics[6], Karen Walton-Bowen[7], Paul Wang[8], Mark F. Bear[9] and Randall L. Carpenter[9,10]

Abstract

Background: Arbaclofen improved multiple abnormal phenotypes in animal models of fragile X syndrome (FXS) and showed promising results in a phase 2 clinical study. The objective of the study is to determine safety and efficacy of arbaclofen for social avoidance in FXS.

Methods: Two phase 3 placebo-controlled trials were conducted, a flexible dose trial in subjects age 12–50 (209FX301, adolescent/adult study) and a fixed dose trial in subjects age 5–11 (209FX302, child study). The primary endpoint for both trials was the Social Avoidance subscale of the Aberrant Behavior Checklist-Community Edition, FXS-specific (ABC-C_{FX}). Secondary outcomes included other ABC-C_{FX} subscale scores, Clinical Global Impression-Improvement (CGI-I), Clinical Global Impression-Severity (CGI-S), and Vineland Adaptive Behavior Scales, Second Edition (Vineland-II) Socialization domain score.

Results: A total 119 of 125 randomized subjects completed the adolescent/adult study ($n = 57$ arbaclofen, 62 placebo) and 159/172 completed the child study (arbaclofen 5 BID $n = 38$; 10 BID $n = 39$; 10 TID $n = 38$; placebo $n = 44$). There were no serious adverse events (AEs); the most common AEs included somatic (headache, vomiting, nausea), neurobehavioral (irritability/agitation, anxiety, hyperactivity), decreased appetite, and infectious conditions, many of which were also common on placebo. In the combined studies, there were 13 discontinuations ($n = 12$ arbaclofen, 1 placebo) due to AEs (all neurobehavioral). The adolescent/adult study did not show benefit for arbaclofen over placebo for any measure. In the child study, the highest dose group showed benefit over placebo on the ABC-C_{FX} Irritability subscale ($p = 0.03$) and Parenting Stress Index (PSI, $p = 0.03$) and trends toward benefit on the ABC-C_{FX} Social Avoidance and Hyperactivity subscales (both $p < 0.1$) and CGI-I ($p = 0.119$). Effect size in the highest dose group was similar to effect sizes for FDA-approved serotonin reuptake inhibitors (SSRIs).

Conclusions: Arbaclofen did not meet the primary outcome of improved social avoidance in FXS in either study. Data from secondary measures in the child study suggests younger patients may derive benefit, but additional studies with a larger cohort on higher doses would be required to confirm this finding. The reported studies illustrate the challenges but represent a significant step forward in translating targeted treatments from preclinical models to clinical trials in humans with FXS.

Keywords: Fragile X syndrome, Arbaclofen, GABA agonist, FMR1, Targeted treatment, Neurodevelopmental disorder

Hello!
I am a Fragile X mouse

Good news !

Regardless of the drug you try, you can « cure » me!

JMG

Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model

T V Bilousova, L Dansie, M Ngo, et al.

J Med Genet 2009 46: 94-102 originally published online October 3, 2008
doi: 10.1136/jmg.2008.061796

Hébert *et al. Orphanet Journal of Rare Diseases* 2014, **9**:124
http://www.ojrd.com/content/9/1/124

ORPHANET JOURNAL OF RARE DISEASES

Rescue of fragile X syndrome phenotypes in *Fmr1* KO mice by a BKCa channel opener molecule

Betty Hébert[1,2†], Susanna Pietropaolo[3,4†], Sandra Même[5], Béatrice Laudier[1,2,6], Anthony Laugeray[1,2], Nicolas Doisne[1,2], Angélique Quartier[1,2], Sandrine Lefeuvre[6], Laurence Got[6], Dominique Cahard[7], Frédéric Laumonnier[8,9], Wim E Crusio[3,4], Jacques Pichon[1,2], Arnaud Menuet[1,2], Olivier Perche[1,2,6] and Sylvain Briault[1,2,6*]

Neuropsychopharmacology (2009) 34, 1011–1026

α-Tocopherol Protects Against Oxidative Stress in the Fragile X Knockout Mouse: an Experimental Therapeutic Approach for the *Fmr1* Deficiency

Yolanda de Diego-Otero*[,1], Yanina Romero-Zerbo[1], Rajaa el Bekay[1,2], Juan Decara[1], Lourdes Sanchez[1], Fernando Rodriguez-de Fonseca[1] and Ignacio del Arco-Herrera[1,3]

Fam a [illegible] e

NATURE MEDICINE VOLUME 19 | NUMBER 5 | MAY 2013

Targeting the endocannabinoid system in the treatment of fragile X syndrome

Arnau Busquets-Garcia[1], Maria Gomis-González[1], Thomas Guegan[1], Carmen Agustín-Pavón[2,3,11], Antoni Pastor[4,5], Susana Mato[6–8], Alberto Pérez-Samartín[6–8], Carlos Matute[6–8], Rafael de la Torre[1,4,9], Mara Dierssen[2,3,10], Rafael Maldonado[1] & Andrés Ozaita[1]

PNAS | July 3, 2007 | vol. 104 | no. 27 | 11489–11494

Inhibition of p21-activated kinase rescues symptoms [illegible] s !

Mansuo L. Hayashi*†, B. S. Shankaranarayana Rao‡, Jin-Soo Seo§, Han-Saem Choi§, Bridget M. Dolan*, Se-Young Choi§, Sumantra Chattarji¶, and Susumu Tonegawa*‖

Regardless of the drug you try,

[illegible] can « cure

Neuron
Report

Neuron 77, 243–250, January 23, 2013 ©2013 Elsevier Inc.

Lovastatin Corrects Excess Protein Synthesis and Prevents Epileptogenesis in a Mouse Model of Fragile X Syndrome

Emily K. Osterweil,[1] Shih-Chieh Chuang,[2] Alexander A. Chubykin,[1] Michael Sidorov,[1] Riccardo Bianchi,[2] Robert K.S. Wong,[2] and Mark F. Bear[1,*]

The Journal of Neuroscience, October 3, 2007 • 27(40):10685–10694 • 10685

Brain-Derived Neurotrophic Factor Rescues Synaptic Plasticity in a Mouse Model of Fragile X Syndrome

Julie C. Lauterborn,[1] Christopher S. Rex,[2] Eniko Kramár,[3] Lulu Y. Chen,[1] Vijay Pandyarajan,[1] Gary Lynch,[3] and Christine M. Gall[1,2]
Departments of [1]Anatomy and Neurobiology, [2]Neurobiology and Behavior, and [3]Psychiatry and Human Behavior, University of California, Irvine, California 92697-4292

Why these trials did not confirm previous results of the PoC Study?

1. **Different study designs**
   parallel groups, samples, trial duration
2. **Outcome measures**
   pitfalls of ABC-C and other scales
3. **Choice of accurate therapeutic target**
   glutamate vs GABA
4. **Reproducibility of preclinical data**
   Animal model / publication bias

Structural abnormalities of Fmr1 KO are subtle and difficult to reproduce

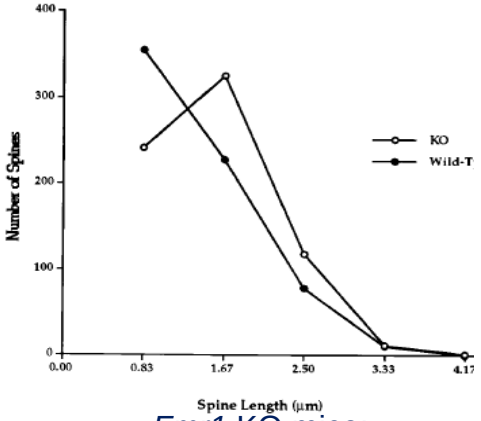
Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 5401–5404, May 1997
Neurobiology

Fmr1 KO mice

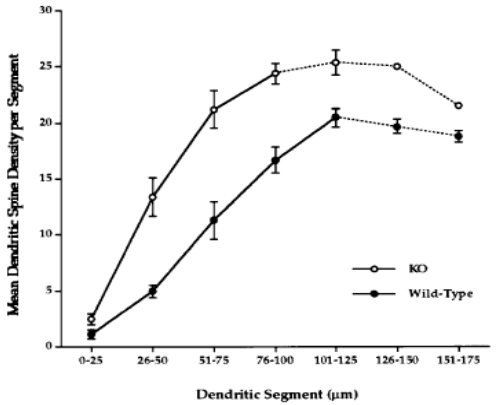
Abnormal dendritic spines in fragile X knockout mice: Maturation and pruning deficits

THOMAS A. COMERY*†‡, JENNIFER B. HARRIS†‡, PATRICK J. WILLEMS§, BEN A. OOSTRA¶, SCOTT A. IRWIN*‡, IVAN JEANNE WEILER‡‖, AND WILLIAM T. GREENOUGH*‡‖**††‡‡

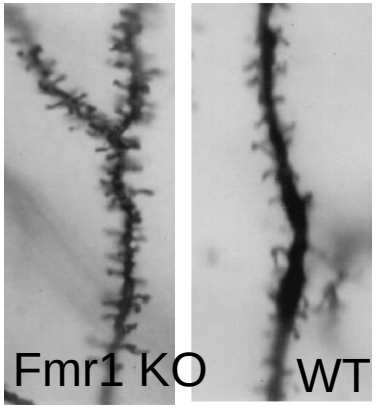
*Neuroscience Program, Departments of †Biology, ‖Psychology, **Psychiatry, and ††Cell and Structural Biology, and ‡Beckman Institute, University of Illinois, Urbana-Champaign, IL 61801; §Department of Medical Genetics, University of Antwerp, 2610 Antwerp, Belgium; and ¶Department of Clinical Genetics, Erasmus University, 3015 GE, Rotterdam, The Netherlands



Fmr1 KO mice:
fewer short spines
more long spines



Fmr1 Ko mice:
greater spine density



Fmr1 KO: « immatures »
dendritic spines

Results of therapeutic effects need to be replicated

- in at least two independant laboratories
- and in two distinct genetic backgrounds

Fmr1 KO mice have NORMAL performances in most cognitive tests, even challenging testing procedures

eNeuro

Normal Performance of *Fmr1* Mice on a Touchscreen Delayed Nonmatching to Position Working Memory Task[1,2,3]

Prescott T. Leach, Jane Hayes, Michael Pride, Jill L. Silverman, and Jacqueline N. Crawley

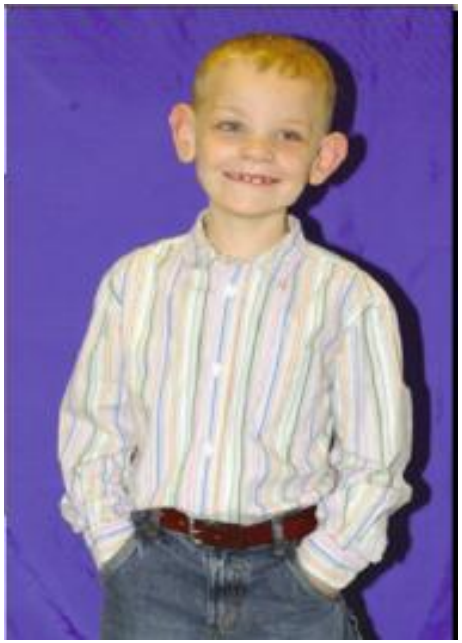
DOI:http://dx.doi.org/10.1523/ENEURO.0143-15.2016

Department of Psychiatry and Behavioral Sciences, MIND Institute, University of California, Davis, School of Medicine, Sacramento, California 95817

Abstract

Fragile X syndrome is a neurodevelopmental disorder characterized by mild-to-severe cognitive deficits. The complete absence of *Fmr1* and its protein product in the mouse model of fragile X (*Fmr1* KO) provides construct validity. A major conundrum in the field is the remarkably normal performance of *Fmr1* mice on cognitive tests in most reports. One explanation may be insufficiently challenging cognitive testing procedures. Here we developed a delayed nonmatching to position touchscreen task to test the hypothesis that paradigms placing demands on working memory would reveal robust and replicable cognitive deficits in the *Fmr1* KO mouse. We first tested *Fmr1* KO mice (*Fmr1*) and their wild-type (WT) littermates in a simple visual discrimination task, followed by assessment of reversal learning. We then tested *Fmr1* and WT mice in a new touchscreen nonmatch to position task and subsequently challenged their working memory abilities by adding delays, representing a higher cognitive load. The performance by *Fmr1* KO mice was equal to WTs on both touchscreen tasks. Last, we replicated previous reports of normal performance by *Fmr1* mice on Morris water maze spatial navigation and reversal. These results indicate that, while the *Fmr1* mouse model effectively recapitulates many molecular and cellular aspects of fragile X syndrome, the cognitive profile of *Fmr1* mice generally does not recapitulate the primary cognitive deficits in the human syndrome, even when diverse and challenging tasks are imposed.

Hello!
I am a Fragile X boy



Bad news !

Regardless to the drug you try,
It does not help me more than placebo!

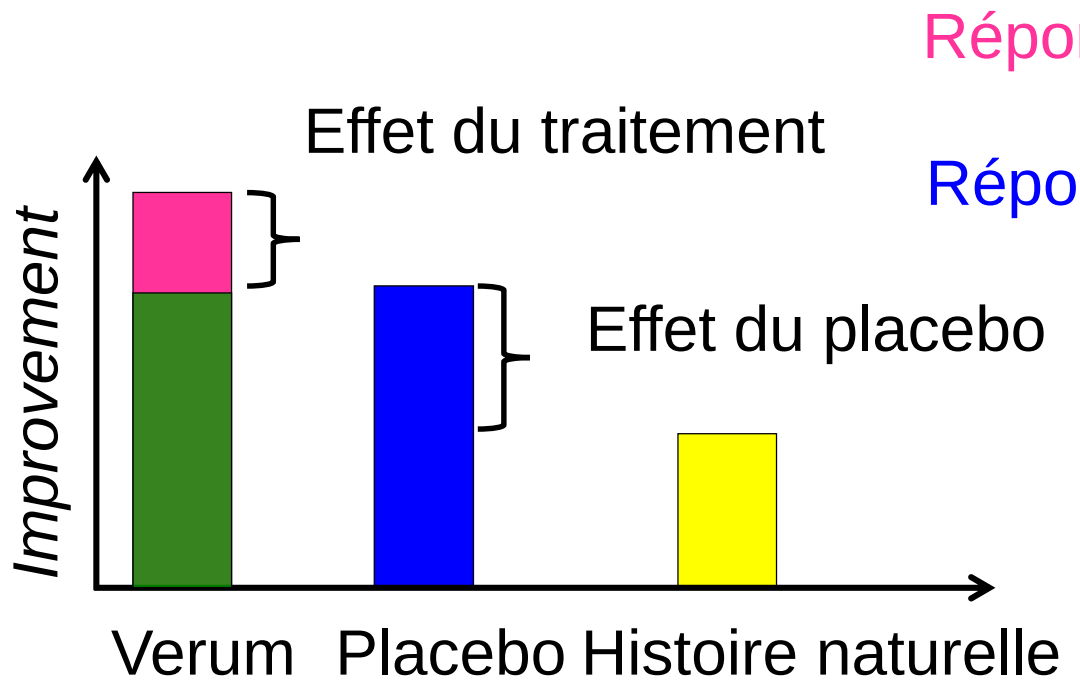
How may we explain this failure to demonstrate any drug effect despite robust preclinical findings ?

Do we correctly take into account the placebo effect?

4. Effet placebo

Placebo : définition

Un traitement inerte qui a un effet positif à travers le fait que le patients s'attende à s'améliorer



Méta-analyse

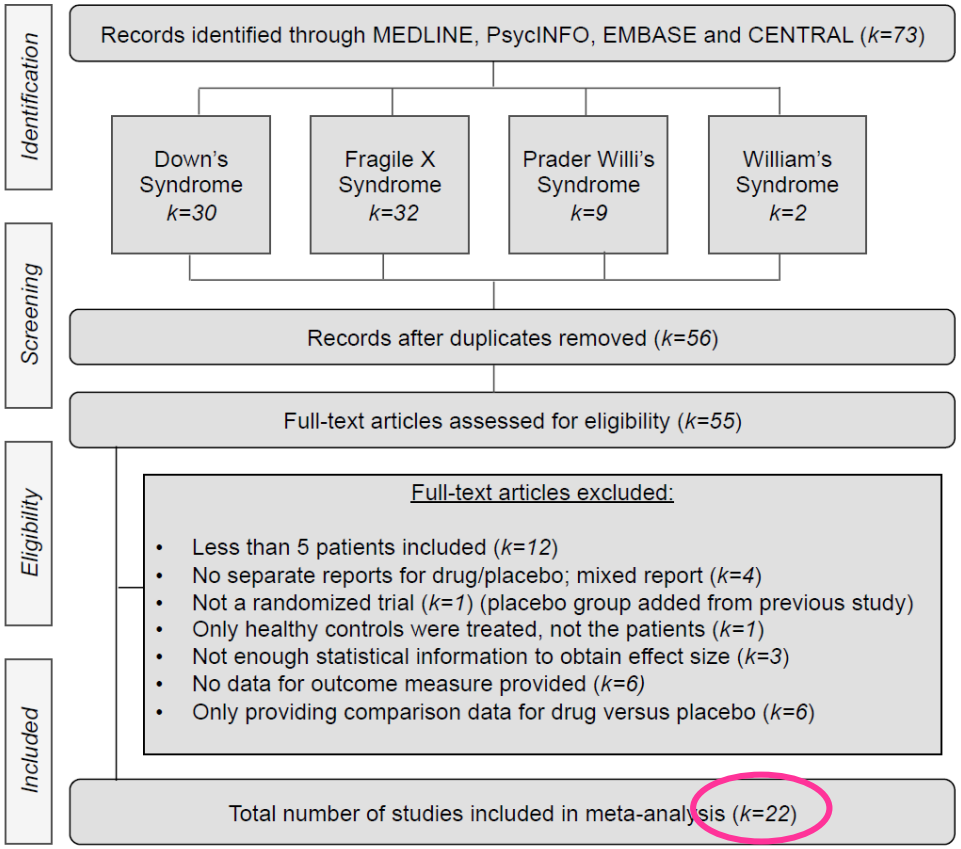
PLOS ONE | July 30, 2015

RESEARCH ARTICLE

Placebo Responses in Genetically Determined Intellectual Disability: A Meta-Analysis

Aurore Curie[1,2,3,4,5]*, Kathy Yang[1], Irving Kirsch[6,7], Randy L. Gollub[1], Vincent des Portes[2,3,4], Ted J. Kaptchuk[6], Karin B. Jensen[1,6,8]

- Incluant tous les essais randomisés contrôlés contre placebo, en double aveugle chez les patients DI d'origine génétique publiés en anglais avant avril 2013



- n=721
- Âge moyen=17.1 ans [0-55]
- 62% garçons
- Durée des essais=35 semaines

Résultats

- **Réponse au placebo significative chez les patients DI**

  from pre to post treatment (g=0.5, p=0.002)

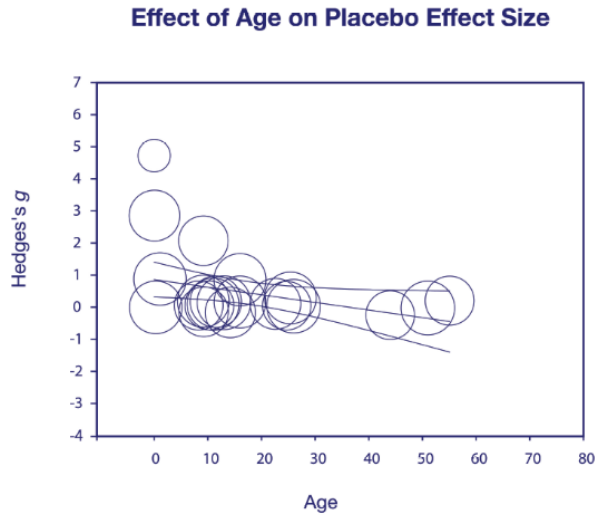
- **Pour les critères de jugement subjectifs** (évaluation par une tierce personne): p=0.02

et objectifs (évaluation directe des capacités des patients): p=0.036

- **Patients avec QI plus élevés ont des réponses au placebo plus importantes** (p=0.02)

- **Pas de réponse au placebo chez les patients DI avec démence associée**

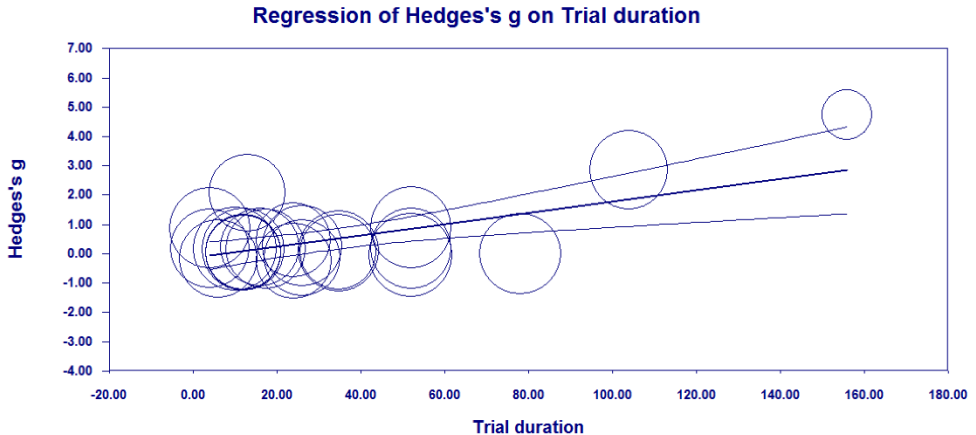
- **Effet significatif de l'âge sur les réponses au placebo** : réponse plus forte chez les patients plus jeunes



p=0.02

- **Réponse au Placebo stable dans le temps**

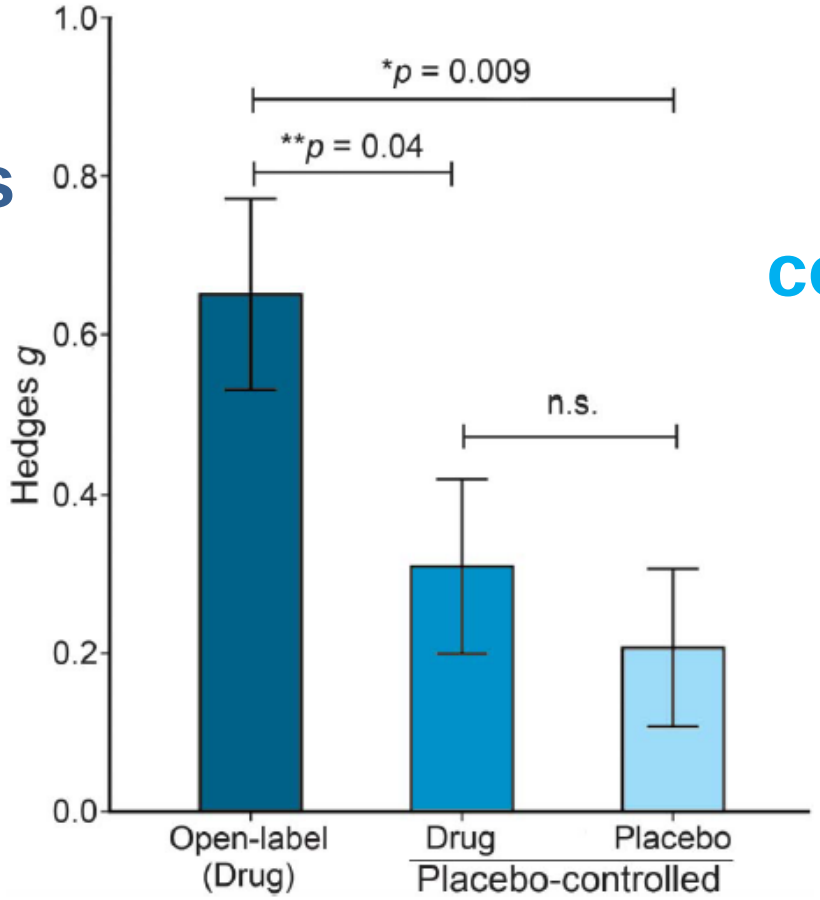
Contrairement à la croyance générale que la réponse au placebo est de courte durée et diminue avec le temps!



Effet de l'attente concernant le traitement

Essais ouverts

100 % de chances d'avoir la drogue active



Essais contrôlés contre Placebo

Seulement 50% de chance d'avoir la drogue active

Jensen et al., 2017

Les essais ouverts ne devraient plus être utilisés pour évaluer l'efficacité d'un traitement

La taille de l'effet de la réponse au placebo diffère significativement en fonction des catégories de processus mentaux évalués

	Placebo response			Drug response			Drug / Placebo comparison		
Mental process	***g***	**SE**	**p-value**	***g***	**SE**	**p-value**	**Q**	**df(Q)**	**p-value**
Attention (*k* = *2*)	0.056	0.229	0.81	0.180	0.234	0.441	0.143	1	0.71
Language (*k* = *8*)	0.017	0.064	0.79	0.121	0.063	0.05*	1.327	1	0.25
Memory (*k* = *4*)	0.174	0.132	0.19	0.282	0.159	0.08	0.272	1	0.60
Cognitive and developmental (*k* = *17*)	0.305	0.121	0.01*	0.521	0.137	0.0001****	1.394	1	0.24
Abnormal Behavior (*k* = *7*)	0.278	0.09	0.002***	0.480	0.127	0.0001****	1.686	1	0.19
Autistic traits (*k* = *3*)	0.336	0.161	0.037*	0.394	0.142	0.006**	0.073	1	0.79
CGI (*k* = *3*)	2.215	1.049	0.035*	2.004	0.800	0.01*	0.026	1	0.87

Curie et al., 2015

How may we explain this failure to demonstrate any drug effect despite robust preclinical findings ?

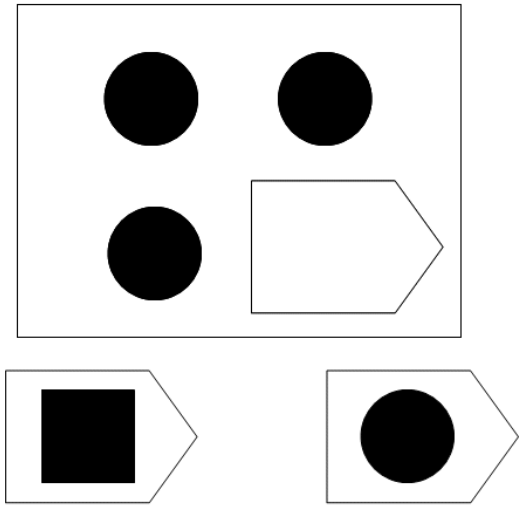
Do we use reliable outcome measures?

5. Importance des critères de jugement

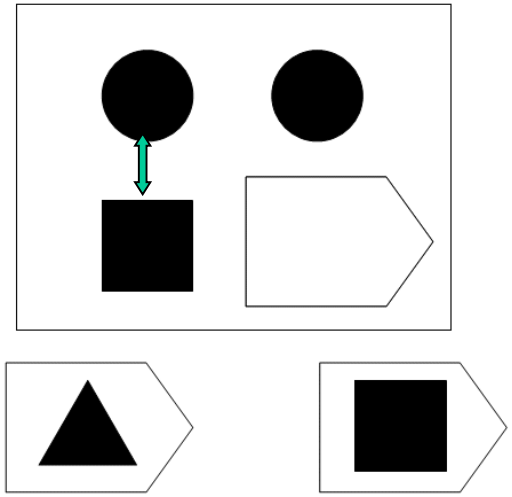
Etude du raisonnement visuel analogique

Tâche issue des matrices de Raven, mais plus simple

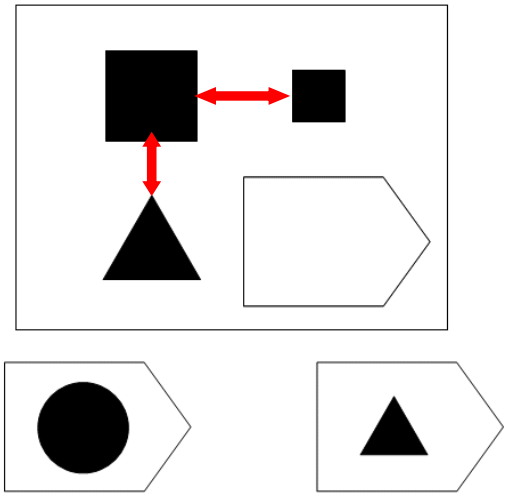
3 niveaux de difficultés:



Matrice identique
0 relation



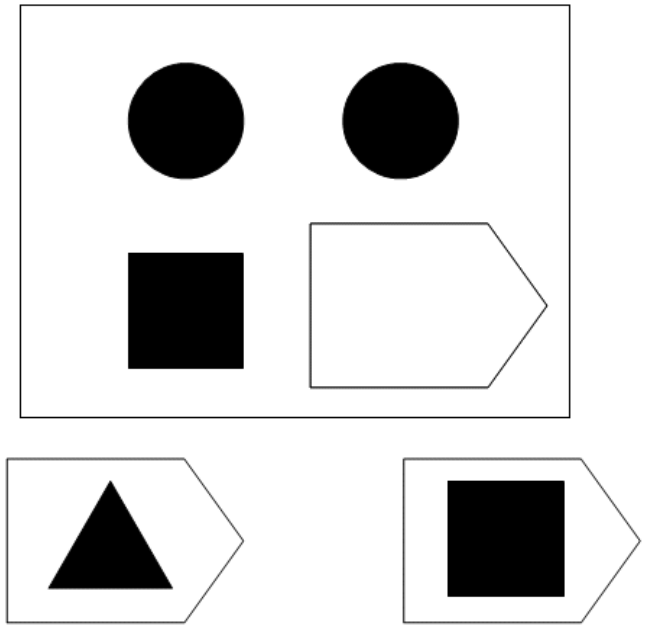
Matrice 1 relation



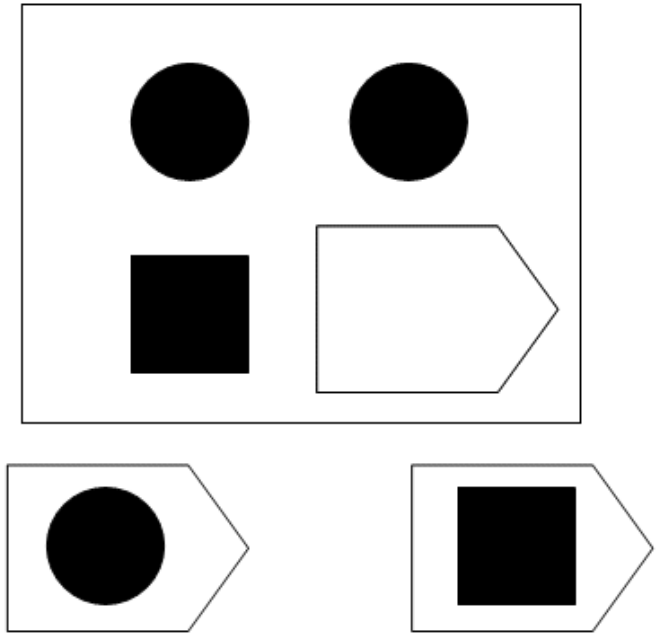
Matrice 2 relations

Brevet HCL / CNRS (ICG10755): « Dispositif d'évaluation des capacités cognitives d'un patient »

2 types de fausses réponses



Fausse réponse neutre

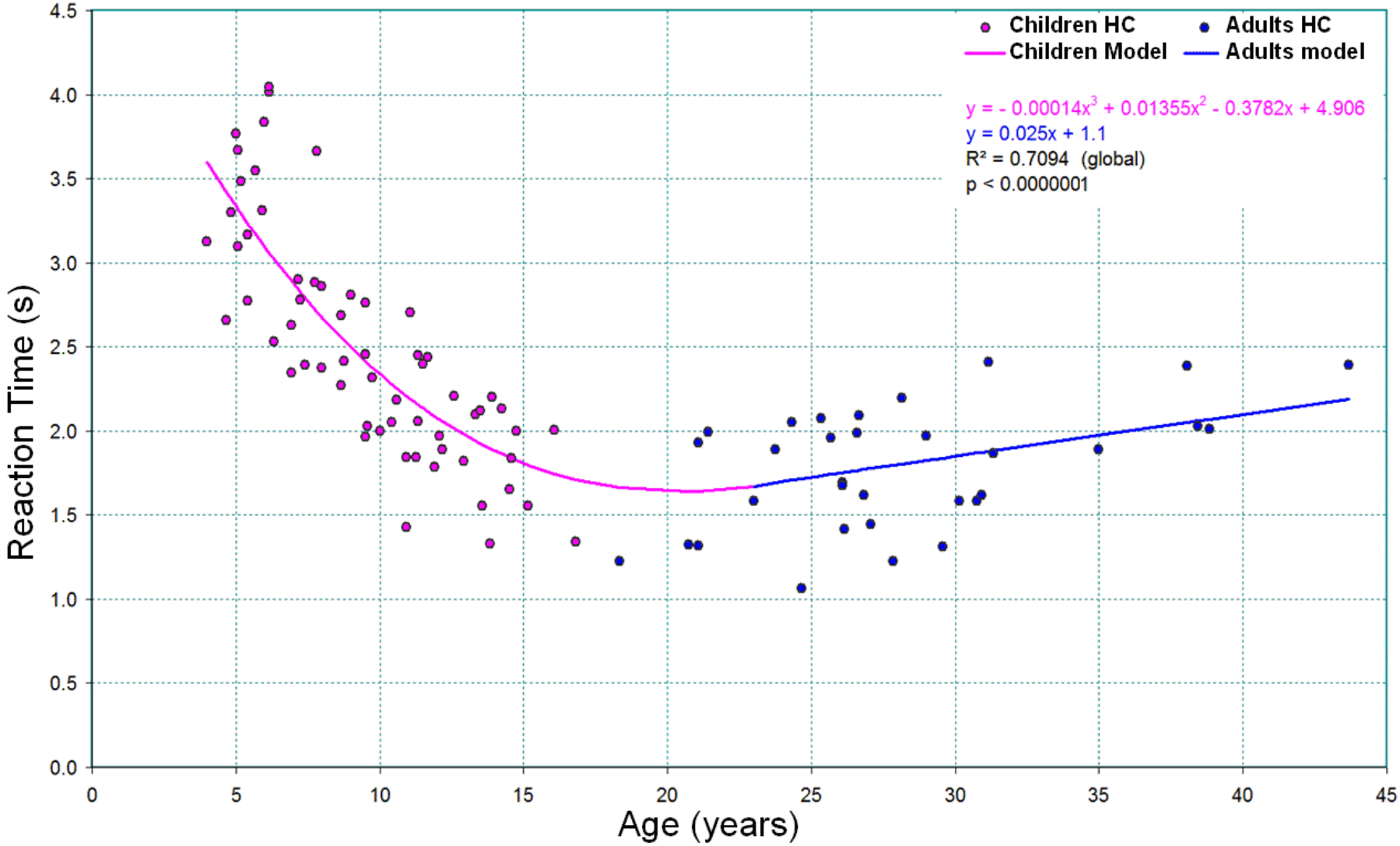


Fausse réponse « à inhiber »

Brevet HCL / CNRS (ICG10755)

Trajectoires Dévelopementales

Sujets sains
n=96

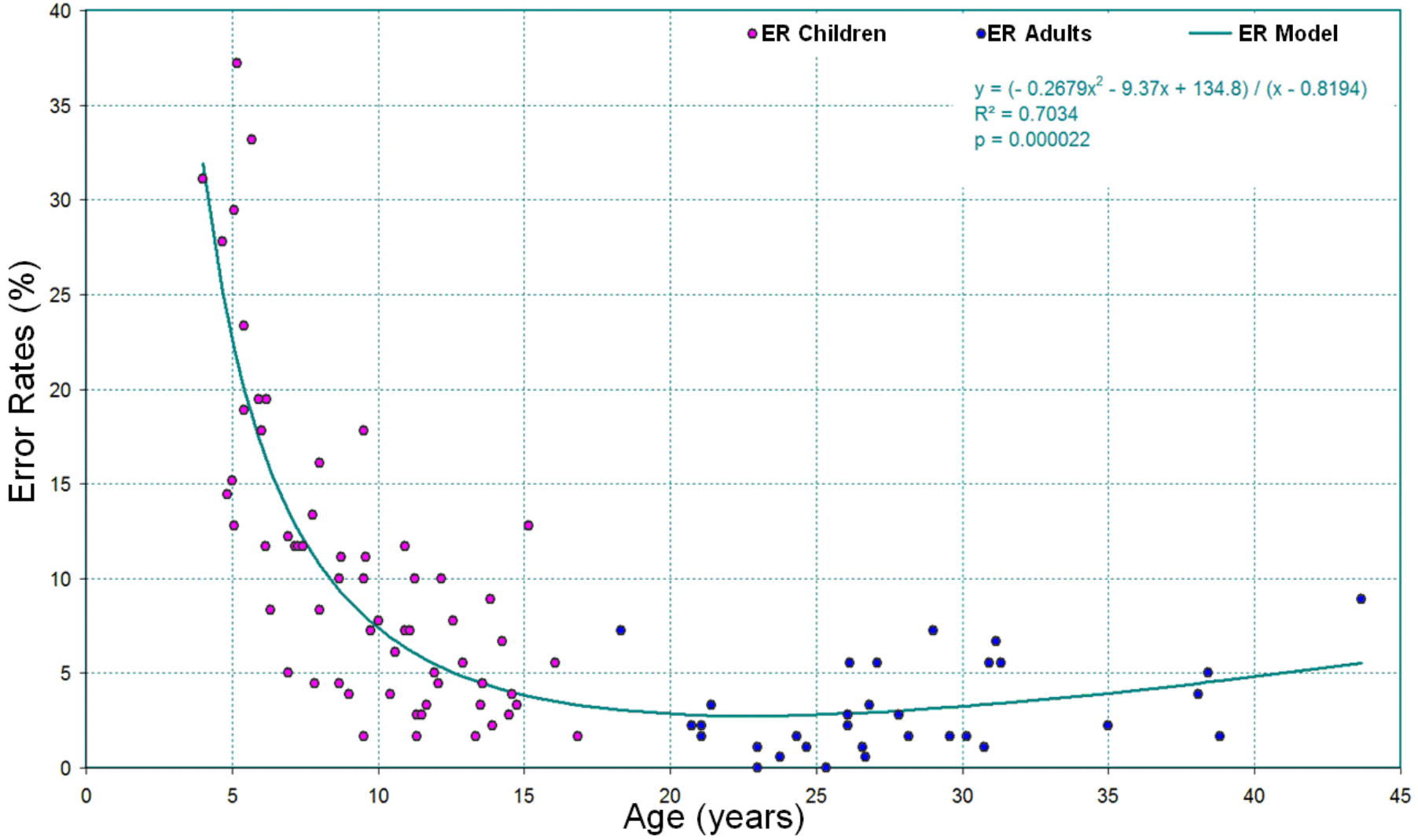


Temps de réaction

Curie et al., 2016

Trajectoires Dévelopementales

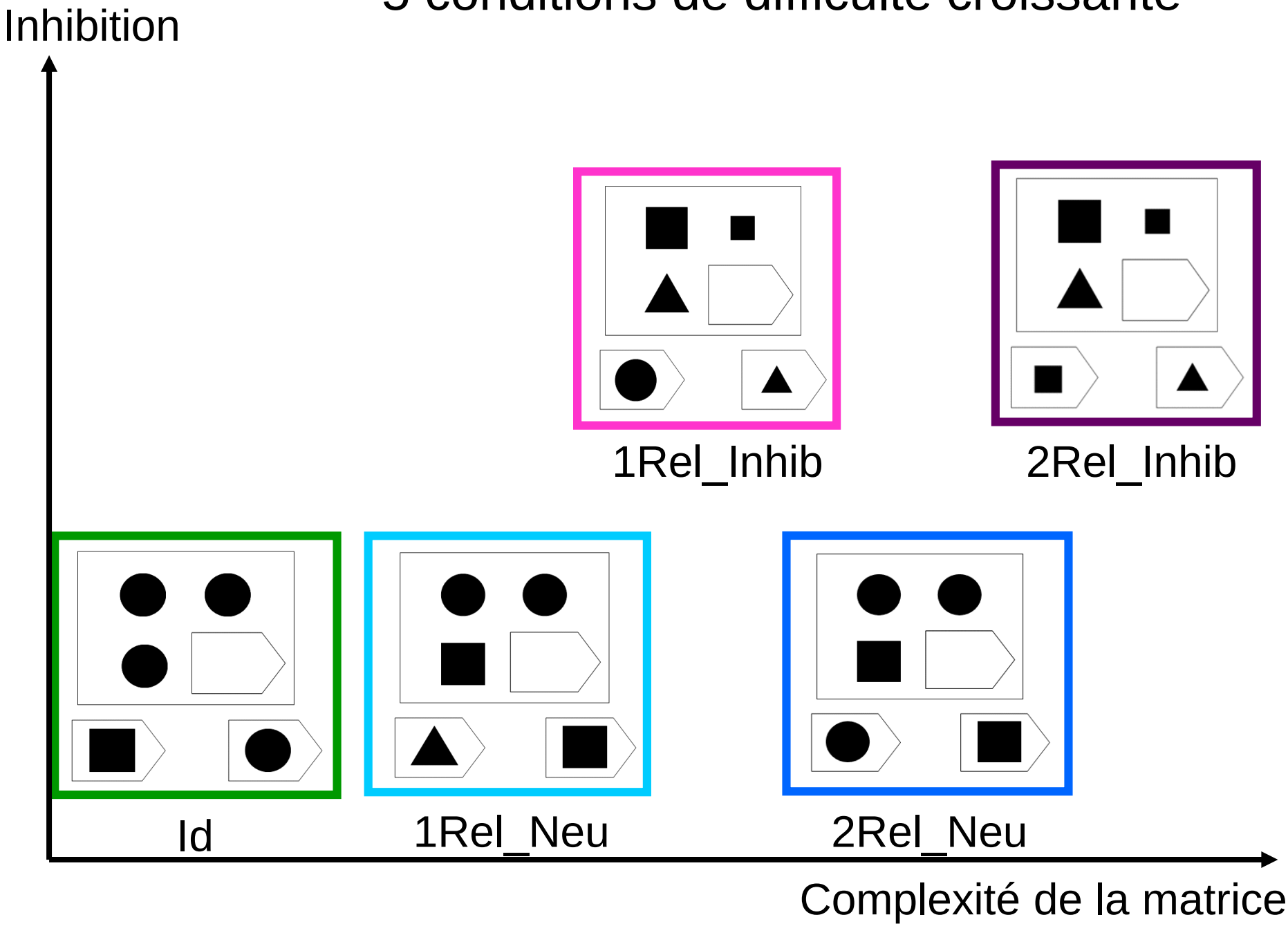
Sujets sains
n=96



Taux d'erreurs

Curie et al., 2016

5 conditions de difficulté croissante



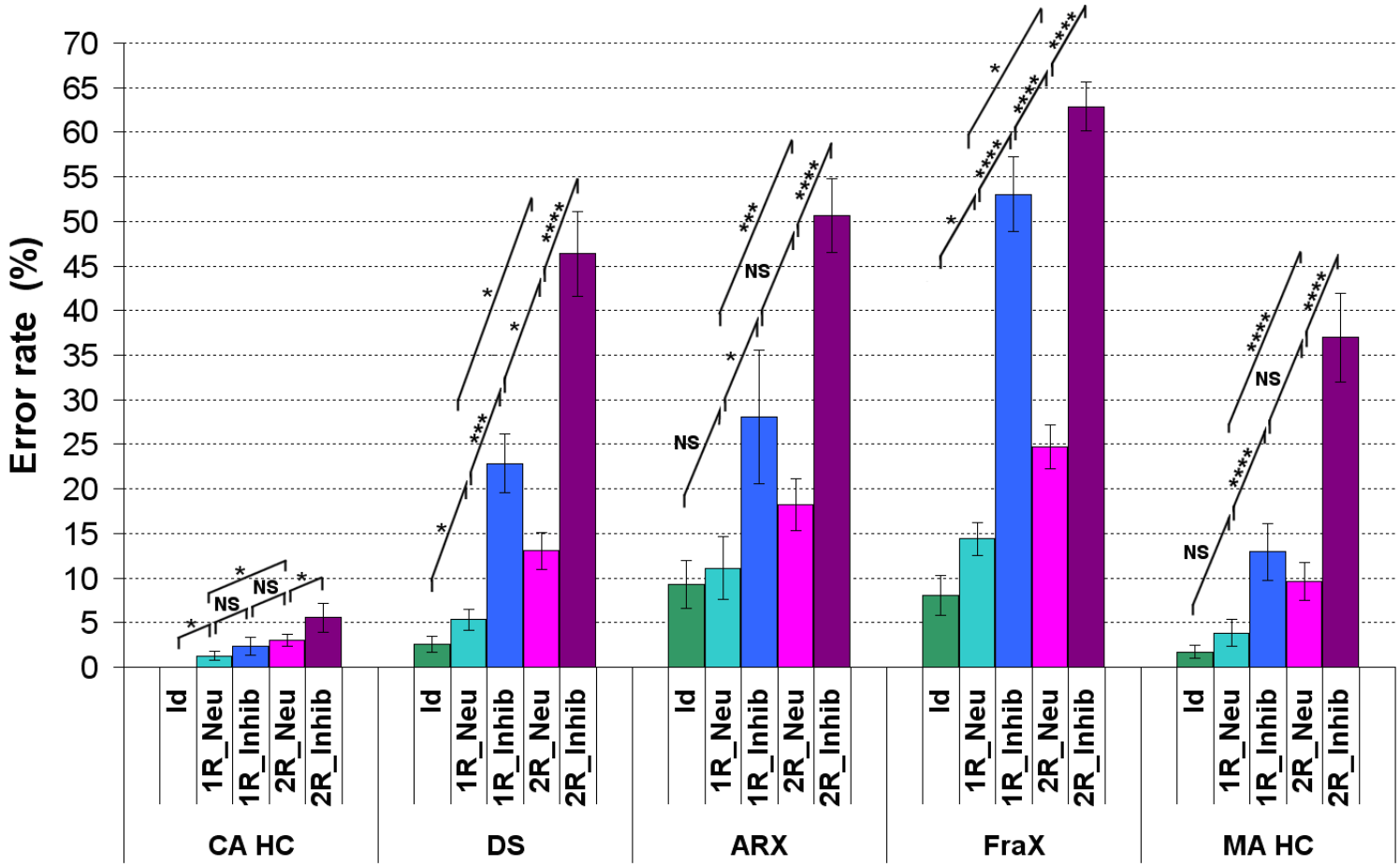
Analyse comportementale

41 patients DI / CA HC / MA HC

- Patients DI : plus lents et font significativement plus d'erreurs que les témoins sains appariés en âge chronologique

- Effet significatif de la complexité de la matrice

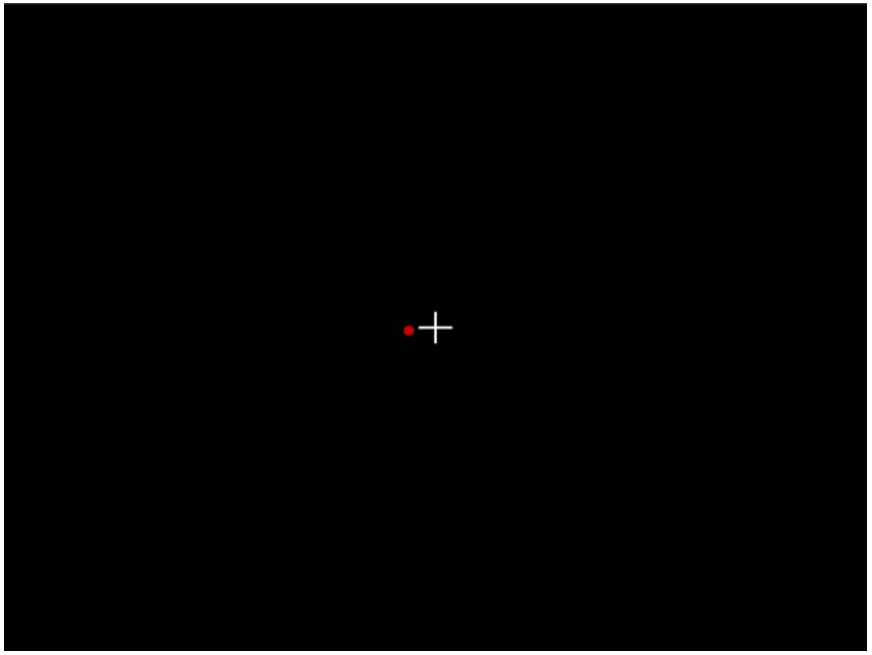
- Effet significatif de l'inhibition chez les patients DI



Curie et al., 2016

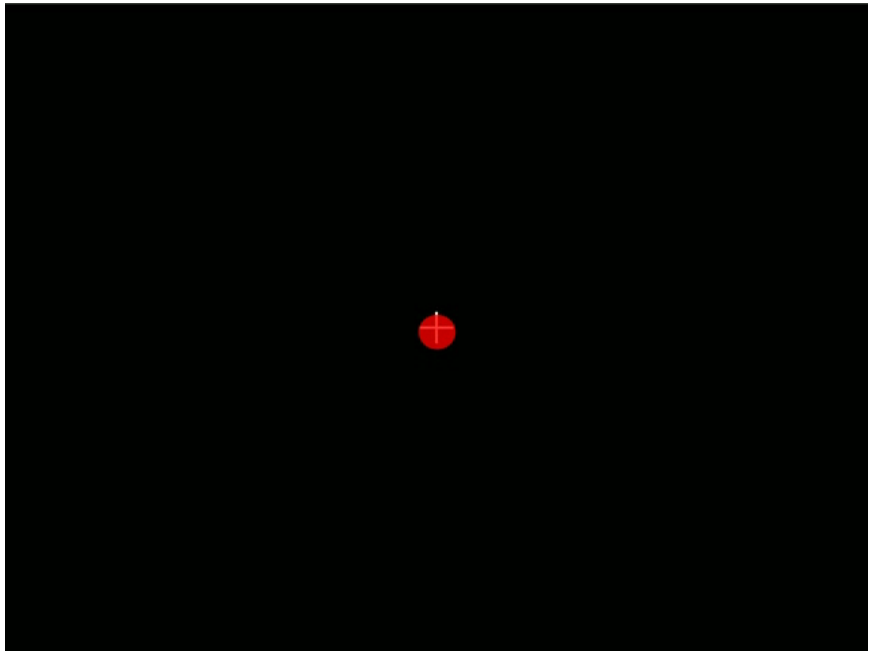
Eye-tracking

Paradigme de raisonnement visuel analogique



Sujet Sain

Stratégie par appariement constructif

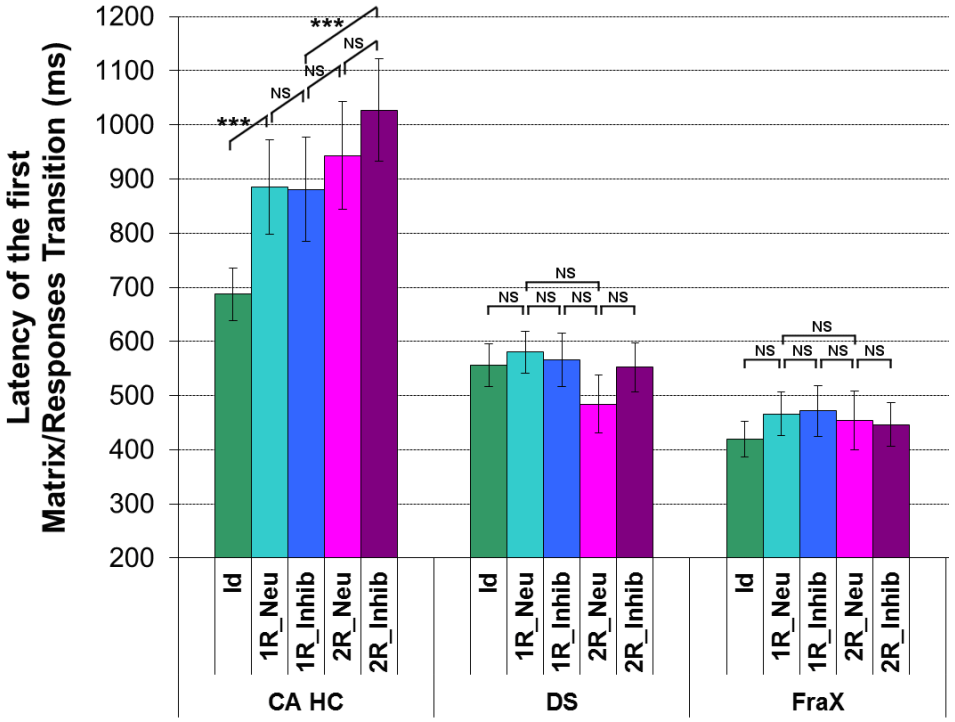


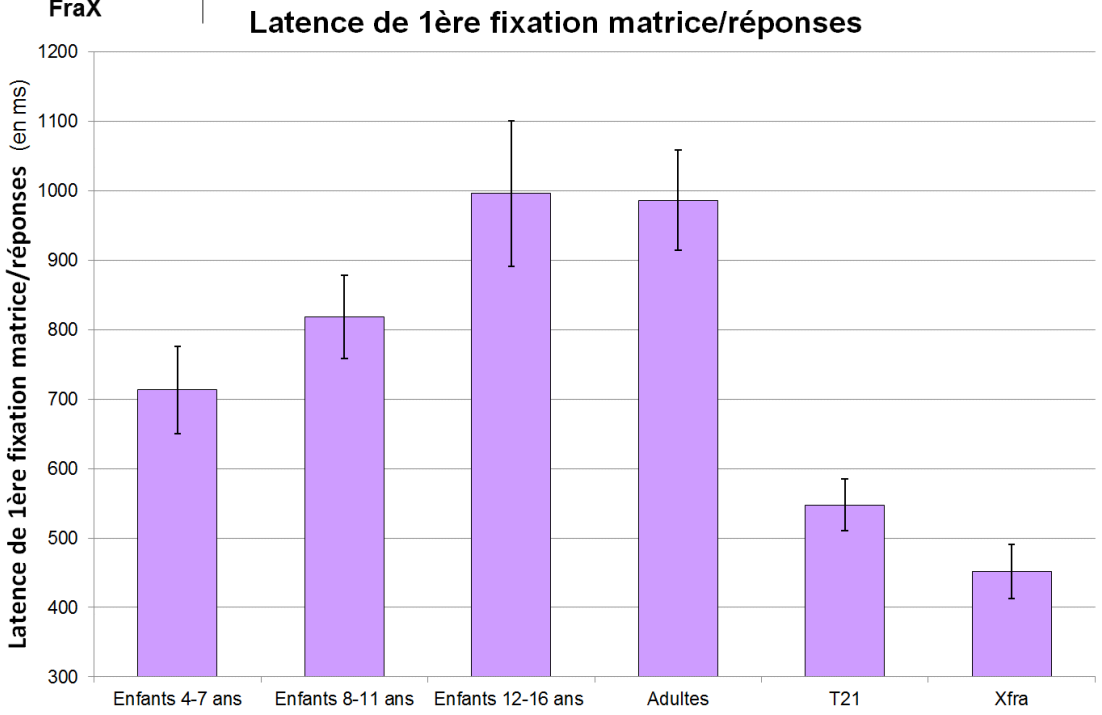
Patient X fragile

Stratégie par élimination

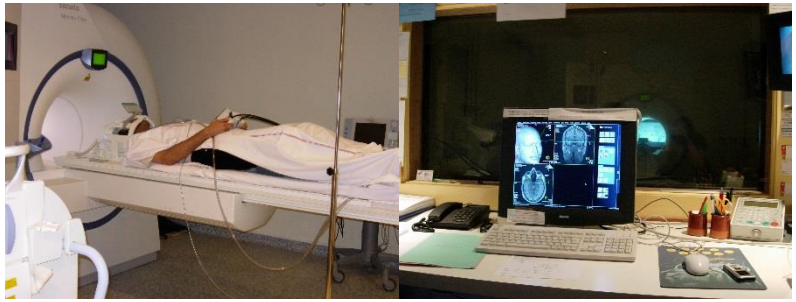
Curie et al., 2016

Eye-tracking

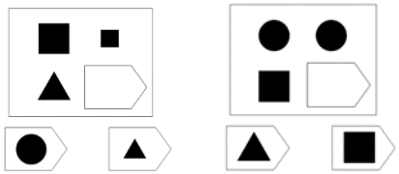




Curie et al., 2016



fMRI analysis



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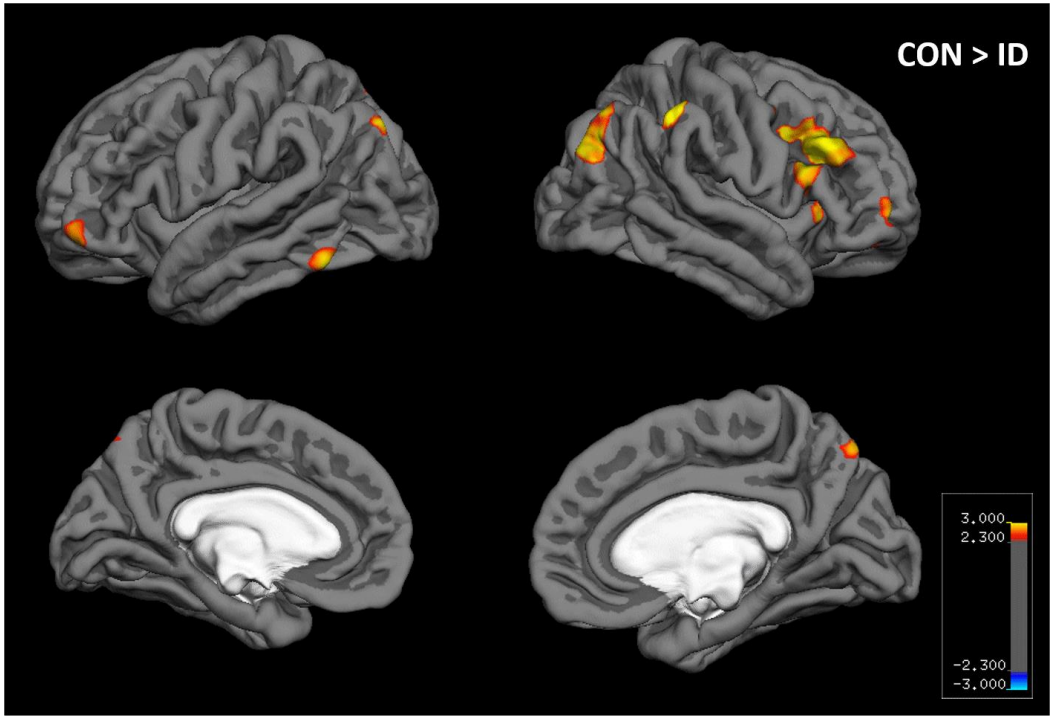
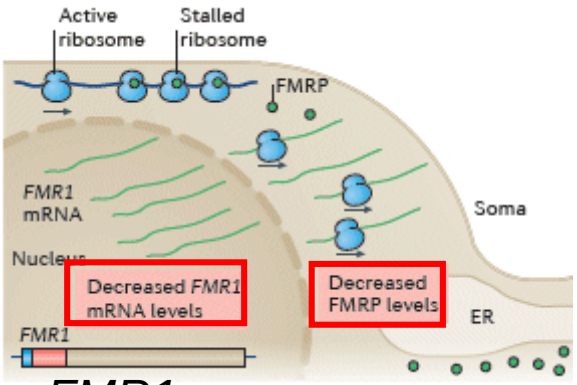
n=17 n=17



Curie et al., in preparation

6. Nombreuses pistes thérapeutiques possibles



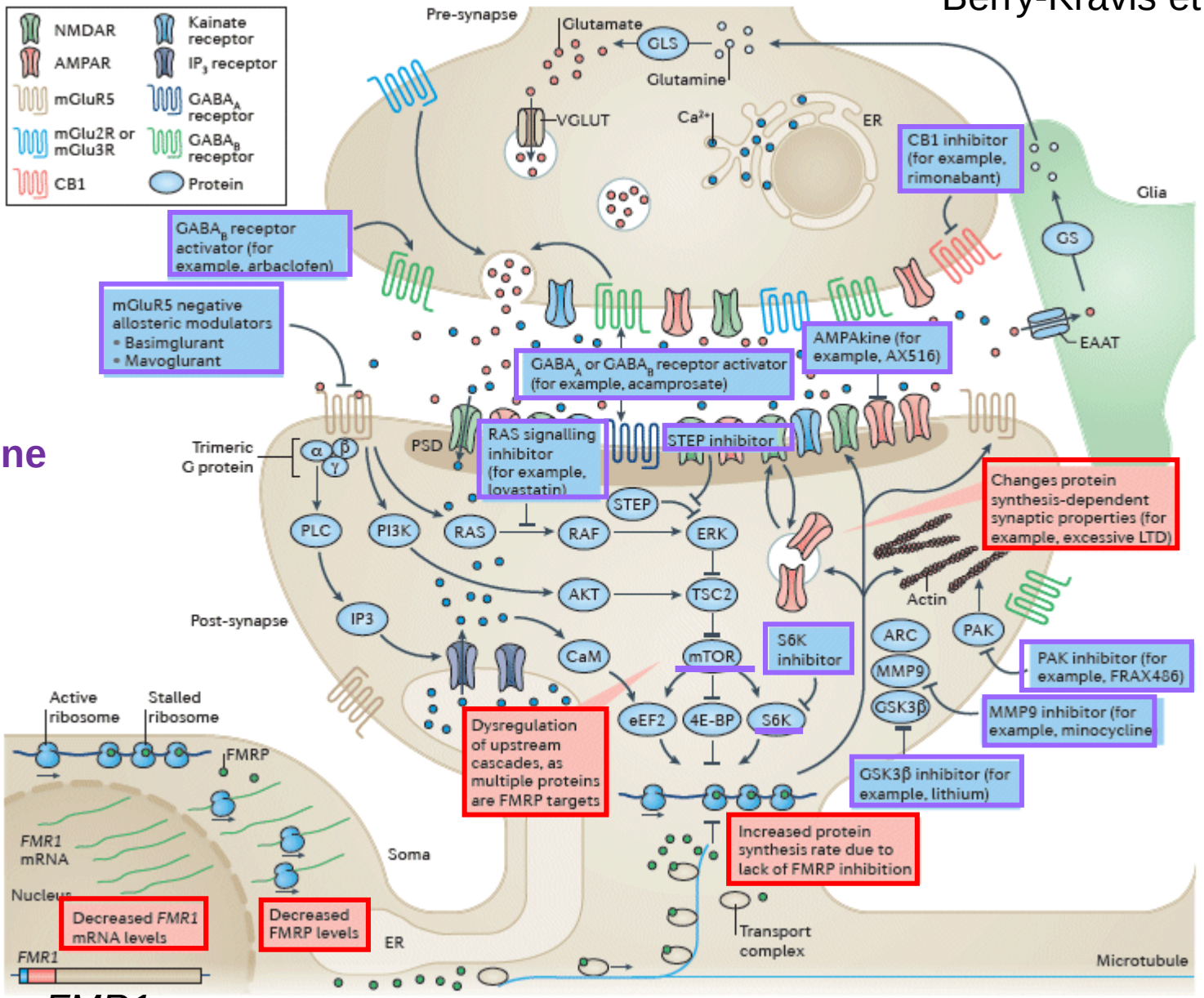
Gène *FMR1*

Berry-Kravis et al., 2017

6. Nombreuses pistes thérapeutiques possibles

Berry-Kravis et al., 2017

Metformine
ERK



Gène *FMR1*

STEP: Striatal Enriched Protein-tyrosine phosphatase

REVIEWS

Drug development for neurodevelopmental disorders: lessons learned from fragile X syndrome

Elizabeth M. Berry-Kravis[1], Lothar Lindemann[2], Aia E. Jønch[3,4], George Apostol[5], Mark F. Bear[6], Randall L. Carpenter[6], Jacqueline N. Crawley[7,8], Aurore Curie[9], Vincent Des Portes[9], Farah Hossain[10], Fabrizio Gasparini[11], Baltazar Gomez-Mancilla[11,12], David Hessl[7,8], Eva Loth[13], Sebastian H. Scharf[14], Paul P. Wang[15], Florian Von Raison[16], Randi Hagerman[7,17], Will Spooren[2] and Sébastien Jacquemont[18,19]

NATURE REVIEWS | DRUG DISCOVERY

2017

Etudes pré-cliniques

Berry-Kravis et al., 2017

Outcome measure	mGluR5*	mGluR5‡	$GABA_BR$ activation§	Statins‖	Lithium	STEP‡	MMP9¶	S6K#	S6K‡	CB1**	PAK‡‡	AMPAR modulation§§
Molecular												
Increased protein synthesis	>3	1	1	1	>3	ND	ND	1	1	ND	ND	ND
Increased ERK–mTOR–PI3K activity	>3	ND	ND	1	>3	ND	1	1	1	1	ND	ND
Synapse												
Altered synapse architecture	>3	1	1	ND	2–3	ND	>3	1	1	1	1	ND
Altered synaptic plasticity	>3	1	ND	1	>3	ND	ND	ND	1	ND	ND	ND
Behaviour												
Increased seizure incidence	>3	1	1	1	>3	1	1	ND	ND	1	1	ND
Impaired sensorimotor gating	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hyperactivity	1	ND	1	ND	>3	1	1	ND	ND	ND	1	ND
Impaired memory and cognition	>3	1	ND	ND	>3	ND	ND	1	1	1	ND	ND
Impaired social interactions	>3	ND	ND	ND	>3	1	ND	1	1	ND	ND	ND
Physiology												
Macroorchidism	1	–	ND	ND	1	ND	ND	1	1	ND	ND	ND
Elevated body growth	>3	1	ND	ND	ND	ND	ND	1	1	ND	ND	ND
Clinical research?	Yes	No	Yes	Yes	Yes	No	Yes	No	No	No	No	Yes

Numbers indicate the number of laboratories independently reporting the phenotype correction. A dash (–) indicates a lack of phenotype correction reported for

Etudes pré-cliniques

- Metformine (antidiabétique oral, biguanide)

Régulation de la voie ERK hyperactivé dans X fragile
Début phase clinique (USA, Canada)

- OV101 (gaboxadol): petite molécule spécifique pour sous-unité delta des récepteurs GABA extrasynaptiques

Modèle souris

- Pioglitazone (Hervé Moine): dysrégulation de l'activité diacylglycérolkinase kappa (DGKk), cible importante de FMRP dans les neurones

Modèle souris
Partenariat avec Lysogène pour projet de thérapie génique pour DGKk

- BKCa canal K activé par le Ca (BMS-204352, Sylvain Briault)

In vitro et modèle souris

Etudes cliniques

Berry-Kravis et al., 2017

- **Récepteurs mGluR5**

Design	Drug	Phase	Treatment duration (months)	n (F/M)	Age (years)	Status	Efficacy	Primary outcomes and biomarkers	Refs
mGluR5 receptor									
OL	Fenobam	IIa	NA, single dose	12 (6/6)	18–31	Completed	Changes reported on a biomarker	PPI (improved over test–retest controls)	150
RCT*	Mavoglurant†	IIa	1	30 (0/30)	18–35	Completed	Efficacy reported in a post-hoc analysis	• ABC • ERK activation rate, ET, PPI (results not published)	71
	Mavoglurant†	IIb	3	175 (11/164)	18–45	Completed	Lack of efficacy reported	• ABC • CGI-I • ET (no meaningful conclusions)	69
				139 (15/124)	12–17	Completed	Lack of efficacy reported	• ABC • CGI-I • ET (no meaningful conclusions)	69
OLES*	Mavoglurant	II/III	>12	148 (10/138)	18+	Terminated	Lack of efficacy reported	CGI-I	151
	Mavoglurant	II/III	>12	119 (13/106)	12–18	Terminated	Lack of efficacy reported	CGI-I	152
RCT	Basimglurant	IIa	1.5	40	18–50	Completed	Results not yet published	ADAMS	76
	Basimglurant	IIb	3	185 (34/151)	14–50	Completed	Lack of efficacy reported	• ADAMS • *FMR1* mRNA, repeat size, methylation status	73
	Basimglurant	IIa	3	47	5–13	Completed	Results not yet published	No outcomes at this point	74

Etudes cliniques

Berry-Kravis et al., 2017

- **Voie de signalisation intra-cellulaire**

Intracellular signalling									
OL	Lithium	IIa	2	16	6–30	Completed	Efficacy reported (ERK and other outcome measures such as ABC-T, CGI, VAS and RBANS)	• ABC • ERK activation rate • AP • ET • HR/HRV • RSA	130
RCT	NNZ-2256	II	1.5	72 (0/72)	12–45	Completed	Results not yet published	• AE • PK • ET	153
	Metadoxine[§]	II	1.5	62 (15/47)	15–55	Completed	Results not yet published	• ADHDRS • ET	154
	Lovastatin and PILI	II	5	60	10–17	Ongoing	Ongoing study	• ELS • AKT • ERK • MMP9	123

- **Protéines régulées par FMRP et AMPA récepteurs**

Proteins regulated by FMRP									
OL	Minocycline and lovastatin	II	3	26	13–45	Ongoing	Ongoing study	• ABC • Neuroimaging[¶]	124
	Minocycline	IIa	2	20 (2/18)	13–35	Completed	Efficacy reported on ABC	ABC	155
RCT	Minocycline	II	3	55 (8/47)	3.5–16	Completed	Modest efficacy reported on CGI-I	• CGI-I • MMP9 • VAS	156
AMPA receptor									
RCT	CX516[¶]	II	1	49 (11/38)	18–50	Completed	Lack of efficacy reported	Memory[#]	147

Etudes cliniques

Berry-Kravis et al., 2017

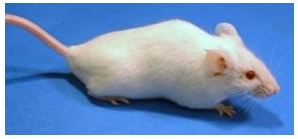
- **Modulateurs de GABA**

Design	Drug	Phase	Treatment duration (months)	n (F/M)	Age (years)	Status	Efficacy	Primary outcomes and biomarkers	Refs
GABA modulators									
RCT*	Arabaclofen**	II	1	63 (8/55)	6–40	Completed	Efficacy reported in post-hoc analysis on ABC	• ABC • APP‡‡	68
	Arabaclofen	III	2	125 (26/99)	12–50	Completed	Lack of efficacy reported	ABC	72
	Arabaclofen	III	2	172 (25/144)	5–11	Completed	Lack of efficacy reported	ABC	72
OL ES*	Arbaclofen	II	12	45	6–40	Terminated	Results not yet published	ABC	157
	Arbaclofen	III	>12	357	5–50	Terminated	Results not yet published	Open-label study for safety	158
OL	Acamprosate	III	2.5	12 (2/10)	5–17	Completed	Efficacy reported on the CGI and behavioural scales	• CGI-I • APP • BDNF‡‡	159
	Donepezil	I	1.5	8 (2/6)	14–44	Completed	Efficacy reported	CNT	160
RCT	Acamprosate	II/III	2.5	48	5–23	Ongoing	Ongoing study	• ABC • APP • ERK • ET	46
	Ganaxolone	II	1.5	59 (9/50)	6–17	Completed	Lack of efficacy	• CGI-I • ABC • ERP	161
	Donepezil	II	3	42 (15/27)	12–29	Completed	Results not yet published	CNT	162
	Donepezil	II	3	20 (0/20)	6–15	Completed	Lack of efficacy reported	IQ§§	163

- Phosphodiestérase 4 (Berry-Kravis)

Correction AMPc

Modèle souris pour le syndrome de l'X fragile



Fmr1 KO mice

- **Critères de jugement mesurés**

  - Synthèse protéique
  - Densité et morphologie des épines dendritiques
  - Long-Term Depression (LTD)
  - Crises d'épilepsie audiogéniques
  - Tâches comportementales (apprentissage et mémoire) pour souris: Water maze de Morris, fear conditioning, novel object recognition, visual discrimination, …
  - Autres: open field, rotarod, marble burrying, self-grooming, social paradigms

Moins robuste

- En oncologie:

8% des traitements semblant efficaces sur les modèles précliniques aboutiront à une réelle application clinique

- **Avis des experts partagés (pas de consensus):**

- Validité du modèle souris KO *Fmr1?*

- Nécessité de développer d'autres modèles animaux

- Problème de la transposabilité des critères de jugement de la souris à l'homme (EEG?, IRMf?)

- chez l'homme: cœur du problème=DI

- chez la souris: déficit cognitif inconstant, et effet de petite taille

- durée, âge de traitement?
- Chez l'enfant: faut-il traiter plus jeunes même si pas d'effet démontré chez l'adulte? (ex: mavoglurant 3-6 ans)

Nouvelles stratégies

- Tester plusieurs molécules à la fois

- Réactivation de l'expression de gènes sur le chromosome X (régulation épigénétique)

A mixed modality approach towards Xi reactivation for Rett syndrome and other X-linked disorders

Lieselot L. G. Carrette[a,b,c,d], Chen-Yu Wang[a,b,c], Chunyao Wei[a,b,c], William Press[a,b,c], Weiyuan Ma[e,f], Raymond J. Kelleher III[e,f], and Jeannie T. Lee[a,b,c,1]

[a]Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA 02114; [b]Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114; [c]Department of Genetics, Harvard Medical School, Boston, MA 02115; [d]Center for Medical Genetics, Ghent University, 9000 Ghent, Belgium; [e]Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA 02114; and [f]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114

Contributed by Jeannie T. Lee, December 1, 2017 (sent for review August 28, 2017; reviewed by Ingolf Bach and Gyorgyi Csankovszki)

The X-chromosome harbors hundreds of disease genes whose associated diseases predominantly affect males. However, a subset, including neurodevelopmental disorders, Rett syndrome (RTT), fragile X syndrome, and CDKL5 syndrome, also affects females. These disorders lack disease-specific treatment. Because female cells carry two X chromosomes, an emerging treatment strategy has been to reawaken the healthy allele on the inactive X (Xi). Here, we focus on methyl-CpG binding protein 2 (MECP2) restoration for RTT and combinatorially target factors in the interactome of Xist, the noncoding RNA responsible for X inactivation. We identify a mixed modality approach combining an Xist antisense oligonucleotide and a small-molecule inhibitor of DNA methylation, which, together, achieve 30,000-fold MECP2 up-regulation from the Xi in cultured cells. Combining a brain-specific genetic *Xist* ablation with short-term 5-aza-2′-deoxycytidine (Aza) treatment models the synergy in vivo without evident toxicity. The Xi is selectively reactivated. These experiments provide proof of concept for a mixed modality approach for treating X-linked disorders in females.

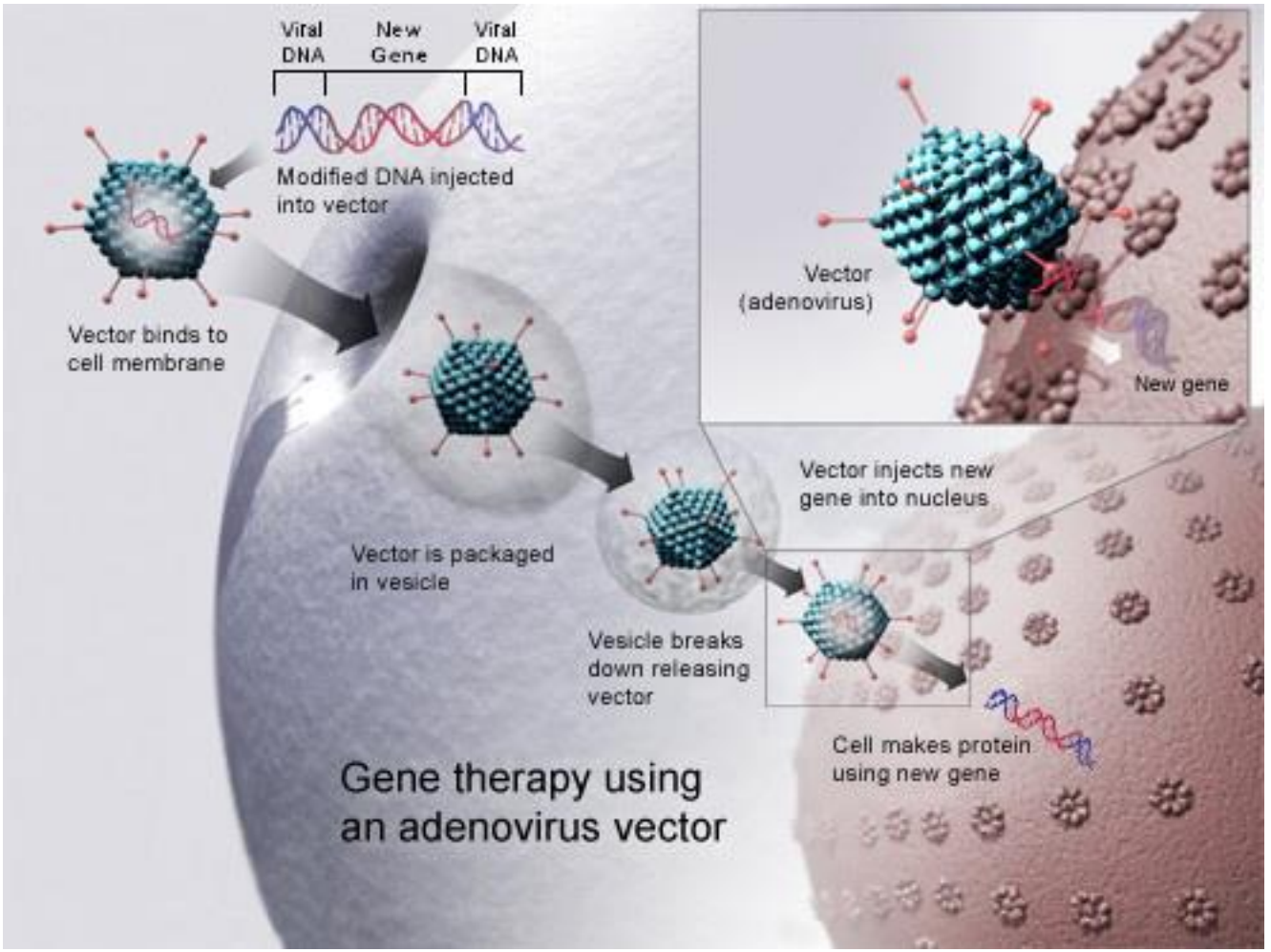
X reactivation | antisense oligonucleotides | LNA | Rett syndrome | Xist

however, two obstacles to an Xi-reactivation strategy. First, sex chromosomal dosage compensation is known to be important throughout development and life: Perturbing XCI by a germline deletion of the master regulator *Xist* resulted in inviable female embryos (8), an epiblast-specific deletion of *Xist* caused severely reduced female fitness (9), and a conditional deletion of *Xist* in blood caused fully penetrant hematological cancers (10). Perturbing dosage balance via Xi reactivation could therefore have untoward physiological consequences. On the other hand, loss of Xist and partial reactivation occur naturally in lymphocytes (11), and may therefore be tolerated in vivo under controlled circumstances. A second challenge is that the Xi has been difficult to reactivate via pharmacological means due to multiple parallel mechanisms of epigenetic silencing (1–3, 12). Progress has been made in recent years, however. Several siRNA screens identified several factors regulating Xi stability, but no overlap of candidates was observed between them (13, 14), perhaps because the screens were not saturating. Others have identified the TGF-β pathway (15), a synergism between Aurora kinase and DNA methylation in a primed small-molecule screen (16), as well as a

Nouvelles stratégies

- Thérapie génique

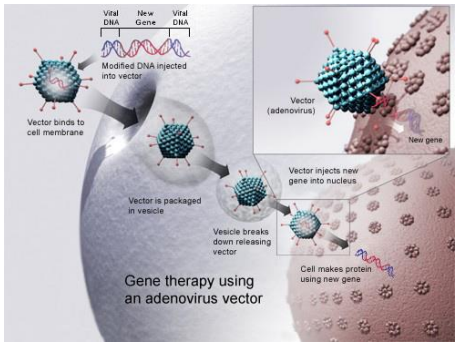
  - administration de vecteurs adéno-viraux exprimant la protéine FMRP (« brain administration »)



Nouvelles stratégies

- Thérapie génique

  - administration de vecteurs adéno-viraux exprimant la protéine FMRP (« brain administration »)



Reduced Phenotypic Severity Following Adeno-Associated Virus-Mediated Fmr1 Gene Delivery in Fragile X Mice

Shervin Gholizadeh[1,3], Jason Arsenault[1,3], Ingrid Cong Yang Xuan[1], Laura K Pacey[1] and David R Hampson*[,1,2]

[1]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada; [2]Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada

Fragile X syndrome (FXS) is a neurodevelopmental disorder caused by a trinucleotide repeat expansion in the FMR1 gene that codes for fragile X mental retardation protein (FMRP). To determine if FMRP expression in the central nervous system could reverse phenotypic deficits in the Fmr1 knockout (KO) mouse model of FXS, we used a single-stranded adeno-associated viral (AAV) vector with viral capsids from serotype 9 that contained a major isoform of FMRP. FMRP transgene expression was driven by the neuron-selective synapsin-1 promoter. The vector was delivered to the brain via a single bilateral intracerebroventricular injection into neonatal Fmr1 KO mice and transgene expression and behavioral assessments were conducted 22–26 or 50–56 days post injection. Western blotting and immunocytochemical analyses of AAV–FMRP-injected mice revealed FMRP expression in the striatum, hippocampus, retrosplenial cortex, and cingulate cortex. Cellular expression was selective for neurons and reached ~50% of wild-type levels in the hippocampus and cortex at 56 days post injection. The pathologically elevated repetitive behavior and the deficit in social dominance behavior seen in phosphate-buffered saline-injected Fmr1 KO mice were reversed in AAV–FMRP-injected mice. These results provide the first proof of principle that gene therapy can correct specific behavioral abnormalities in the mouse model of FXS.

Neuropsychopharmacology (2014) **39,** 3100–3111; doi:10.1038/npp.2014.167; published online 6 August 2014

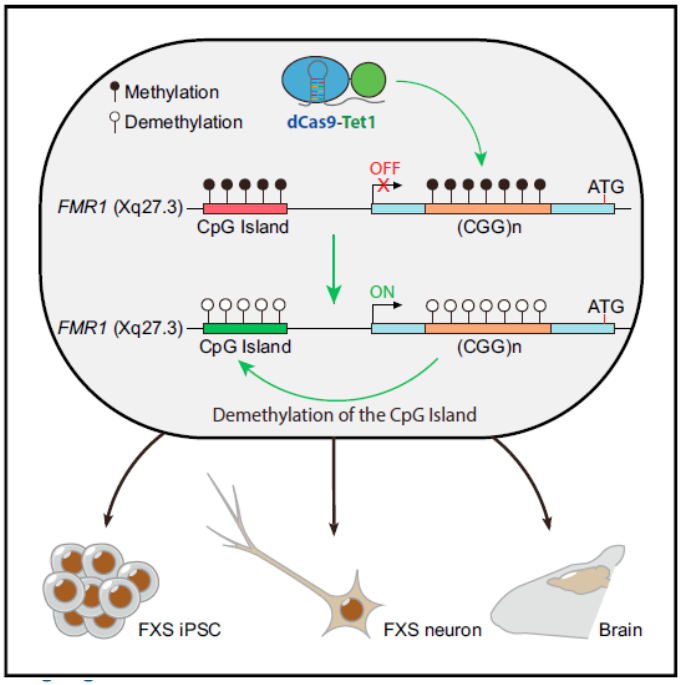
Nouvelles stratégies

- Thérapie génique

  - réactivation ciblée de l'expression du gène *FMR1*

CRISPR-Cas 9

« ciseaux moléculaires »



- Targeted demethylation of CGG repeats by dCas9-Tet1 reactivates *FMR1* in FXS cells
- Demethylation of CGG repeats induces an active chromatin status for *FMR1* promoter
- Methylation-edited FXS neurons behave similarly as wild-type neurons
- *FMR1* reactivation by dCas9-Tet1 is sustainable in a human/mouse chimeric model

Article

Cell

Rescue of Fragile X Syndrome Neurons by DNA Methylation Editing of the *FMR1* Gene

X. Shawn Liu,[1] Hao Wu,[1,3] Marine Krzisch,[1] Xuebing Wu,[1] John Graef,[3] Julien Muffat,[1] Denes Hnisz,[1] Charles H. Li,[1,2] Bingbing Yuan,[1] Chuanyun Xu,[1,5] Yun Li,[1] Dan Vershkov,[4] Angela Cacace,[3] Richard A. Young,[1,2] and Rudolf Jaenisch[1,2,6,*]
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https://doi.org/10.1016/j.cell.2018.01.012

SUMMARY

Fragile X syndrome (FXS), the most common genetic form of intellectual disability in males, is caused by silencing of the *FMR1* gene associated with hypermethylation of the CGG expansion mutation in the 5′ UTR of *FMR1* in FXS patients. Here, we applied recently developed DNA methylation editing tools to reverse this hypermethylation event. Targeted demethylation of the CGG expansion by dCas9-Tet1/single guide RNA (sgRNA) switched the heterochromatin status of the upstream *FMR1* promoter to an active chromatin state, restoring a persistent expression of *FMR1* in FXS iPSCs. Neurons derived from methylation-edited FXS iPSCs rescued the electrophysiological abnormalities and restored a wild-type phenotype upon the mutant neurons. *FMR1* expression in edited neurons was maintained *in vivo* after engrafting into the mouse brain. Finally, demethylation of the CGG repeats in post-mitotic FXS neurons also reactivated *FMR1*. Our data establish that demethylation of the CGG expansion is sufficient for *FMR1* reactivation, suggesting potential therapeutic strategies for FXS.

Au total:

- Prioriser les essais sur données pré-cliniques solides, reproductibles, chez plus d'une espèce

- Essais en double aveugle, contrôlés contre placebo, avec puissance suffisante

- Si possible démonstration d'une modification de la maladie « disease modifier »
  Enfants plus jeunes?
  Modification de la trajectoire développementale

- Etudes nécessaire pour évaluer la variabilité inter-patient, la validation test-retest et le développement de nouveaux critères de jugement

Disease modifying treatment ?

Intensive Rehabilitation

Speech therapy
Reading training

AND

Drugs

Anti mGluR +
GABA ergic...

7. Conclusions

- Nombreuses nouvelles perspectives thérapeutiques
- Enthousiasmant mais rester prudent
- Questions soulevée par le modèle souris
- Problème des critères de jugement

18th International Fragile X and related neurodevelopmental disorders, octobre 2017, Sacacomie, Canada.

Remerciements

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- Collaborators

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Xtraordinaire

NOVARTIS

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