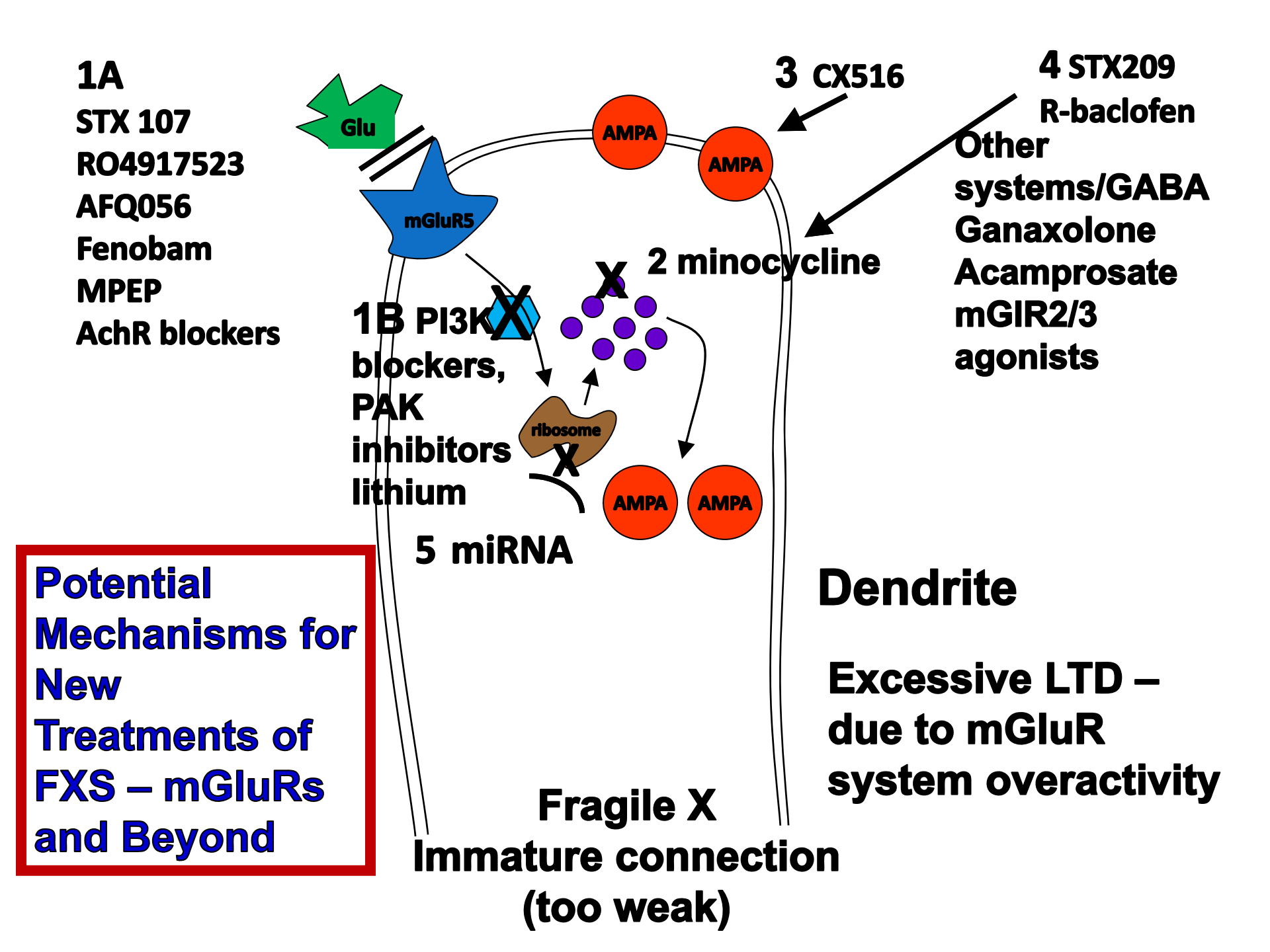
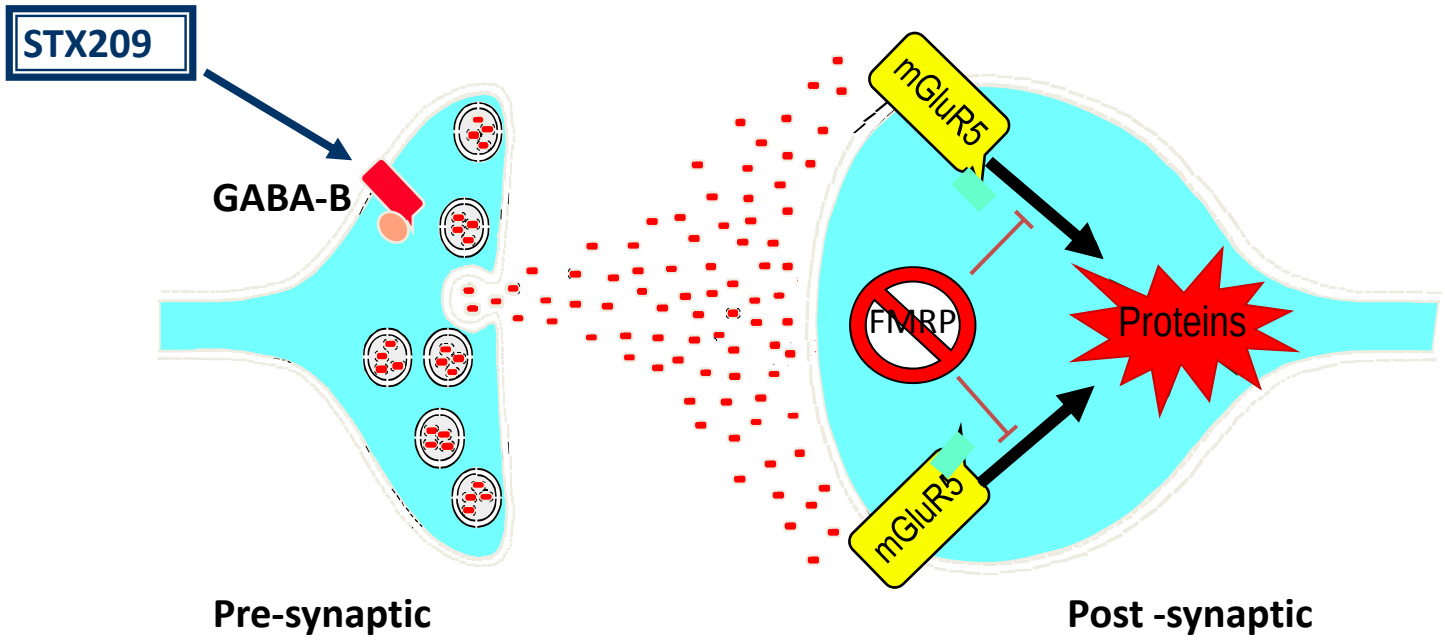
Clinical Trials of New Medications Targeting Brain Mechanisms in Fragile X Syndrome

Elizabeth Berry-Kravis
Rush University Medical Center
Randi Hagerman
UC Davis MIND Institute


1A
STX 107
RO4917523
AFQ056
Fenobam
MPEP
AchR blockers
Glu
mGluR5
AMPA
AMPA
3 CX516
4 STX209
R-baclofen
Other systems/GABA
Ganaxolone
Acamprosate
mGlR2/3 agonists
2 minocycline
1B PI3K blockers, PAK inhibitors lithium
ribosome
AMPA
AMPA
5 miRNA
Potential Mechanisms for New Treatments of FXS – mGluRs and Beyond
Dendrite
Excessive LTD – due to mGluR system overactivity
Fragile X
Immature connection
(too weak)

Arbaclofen (STX209) GABA-B Agonist

Mechanism 4 : Correct Connectivity Through Systems Effect: R-baclofen – Decrease Excessive Glutamatergic Transmission



Model Courtesy of Seaside Therapeutics

STX209 (Arbaclofen)

- GABA-B agonist (activator), decrease glutamate release
- More potent isoform of racemic baclofen
- GABA system abnormal in mouse
- Mouse/fly – reverse audiogenic seizures, spine density, AMPA internalization, behavior and survival phenotypes
- 22001: Placebo-controlled crossover trial with 4 weeks of treatment each arm – 63 subjects (Seaside – 12 sites)
- Good safety/side effect profile

Results from Initial (22001) Seaside Arbaclofen (STX209) Study

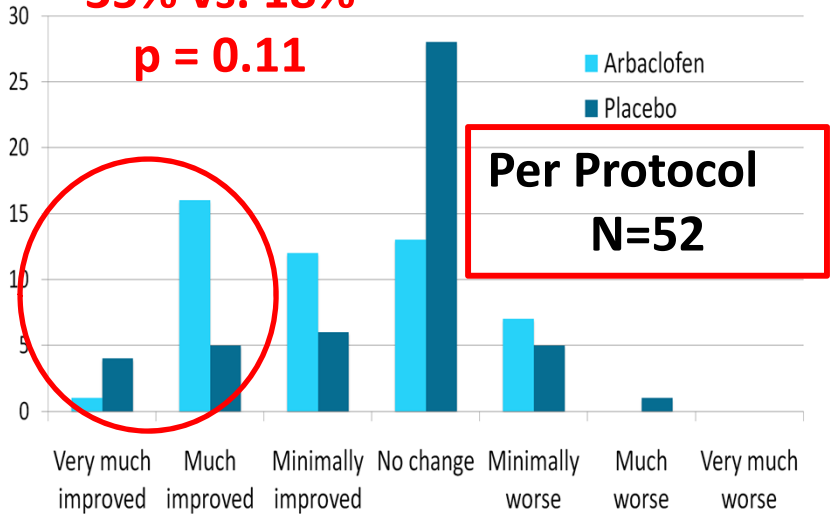
Per Protocol Population (N=52)	STX209 (mean ± SD)	Placebo (mean ± SD)	p-value
ABC-Irritability	-4.2 ± 6.47	-4.5 ± 6.58	ns
CGI-I	3.3 ± 0.93	3.5 ± 0.94	0.181
CGI-S	-0.6 ± 0.86	-0.3 ± 0.87	< 0.10
Treatment preference (clinician)	57%	28%	< 0.10
Treatment preference (parent)	59%	33%	< 0.10
Visual analog scales	*-2.2 ± 2.24*	*-1.2 ± 2.36*	*< 0.05*

22001: ABC-Social Avoidance Analysis

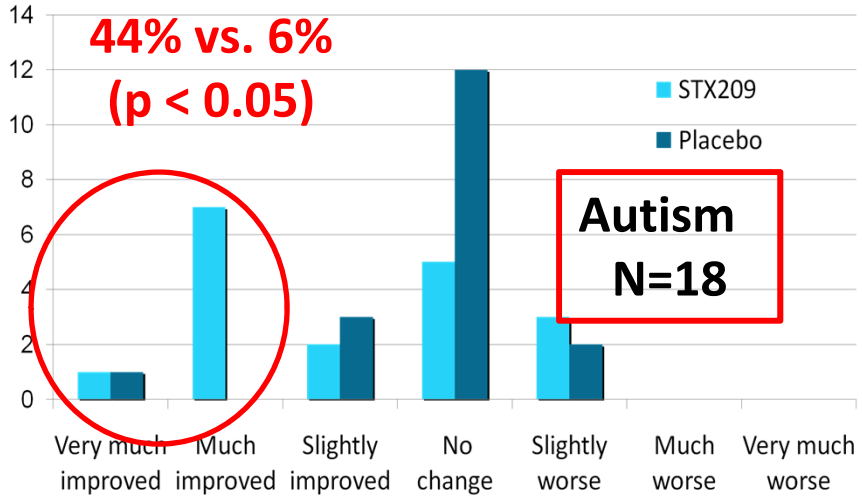
- Aberrant Behavior Checklist originally validated in MR/ID population
  - 5 factors, including “Lethargy/Social Withdrawal”
- New validation study in FXS population – 6 factors
  - Sansone et al. 2012 JADD
  - >600 subjects (MIND, Stanford, KKI/Hopkins, Rush, Duke)
  - New FXS-specific factor: “Social Avoidance”
- Study 22001 analysis using ABC-Social Avoidance scale
  - ITT population
  - STX209: -1.2 ± 2.37 (mean ± SD)
  - Placebo: -0.1 ± 2.53
  - p-value < 0.01

CGI-I (Improvement) Results in Arbaclofen (STX209) Trial

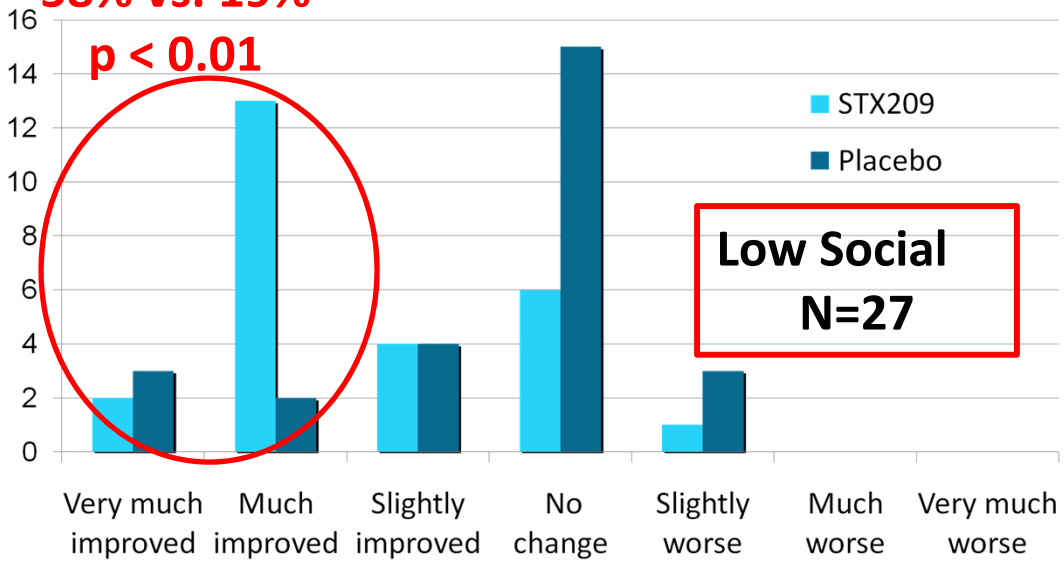
"Responders"
35% vs. 18%
p = 0.11



44% vs. 6%
(p < 0.05)



58% vs. 19%
p < 0.01



Efficacy scores: "Lower sociability" Subgroup

	STX209 n=27 (mean ± SD)	Placebo n=27 (mean ± SD)	p-value
CGI-I	2.7 ± 1.1	3.5 ± 1.2	< 0.01
CGI-S	-1.0 ± 1.1	-0.3 ± 0.9	= 0.01
Treatment preference (clinician)	63%	19%	< 0.01
Treatment preference (parent)	67%	19%	= 0.001
ABC-Social Withdrawal	-4.3 ± 6.3	-0.4 ± 7.1	< 0.05
Vineland Socialization domain (raw score)	14.2 ± 19.0	4.6 ± 10.8	< 0.05
Responders (CGI-I =1 or 2, and ABC-SW improvement ≥ 25%)	42%	7%	< 0.01

Next trials now in progress designed based on 22001 results

Current Trials STX209 Arbaclofen (Seaside)
GABA-B (Harbor Trials)

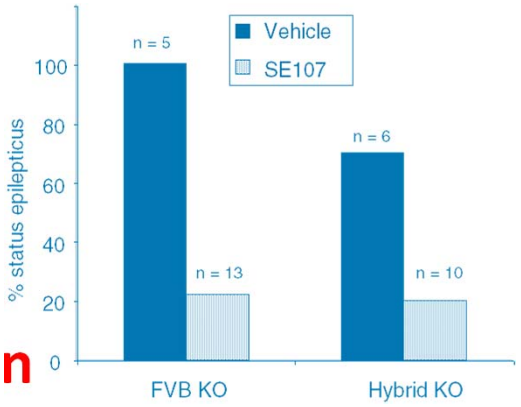
- Target social withdrawal/anxiety – measure with scale to qualify for entry
- "301" Placebo-controlled trial for adolescents and adults ages 12-50
  - 50% chance placebo, 50% chance arbaclofen
  - flexible dose titrated to best dose for 4 weeks then 4 weeks on "best dose" then wean off (total treatment period 8 weeks + wean)
  - 5-6 visits over 12-15 weeks, 5 blood draws, 3 EKGs, questionnaires
  - Up to 3 other meds allowed but NO SSRIs (Prosac, Zoloft, Celexa...)
- "302" Placebo-controlled trial for kids ages 5-11
  - 25% placebo, 25% each low, med, high dose arbaclofen
  - fixed dose – titrate up to set dose, 4 or more weeks on assigned dose, then wean off (total treatment period 8 weeks + wean)
  - 5-6 visits over 12-15 weeks, 5 blood draws, 3 EKGs, questionnaires
  - Up to 3 other meds allowed but NO SSRIs
- "303" Extension that everyone can join after the placebo-controlled trial to go on treatment with arbaclofen; medication titrated like in clinic to best effect; visits after 1 month, 2 months, every 3 months; 2 blood tests/yr

mGluR5 Negative Allosteric Modulators ("Blockers")
AFQ056, RO4917523

Mechanism 1A: mGluR5 Blockers in FXS Models Reverse Phenotypes at all Ages

Mouse

- Audiogenic seizures
- Epileptiform bursts
- Open field behavior
- Dendritic spine shape
- AMPA receptor internalization
- Excessive LTD
- Excess protein synthesis
- Behavioral phenotypes



Yan et al. Neuropharmacology 2005

Chuang et al. J Neurosci 2005

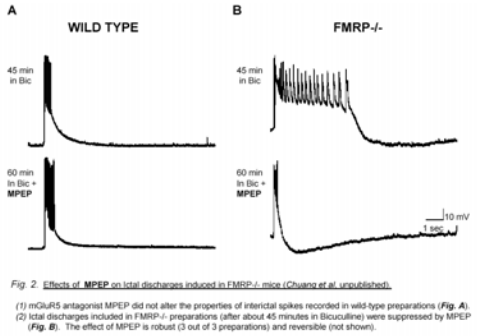


Fig. 2. Effects of **MPEP** on ictal discharges induced in FMRP-/- mice (*Chuang et al.* unpublished).

(1) mGluR5 antagonist MPEP did not alter the properties of interictal spikes recorded in wild-type preparations (**Fig. A**).
(2) Ictal discharges included in FMRP-/- preparations (after about 45 minutes in Bicuculline) were suppressed by MPEP (**Fig. B**). The effect of MPEP is robust (3 out of 3 preparations) and reversible (not shown).

Many phenotypes in the FXS fly (eg. courtship and odor-shock memory) reversed by mGluR5 blockers

Phenotypes in the fmr1 K/O mouse also all reversed by crossing fmr1 K/O to mGluR5 heterozygous mutant (half the mGluR5 receptors)

Mechanism 1A: mGluR5 Blockers New Study April 2012

Collaboration between Roche and Mark Bear lab

17 week chronic treatment with mGluR5 blocker CTEP in ADULT K/O mouse reverses:

- learning and memory deficits
- sensory hypersensitivity
- hyperactivity
- audiogenic seizures
- dendritic spine shape
- exaggerated LTD
- ERK/mTOR signaling abnormalities
- macroorchidism

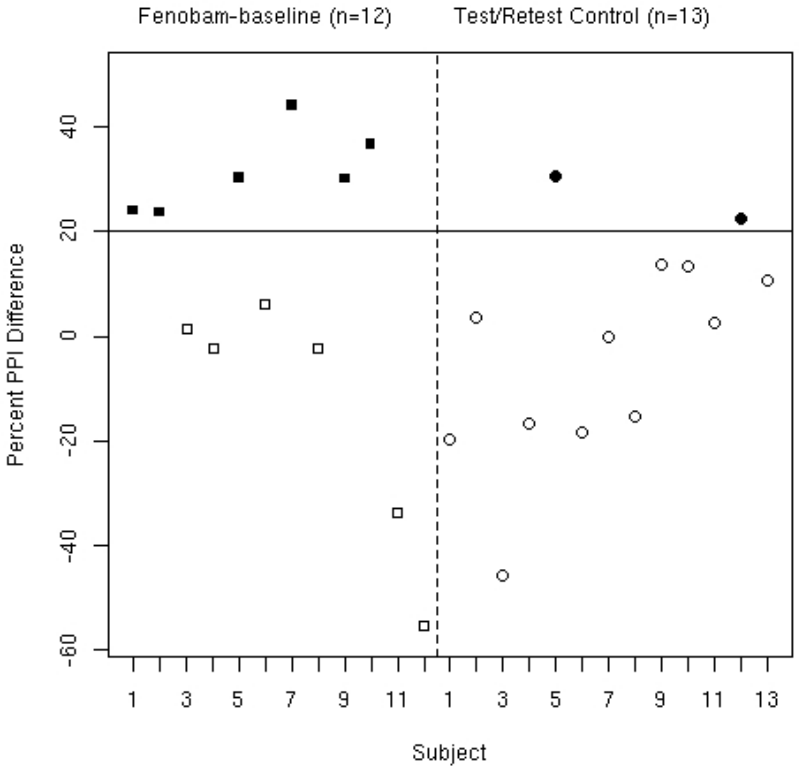
Michalon et al. Neuron 2012

Trials of mGluR5 Blockers in FXS: Fenobam

RUSH and UC Davis (Neuropharm and FRAXA) safety trial of 1 dose (50-150 mg), 12 adult FXS (6M, 6F),age 18-38, IQ 36-85

- **PPI improved 20% in 6/12 subjects (control test-retest group 2/13, p=0.03)**
- **Positive behavioral changes in 9/12 subjects**
- **No fenobam-related AEs**
- **Erratic PK**

Berry-Kravis et al. JMG 2009



Trials of mGluR5 Blockers in FXS: AFQ056 - Results of First Multiple-Dose Trial

Figure 2. The response of individual patients to AFQ056 and placebo treatment on the ABC-C at Day 19/20, grouped by methylation status at the *FMR1* promoter.

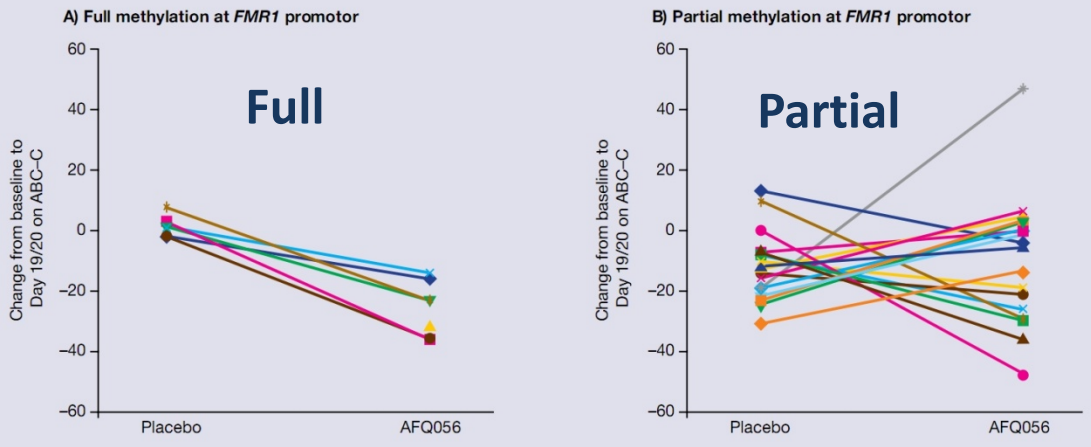


Table 2. Treatment difference* on ABC-C between AFQ056 and placebo from baseline to Day 19/20 by *FMR1* promoter methylation status.

	Subpopulation with full methylation at *FMR1* promoter (n = 7) — Full		Subpopulation with partial methylation at *FMR1* promoter (n = 18) — Partial	
	Difference* (90% CI)	P-value	Difference* (90% CI)	P-value
ABC-C	−27.82 (−39.05, −16.59)	< 0.001	3.10 (−5.61, 11.82)	0.554
CGI-I	−1.78 (−2.34, −1.22)	< 0.001	0.58 (0.04, 1.11)	0.079
CGI efficacy index	1.76 (1.13, 2.39)	< 0.001	−0.43 (−0.96, 0.11)	0.193
RBS-R	−9.81 (−16.57, −3.05)	0.038	−0.81 (−5.06, 3.43)	0.747
SRS	−17.91 (−30.04, −5.77)	0.031	3.22 (−6.54, 12.99)	0.582
VAS	31.84 (14.01, 49.67)	0.006	−4.15 (−16.73, 8.44)	0.584
VABS	2.02 (−16.84, 20.88)	0.769	2.03 (−1.52, 5.58)	0.333

AFQ056 mgluR5 blocker most effective in subgroup of fully methylated subjects in small exploratory trial – methylation status may define treatment response to targeted treatments

These results prompted larger ongoing trials

Jacquemont et al. Sci Trans Med 2010

RUSH UNIVERSITY MEDICAL CENTER

AFQ056 (Novartis) mGluR5 "Blocker"

- Targets global behavior – measure on scales to qualify for entry – need significant behavioral issues

- "2212" Placebo-controlled trial adults age 18-45
  - 25% chance placebo, 25% each low, med, high dose AFQ
  - fixed dose – titrate to set dose, 2 mo on assigned dose (total treatment 4 mo)
  - 8 visits over 4-5 mo, 4 blood draws, 6 EKGs, thinking tests, questionnaires
  - Up to 2 other meds allowed, have to meet methylation criteria, subnormal IQ

- "2214" Placebo-controlled trial adolescents age 12-17
  - 25% chance placebo, 25% each low, med, high dose AFQ
  - fixed dose – titrate to set dose, 2 mo on assigned dose (total treatment 4 mo)
  - 8 visits over 4-5 mo, 4 blood draws, 6 EKGs, thinking tests, questionnaires
  - Up to 2 other meds allowed, have to meet methylation criteria, subnormal IQ

AFQ056 (Novartis) mGluR5 “Blocker”

- “2279” Extension
  adults can join at last visit of placebo-controlled trial to go on treatment with AFQ;
  AFQ titrated to best effect;
  visits 2, 4 wk, 2, 3 mo, every 3 mo to 2+ yrs, 4 blood draws

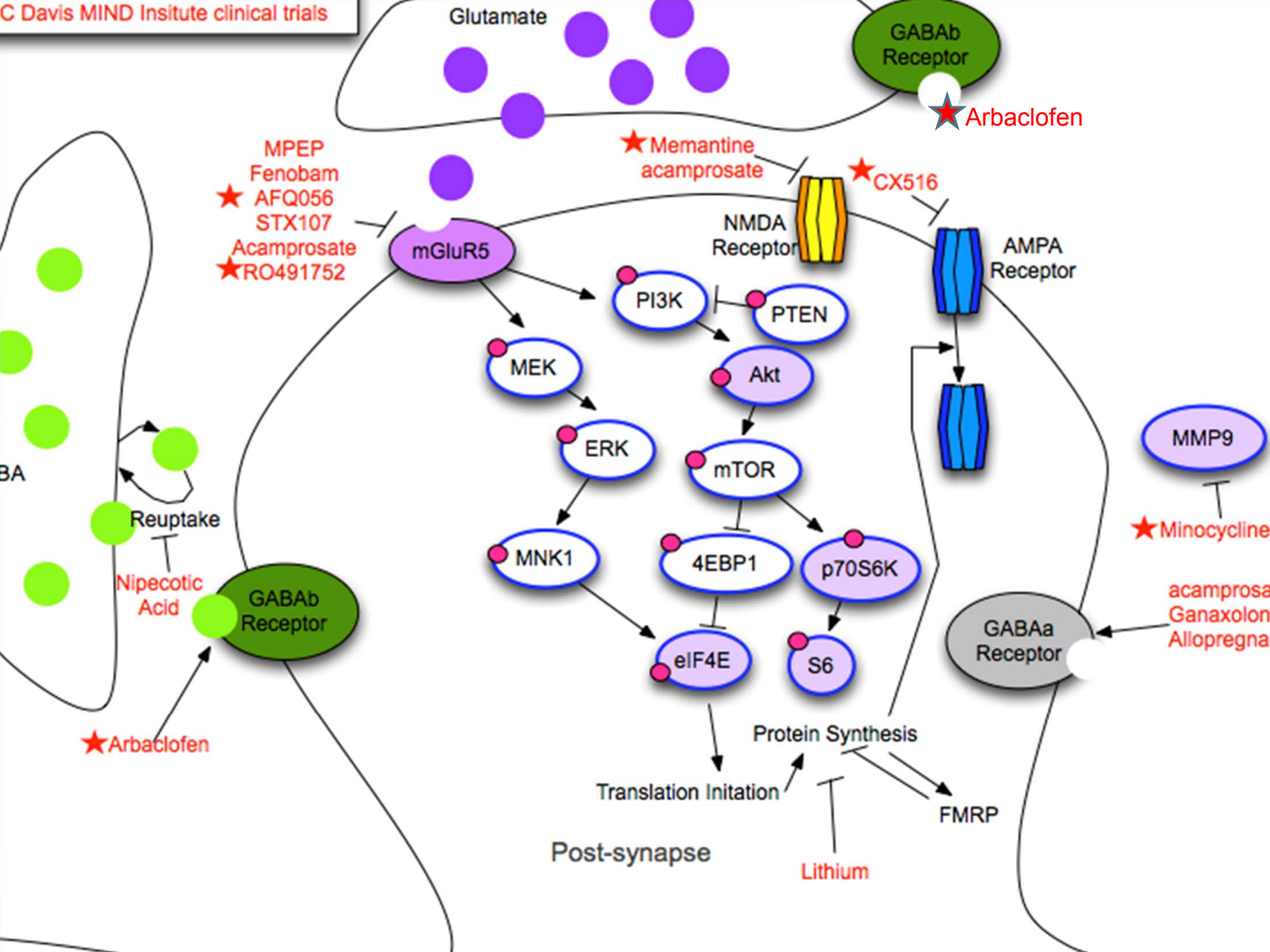
- “2278” Extension
  adolescents can join at last visit of placebo-controlled trial to go on treatment with AFQ;
  AFQ titrated to best effect;
  visits 2, 4 wk, 2, 3 mo, every 3 mo to 2+ yrs, 4 blood draws

AFQ056 (Novartis) mGluR5 "Blocker"

- "2154" Child PK Study, kids age 5-11
  - To evaluate safety and blood levels
  - No IQ, behavior, methylation criteria for entry
  - Period 1 single dose followed by all day PK (blood draws), then Period 2 a week of treatment with all day PK at end
  - 4 visits: screen, 3-day visit (2 overnights), two 2-day visits (1 overnight each although can stay all week)
  - 6-7 days of blood tests – IV when multiple, several EKGs, thinking tests, questionnaires
  - PK participants have option to go into extension directly without doing placebo-controlled study if/when child study opened
  - May be 3-4 year old study when 5-11 done

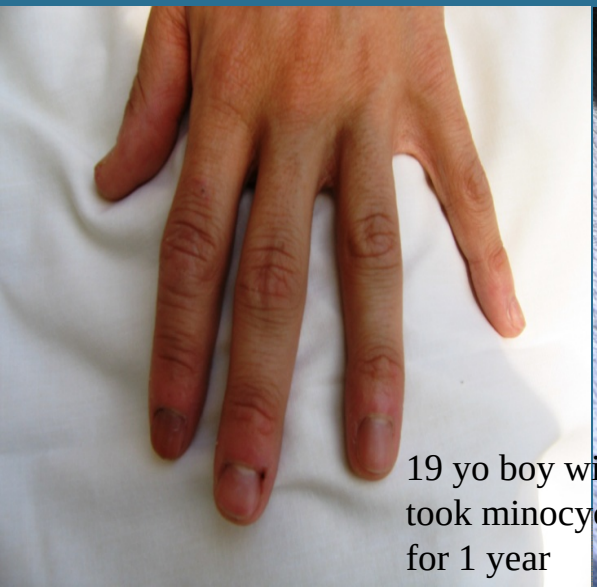
RO4917523 (Roche) mGluR5 "Blocker"

- Target is anxiety-related behavior - measure on scales to qualify for entry – can have mild behavioral issues
- NP27936 – Placebo-controlled trial age 16-50
  - 1/3 chance each placebo, medium dose, high dose
  - Fixed dose, 3 mo on assigned dose (total treatment period 3 mo)
  - 7 visits over about 4 months, 7 blood draws, 4 EKGs, thinking tests, questionnaires
  - No medication restrictions, no IQ or methylation restrictions
- No extension with treatment on drug – but hope is that will occur in future and participants from NP27836 will be able to enroll

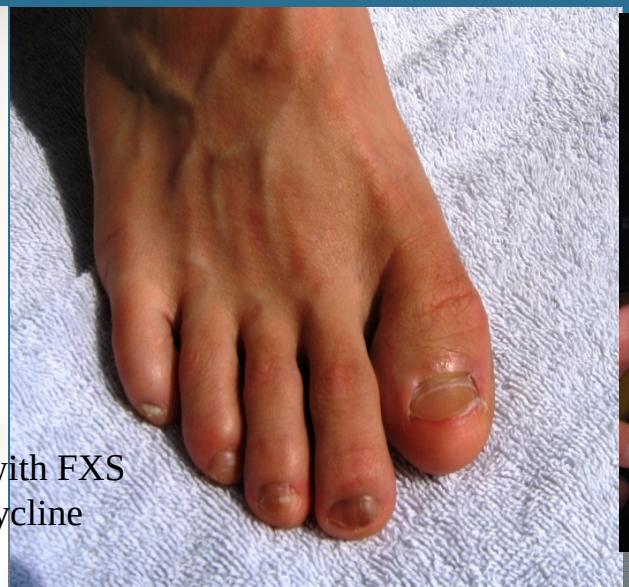

C Davis MIND Insitute clinical trials
Glutamate
GABAb Receptor
Arbaclofen
MPEP
Fenobam
AFQ056
STX107
Acamprosate
RO491752
Memantine
acamprosate
CX516
NMDA Receptor
AMPA Receptor
mGluR5
PI3K
PTEN
MEK
Akt
ERK
mTOR
MMP9
Minocycline
Reuptake
Nipecotic Acid
GABAb Receptor
MNK1
4EBP1
p70S6K
acamprosa
Ganaxolon
Allopregna
GABAa Receptor
eIF4E
S6
Arbaclofen
Protein Synthesis
Translation Initation
FMRP
Post-synapse
Lithium

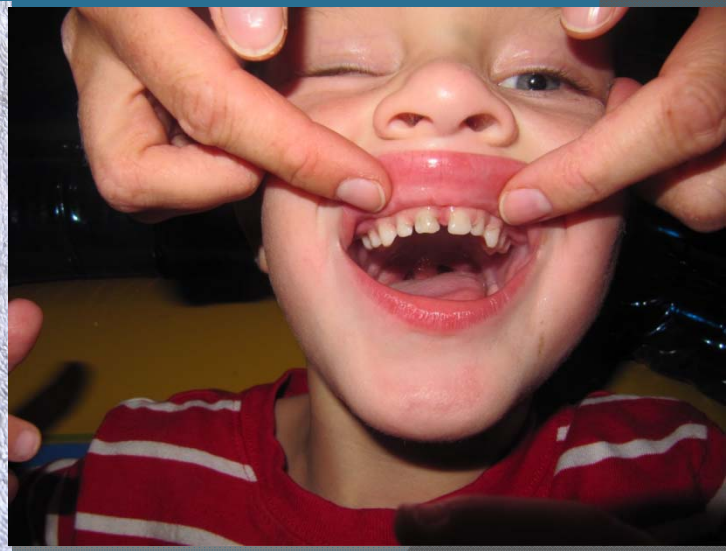
Minocycline Studies in FXS or Autism

- Bilousova et al 2009 demonstrated that minocycline lowers MMP9 levels in FXS and improved behavior and cognition in the FX mouse
- Agustini Utari MD surveyed 50 families whose child was Tx with minocycline for >2wks and found 70% positive response especially in language and limited side effects (Utari et al 2010 AJIDD).
- Positive open trial in FXS in Toronto with age ≥ 13 years (Paribello et al 2010)
- Trial of minocycline in autism at NIMH (Sue Swedo PI)



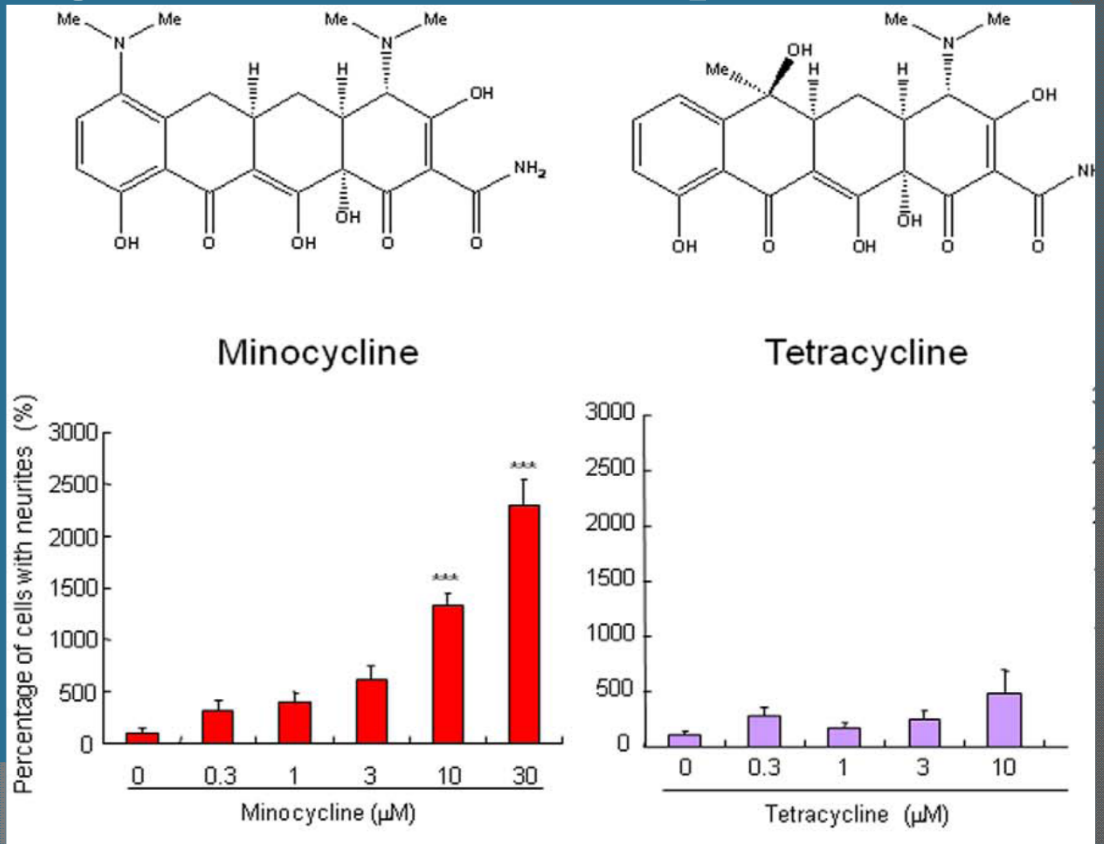
19 yo boy with FXS took minocycline for 1 year



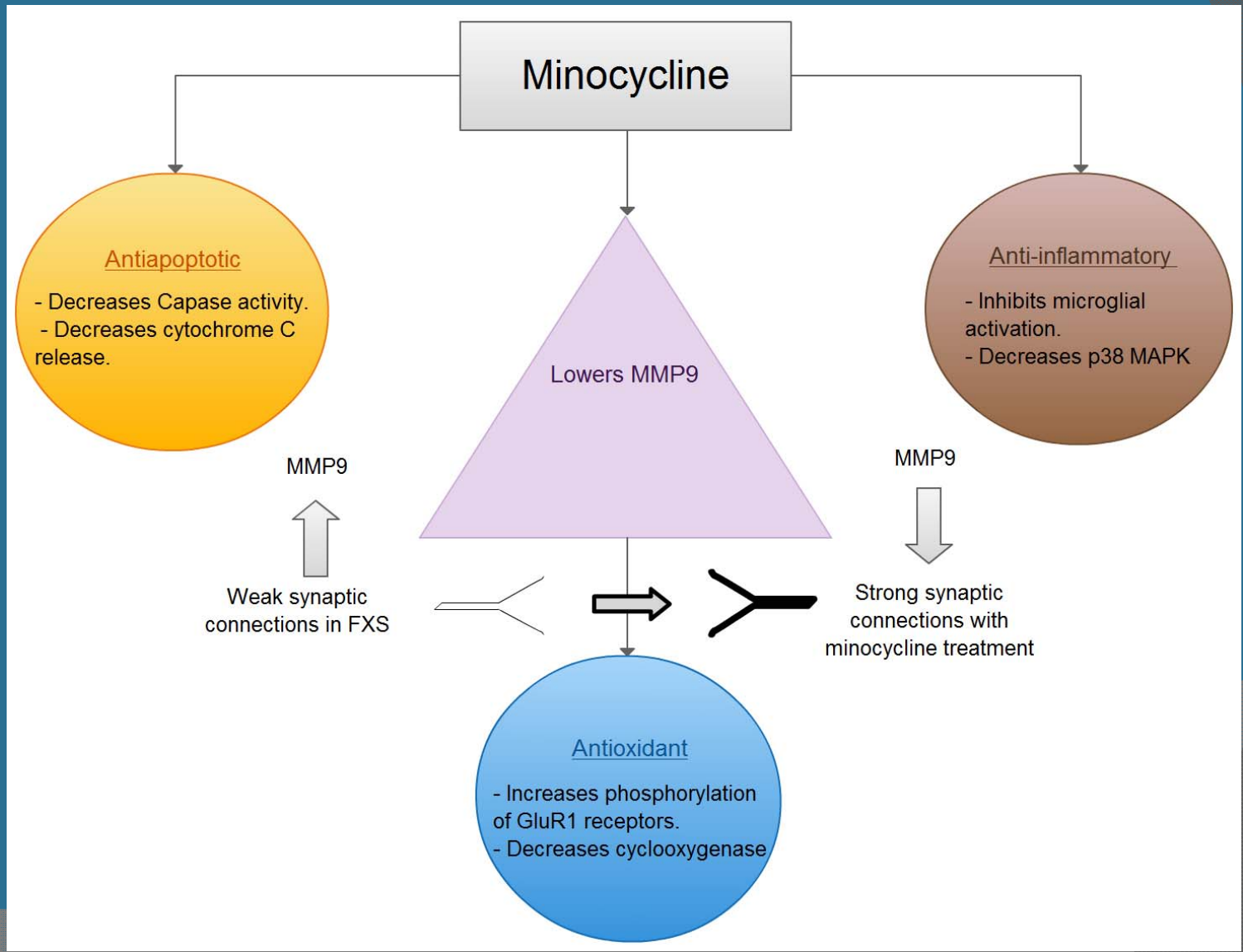


Minocycline Hydrochloride

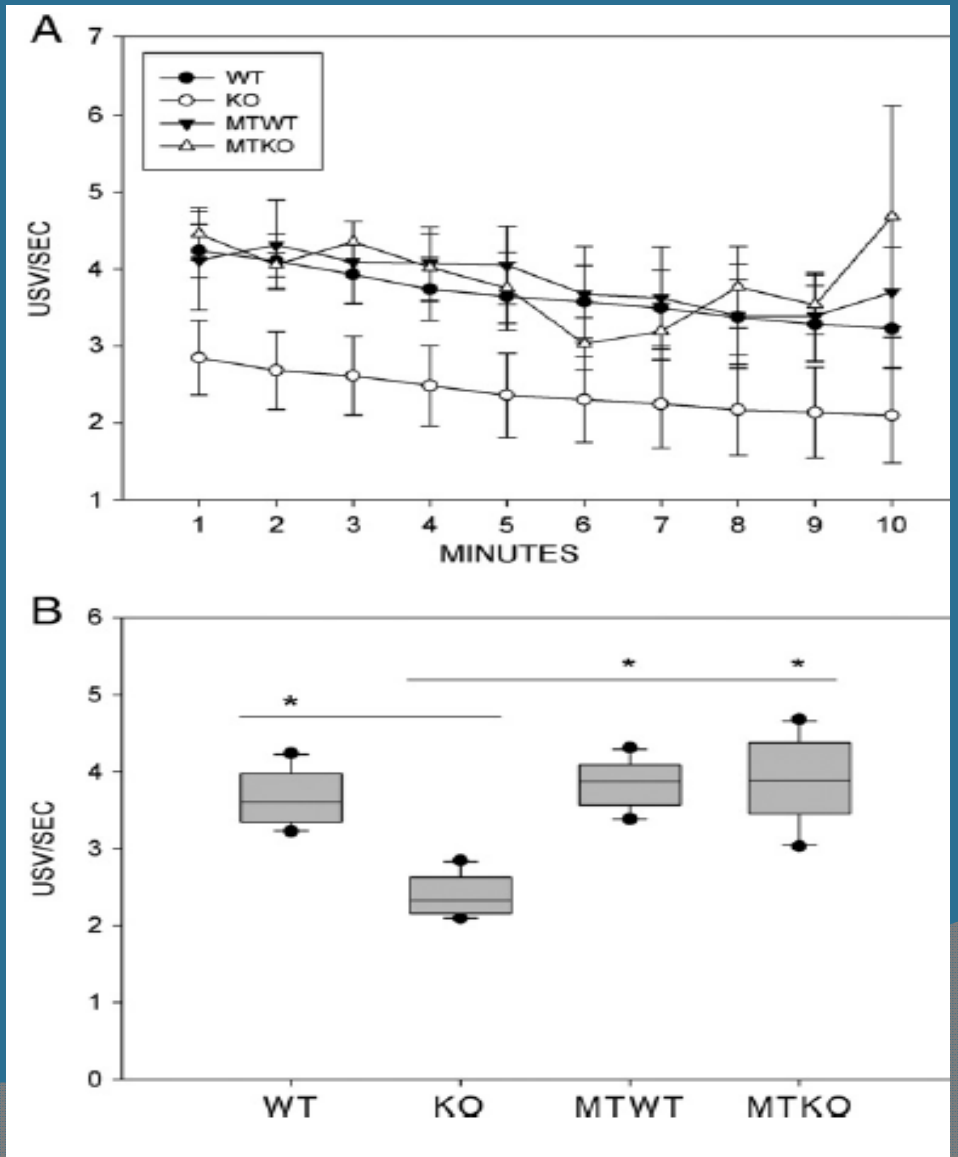
- Semisynthetic tetracycline derivative
- lowers MMP9 and strengthens synaptic connections in FXS
- Commonly used in treatment of acne
- Investigated in Huntington's Disease, multiple sclerosis, ALS
- Enhances neurite outgrowth with NGF treatment



Mechanisms of Action of Minocycline

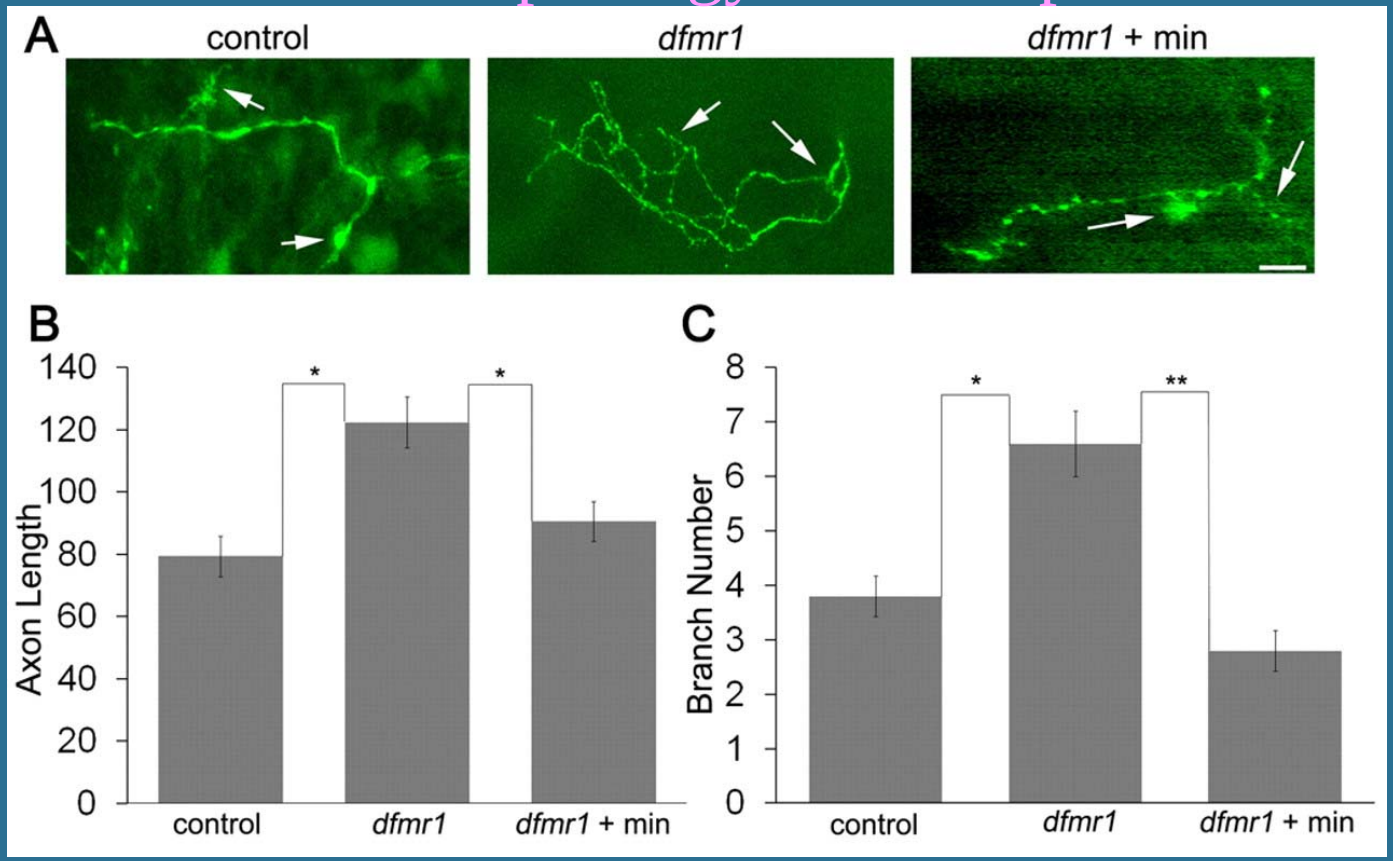


Ultrasonic vocalizations are reduced in KO mouse and minocycline rescues this



Rotschafer et al 2012
Brain Research

Minocycline restores dfmr1 null mushroom body neuron morphology in Drosophila



Also the morphology of neurons in the clock circuit and neurons in the neuromuscular system were rescued by minocycline

Siller S S , Broadie K Dis. Model. Mech. 2011;4:673-685

Minocycline Study Design

- Randomized
- Double blind Placebo controlled trial 3.5-16y
- Crossover : 3 months for each arm
- Voluntary recruitment from UC Davis MIND Institute Fragile X Research and Treatment Center 66 entered 48 completed www.clinicaltrials.gov

Minocycline Dosing

Weight	Minocycline Daily Dose
<25kg	25mg
25-45kg	50mg
>45kg	100mg

Dr Mary Jae Leigh will present the whole trial at the minocycline workshop

Controlled Trial of Minocycline

Visit 1
Cognitive Assessment
Autism Assessment
VABS-II
ABC-C
CGI
EVT-II
VAS
Hx & PE
Con Meds

Visit 2
VABS-II
ABC-C
CGI
EVT-II
VAS
Hx & PE
Con Meds
AEs

Visit 3
VABS-II
ABC-C
CGI
EVT-II
VAS
Hx & PE
Con Meds
AEs

Study Period: January 2010-December 2011

ANA+ Titers is one side effect of minocycline treatment but also + in the general population

Table 2 - Prevalence of ANA+ of the volunteer blood donors included in the study, according to different titers (n=500).

ANA (titer)	N	Prevalence (%)	$CI_{95\%}$ (%)
1:40	73	14.6	11.5 to 17.7
1:80	23	4.6	2.8 to 6.4
1:160	10	2.0	0.8 to 3.2
1:320 or more	7	1.4	0.3 to 2.4
Any titer	113	22.6	18.9 to 26.3

Fernandez SA., AZ Lobo, ZN Oliveira, LM Fukumori, AM Périgo, EA Rivitti. Prevalence of antinuclear autoantibodies in the serum of normal blood donors. *Rev Hosp Clin Med Sao Paulo*. 2003; 58(6):315-9.

Antinuclear Antibodies in FXS Patients Treated with Minocycline (n = 40) (Nuva Rafika will present at this conference)

ANA	Titer	Total	Percentage	Total Percentage
Positive	≥ 1 : 640	2	5%	25%*
	1 : 320	2	5%	
	1 : 160	4	10%	
	1 : 80	2	5%	
Borderline	1 : 40	4		12.5%
Negative		26		62.5%
Total		40		

*ANA positivity did not associate with symptoms

Rafika, N., MJS Leigh, JE Goodrich & RJ Hagerman. Antinuclear Antibodies in Fragile X Syndrome Patients Treated with Minocycline. 2012; Unpublished .

Severe involvement from FXS
Autistic , non verbal, aggressive, would not tolerate clothes
could not go outside

After 2 years on minocycline 200 mg/d

He can talk and dress
He drinks from a cup
He walks with his social worker
Aggression is gone
He can come to clinic
Looks at magazines and TV

Minocycline can be prescribed by physicians and often used for acne

- Use probiotic to avoid loose stools or diarrhea
- Do not give minocycline at the same time as milk; wait 1 hour pre and post minocycline
- Minocycline comes in a 50 mg capsule and half can be mixed with applesauce for dosing kids
- Discontinue minocycline if rash, swollen joints ie lupus like picture or severe headache (pseudotumor cerebri) or cosmetic problems of dark nails or gums
- Check ANA every 6 months and with escalating titers discontinue minocycline

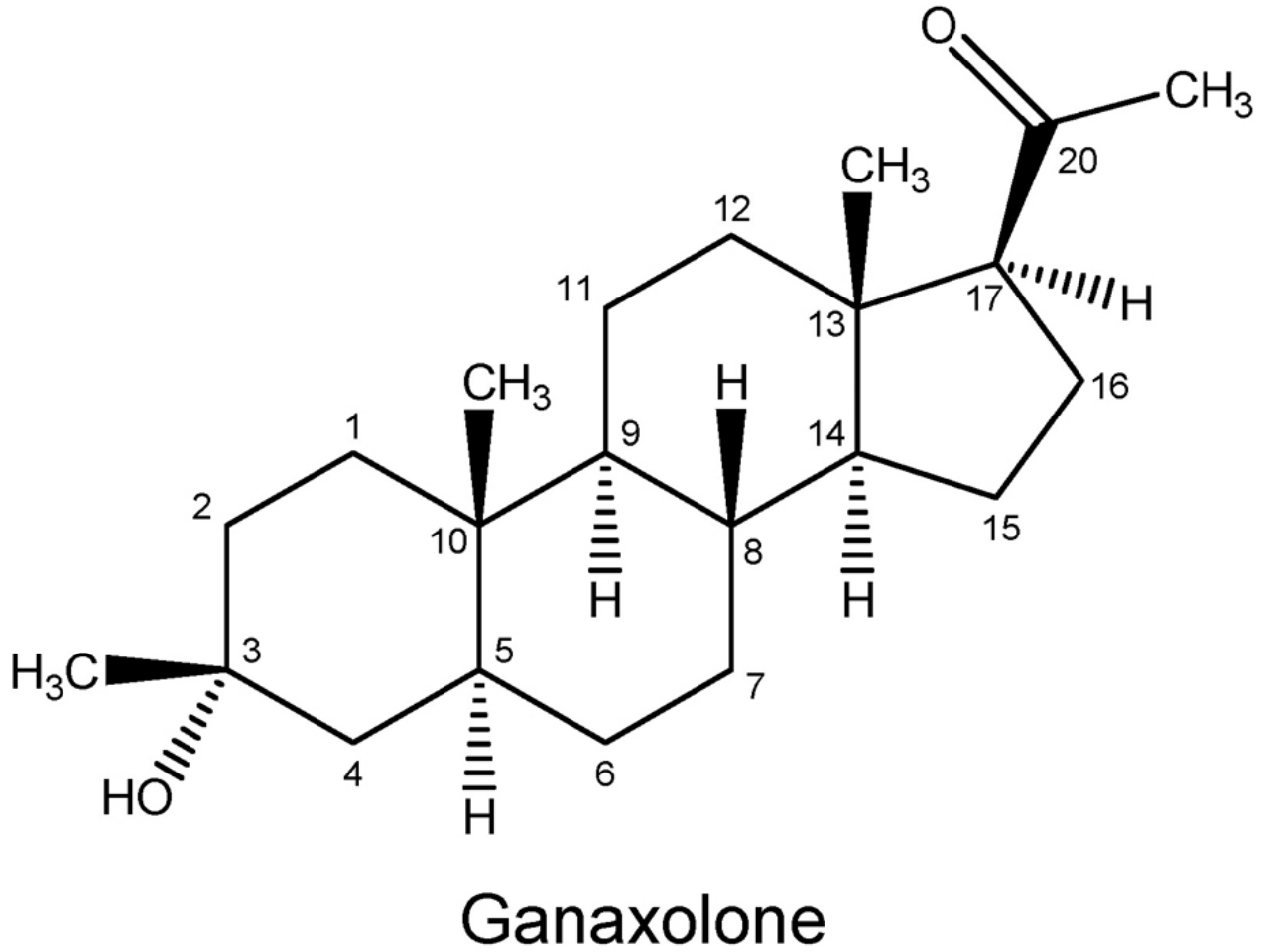
MMP9 effects at the synapse may be additive to other treatments

- Data from Kendal Broadie suggests that lowering MMP9 at the synapse has significant effects on heparin sulfate proteoglycans and other proteins to facilitate the signal across the synapse
- Therefore minocycline's effect may be additive to other targeted treatments in FXS and the mGluR5 protocols allow minocycline's use
- Other targeted treatments may also lower MMP9 levels and there may be additive effects on MMP9 levels

$GABA_A$ receptor expression is down in FXS

- $GABA_A$ receptors and expression are down regulated in the KO mouse (D'Hulst et al 2007; Kooy et al 2005;Heulens et al 2012)
- Treatment with ganaxolone rescues seizures in the KO mouse
- $GABA_A$ agonists: Ganaxolone
  - Investigational neurosteroid with efficacy in infantile spasms and other types of epilepsy: A controlled trial in children with FXS (6 -18y) funded by DOD is taking place at the MIND Institute; Marinus is supplying the ganaxolone
  - Targeting improvement in anxiety, behavior and seizure frequency

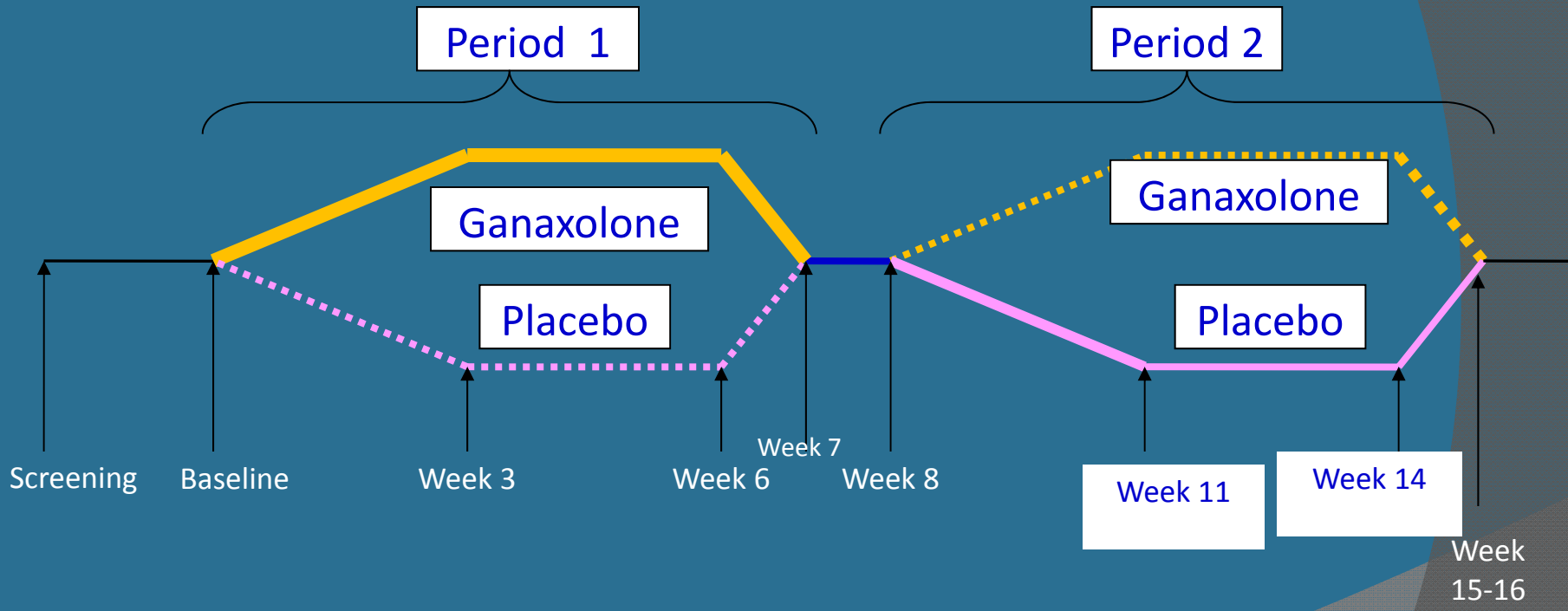
Ganaxolone is a neurosteroid which works as an anticonvulsant and upregulates the GABAA pathways



Ganaxolone

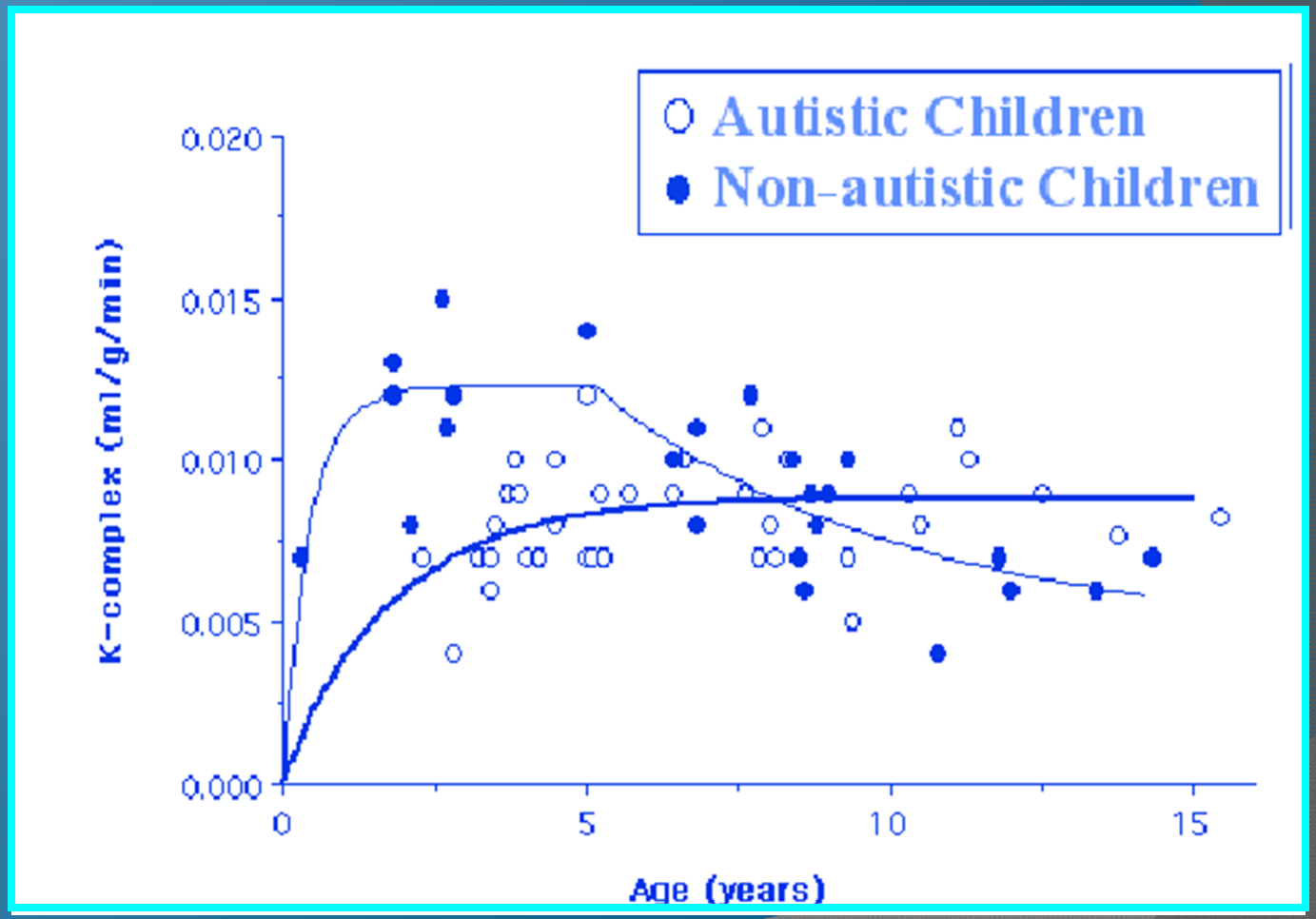
Ganaxolone treatment timeline

doubleblind crossover controlled trial



Yoga and Mindfulness Meditation improves GABA inhibition

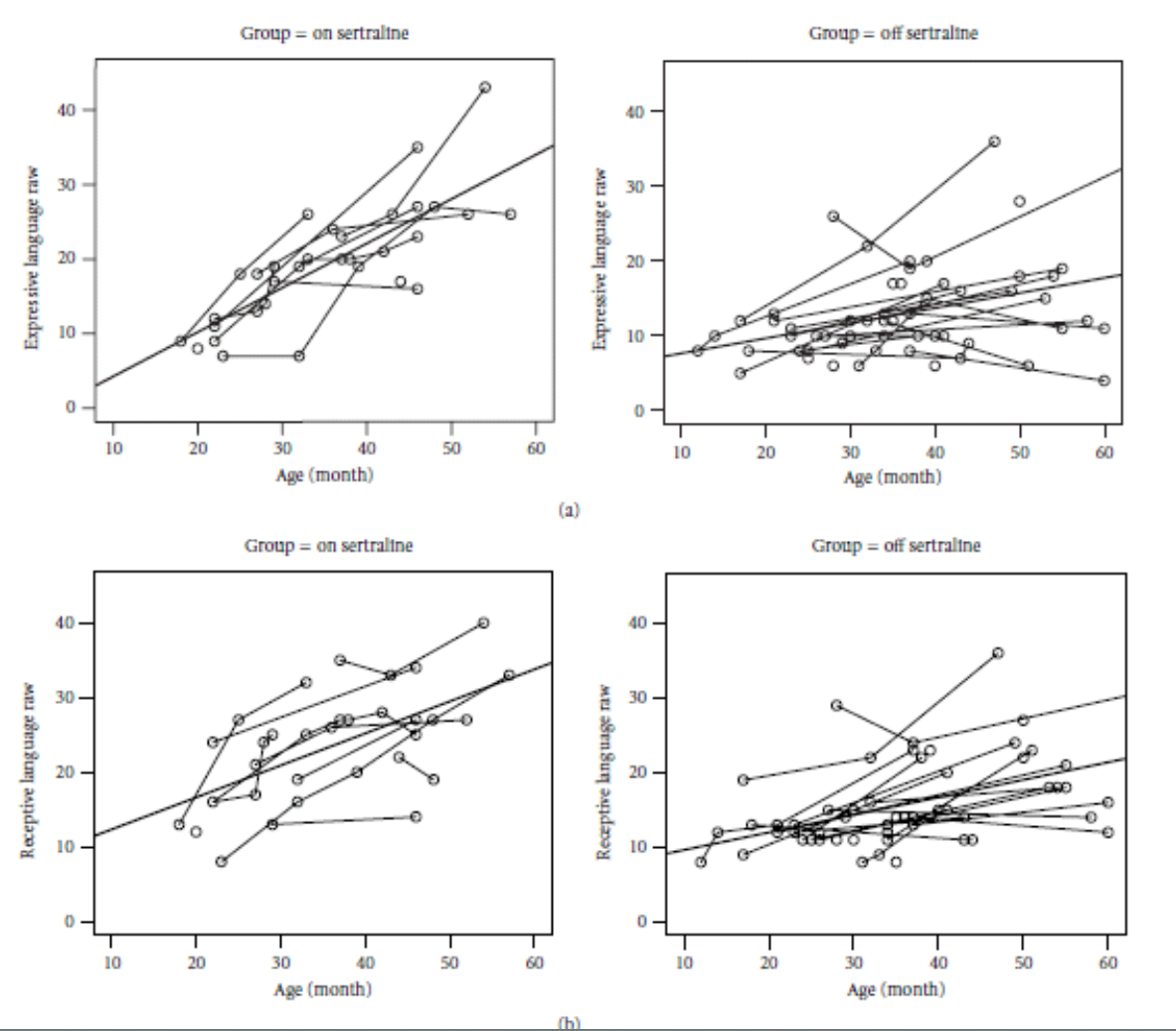
In autism serotonin synthesis is reduced frontally.
This may be true for FXS
since clinically they respond to early sertraline Tx



Chugani et al., 1999

Sertraline Treatment in Early Childhood in FXS

A retrospective study of 45 children followed 12 to 50 months and 11 treated with sertraline: significant differences in expressive and receptive language in TX vs non treated (p=0.0001 and p=0.0071 respectively)



Winarni et al 2012 Autism Treatment and Research

Sertraline is an SSRI with less activation compared to fluoxetine

- SSRIs stimulate neurogenesis in the mouse and in humans (Malberg et al 2004; Warner-Schmidt et al 2006)
- In mouse model of Down syndrome: fluoxetine after birth stimulated neurogenesis, increased BDNF, and enhanced cognition (Bianchi et al 2010)
- Sertraline in FXS has a calming effect to the anxiety and may directly stimulate speech or improve language as a secondary effect from less anxiety

Targeted Treatments must be combined with innovative educational programs

- If synaptic connections are improved with targeted treatment we must enhance these connections with educational interventions
- Combine treatment trials with educational interventions, computer programs such as CogMed, AT devices, iPAD apps.

FX tracking game

iPAD apps

Co-Writer and write out loud

Our goal is to provide interventions to reverse the FXS phenotype leading to normal development and cognition. We are closer than ever!

For More Information

- Clinicaltrials.gov – search “fragile X syndrome”
- NFXF website
- Fraxa.org – clinical trials button
- Fragilex.org podcast – “Latest News”
- Individual clinic coordinators (listed on all 3 sites)