



A pilot open-label single-dose trial of fenobam in adults with fragile X syndrome

Elizabeth M Berry-Kravis, David Hessler, Sarah Coffey, Crystal Hervey, Andrea Schneider, Jennifer Yuhua, Julie Hutchison, Michael Snape, Michael Tranfaglia, Danh V. Nguyen and Randi Hagerman

J. Med. Genet. published online 6 Jan 2009;
doi:10.1136/jmg.2008.063701

Updated information and services can be found at:
<http://jmg.bmj.com/cgi/content/abstract/jmg.2008.063701v1>

These include:

- | | |
|-------------------------------|--|
| Open Access | This article is free to access |
| Rapid responses | You can respond to this article at:
http://jmg.bmj.com/cgi/eletter-submit/jmg.2008.063701v1 |
| Email alerting service | Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article |
-

Notes

Online First contains unedited articles in manuscript form that have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Online First articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Online First articles must include the digital object identifier (DOIs) and date of initial publication.

To order reprints of this article go to:
<http://journals.bmj.com/cgi/reprintform>

To subscribe to *Journal of Medical Genetics* go to:
<http://journals.bmj.com/subscriptions/>

A PILOT OPEN-LABEL SINGLE-DOSE TRIAL OF FENOBAM IN ADULTS WITH FRAGILE X SYNDROME

Elizabeth Berry-Kravis¹, David Hessl^{2,3}, Sarah Coffey^{3,8}, Crystal Hervey⁴, Andrea Schneider^{2,3}, Jennifer Yuhas², Julie Hutchison⁵, Michael Snape⁵, Michael Tranfaglia⁶, Danh V. Nguyen⁷, Randi Hagerman^{3,8}

¹Departments of Pediatrics, Neurological Sciences, Biochemistry, Rush University Medical Center

²Department of Psychiatry and Behavioral Sciences, University of California Davis

³M.I.N.D. Institute, University of California Davis Medical Center

⁴Department of Pediatrics, Rush University Medical Center

⁵Neuropharm LTD

⁶FRAXA Research Foundation

⁷Department of Public Health Sciences, University of California Davis

⁸Department of Pediatrics, University of California Davis Medical Center

Address Correspondence to:

Elizabeth Berry-Kravis MD PhD
Rush University Medical Center
1725 West Harrison Street, Suite 718
Chicago, IL 60612

Key words: fragile X syndrome, fragile X mental retardation protein, fenobam, metabotropic glutamate receptor, prepulse inhibition

Running head title: Fenobam in fragile X syndrome

Word Count: 3260

Abstract

Objective: A pilot open-label, single-dose trial of fenobam, an mGluR5 antagonist, was conducted to provide an initial evaluation of safety and pharmacokinetics in adult males and females with fragile X syndrome (FXS). *Methods:* Twelve subjects, recruited from two Fragile X Clinics, received a single oral dose of 50 to 150 mg of fenobam. Blood for pharmacokinetic (PK) testing, vital signs and side effect screening was obtained at baseline and numerous time points for 6 hours after dosing. Outcome measures included prepulse inhibition (PPI) and a continuous performance test (CPT) obtained before and after dosing to explore the effects of fenobam on core phenotypic measures of sensory gating, attention and inhibition. *Results:* There were no significant adverse reactions to fenobam administration. Pharmacokinetic analysis showed that fenobam levels were dose dependent but variable, with mean peak levels of 39.7 ± 18.4 ng/mL at 180 minutes after the 150 mg dose. PPI met a response criterion of an improvement of at least 20 percent over baseline in 6 of 12 individuals (4/6 males and 2/6 females). The CPT did not display improvement with treatment due to ceiling effects. *Conclusions:* Clinically significant adverse effects were not identified in this study of single-dose fenobam across the range of dosages utilized. The positive effects seen in animal models of FXS treated with fenobam or other mGluR5 antagonists, the apparent lack of clinically significant adverse effects, and the potential beneficial clinical effects seen in this pilot trial support further study of the compound in adults with FXS.

Introduction

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability, autism, and learning disability, with a broad range of severity and full mutation gene frequency of 1/2500[1]. FXS results from an unstable trinucleotide repeat expansion of >200 CGG repeats (full mutation) in the promoter of the *FMR1* (Fragile X Mental Retardation-1) gene[2] which leads to transcriptional silencing of *FMR1* and thus, absence or significant reduction of the *FMR1* Protein (FMRP)[3]. Because *FMR1* is located on the X chromosome, females with a full mutation are more mildly affected than males, due to production of FMRP from the normal *FMR1* allele on the non-mutated X chromosome. FMRP is an RNA binding protein which modulates dendritic maturation and synaptic plasticity through mechanisms including inhibition of group 1 metabotropic glutamate receptor (mGluR1 and mGluR5)-mediated mRNA translation in dendrites[4, 7]. Numerous expected consequences of excessive activation of mGluR-mediated dendritic protein synthesis due to loss of inhibitory control by FMRP are found in the *fmr1* knockout mouse, including enhanced mGluR-activated hippocampal[8] and cerebellar[9] long-term depression (LTD), reduction of synaptic AMPA receptors[10], immature-appearing elongated dendritic processes[11,12] and abnormal epileptiform discharges[13]. Further, many phenotypic features of FXS are predicted effects that would occur in a setting of enhancement of mGluR-mediated processes, including seizures, epileptic abnormalities on EEGs, cognitive problems, strabismus, enhanced anxiety, perseverative behaviors, coordination problems, hypersensitivity to tactile stimuli and even loose stools[10].

Consistent with this underlying mechanism of mGluR overactivity in FXS, MPEP (2-Methyl-6-(Phenylethynyl)-Pyridine) and other mGluR negative modulators have been shown to reverse multiple phenotypes in the *fmr1* knockout mouse including audiogenic seizures, epileptiform discharges and open field hyperactivity[13,14] as well as impairments in courtship memory in *dfxr* mutant *Drosophila*[15] models. Genetic down regulation of mGluR5 expression by crossing the *fmr1* KO mouse with mGluR5 heterozygous knockouts[16] also reverses these and other phenotypes including dendritic spine changes, ocular dominance plasticity, and excessive protein synthesis.

Although mGluR5 negative modulators are not currently available for treatment of humans with FXS, during recent high throughput lead-finding screens, Porter *et al.*[17], discovered that fenobam is a high potency and highly selective mGluR5 antagonist, comparable to MPEP, with no relevant effects on a panel of 86 CNS receptors assayed in a commercial receptor-binding screen, including other mGluRs. Fenobam was previously investigated as an anxiolytic in a number of Phase II studies in the early 1980s[18-20]. These studies revealed a mixed picture of anxiolytic efficacy, with double-blind, placebo-controlled trials variously reporting the compound as active or inactive. This discrepancy was not easily reconciled based on patient numbers, dose level, duration of treatment, or outcome measures. There were no major safety concerns although a number of subjects taking doses of 150 mg QID (four times daily) of fenobam for up to 4 weeks did describe odd CNS-related perceptual phenomena, such as hallucinations, vertigo, paraesthesias, and insomnia.

Given the likelihood that fenobam would target a specific underlying mechanism of neural dysfunction in FXS, we initiated an open-label single-dose study to provide an initial evaluation of the safety and pharmacokinetics of fenobam in adult males and females with FXS and to explore effects of fenobam on core phenotypic measures of sensory gating, attention and inhibition in FXS. The CNS side effects previously reported in clinical studies with fenobam have the potential to be subjectively distressing. FXS represents a vulnerable patient population with impaired understanding and communication skills. Negative modulators of the mGluR5 receptor are capable of producing morphological and behavioral changes after a single dose[21, 22]. Accordingly we felt it most appropriate to examine the effects of fenobam in patients with FXS with a single dose in this first clinical trial of an mGluR5 antagonist.

Methods

Subjects were recruited from Fragile X Clinics at Rush University Medical Center (RUMC) and the MIND Institute at University of California at Davis Medical Center (UCDMC). Inclusion criteria required a DNA-based diagnosis of FXS, stable medication doses for at least 6 weeks prior to study, and ability to tolerate an intravenous catheter (IV) for 6 hours for PK studies. Exclusion criteria included concurrent treatment with lithium, typical antipsychotics, tricyclic antidepressants, NMDA antagonists, or enzyme-inducing anticonvulsants, concurrent or recent initiation of cognitive behavioral therapy, significant disease in another organ system, hearing or vision impairments,

psychosis, major depressive symptoms, pregnancy, drug abuse disorder, Tourette syndrome, and significant abnormalities in baseline laboratory tests. Subjects with well-controlled seizures were not excluded although none of the subjects enrolled had a seizure history. Informed written consent was obtained from either the subject or the parent prior to participation. Assent from the subject was obtained when the subject was not his/her own legal guardian. The study was approved by the Institutional Review Boards at RUMC and UCDCM. The sequence of subject enrollment and treatment was random, depending only on when subjects contacted the study center and when they could be scheduled.

At the Screening Visit medical history, exam, vital signs, laboratory testing including routine chemistries, blood counts, thyroid functions, EKG, and a pregnancy test (females) were evaluated, and baseline CPT (Carolina Project Fragile X continuous performance test) and prepulse inhibition (PPI) outcome measures were obtained. At the Treatment Visit, 14-28 days after screening, an IV was inserted for blood drawing and the subject received the study medication orally. Blood for pharmacokinetic (PK) testing, blood pressure, heart rate and side effect screening was obtained at 0, 15, 30, 45, 60, 120, 180, 240, 300, and 360 minutes after dosing. Our side effects screening protocol involved asking both the subject and family members if the subject was having any of a structured list of symptoms relevant to potential effects of fenobam (aggression, fatigue, hyperactivity, anxiety and fidgetiness, increase in self-stimulation, odd behavior, inappropriate laughter, dizziness, vertigo, nausea, paraesthesia, and headache), and direct observation by the physician and family for the above symptoms and other behavioral changes. Subjects were all verbal and sufficiently high functioning to report side effects in response to simple questions. Post-treatment PPI was performed after the 60 min blood sampling, followed by CPT. The first subject for each gender was dosed with 50 mg fenobam, the second with 100 mg, and all subsequent subjects with 150 mg. The final dosage of 150 mg fenobam was at the middle range of individual doses in previous studies where CNS side effects were observed. Conference calls were held after each subject was dosed, to confirm absence of adverse events and justify dose escalation or maintenance for the subsequent subject of that gender.

PPI was chosen as an outcome measure to assess sensorimotor gating and inhibitory control because: a) there is significant deficit of PPI in males and females with FXS[23,24], b) PPI at 120 ms has excellent test-retest reliability with intraclass correlations of 0.85 for FXS and 0.88 for controls[24], c) PPI is responsive to medication effects[25,26] d) PPI in the mouse model of FXS can be corrected with MPEP[21], and e) PPI represents an electrophysiological measure that is expected to be less amenable to placebo effects than other measures. The PPI protocol used for this study was a slightly modified version of the procedure previously described[24]. Startle stimuli (SS) were 50 ms 105 db SPL white noise pulses and acoustic prepulses (PP) are 25 ms 75 db SPL 1 kHz tones. These trials are delivered while participants watch a silent movie to maintain compliance and interest in the procedure. Test-retest studies have demonstrated good to excellent reliability for PPI at 120 ms and 240 ms, but inadequate reliability for PPI at 60 ms. Therefore, for the current study, we eliminated the 60 ms trial type and added two trials per type, resulting in a protocol with 30 total trials (10 with SS alone, 10 with 120 ms prepulse and 10 with 240 ms prepulse). Digitized obicularis oculi electromyographic peak magnitudes recorded between 20 ms and 200 ms post startle probe onset were averaged across trials within each type. PPI was calculated as: $100 \times [(\text{mean response magnitude in the startle stimulus alone trials} - \text{mean response magnitude in the prepulse trials}) / \text{mean response magnitude in the startle stimulus alone trials}]$. Based on group differences obtained in our prior study and review of the literature on PPI reliability and its response to psychopharmacological intervention, we determined a priori that subjects with PPI improvement of 20% on the 120ms trials (the most reliable trial latency), or more would be considered responders.

The Carolina Fragile X Project Continuous Performance Test (FXCPT), developed for individuals with cognitive impairment, was chosen to assess attention, impulsivity, and inhibition, as other more standard CPT measures are too difficult for individuals with FXS[27]. The FXCPT showed a significant deficit in response inhibition in males with FXS, compared to mental age matched controls[28] and in a test-retest reliability study, had a weighted kappa of 0.7, suggesting a good reproducibility[29]. The FXCPT was administered as previously described[28,29] and the number of correct hits on a target stimuli, omissions involving lack of reaction to a target (attention deficit) and commissions involving reacting to a non-target (impulsivity, poor inhibitory control) were tabulated. Individuals with FXS often hit the mouse button repeatedly and impulsively, thus achieving a perfect

number of hits with low omission scores, but a poor overall performance due to a high number of commission errors[29]. Thus, the commission score was the main focus of the analysis, as the more reliable marker for abnormal performance on the FXCPT.

Fenobam levels from plasma samples were measured with MS-MS based assays validated for human application, using positive ion liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). For comparison, plasma samples over 24 hours were obtained from three healthy adult male volunteers.

For statistical analysis of PPI data, a positive response was defined as a 20% or greater improvement over baseline. One-sample exact binomial 95% confidence interval was computed for the proportion of positive response. A one-sample two-sided exact hypothesis test was used to compare the proportion of positive response to the null proportion of 2/13 (15.4%), obtained from our prior PPI study with untreated individuals with FXS[24].

Results

Six males and six females with FXS (3 males and 3 females at each site; mean age; 23.9 ± 5.4 years, range 18.7-30.7 years) were screened and enrolled. There was wide variability in cognitive ability, with IQs ranging from 36-85, as would be expected in a study enrolling both males and females with FXS. No subjects dropped out or failed to meet entry criteria. There were no clinically significant abnormalities of the examination, vital signs, or laboratory or EKG parameters at baseline. Demographic data are given in Table 1.

There were no significant adverse reactions to fenobam. Particularly, adverse events (listed in Methods) in relation to altered CNS function were not seen. Three subjects experienced mild sedation (Table 1). In 9 of the 12 cases calmed behavior was observed with improvement in eye contact, ability to interact, anxiety and/or motor over-activity (Table 1).

PPI for the 120 ms trials met response criterion of at least 20% improvement over baseline in 6 of 12 individuals (4 of 6 males and 2 of 6 females, Table 1) with 95% confidence interval of 21.1% to 78.9%. Improvements ranged from 23.7% to 44.2%(Figure 1). The proportion of positive response (at least 20% improvement after fenobam) was significantly greater than that expected in untreated individuals ($p=0.01$), which was found to be 15.4% (2/13) based on a prior study[24] consisting of a similar cohort of individuals with FXS who underwent test-retest on the PPI utilizing the same methodology in the absence of intervention (Figure 1).

As expected, most subjects (10 on auditory FXCPT, 9 on visual FXCPT) had a 100% rate of hits at baseline. The rate of commissions was also low, with only one subject on auditory FXCPT and one on the auditory and visual FXCPT showing more than 2 commission errors at baseline. Thus, there were no significant changes between baseline and treatment in FXCPT performance due to ceiling effects. However, the most impaired subject on this task did show a decrease in commissions (from 18 to 12 on visual FXCPT and 26 to 6 on auditory FXCPT).

Pharmacokinetic analysis in subjects with FXS showed that fenobam levels were dose dependent but variable, with mean peak levels following administration of 150 mg of fenobam being 39.7 ± 18.4 ng/mL at 180 minutes post dose. The mean peak plasma level of fenobam did not differ from that seen in normal volunteers ($n=3$). Upon visual inspection the timing to mean peak levels was similar in fragile X syndrome patients and in normal controls(Figure 2). Neither fenobam dose nor levels after 60 or 120 minutes correlated with improvement in PPI.

TABLE 1
CHARACTERISTICS AND RESPONSES OF FXS SUBJECTS TREATED WITH SINGLE-DOSE FENOBAM

Study ¹	Gender	Ethnicity	IQ	Concomitant Medications ²	Fenobam Dose (mg)	Side Effects ⁸	PPI ⁹	Type of Improvement noted clinically ⁸
01-001	M	C ³	53	aripiprazole quetiapine	50	none	+	Improved eye contact and interaction
01-002	M	C	55	venlafaxine aripiprazole	150	none	+	Improved interaction
01-003	F	AA ⁴ /H ⁵	85	venlafaxine	150	mild sedation	-	No improvement
01-004	M	C	52	none	150	mild sedation	-	Calmed behavior, improved eye contact, less perseveration
01-005	F	C	71	none	150	none	+	Improved interaction
01-006	F	C	66	none	150	mild sedation	-	Calmed behavior, less nervous giggling, improved eye contact
02-001	M	C	N/A ⁶	none	100	none	-	Improved eye contact
02-002	F	C	54	dextro- amphetamine fluoxetine	50	none	+	Calmed behavior and improved eye contact
02-003	F	C	53	methylphenidate	100	none	+	No improvement
02-004	M	C	36	fluoxetine	150	none	+	Improved eye contact and interaction
02-005	M	B	36	dextro- amphetamine	150	none	-	No improvement
02-006	F	C	50	escitalopram	150	Anxious, tremulous, clammy. Lightheaded when IV was put in.	-	Calmed behavior, able to tolerate blood draws better, much more tolerant of IV placement
Average ± SD ⁷ range	55.5 ± 13.6		36 - 85					

¹01=MIND Institute UCDMC; 02=Rush University Medical Center

²Only psychoactive concomitant medications; 3 subjects on stimulants, 3 on selective serotonin reuptake inhibitors, 2 on other antidepressant, 2 on atypical antipsychotics

³Caucasian

⁴Black/ African American

⁵Hispanic

⁶IQ measurements were not available for this subject

⁷standard deviation

⁸Side effects and clinical improvement were characterized by observations at designated timepoints (0, 15, 30, 45, 60, 120, 180, 240, 300, 360 min) by the PI and by formal questioning of the subject/guardian throughout the visit for the occurrence of CNS side effects relevant to fenobam from a checklist (see Methods) new, or worsening of existing signs, symptoms or behaviors.

⁹Result of PPI after fenobam relative to baseline; "+" denotes achieved response criterion of 20% improvement in PPI and "-" represents less than 20% improvement

Conclusion

To our knowledge this is the first study assessing safety and pharmacokinetic metabolism of an mGluR5 antagonist in humans with FXS. Administration of a single dose to this cohort of 12 adults did not result in significant adverse events. Although the doses of 100 to 150 mg are within the range (100-600 mg) of single fenobam doses previously investigated in patients with anxiety disorders, and despite extensive questioning of subjects and parents, side effects described in previous studies were not observed. The pharmacokinetics of fenobam showed wide intersubject variability, as previously noted in normal volunteers and patients with anxiety disorders. Peak plasma levels in this study ranged from 3.28-113 ng/mL (5.3 – 220 ng/mL in prior studies[20]) which overlays the concentration range at which fenobam interacts with the mGluR5 receptor[17]. Peak plasma times occurring around 2-3 hours were slightly longer than reported previously (0.33 – 2 hrs)[20].

In a subset of subjects, calmed behavior was observed within an hour after fenobam dosing, prior to the mean peak plasma level of fenobam. The observation of rapid reduction in hyperactivity and anxiety after fenobam dosing in the most affected subjects with FXS was particularly surprising. Similarly, it was remarkable that 50% of our cohort demonstrated at least a 20% improvement in PPI whereas only 15.4% (2 of 13) of untreated individuals with FXS and 0% of normal controls (0 of 16) had 20% or more increase in PPI in test-retest studies. PPI improved in subjects treated with 50, 100, or 150 mg of fenobam (Table 1), consistent with variable pharmacokinetics of the drug and suggesting that, as with many psychoactive drugs, effective dosing may be highly variable in different individuals. Similarly, clinical responsiveness was variable and not specific to the dosage used in this study. Five of the 6 individuals with improved PPI also showed a subjective clinical improvement and 1 did not, and 4 individuals showed clinical improvement but not an improved PPI, so there was not complete concordance between PPI and clinical improvement. However, PPI was only done at 1 hour post dosing, when fenobam levels had not yet peaked, and clinical assessment was continuous for 6 hours after the dosing, so clinical observations were likely more sensitive, given the PK variability of fenobam, but the PPI data is less subject to placebo effect and thus provides a useful additional indicator of response.

The rapid improvement in PPI is consistent with prior studies suggesting that PPI can respond acutely to drug treatments: children with ADHD showed improvement in PPI after a single dose of methylphenidate[25] and mGluR agonists produce rapid, within 30 minutes of treatment, normalization of PPI in the knockout mouse model of FXS[21]. Thus, although more permanent synaptic re-organization associated with cognitive improvement would be expected to take much longer, PPI may be at least partially dependent on rapid changes in neurotransmitter and neuroreceptor expression modulating short-term synaptic plasticity.

Fenobam peak levels occurred later than expected based on prior PK results and thus the PPI was done when levels were still increasing on the PK curve. Therefore, the PPI results presented may represent an underestimate of the effect of fenobam on PPI that would have been measured if PPI was done at 2-3 hours post-dose. Further, correlations between PPI and fenobam levels may have been masked due to variable rates of increase in different subjects. In future study design for fenobam trials, PPI should be administered 2-3 hours post-dose to optimize drug impact. The PPI results shown here are consistent with a recent study using eyeblink startle PPI methodology, similar to methods used in human studies, which demonstrated a significant PPI deficit in the *fmr1* knockout mouse that was rescued to wild-type levels by MPEP, an mGluR5 antagonist[21].

The poor correlation of PPI improvement with fenobam levels, and the observation of behavioral and PPI responses well before the peak level of fenobam was reached, could be due to rapid generation of an active metabolite with a variable rate of conversion or because the observed change was unrelated to fenobam. As expected, PPI results for females are more variable than males, likely due to neuronal and brain circuit mosaicism for FMRP production. Thus, depending on brain patterns of FMRP mosaicism, PPI deficits (and rescue) would not necessarily be correlated with cognition in females with FXS.

Although the FXCPT did not show significant changes from baseline to treatment conditions in the averaged group results, some individual improvements in commission errors were observed. This CPT is not sufficiently sensitive to be used as an outcome measure in relatively high functioning cohorts of subjects with FXS due to ceiling effects. Additional work is needed to identify a CPT that functions as a measure of attention and inhibition across the entire spectrum of involvement in FXS

as well as other outcome measures, such as a validated FXS-specific scale directed at measuring change in behavioral phenotypes prominent in FXS.

Limitations of this study include the lack of placebo control, the small sample size, and the biasing of the subject sample to include only those who were higher functioning behaviorally and less anxious so that they would be able to tolerate the study procedures including the intravenous catheter. More anxious individuals with FXS might be expected to show a more obvious response to the drug, but would not have tolerated this protocol. Also, there may have been limitations in the ability of subjects to describe side effects despite the extensive measures taken to elicit these, and the study does not rule out side effects that might emerge with chronic dosing, as in cohorts treated with fenobam in the 1980s. Nonetheless, the lack of safety problems seen in this study should encourage further studies of fenobam, starting at low doses and with careful titration based on efficacy and tolerability, in patients with FXS, given the remarkable benefits of mGluR5 negative modulators with respect to behavior, cognition and even dendritic structure in animal models of FXS. It is hoped that long-term use of fenobam will similarly rescue synaptic plasticity deficits in humans with FXS, and that PPI improvement observed here signals enhanced frontal gating, which with long-term treatment could lead to improved information processing and cognition. To effect long term improvements in cognition, pharmacological treatment will likely require concomitant learning programs to provide the substrate for the stimulus-dependent synaptic remodeling presumably facilitated by the mGluR5 blocker. This will necessitate re-initiation of learning programs for adults and emphasizes the need to work toward trials of fenobam in children, who are still in school, and are earlier in the course of the effects of FXS on synaptic plasticity.

In summary this trial did not find major safety concerns to a single-administration of fenobam in FXS, and suggested that clinical improvements in behavior and PPI may be seen even after a single dose. This would indicate that placebo-controlled trials of fenobam and other mGluR5 antagonists involving longer-term treatment of individuals with FXS should be considered to investigate whether rescue of the FXS phenotype observed in animal models can be extended to humans.

Acknowledgements

The authors wish to thank the subjects with FXS and their families for their participation and dedication. This study was sponsored and supported by Neuropharm, LTD with supplementary support from the FRAXA Research Foundation, Administration of Developmental Disabilities grant 90DD0596 (RJH), and NIH grants UL1DE019583, RL1AG032119, RL1AG032115, HD036071, UL1RR024146 (DN and RJH) and MH77554 (DH). This publication was also made possible by Grant Number UL1 RR024146 from the National Center for Research for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. Information on Re-engineering the Clinical Research Enterprise can be obtained from <http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp>

Figure Legends

Figure 1. Change in PPI in adults with FXS after single dose fenobam compared to variation in a prior control group with FXS (18.70 ± 9.56 years) that underwent test-retest with a similar PPI protocol performed at the same study sites over the same time frame[24]. Four of six males (67%) and two of six females (33%) had PPI increases of 20% or more (improvement criterion) after fenobam compared to 2 of 13 with FXS (15%) in the test-retest control group.

Figure 2. Plasma levels of fenobam following oral administration. Upper Panel (A) shows plasma levels in $n = 3$ male healthy adult volunteers administered a single dose of 150 mg fenobam monohydrate. The mean \pm standard error of the mean (SEM) plasma level achieved was 67.1 ± 37.8 ng.ml at 120 minutes post dose. Healthy volunteers were tested through a different Phase I PK study of the formulation of fenobam to be used in the FXS study. These individuals were tested at Qualia Clinical Services, Inc, Nebraska, US during 2007 after they signed informed consent. The protocol for PK testing in the volunteers was approved by Qualia Clinical Services Inc independent IRB and submitted to FDA. Time points for collection of the PK data were slightly different from those used in the subjects with FXS. Middle Panel (B) shows plasma levels of fenobam following administration of 50 mg ($n = 2$), 100 mg ($n = 2$) and 150 mg ($n = 8$) to male and female adults with FXS. Samples were obtained from patients during the duration of a single outpatient visit. Following administration of 150

mg fenobam to patients the mean \pm SEM plasma level achieved was 39.7 ± 18.4 ng / ml. at 180 minutes post dose. The difference between mean peak levels after administration of 150 mg fenobam was not significant when normal healthy adults males were compared to subjects with FXS ($t = 0.47$, d.f. = 2 $p = 0.67$). The mean peak plasma levels obtained after administration of 150 mg fenobam to males and females with FXS was also not significant ($t = 0.28$, d.f. = 6, $p = 0.79$). Lower Panel (C) shows mean \pm SEM plasma levels achieved following administration of 50 mg ($n = 2$), 100 mg ($n = 2$) and 150 mg ($n = 8$) fenobam monohydrate in male and female adult patients with FXS. The mean peak plasma level achieved was related to dose.

References

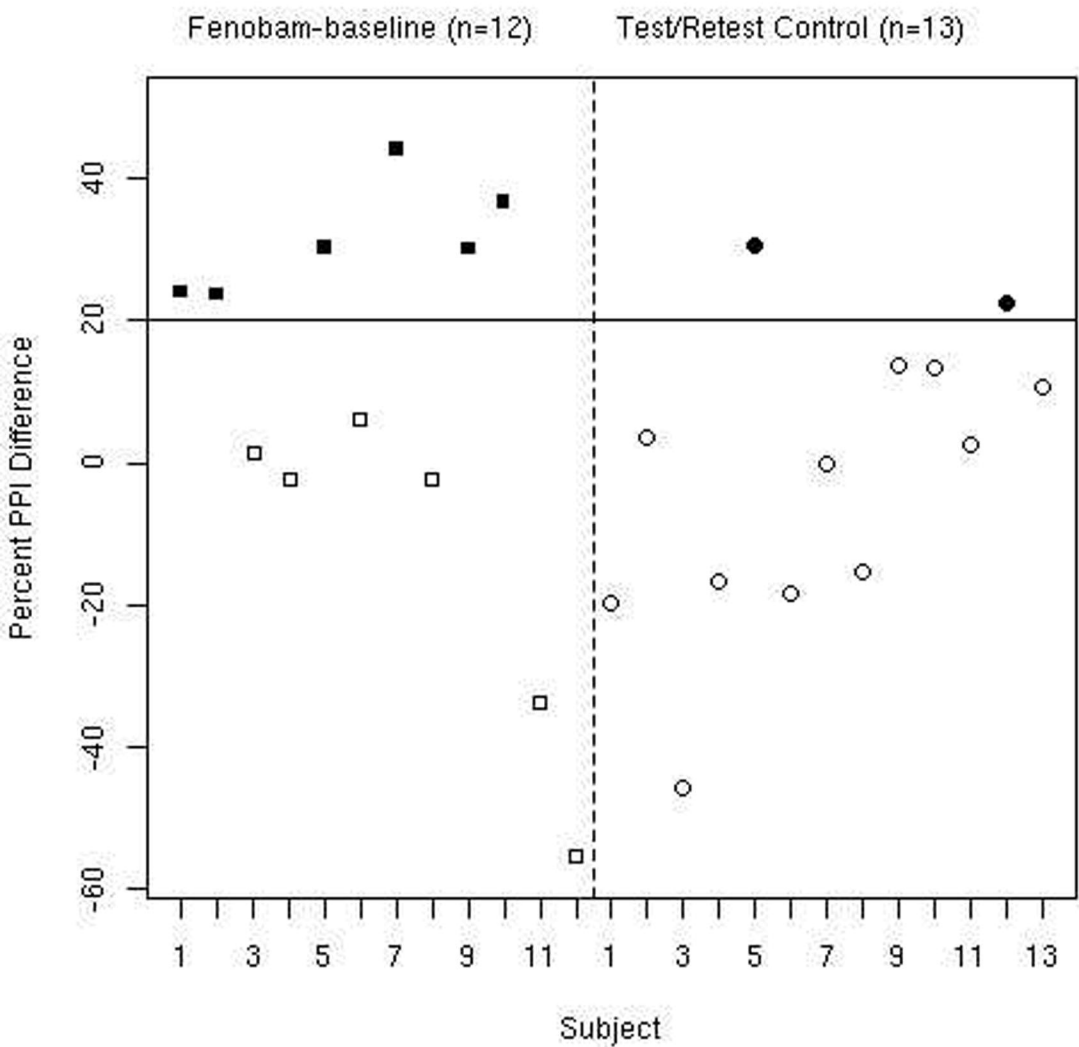
1. Hagerman PJ, The fragile X prevalence paradox. *J Med Genet*, 2008;**45**(8):498-9
2. Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, Eussen BE, van Ommen GJB, Blonden LA, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA, Warren ST. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*, 1991;**65**(5): 905-914.
3. Devys D, Lutz Y, Rouyer N, Bellocq JP, Mandel JL. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nat Genet*, 1993;**4**(4):335-40.
4. Grossman AW, Aldridge GM, Weiler IJ, Greenough WT. Local protein synthesis and spine morphogenesis: Fragile X syndrome and beyond. *J Neurosci*, 2006;**26**(27):7151-5.
5. Antar LN, Afroz R, Dichtenberg JB, Carroll RC, Bassell GJ. Metabotropic glutamate receptor activation regulates fragile x mental retardation protein and FMR1 mRNA localization differentially in dendrites and at synapses. *J Neurosci*, 2004;**24**(11):2648-55.
6. Aschrafi A, Cunningham BA, Edelman GM, Vanderklish PW. The fragile X mental retardation protein and group I metabotropic glutamate receptors regulate levels of mRNA granules in brain. *Proc Natl Acad Sci U S A*, 2005;**102**(6):2180-5.
7. Weiler IJ, Spangler CC, Klintsova AY, Grossman AW, Kim SH, Bertaina-Anglade V, Khaliq H, de Vries FE, Lambers FA, Hatia F, Base CK, Greenough WT. Fragile X mental retardation protein is necessary for neurotransmitter-activated protein translation at synapses. *Proc Natl Acad Sci U S A*, 2004;**101**(50):17504-9.
8. Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci U S A*, 2002;**99**(11): 7746-50.
9. Koekkoek SK, Yamaguchi K, Milojkovic BA, Dortland BR, Ruigrok TJ, Maex R, De Graaf W, Smit AE, VanderWerf F, Bakker CE, Willemsen R, Ikeda T, Kakizawa S, Onodera K, Nelson DL, Mientjes E, Joosten M, De Schutter E, Oostra BA, Ito M, De Zeeuw CI. Deletion of FMR1 in purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in fragile X syndrome. *Neuron*, 2005;**47**(3):339-52.
10. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci*, 2004;**27**(7):370-77.
11. Irwin SA, Galvez R, Weiler IJ, Beckel-Mitchener A, Greenough WT. Brain structure and functions of FMR1 protein, in *Fragile X Syndrome: Diagnosis, Treatment and Research*, 3rd

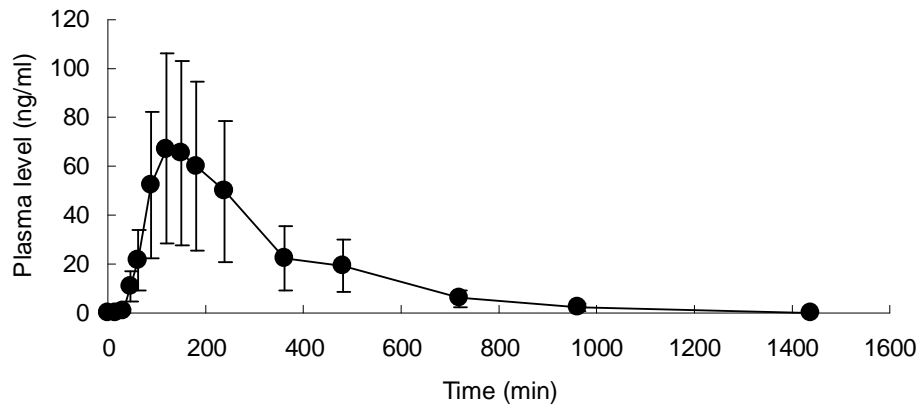
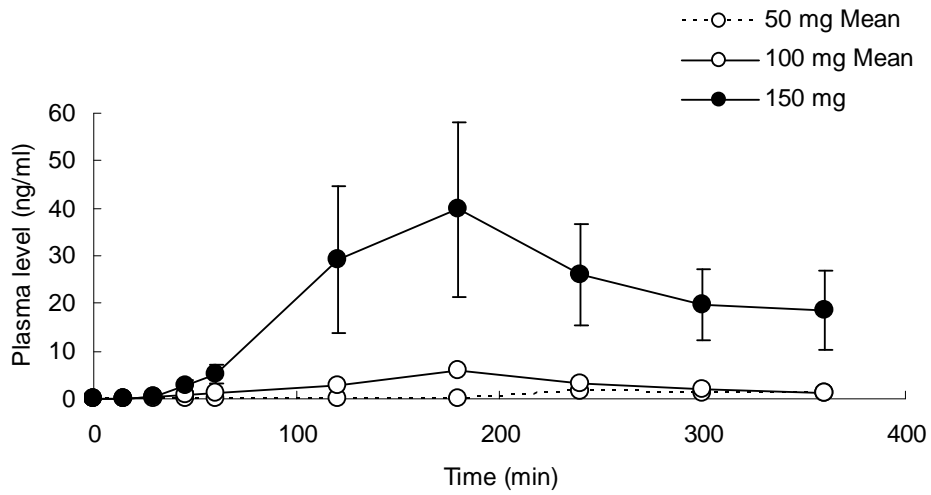
Edition, Hagerman RJ and Hagerman PJ Editors. 2002, The Johns Hopkins University Press:191-205: Baltimore.

12. Beckel-Mitchener A, Greenough WT. Correlates across the structural, functional, and molecular phenotypes of fragile X syndrome. *Ment Retard Dev Disabil Res Rev*, 2004;**10**(1):53-9.
13. Chuang SC, Zhao W, Bauchwitz R, Yan Q, Bianchi R, Wong RK. Prolonged epileptiform discharges induced by altered group I metabotropic glutamate receptor-mediated synaptic responses in hippocampal slices of a fragile X mouse model. *J Neurosci*, 2005;**25**(35):8048-55.
14. Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major fragile X syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology*, 2005;**49**(7):1053-66.
15. McBride SM, Choi CH, Wang Y, Liebelt D, Braunstein E, Ferreiro D, Sehgal A, Siwicki KK, Dockendorff TC, Nguyen HT, McDonald TV, Jongens TA. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. *Neuron*, 2005;**45**(5):753-64.
16. Dölen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, Bear MF. Correction of fragile X syndrome in mice. *Neuron*, 2007;**56**(6):955-962.
17. Porter RH, Jaeschke G, Spooren W, Ballard TM, Büttelmann B, Kolczewski S, Peters JU, Prinssen E, Wichmann J, Vieira E, Mühlemann A, Gatti S, Mutel V, Malherbe P. Fenobam: A clinically validated nonbenzodiazepine anxiolytic is a potent, selective, and noncompetitive mGlu5 receptor antagonist with inverse agonist activity. *J Pharmacol Exp Ther*, 2005;**315**(2):711-21.
18. Friedmann C, Davis L, Ciccone P, Rubin R. Phase II double-blind controlled study of a new anxiolytic, fenobam (McN-3377) vs placebo. *Current Therapeutic Research*, 1980;**27**(2):144-151.
19. Pecknold JC, McClure DJ, Appeltauer L, Wrzesinski L, and Allan T. Treatment of anxiety using fenobam (a nonbenzodiazepine) in a double-blind standard (diazepam) placebo-controlled study. *J Clin Psychopharmacol*, 1982;**2**(2):129-33.
20. Itil TM, Seaman BA, Huque M, Mukhopadhyay S, Blasucci D, Nq KT, and Ciccone PE. The clinical and quantitative EEG effects and plasma levels of fenbam (McN-3377) in subjects with anxiety: an open rising dose tolerance and efficacy study. *Current Therapeutic Research*, 1978;**24**(6):708-724.

21. de Vrij FMS, Levenga J, van der Linde HC, Koekkoek SK, De Zeeuw CI, Nelson DL, Mientjes E, Oostra BA, Willemsen R. Rescue of behavioral phenotype and neuronal protrusion morphology in Fmr1 KO mice. *Neurobiology of Disease*, 2008;**31**:127-132.
22. Nakamoto M, Nalavadi V, Epstein MP, Narayanan U, Bassell GJ, Warren ST. Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. *Proc Natl Acad Sci U S A*, 2007;**104**(39):15537-42.
23. Frankland PW, Wang Y, Rosner B, Shimizu T, Balleine BW, Dykens EM, Ornitz EM, Silva AJ. Sensorimotor gating abnormalities in young males with fragile X syndrome and FMR1-knockout mice. *Mol Psychiatry*, 2004;**9**(4):417-25.
24. Hessler D, Cordeiro L, Yuhas J, Campbell A, Ornitz E, Berry-Kravis E, Elizabeth Chruscinski E, Hervey C, Long JM, Hagerman RJ. Prepulse inhibition in fragile X syndrome: feasibility, reliability, and implications for treatment. *Am J Med Genet B Neuropsychiatr Genet*, 2008. (Epub ahead of print PMID: 18785205)
25. Hawk Jr LW, Yartz AR, Pelham Jr. WE, Lock TM. The effects of methylphenidate on prepulse inhibition during attended and ignored prestimuli among boys with attention-deficit hyperactivity disorder. *Psychopharmacology (Berl)*, 2003;**165**(2):118-27.
26. Minassian A, Feifel D, Perry W. The relationship between sensorimotor gating and clinical improvement in acutely ill schizophrenia patients. *Schizophr res*, 2007;**89**:225-231.
27. Berry-Kravis E, Krause SE, Block SS, Guter S, Wu J, Leurgans S, Declé P, Potanos K, Cook E, Salt J, Maino D, Weinberg D, Lara R, Jardini T, Cogswell J, Johnson SA, Hagerman R. Effect of CX516, an AMPA-modulating compound, on cognition and behavior in fragile X syndrome: A controlled trial. *J Child Adolesc Psychopharmacol*, 2006;**16**(5):525-40.
28. Sullivan K, Hooper S, Hatton D. Behavioural equivalents of anxiety in children with fragile X syndrome: parent and teacher report. *J Intellect Disabil Res*, 2007;**51**(Pt 1):54-65.
29. Berry-Kravis E, Sumis A, Kim OK, Lara R, Wu J. Characterization of potential outcome measures for future clinical trials in fragile X syndrome. *J Autism Dev Disord*, 2008.

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive license (or non-exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in Journal of Medical Genetics and any other BMJPGJL products to exploit all subsidiary rights, as set out in our license (<http://jmg.bmj.com/iforalicence.pdf>).



A**B****C**