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Molecular biomarkers predictive of sertraline treatment response in young children with fragile X syndrome

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Abstract

Objectives—Several neurotransmitters involved in brain development are altered in fragile X syndrome (FXS), the most common monogenic cause of autism spectrum disorder (ASD). Serotonin plays a vital role in synaptogenesis and postnatal brain development. Deficits in serotonin synthesis and abnormal neurogenesis were shown in young children with autism, suggesting that treating within the first years of life with a selective serotonin reuptake inhibitor might be the most effective time. In this study we aimed to identify molecular biomarkers involved in the serotonergic pathway that could predict the response to sertraline treatment in young children with FXS.

Methods—Genotypes were determined for several genes involved in serotonergic pathway in 51 children with FXS, ages 24 to 68 months. Correlations between genotypes and deviations from baseline in primary and secondary outcome measures were modeled using linear regression models.

Results—A significant association was observed between a BDNF polymorphism and improvements for several clinical measures, including the Clinical Global Impression scale (P= 0.008) and the Cognitive T Score (P= 0.017) in those treated with sertraline compared to those in the placebo group. Additionally, polymorphisms in the MAOA, Cytochrome P450 2C19 and 2D6, and in the 5-HTTLPR gene showed a significant correlation with some of the secondary measures included in this study.

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Author Disclosure Statement

RH has carried out treatment studies in FXS funded by Novartis, Roche, Alcobra, and Neuren and she has consulted with Zynerba and Roche regarding treatment studies in FXS. The other authors have no competing financial interests exist.

Conclusion—This study shows that polymorphisms of genes involved in the serotonergic pathway could play a potential role in predicting response to sertraline treatment in young children with FXS. Larger studies are warranted to confirm these initial findings.

Keywords

Fragile X Syndrome; Serotonin; Sertraline; Selective Serotonin Reuptake Inhibitor; BDNF; Cytochrome P450; Neurotransmitters; Molecular Biomarkers

Introduction

Fragile X syndrome (FXS) is the most commonly inherited form of intellectual disability caused by methylation, subsequent to an expansion greater than 200 CGG repeats, in the 5′UTR of the *FMR1* gene. The consequent deficit/absence of the fragile X mental retardation protein (FMRP) affects brain development and results in significant behavioral, cognitive, and emotional problems. Importantly FXS is also the most common monogenic cause of autism spectrum disorder (ASD) with approximately 60% of those with the full mutation presenting with ASD (1).

Individuals affected by FXS have phenotypic and behavioral features including macroorchidism, aggression, seizures, attention deficit hyperactivity disorder, anxiety, and deficits in sensory integration, language and attention (2).

Several neural pathways and neurotransmitters involved in the brain development are altered in FXS (3), including serotonin which plays a vital role in synaptogenesis and postnatal brain development. A study by Chugani et al. (4), revealed that children with and without ASD had differences in serotonin synthesis capacity for the first five years of life. In children without ASD serotonin synthesis capacity was 200% more than that of adults compared to 1.5 times the adult normal values in children with ASD suggesting that brain serotonin synthesis capacity during early childhood is disrupted in ASD.

Thus, introducing selective serotonin reuptake inhibitors (SSRI) within the first years of life might be effective for those with ASD (5,6). Further, treatment with SSRIs early in postnatal development, paired with appropriate behavioral intervention may have the ability to stimulate neurogenesis and improve clinical symptoms. Indeed, to alleviate maladaptive or disruptive behaviors and social deficits that manifest as anxiety-related symptoms, SSRIs are often prescribed to patients with FXS (7,8). According to medication usage surveys, approximately 50% of patients over five years old with FXS are prescribed an SSRI to treat in particular, anxiety, irritability, and socialization deficits (6–8). Similarly, several studies demonstrated positive responses in anxiety, mood and irritability, with minimal adverse effects in children with ASD, with and without FXS, following low-dose sertraline treatment (9–11). A retrospective study showed improvements in receptive and expressive language, on the Mullen Scales for Early Learning in young patients with FXS treated with sertraline compared to those not treated, suggesting that sertraline may improve language developmental trajectory in young children with FXS (8). To further support the role of serotonin in FXS, two studies, in both humans and mouse, showed that the alteration in the

GluA1-dependent long-term potentiation (LTP) and long-term depression (LTD) in patients with FXS can be partly corrected by serotonin (12,13).

Patients with FXS are often prescribed a multitude of medications to treat specific symptoms (14,15). The neurobiological consequences resulting from the loss of FMRP, have led efforts to identify targeted treatments for FXS (16).

Clinical trials in FXS are usually carried out in adolescents and adult subjects, yet, none have studied the effect of targeted medications in children younger than 5 years old (Reviewed in (17)) with the exception of this recent controlled trial of sertraline in children aged 24 to 72 months described below on which our study is based (18). To be more effective, targeted treatment should likely occur early within this developmental window of brain development to correct any alterations in neurotransmission, and to enhance neuroplasticity and experience-dependent change. The behavioral overlap between ASD and FXS suggests that there might be overlapping bio-molecular pathways. Thus, targeted treatments that were found effective against ASD might be effective in FXS and vice versa.

Based on this evidence a double blind, randomized, placebo-controlled 6-month clinical trial of low-dose sertraline in children ages 24–72 months old with FXS was conducted at the UC Davis MIND Institute to evaluate the efficacy and benefit with respect to early expressive language development and global clinical improvement.

Here, we further investigated the participants of this clinical trial to identify molecular biomarkers predictive of efficacy of responsiveness to sertraline treatment. Candidate genes were selected specifically on the basis of their role in serotonin metabolism, uptake and transport, including Serotonin Transporter-linked polymorphic region (5-HTTLPR), Brain-Derived Neurotrophic Factor (BDNF), variable number of tandem repeat promoter of Monoamine Oxidase A (MAOA-VNTR), Cytochrome P450 2D6 (CYP2D6), and Cytochrome P450 2C19 (CYP2C19) (19). As plasma levels of APP, MMP-9 and BDNF were found to be elevated in FXS and ASD (20–22), we also investigated if the use of sertraline would normalize the plasma levels of these biomarkers.

In this study, we aimed to identify molecular biomarkers that play an important role in the serotonergic pathway and might be predictive of clinical response to sertraline.

Methods

Study Design

A double-blind placebo controlled clinical trial was conducted on 57 subjects aging 24 to 72 months at the UC Davis MIND Institute. Out of the 57 subjects enrolled in the clinical trial, five discontinued and of the remaining 52 subjects who completed the sertraline clinical trial, we received biological samples, at both baseline and follow up visit, for 51 of them. The cohort consisted of 6 females and 45 males. Details of this clinical trial are reported in (18). All patients were randomized and either received a placebo or sertraline. Sertraline was administered in liquid form in a dose of 2.5 mg per day in patients ages 2 to 3 years and 5.0 mg per day in those 4 years to 5 years 8 months. Plasma samples from 19 typically

developing male controls were used to compare their BDNF (Age range: 4–18 years old), APP and MMP9 (Age range: 4–9 years old) plasma levels to children with FXS. Biological samples collected at baseline and post-treatment were under protocols approved by the UC Davis Institutional Review Board and all caregivers signed consent for this study.

Clinical Measures

Clinical assessment of study participants involved primary outcome measures: Mullen Scales of Early Learning (MSEL) expressive language raw score, expressive language standard score and Clinical Global Impression Scale-Improvement (CGI-I). The CGI-I score is a follow-up measure scored as follows: 1=very much improved since the initiation of treatment; 2=much improved; 3=minimally improved; 4=no change from baseline; 5=minimally worse; 6= much worse; 7=very much worse since the initiation of treatment (23). Additionally, the following secondary outcome measures were used: MSEL subscales: fine motor, visual reception and receptive language score; social affect, restricted and repetitive behavior scores and total score of the ADOS 2; Visual Analog Scale (VAS); Sensory Processing Measures (SPM; Pre-school and Home); and Preschool Language Scale (PLS; fifth edition). For each participant, all assessments were completed both at baseline and at the six-month follow up visit. Side effects were monitored as described in (18).

Molecular measures

Genomic DNA was isolated from 3ml of peripheral blood using Gentra Puregene Blood Kit (Qiagen) and following standard procedure.

Plasma collection was done using EDTA, followed by centrifugation for 10 minutes at 1000×g within 30 minutes of blood collection.

5-HTTLPR and MAOA genotyping were performed using 100–200ng genomic DNA and 20 μM of the following specific primers, forward HTTP2A (5′-TGA ATG CCA GCA CCT AAC CC-3′), reverse HTTP2A (5′-TTC TGG TGC CAC CTA GAC GC-3′), MAOA forward (5′-ACA GCC TGA CCG TGG AGA AG-3′) and MAOA reverse (5′-GAA CGG ACG CTC CAT TCG GA-3′) following PCR conditions as detailed in (24). BDNF genotype was determined using Taqman SNP Genotyping Assay (RS6265, Applied Biosystem) and the 7900HT Sequencer and Sequence Detection System Software (Applied Biosystems, Inc. Foster City, CA). Analysis of Cytochrome P450s 2D6 and 2C19 genotypes was achieved using the xTAG 2C6 and 2C19 v3 Kits (Luminex Corporation, Austin, TX) on a Luminex 100/200 instrument. The qualitative genotyping assays, consisting of multiplex PCR reactions with amplicon treatment, allele specific primer extension, hybridization and detection, were utilized to detect and identify nucleotide variants and copy number variants for the polymorphic regions of both genes. *1, *2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *15, *17, *29, *35, *41 variants were identified for 2D6. *1, *2, *3 and *17 variants were identified for 2C19. PCR reactions and conditions were as recommended by the manufacturer and allelic variants were classified into one of four metabolic phenotypes: poor (PM), intermediate (IM), extensive (EM) and ultra-rapid (UM) metabolizers as indicated (Luminex Corporation, Austin, TX).

To determine the total plasma sAPP, sAPPα and sAPPβ levels, the Humans total sAPP, sAPPα and sAPPβ highly sensitive Assay kit (IBL-America, Minneapolis, MN) were used. Wash buffer, labeled antibody, standards and samples were prepared according to the protocol provided by the supplier. The reagent blank, the test sample blank, the test sample and dilutions of standard were all included in each run. Measurement at 450 nm against a reagent blank was obtained on the plate reader. Calculations were done according to the protocol provided by the manufacture.

To BDNF plasma levels were measured using a Milliplex assay (EMD-Millipore-Bellerica/MA). Samples were diluted 100 fold with Assay Buffer. Overnight incubation was carried out for 17 hours at 4°C with shaking. Samples were measured within one hour of finishing protocol using Luminex bead reader.

To determine the MMP-9 plasma activity, the Human MMP Magnetic Bead Panel 2 96-Well Plate Assay (Merck Millipore, Billerica, MA) was used. Preparation of plasma samples and reactions were performed according to the manufacturer's protocol.

Statistical Analysis

For analysis, the met/met and val/met BDNF genotypes, the 2/2, 3/3, 3.5/3.5, and 3/4 MAOA and 4/4, 4/5, and 5/5 MAOA genotypes, the EM or IM-EM and the IM or PM 2C19 genotypes, and the IM and PM 2D6 genotypes were grouped.

The differences between 5-HTTLPR, BDNF, MAOA, CYP2D6, and CYP2C19 genotypes in changes from baseline in ELC, receptive language, and expressive language were modeled using linear regression models including genotype, treatment, the genotype by treatment interaction, baseline score, molecular category (full mutation vs. mosaic), and gender.

The difference between genotypes in CGI-I score post treatment was modeled using proportional odds logistic regression models. These models included the covariates genotype, treatment arm, genotype-treatment interaction, pre-treatment CGI-S score, gender, and category (mosaic vs. full). Analyses were conducted using the statistical software environment R, version 3.2.1 (25).

Log transformed baseline APP and BDNF data were compared between TD and FXS subjects using ANOVA. (Data were log transformed in order to more closely satisfy normality assumptions). P-values for pairwise comparisons were obtained using the Tukey HSD method.

In FXS subjects, for each form of sAPP, the change from baseline was analyzed using a linear model in which the response was the change from baseline in the log expression and covariates included baseline expression of that form of sAPP, gender, age, and clinical trial treatment arm. The estimated changes from baseline reported are those for a hypothetical 5-year-old male subject with baseline expression.

Data were log transformed prior to analysis. Changes from baseline in MMP9 or its ratio were analyzed using a linear model in which the response was the change from baseline in

log transformed biomarker and using treatment group, age, gender, and log baseline biomarker value as covariates.

Results

Study Subjects

Biological samples, at both baseline and follow up visits, were received for 51 subjects who completed the sertraline clinical trial; 26 were on placebo and 25 were treated with sertraline. 58.8% of the subjects had ASD, 37.3% of them were non-ASD and 2% had an unknown ASD status. Approximately 54.9% of the subjects had full mutation and 45.1 % were mosaic. There were no significant demographic differences between the two treatment arms as shown in Table 1.

Molecular Measures

Genotypes distribution—Allelic distribution for the 5 genes for the 51 participants is shown in Table 2.

Candidate genes associated with differential response to sertraline treatment on CGI-I—The clinical global impression scale (CGI-I) was used to quantify and track the subjects' treatment response, progress and response over time. Three allelic variants of the candidate genes were found to be significantly associated with a beneficial effect on the CGI-I.

For the CYP2C19 genotype-based response to sertraline in CGI-I, subjects with the IM/PM genotypes showed a significant percentage in the very much improved/much improved of those on the active arm relative to placebo (P=0.007) (Figure 1A).

In case of MAOA, only a trend towards a differential treatment effect between genotypes was observed (P=0.085, Table 3). Subjects with the 2/2, 3/3, 3/4, or 3.5/4 genotypes showed significant lower odds of a higher CGI-I on the active arm relative to placebo (P=0.045) (Figure 1B).

BDNF showed a statistical significant difference between genotypes in response to treatment as measured by CGI-I (P=0.008, Table 3). Furthermore, subjects with the val/val genotype had significantly lower odds of a higher CGI-I on the active arm relative to placebo (P=0.019) (Figure 1C).

Candidate genes associated with differential response to sertraline treatment on Cognitive T score—Using a linear regression model, the effect of sertraline on the cognitive T score sum differed significantly by BDNF genotype (P=0.017). In addition, a significant difference in response between the baseline and after treatment in the active arm was observed for genotypes met/met and val/met (P=0.009), while the val/val genotype did not correlate with a positive response (P=0.7) (Figure 2).

Candidate genes associated with differential response to sertraline treatment on Social Participation Raw Score—5-HTTLPR showed a significantly different

change from baseline in SPRS in the active arm relative to placebo in those with the L/L genotype (P=0.005) but no significant difference between treatment and placebo was seen for the S/L (P=0.422) or S/S (P=0.997) genotypes.

The MAOA 2/2, 3/3, 3/4, and 3.5/4 genotypes showed a significant difference from baseline and after treatment in those treated with sertraline compared to those on placebo in social participation score (P=0.014). No significant difference was seen between treatment and placebo for the 4/4 genotype (P=0.953).

The CYP450 2D6 IM and PM genotypes showed a significance difference from baseline to after treatment in the active arm compared to placebo (P=0.014). No significant difference was seen between treatment and placebo for the EM genotype (P=0.375). For CYP450 2C19 polymorphisms, the change from baseline in social participation score (SPRS) differed significantly between treatment and placebo for the EM/IM-EM genotypes (P=0.046) but no significant difference was seen between treatment and placebo for the other genotypes.

As for BDNF, a significant positive response was observed for the val/val genotype in those on the active arm compared to those on placebo (P=0.043) whereas no significant difference was observed for the other BDNF genotypes.

Candidate genes associated with differential response to sertraline treatment on Early Learning Composite—Our findings showed an association between the overall BDNF genotypes and response to sertraline in ELC (P=0.015). Patients with a val/met or met/met BNDF genotype who were on the active arm showed a significant increase from baseline, and that increase from baseline was significantly higher than the change from baseline in the placebo arm (P=0.013). Patients with a val/val BDNF genotype showed no significant change from baseline in ELC on active treatment and no difference in change from baseline between active and placebo.

Candidate genes associated with differential response to sertraline treatment on Fine Motor Raw Score—MAOA showed a significant difference in the active arm compared to placebo for genotypes 2/2, 3/3, 3/4 and 3.5/4 (P=0.025) whereas no significant difference was shown for genotype 4/4 (P=0.592).

2C19 showed a significant difference between the active arm and placebo for genotypes IM and PM (P=0.018) whereas no significant difference was found for other genotypes.

Candidate genes associated with differential response to sertraline treatment on primary clinical outcome measures—The analysis of the data showed that the effect of sertraline on MSEL receptive language raw score (RLRS), MSEL expressive language raw score (ELRS), MSEL visual reception age equivalent score (VRAES), and MSEL visual reception raw score (VRRS) did not differ significantly between genotypes for any of the analyzed markers.

Plasma levels of BDNF, APP and MMP-9 in response to sertraline treatment—BDNF and APP plasma levels didn’t change in response to sertraline treatment. Indeed, we didn’t observe a significant difference between the baseline and after treatment with

sertraline. BDNF plasma levels were not significantly different in those treated with sertraline compared to those on placebo (P= 0.1048), suggesting that sertraline does not affect BDNF plasma levels. However, BDNF plasma levels at baseline showed a trend towards the FXS cohort (n=30) having an increased BDNF levels compared to typical developing controls (P=0.059). However, after correcting for age, the 2 groups did not show a significant difference (P=0.119). In addition, plasma BDNF levels were not significantly associated with BDNF genotypes (P=0.645).

As for the plasma APP level, levels at baseline did not differ significantly between FXS and TD subjects for both forms of APP (α and βAPP) and also before and after treatment of sertraline in those with FXS.

Finally, plasma MMP-9 activity was elevated in FXS compared to age matched normal controls, confirming our previous findings (21). However, the observed elevated levels were not normalized by treatment with sertraline.

Discussion

Several studies support the benefit of targeted psychopharmacological treatment in neurodevelopmental disorders including FXS, particularly when used as an early intervention compared to a later one (6,16,26). These data suggest that the use of an SSRI could be highly beneficial in young children with FXS as serotonin levels may be low in the first few years of life in children with FXS and in children with ASD (6). To further support the role of serotonin in FXS, a retrospective study showed an improvement in receptive and expressive language trajectories in young children with FXS associated with a low-dose sertraline treatment (8).

Based on these findings, a controlled trial of sertraline in FXS to determine efficacy of treatment in young children with FXS was recently conducted at the MIND Institute, UC Davis (18). Several primary and secondary clinical measurements were used to assess improvement post-treatment compared to pre-treatment with sertraline and placebo. The results of this study showed that children with FXS demonstrated significant improvements in visual perception, fine motor and overall composite scores on the MSEL when on sertraline compared to placebo. In addition, those with FXS and ASD demonstrated significant improvements in expressive language raw scores after treatment with sertraline compared to placebo. The social participation raw score was also improved. Hence, sertraline not only demonstrated a significant effect on overall development and cognition, but also showed evidence of social improvement (18).

In this study we investigated if polymorphisms in five gene candidates, involved in the serotonin pathway could help to predict the response to sertraline in children with FXS. The candidate genes included the 5-HTTLPR gene which plays a key role in the serotonergic system, whose dysfunction can lead to failure of the central nervous system to properly moderate mood, anxiety, and impulsive behavior (27–30), the MAOA gene regulates intracellular levels of 5-HT and the variable number of tandem repeat functional polymorphism within the gene plays an important regulatory role as a direct or indirect gene

modifier of neurodevelopmental disorders (31), the BDNF gene, which encodes for the protein BDNF protein is responsible for the maintenance and growth of neurons and synapses (32). Treatment with sertraline has shown pro-cognitive effects, increased brain BDNF, and neuroprotective effects (27). Additionally, MAOA, BDNF and 5-HTTLPR appeared to be significant pharmacodynamics targets. The genetic variants of each of those genes, were found to contribute to the modulation of the response of patients using antidepressants (33,34). Additionally, some of these genes are known to contribute to the pathology of several neurological disorders when mutated (35,36). Finally, pharmacokinetic studies have shown effects on efficacy for genetic variations in genes coding for CYP2C19 and 2D6, which play a major metabolic role in pharmacodynamics and metabolism of many drugs, including the SSRIs.

In our study, we found that BDNF genotypes correlated with CGI-I in children treated with sertraline relative to placebo. Additionally, they showed significantly higher change from baseline in the active arm relative to placebo when assessing the Cognitive T Score. This comes in line with several reported studies. Felmingham et al. (37) showed that patients with posttraumatic stress disorder with Met-66 allele showed poorer response to exposure therapy compared to those carrying the val/val alleles. Another study suggested that the val/met polymorphism of BDNF was associated with citalopram efficacy, in addition to improvements in anxiety symptoms (38). On the other hand, we did not observe a significant difference in BDNF plasma levels between those with FXS with or without ASD and between those in the treatment arm compared to those on placebo. Thus, our findings indicate no effect on BDNF plasma level with sertraline treatment. Interestingly, in a study conducted by Erikson et al. (22), BDNF levels showed a steady increase with the use of acamprosate, a $GABA_A$ agonist approved for treatment of alcohol withdrawal. Yet, they mentioned several limitations including a small sample size and only one treatment non-responder had pre- and post-treatment BDNF data available. Additionally, it was found that the use of concomitant medications made it difficult to interpret the BDNF findings (22). Furthermore, in our study we used different age groups compared to the Erickson's study (22) and we included children with FXS both with and without ASD.

Our study also indicates that CYP2C19 IMs and PMs have significantly lower odds of a higher CGI-I on sertraline relative to placebo. As for CYP2D6, the change from baseline in SPRS differed significantly between treatment and placebo for the IMs and PMs. Conversely, a study conducted by Obach et al. (39) which aimed at understanding the role of CYP450 in sertraline metabolism showed that sertraline pharmacokinetics were not different in CYP2D6 EMs and PMs whereas CYP2C19 PMs had 40% higher sertraline exposure. 2D6 poses unique challenges because new variants are constantly arising and unidentified haplotype mutations can also be present. Thus, understanding allelic constitution of each individual may be important for drug dosage.

We also observed a differential response to sertraline when assessing CGI-I, SPRS and VRRS in patients with different MAOA genotypes. Our results are in line with the findings of several studies that showed association of low activity alleles (2,3,5) with increased severity of sensory behavior, aggression, social communication skills, lower IQ and arousal regulation in addition to enlargement of the cerebral cortex in patients with ASD (40–42).

A differential response to sertraline via change from baseline in SPRS relative to placebo was observed in the 5-HTTLPR LL genotype. A study conducted by Brune et al. (43) showed that there was a 5-HTTLPR allelic variation-dependent phenotype in children with ASD assessed by ADI-R and ADOS. Patients with S/L and S/S alleles showed “failure to use nonverbal communication to regulate social interaction,” whereas those with L/L allele showed “stereotyped and repetitive mannerisms”, aggression, alterations in directed facial expressions and unusual sensory interests. Since the literature is mixed regarding which allele is most favorable for children with ASD, more studies are needed to determine if the LL genotype will have the most favorable social response with serotonin treatment in FXS.

A recent study conducted by Erickson et al. (20) showed that youth with ASD treated with Acamprosate had significant reduction in plasma total sAPP. In our study, we did not observe any increase in APP plasma levels in those with FXS compared to TD and no differences were detected before and after treatment with sertraline.

Dziembowska et al. (21) reported that individuals with FXS had a high plasma activity of MMP-9 which was found to be lowered by minocycline treatment in a double blind controlled clinical trial (21). Moreover, minocycline demonstrated efficacy in children with FXS ages 3.5 to 16 years compared to placebo on the CGI-I and on the visual analogue scale (26) and has been shown to reduce elevated levels of MMP-9 in mice and rescue spine morphology (44). In this study, although we confirmed our previous study on elevated plasma MMP-9 activity in children with FXS compared to age-matched controls, we didn’t observe any change in those treated with sertraline before and after treatment whether they had FXS alone or FXS and ASD indicating that sertraline has no effect on MMP-9 activity.

In conclusion, this study indicates an association between polymorphisms in genes related to the serotonin pathway and positive outcomes in young children with FXS treated with sertraline. The molecular biomarker that was most responsive to the effects of sertraline from the CGI-I perspective was the BDNF genotype (Figure 1). Other outcome measures demonstrated that additional biomarkers could be playing a role in predicting responsiveness to an SSRI. However, the effect of any single given biomarker is likely to be small and therefore a combination of them may be more useful for predicting responsiveness to sertraline. Thus, further studies are warranted to consolidate the results of this study and confirm the association of the discussed gene candidates’ genotypes to effective response to sertraline treatment.

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References

1. Harris SW, Hessl D, Goodlin-Jones B, Ferranti J, Bacalman S, Barbato I, et al. Autism profiles of males with fragile X syndrome. Am J Ment Retard. 2008; 113:427–38. [PubMed: 19127654]

2. Hagerman, RJ., Hagerman, PJ. Fragile X Syndrome: Diagnosis, Treatment, and Research. 3. Baltimore: The Johns Hopkins University Press; 2002. p. 3-109.
3. Contractor A, Klyachko VA, Portera-Cailliau C. Altered neuronal and circuit excitability in fragile X syndrome. Neuron. 2015; 87:699–715. [PubMed: 26291156]
4. Chugani DC, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J, et al. Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. Ann Neurol. 1999; 45:287–95. [PubMed: 10072042]
5. Hagerman RJ, Polussa J. Treatment of the psychiatric problems associated with fragile X syndrome. Curr Opin Psychiatry. 2015; 28:107–12. [PubMed: 25602250]
6. Hanson AC, Hagerman RJ. Serotonin dysregulation in fragile X syndrome: implications for treatment. Intractable Rare Dis Res. 2014; 3:110–7. [PubMed: 25606361]
7. Valdovinos MG, Parsa RA, Alexander ML. Results of a nation-wide survey evaluating psychotropic medication use in fragile X syndrome. J Dev Phys Disabil. 2009; 21:23–37.
8. Indah Winarni T, Chonchaiya W, Adams E, Au J, Mu Y, Rivera SM, et al. Sertraline may improve language developmental trajectory in young children with fragile X syndrome: A retrospective chart review. Autism Res Treat. 2012; 2012:104317. [PubMed: 22934167]
9. Steingard RJ, Zimnitzky B, DeMaso DR, Bauman ML, Bucci JP. Sertraline treatment of transition-associated anxiety and agitation in children with autistic disorder. J Child Adolesc Psychopharmacol. 1997; 7:9–15. [PubMed: 9192538]
10. Schaefer TL, Davenport MH, Erickson CA. Emerging pharmacologic treatment options for fragile X syndrome. Appl Clin Genet. 2015; 8:75–93. [PubMed: 25897255]
11. Davenport MH, Schaefer TL, Friedmann KJ, Fitzpatrick SE, Erickson CA. Pharmacotherapy for fragile X syndrome: Progress to date. Drugs. 2016; 76:431–45. [PubMed: 26858239]
12. Costa L, Spatuzza M, D'Antoni S, Bonaccorso CM, Trovato C, Musumeci SA, et al. Activation of 5-HT7 serotonin receptors reverses metabotropic glutamate receptor-mediated synaptic plasticity in wild-type and FMR1 knockout mice, a model of fragile X syndrome. Biol Psychiatry. 2012; 72:924–33. [PubMed: 22817866]
13. Zhang J, Hou L, Klann E, Nelson DL. Altered hippocampal synaptic plasticity in the FMR1 gene family knockout mouse models. J Neurophysiol. 2009; 101:2572–80. [PubMed: 19244359]
14. Hagerman RJ, Berry-Kravis E, Kaufmann WE, Ono MY, Tartaglia N, Lachiewicz A, et al. Advances in the treatment of fragile X syndrome. Pediatrics. 2009; 123:378–90. [PubMed: 19117905]
15. Wang H, Pati S, Pozzo-Miller L, Doering LC. Targeted pharmacological treatment of autism spectrum disorders: fragile X and Rett syndromes. Front Cell Neurosci. 2015; 9:1–23. [PubMed: 25667569]
16. Berry-Kravis EM, Hessl D, Rathmell B, Zarevics P, Cherubini M, Walton-Bowen K, et al. Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. Sci Transl Med. 2012; 4:152ra127.
17. Ligsay A, Hagerman RJ. Review of targeted treatments in fragile X syndrome. Intractable Rare Dis Res. 2016; 5:158–67. [PubMed: 27672538]
18. Greiss Hess L, Fitzpatrick SE, Nguyen DV, Chen Y, Gaul KN, Schneider A, et al. A randomized double-blind, placebo-controlled trial of low-dose sertraline in young children with fragile X syndrome. J Dev Behav Pediatr. 2016; 37:619–28. [PubMed: 27560971]
19. Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat Neurosci. 2006; 9:1221–5. [PubMed: 17001341]
20. Erickson CA, Ray B, Maloney B, Wink LK, Bowers K, Schaefer TL, et al. Impact of acamprosate on plasma amyloid-β precursor protein in youth: A pilot analysis in fragile X syndrome-associated and idiopathic autism spectrum disorder suggests a pharmacodynamic protein marker. J Psychiatr Res. 2014; 59:220–8. [PubMed: 25300441]
21. Dziembowska M, Pretto DI, Janusz A, Kaczmarek L, Leigh MJ, Gabriel N, et al. High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. Am J Med Genet A. 2013; 161A:1897–903. [PubMed: 23824974]

22. Erickson CA, Wink LK, Ray B, Early MC, Stiegelmeyer E, Mathieu-Frasier L, et al. Impact of acamprosate on behavior and brain-derived neurotrophic factor: An open-label study in youth with fragile X syndrome. Psychopharmacology. 2013; 228:75–84. [PubMed: 23436129]
23. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry. 2007; 4:28–37.
24. Tassone F, Qi L, Zhang W, Hansen RL, Pessah IN, Hertz-Picciotto I. MAOA, DBH and SLC6A4 variants in CHARGE: A case control study of autism spectrum disorders. Autism Res. 2011; 4:250–61. [PubMed: 21538940]
25. R CoreTeam. R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna: 2016.
26. Leigh MJ, Nguyen DV, Mu Y, Winarni TI, Schneider A, Chechi T, et al. A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile X syndrome. J Dev Behav Pediatr. 2013; 34:147–55. [PubMed: 23572165]
27. Taler M, Miron O, Gil-Ad I, Weizman A. Neuroprotective and procognitive effects of sertraline: In vitro and in vivo studies. Neurosci Lett. 2013; 550:93–7. [PubMed: 23827216]
28. Brody GH, Beach SR, Chen YF, Obasi E, Philibert RA, Kogan SM, et al. Perceived discrimination, serotonin transporter linked polymorphic region status, and the development of conduct problems. Dev Psychopathol. 2011; 23:617–27. [PubMed: 23786700]
29. Gatzeva-topalova PZ, Warner LR, Pardi A, Carlos M. 5HTTLPR genotype moderates the longitudinal impact of early caregiving on externalizing behavior. Dev Psychopathol. 2015; 27:7–18. [PubMed: 25640827]
30. Vaske J, Newsome J, Wright JP. Interaction of serotonin transporter linked polymorphic region and childhood neglect on criminal behavior and substance use for males and females. Dev Psychopathol. 2012; 24:181–93. [PubMed: 22293003]
31. Deckert J, Catalano M, Syagailo YV, Bosi M, Okladnova O, Di Bella D, et al. Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum Mol Genet. 1999; 8:621–4. [PubMed: 10072430]
32. Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors. 2004; 22(3):123–31. [PubMed: 15518235]
33. Perlis RH. Pharmacogenetic studies of antidepressant response: how far from the clinic? Psychiatr Clin North Am. 2007; 30:125–38. [PubMed: 17362808]
34. Kato M, Serretti A. Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. Mol Psychiatry. 2010; 15:473–500. [PubMed: 18982004]
35. Gerretsen P, Müller JD, Tiwari A, Mamo D, Pollock BG. The intersection of pharmacology, imaging, and genetics in the development of personalized medicine. Dialogues Clin Neurosci. 2009; 17:24–6. 47.
36. Brett ZH, Sheridan M, Humphreys K, Smyke A, Gleason MM, Fox N, et al. A neurogenetics approach to defining differential susceptibility to institutional care. Int J Behav Dev. 2015; 39:150–60. [PubMed: 25663728]
37. Felmingham KL, Dobson-stone C, Schofield PR, Quirk GJ, Bryant RA. The brain-derived neurotrophic factor Val66Met polymorphism predicts response to exposure therapy in posttraumatic stress disorder. Biol Psychiatry. 2013; 73:1059–63. [PubMed: 23312562]
38. Choi M-J, Kang R-H, Lim S-W, Oh K-S, Lee M-S. Brain-derived neurotrophic factor gene polymorphism (Val66Met) and citalopram response in major depressive disorder. Brain Res. 2006; 1118:176–82. [PubMed: 16979146]
39. Obach RS, Cox LM, Tremaine LM. Sertraline is matabolized by multiple cytrochrome P450 enzymes, monoamine oxidases, and glucuronyl transferases in human: an in vitro study. Drug Metab Dispos. 2005; 33:262–70. [PubMed: 15547048]
40. Cohen IL, Liu X, Schutz C, White BN, Jenkins EC, Brown WT, et al. Association of autism severity with a monoamine oxidase A functional polymorphism. Clin Genet. 2003; 64:190–7. [PubMed: 12919132]
41. Cohen IL, Liu X, Lewis MES, Chudley A, Forster-Gibson C, Gonzalez M, et al. Autism severity is associated with child and maternal MAOA genotypes. Clin Genet. 2011; 79:355–62. [PubMed: 20573161]

42. Davis LK, Hazlett HC, Librant AL, Nopoulos P, Sheffield VC, Piven J, et al. Cortical Enlargement in Autism is Associated With a Functional VNTR in the Monoamine Oxidase A Gene. Am J Med Genet B Neuropsychiatr Genet. 2008; 147B:1145–51. [PubMed: 18361446]

43. Brune CW, Kim S-J, Salt J, Leventhal BL, Lord C, Cook EH Jr. 5-HTTLPR genotype-specific phenotype in children and adolescents with autism. Am J Psychiatry. 2006; 163:2148–56. [PubMed: 17151167]

44. Bilousova TV, Dansie L, Ngo M, Aye J, Charles JR, Ethell DW, et al. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. J Med Genet. 2009; 46:94–102. [PubMed: 18835858]

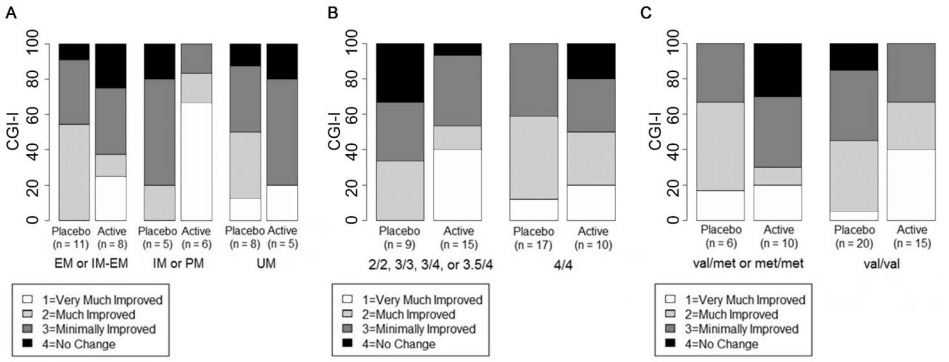

Figure 1.

Candidate genes association with the differential effect of sertraline treatment on CGI-I. A) In the case of 2C19, a higher percent of the intermediate and poor metabolizers were very much improved in the active arm but not in the placebo; B) *MAOA* genotypes 2/2, 3/3, 3/4 and 3.5/4 showed higher percent of very much improved since the initiation of treatment in the active arm compared to placebo; C) Subjects with the BDNF val/val genotype in the active arm had higher rate of very much improved compared to placebo.

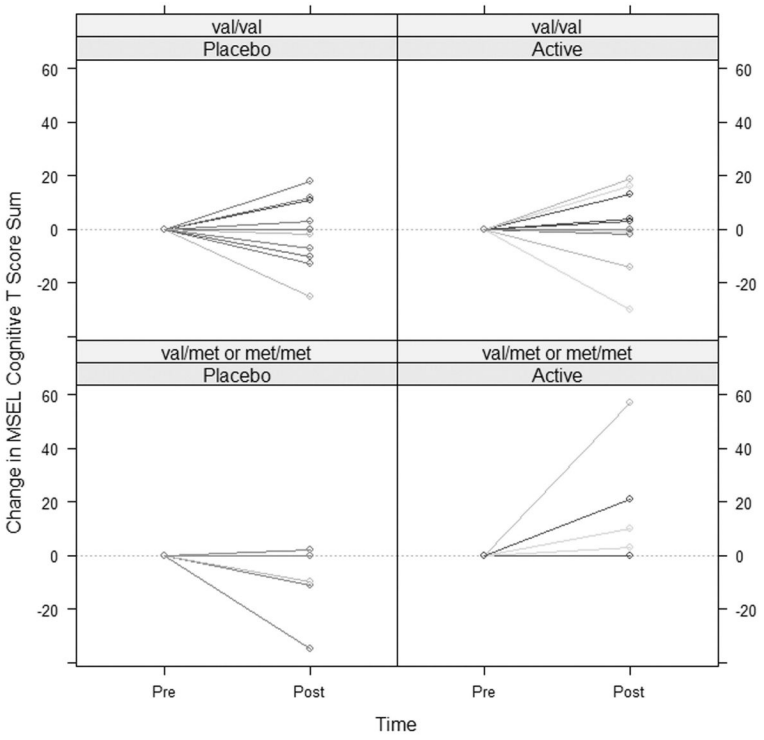

Figure 2.

The graphs show a significant improvement from baseline to after treatment, in the MSEL Cognitive T score, only in those in the active arm with the BDNF val/met or met/met genotype but not with the BDNF val/val genotype. No significant improvement was observed in the placebo group regardless the genotype

Table 1

Subject baseline characteristics by treatment group.

Age at Baseline (Months)	Placebo (n = 26)	Active (n = 25)	All Subjects (n = 51)
N	26	25	51
Mean (SD)	45.5 (13.2)	46.7 (12.1)	46.1 (12.6)
Median (Range)	47.1 (24.8–71.9)	47.8 (24.1–67.1)	47.8 (24.1–71.9)
Gender			
Female (n, %)	1 (3.8%)	5 (20%)	6 (11.8%)
Male (n, %)	25 (96.2%)	20 (80%)	45 (88.2%)
ASD Status			
ASD (n, %)	16 (61.5%)	14 (56%)	30 (58.8%)
Non-ASD (n, %)	10 (38.5%)	9 (36%)	19 (37.3%)
Unknown (n, %)	0	2 (8%)	2 (3.9%)
Molecular Category			
Full (n, %)	14 (53.8%)	14 (56%)	28 (54.9%)
Mosaic (n, %)	12 (46.2%)	11 (44%)	23 (45.1%)

Table 2

Allelic variations in the candidate genes in study subjects.

Genes/Genotypes	Placebo (n = 26)	Active (n = 25)	All Subjects (n = 51)
Serotonin Transporter Genotype			
L/L (n, %)	7 (26.9%)	6 (24%)	13 (25.5%)
S/L (n, %)	14 (53.8%)	13 (52%)	27 (52.9%)
S/S (n, %)	5 (19.2%)	6 (24%)	11 (21.6%)
BDNF Genotype			
met/met (n, %)	0	2 (8%)	2 (3.9%)
val/met (n, %)	6 (23.1%)	8 (32%)	14 (27.5%)
val/val (n, %)	20 (76.9%)	15 (60%)	35 (68.6%)
MAOA Genotype			
2/2 (n, %)	1 (3.8%)	0	1 (2%)
3.5/4 (n, %)	0	1 (4%)	1 (2%)
3/3 (n, %)	8 (30.8%)	11 (44%)	19 (37.3%)
3/4 (n, %)	0	3 (12%)	3 (5.9%)
4/4 (n, %)	17 (65.4%)	10 (40%)	27 (52.9%)
2C19 Score			
EM (n, %)	10 (38.5%)	6 (24%)	16 (31.4%)
IM (n, %)	4 (15.4%)	5 (20%)	9 (17.6%)
IM-EM (n, %)	1 (3.8%)	2 (8%)	3 (5.9%)
PM (n, %)	1 (3.8%)	1 (4%)	2 (3.9%)
UM (n, %)	8 (30.8%)	5 (20%)	13 (25.5%)
Unknown (n, %)	2 (7.7%)	6 (24%)	8 (15.7%)
2D6 Score			
EM (n, %)	21 (80.8%)	17 (68%)	38 (74.5%)
IM (n, %)	4 (15.4%)	2 (8%)	6 (11.8%)
PM (n, %)	1 (3.8%)	6 (24%)	7 (13.7%)

Table 3

Tests for Association Between Genotype and Response to Sertraline. *Linear Regression Models*

Gene / Tests	ELC	RLRS	ELRS	VRRS	FMRS	VRAES	FMAES	CTS	SPRS	CGI-I
Serotonin	0.48	0.345	0.469	0.4	0.912	0.331	0.906	0.356	***0.057***	0.135
BDNF	***0.015***	0.257	0.729	0.234	0.965	0.165	0.796	***0.017***	0.239	***0.008***
MAOA	0.591	0.438	0.724	***0.099***	0.215	0.127	0.31	0.518	***0.086***	0.085
2C19	0.881	0.966	0.706	0.439	0.102	0.545	0.181	0.905	0.524	***0.018***
2D6	0.189	0.533	0.526	0.895	0.8	0.86	0.629	0.256	***0.07***	0.619

*Adjusting for category, gender, and baseline score

ELC, Receptive Language Raw Score (RLRS), Expressive Language Raw Score (ELRS), Visual Reception Raw Score (VRRS), Fine Motor Raw Score (FMRS), Visual Reception Age Equivalent Score (VRAES), Fine Motor Age Equivalent Score (FMAES), Cognitive T Score (CTS), Social Participation Raw Score (SPRS) and Clinical Global Impression Improvement (CGI-I).