Pharmacogenetic Study of Serotonin Transporter and 5HT2A Genotypes in Autism

Fedra Najjar, MD,¹ Thomas Owley, MD,² Matthew W. Mosconi, PhD,³ Suma Jacob, MD, PhD,⁴ Kwan Hur, PhD,⁵ Stephen J. Guter, MA,¹ John A. Sweeney, PhD,³ Robert D Gibbons, PhD,⁵ Edwin H. Cook, MD,¹ and Jeffrey R. Bishop, PharmD⁶

Abstract

Objective: The purpose of this study was to determine whether polymorphisms in the serotonin transporter (SLC6A4) and serotonin-2A receptor (HTR2A) genes are associated with response to escitalopram in patients with autism spectrum disorder (ASD).

Methods: Forty-four participants with ASD were enrolled in a 6 week, forced titration, open label examination of the selective serotonin reuptake inhibitor (SSRI) escitalopram. Doses increased at weekly intervals starting at 2.5mg daily with a maximum possible dose of 20 mg daily achieved by the end of the study. If adverse events were experienced, participants subsequently received the previously tolerated dose for the duration of study. SLC6A4 (5-HTTLPR) and HTR2A (rs7997012) genotype groups were assessed in relation to treatment outcomes and drug doses.

Results: Insistence on sameness and irritability symptoms significantly improved over the course of the 6 week treatment period ($p < 0.0001$) in this open-label trial. There were no significant differences observed in the rate of symptom improvement over time across genotype groups. Similarly, dosing trajectory was not significantly associated with genotype groups.

Conclusions: Previous studies have identified SLC6A4 and HTR2A associations with SSRI response in patients with depression and 5-HTTLPR (SLC6A4) associations with escitalopram response in ASD. We did not observe evidence for similar relationships in this ASD study.

Introduction

INSISTENCE ON SAMENESS (IS) IS ONE ASPECT of the repetitive and restricted behaviors of autism spectrum disorder (ASD) that is common and troublesome in this population (Kanner 1943; Szatmari et al. 2006). Serotonin dysregulation has been hypothesized to be a biological contributor to the underlying pathology and clinical severity of IS. Therefore, it was initially thought that patients with ASD and IS may benefit from treatment with medications that affect serotonin such as serotonin reuptake inhibitors (SSRIs) (McDougle et al. 1996; Owley et al. 2005). Although not formally indicated for ASD, SSRIs such as escitalopram are commonly used in clinical practice. Although effective for many patients with ASD (Hollander et al. 2012), the response to SSRIs in this population has been variable. Some patients with ASD have adverse events, including restlessness, increased irritability, or insomnia (Cook et al.

1992; Owley et al. 2005) whereas others do not experience symptom improvement from SSRIs (King et al. 2009). The variable response to SSRIs may in part be caused by genetic polymorphisms in two genes known to influence serotonin signaling. The serotonin transporter gene (SLC6A4) and the serotonin receptor 2A gene (HTR2A) have both been implicated in treatment response to and tolerability of SSRIs, although these relationships have primarily been examined in adults (McMahon et al. 2006; Serretti et al. 2007).

To our knowledge, two prior studies have examined pharmacogenetic relationships between the serotonin-transporter-linked polymorphic region (5HTTLPR) of SLC6A4 and SSRI response in ASD. In a 12 week, double-blind, placebo-controlled study of fluvoxamine ($n=18$), Sugie et al. (2005) found that the subjects with LL and SL genotypes had better outcomes as measured by the Clinical Global Impressions–Improvement Scale than subjects

¹Institute for Juvenile Research, Department of Psychiatry, University of Illinois at Chicago, Chicago, Illinois.

²Department of Psychiatry, Rush University, Chicago, Illinois.

³Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas Texas.

⁴Department of Psychiatry, University of Minnesota, Minneapolis, Minnesota.

⁵Department of Public Health Sciences, University of Chicago, Chicago, Illinois.

⁶Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, Minnesota.

Funding: This work was supported by National Institute of Child Health and Human Development (NICHD)/National Institute of Neurological Disorders and Stroke (NINDS)/National Institute of Environmental Health Sciences (NIEHS) P50HD055751 (EHC, JAS, MWM, RDG, FN, TO), National Institute of Mental Health (NIMH) P50MH094267 (E.H.C., R.D.G.), K08MH083888 (J.R.B.), K23092696 (M.W.M.), and K23MH082121 (S.J.) and by a Lever Award from the Chicago Biomedical Consortium.

with the SS genotype. In contrast, participants in the SS group showed significant improvement in delayed speech or peculiar or inappropriate speech. In an open label study (blind to genotype) with escitalopram ($n=58$), the LL and SL groups showed a greater reduction in the Aberrant Behavior Checklist-Irritability factor (Aman et al. 1985) scores than did the SS group (Owley et al. 2010). The direction of these findings is generally consistent with those found in pharmacogenetic studies of major depressive disorder (Serretti et al. 2007).

Postsynaptic serotonin receptors, including 5-HT_{2A}, bind serotonin released presynaptically. HTR2A variants have also been implicated in variable response and tolerability to SSRIs. McMahon et al. (2006) found a strong association between the HTR2A single nucleotide polymorphism (SNP), rs7997012, and treatment outcome using SSRIs in depression.

The goal of the present study is to test the hypotheses that SLC6A4 5HTTLPR or HTR2A rs7997012 genotypes are associated with response and tolerability to escitalopram in subjects with ASD.

Methods

Participants

Subjects ($n=44$) were recruited through the Developmental Disorders Clinic of the University of Illinois at Chicago Institute for Juvenile Research, referral from providers of autism services, and by recruitment from the Autism Societies of Illinois and other parent advocacy organizations, as well as a web site providing information about the study.

The study was approved by the University of Illinois at Chicago Institutional Review Board. Informed consent was obtained from the parent or guardian of the study participants, if they were minors or decisionally impaired, or from adult study subjects able to consent for themselves.

All participants had a physical examination by a pediatric neurologist or a child psychiatrist, as well as a psychiatric evaluation by a child psychiatrist experienced in ASD (T.O., F.N., E.H.C.). Subjects were included if they met criteria for American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed., Text Revision (DSM-IV-TR) (American Psychiatric Association 2000) ASD including autism, Asperger disorder, or pervasive developmental disorder not otherwise specified (PDD NOS). Inclusion criteria included meeting classification of autism or ASD on both the Autism Diagnostic Interview-Revised (ADI-R) (Rutter et al. 2003; Risi et al. 2006), and the Autism Diagnostic Observation Scale, 2nd ed. (ADOS-2) (Lord et al. 2012; Hus et al. 2014; Hus & Lord 2014). Cognitive testing was administered; the measure used depended upon the subject's cognitive abilities. Tests used included the Differential Ability Scales, 2nd ed. (DAS II) (Elliott 2006), which was the preferred measure. The Mullen Scales of Early Learning (MSEL) (Mullen 1995) was used for subjects with lower cognitive abilities, and the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999) was used for higher functioning subjects ≥ 18 years of age.

Target symptoms were assessed with the Repetitive Behavior Scale-Revised (RBS-R) (Bodfish et al. 1999) and the Aberrant Behavioral Checklist-Community Version (ABC-CV) (Aman et al. 1985), which were completed by the subject's caregiver based on observations from the previous week. The ABC-CV irritability (ABC-CV-IRR) subscale score and the sum of the Lam and Aman (2007) RBS-R Compulsive Behavior Subscale, and Ritualistic/Sameness Behavior Subscale Scores (RBS-R-CRS) were used as

the primary outcome measures. To be included, subjects had to meet a minimum severity score of 5 on the RBS-R-CRS (see Table 1 for demographic and baseline clinical data). Additionally the Clinical Global Impressions (CGI) Severity Scale was completed (Guy 1976).

Treatment

The treatment aspect of this study was designed as a 6 week, forced-titration, open-label examination of escitalopram. All subjects,

TABLE 1. DEMOGRAPHIC AND BASELINE CLINICAL DATA

<i>Characteristics (n)</i>	
Gender (%)	
Male	34 (77)
Female	10 (23)
Age, mean $\pm$ SD, months	161 $\pm$ 86
Age range, months	61-532
Race/Ethnicity (%)	
African American	8 (18)
African American/Hispanic	1 (2)
Asian	2 (4)
Caucasian/Hispanic	7 (16)
Caucasian/Non-Hispanic	26 (60)
Best estimate diagnosis (%)	
ASD	9 (20)
Autism	35 (80)
ADI-R Algorithm, mean $\pm$ SD (range) ^a	
Reciprocal Social Interaction	23 $\pm$ 4 (13-30)
Communication (Nonverbal) ^b	10 $\pm$ 4 (2-14)
Communication (Verbal)	18 $\pm$ 5 (7-25)
Restricted, Repetitive, and Stereotyped Patterns of Behavior	8 $\pm$ 3 (3-12)
Abnormality of Development	4 $\pm$ 1 (1-5)
ADOS-2 Algorithm, mean $\pm$ SD (range) ^c	
Social Affect (SA)	13 $\pm$ 4 (6-19)
Restricted and Repetitive Behavior (RRB)	4 $\pm$ 2 (0-8)
Overall (SA + RRB) total	16 $\pm$ 5 (8-25)
ADOS-2 Calibrated Severity Score (CSS), mean $\pm$ SD (range) ^d	
Overall total CSS	8 $\pm$ 2 (4-10)
Social Affect CSS	8 $\pm$ 1 (5-10)
Restricted and Repetitive Behavior CSS	7 $\pm$ 2 (1-10)
ADOS-2 diagnosis (%)	
ASD	4 (9)
Autism	40 (91)
Cognitive, mean $\pm$ SD (range)	
Verbal IQ ($n=38$)	78 $\pm$ 25 (30-120)
Nonverbal IQ ($n=44$)	80 $\pm$ 25 (35-130)
Full Scale IQ ($n=38$)	80 $\pm$ 25 (33-130)
RBS-R CRS	20 $\pm$ 10 (5-41)
ABC-CV Irritability Subscale	20 $\pm$ 9 (4-43)

^aOne adult proband did not have an ADI completed.

^bNonverbal total regardless of Overall Level of Language (ADI-R Q30).

^cADOS-2 algorithm (Lord et al. 2012; Hus and Lord 2014).

^dADOS-2 CSS (Gotham et al. 2009; Hus et al. 2014; Hus and Lord 2014).

ABC-CV, Aberrant Behavior Checklist – Community Version; ADI-R, Autism Diagnostic Interview – Revised; ADOS-2, Autism Diagnostic Observation Schedule, 2nd edition; ASD, autism spectrum disorder; IQ, intelligence quotient; RBS-R CRS, Repetitive Behavior Scale – Revised, Compulsive Behavior and Ritualistic/Sameness Behavior Subscales Combined Total.

caregivers, and investigators were aware of the drug dose throughout the study, although investigators were blind to genotype results until after treatment was completed. All subjects were initiated on a dose of 2.5 mg escitalopram at the beginning of week 1 of the study. If no side effects were apparent, the dose was increased to 5 mg for week 2, then to 10 mg for week 3, to 15 mg for week 4, and to 20 mg for weeks 5 and 6. At the end of every week, the parents were asked to complete and send via fax the ABC-CV and RBS-R reflecting the behavior of the subject over the previous week. If the scores reflected an emergence of side effects, then the dose was lowered to the previously tolerated dose for the duration of the study. Side effects considered in this context included an increase of 6 or more on the irritability subscale of the ABC-CV. Participants were evaluated at the research site at three visits. These included the initial visit the day the medication was started, a second visit at end of week 3, and a final visit at end of week 6. Medication adherence was tracked via calendar and ancillary reports from parents or caregivers as needed, although no significant deviations from drug regimens were identified during the course of the study. Additionally, participants were not allowed to take interacting medications during the study.

Genotyping and genetic analyses

Whole blood was used as the source for DNA used in genotyping for the pharmacogenetic analyses of this study. All subjects had blood drawn prior to starting the medication, and genotyping was completed after all participants completed the 6 week treatment study. Genotyping for 5HTTLPR was completed as previously described, examining the commonly studied “long” and “short” versions of the 5HTTLPR as well as the rs25531 SNP within this region as detailed by recently published methods (Klumpp et al. 2014). All SS subjects were SaSa. SL (6 SaLg, 17 SaLa) and LL (4 LgLa, 6 LaLa) groups were combined as the “not-SS” group to create lower (SS) and higher (not-SS) categories for association analyses (SS: $n = 11$; not-SS: $n = 33$).

The HTR2A rs7997012 SNP was assessed according to manufacturer specifications with a predesigned TaqMan Assay (C__1619749_10) from Applied Biosystems/Life Technologies (Grand Island, NY). This polymorphism was previously associated with less favorable response to citalopram in adults with major depressive disorder (McMahon et al. 2006). We assessed this genotype assuming an additive model of inheritance.

Genotypes were consistent with Hardy–Weinberg Equilibrium. Both genotypes were 100% complete (see Table 2 for genotype allele groupings by demographic information).

Statistics

IS was our primary outcome variable and was defined by RBS-R-CRS scores measured at week 0 through week 6. We analyzed whether 5HTTLPR or HTR2A rs7997012 genotype groups contributed to the mean CRS score over time as the patients were treated with their tolerated dose of escitalopram. The hypotheses were that the low expressing 5HTTLPR (SS) or HTR2A rs7997012_AA genotypes would have less symptom improvements than other genotypes. Our additional primary outcome measure was ABC-CV-IRR. A mixed-effects regression model (random intercept and slope)(Hedeker and Gibbons 2006) was used to assess repetitive behaviors (RBS-R-CRS) and irritability (ABC-CV-IRR) change over the course of the study. Primary analyses examined whether symptom trajectory differed as a function of 5HTTLPR or HTR2A genotype groups controlling for age and sex over the course of the 6 week study. Additional analyses assessed the effects of race, pubertal status, race, and ADI-R “ever” IS rating severity (Cuccaro et al. 2003), as well as nonverbal intelligence quotient (IQ) on treatment outcomes. These clinical variables did not influence the significance of any pharmacogenetic findings in the present study. However, as age and sex were pre-defined covariates in the data analysis plan, the reported test statistics in the Results section represent values adjusted for these two variables. Comparison of changes in medication dosage over time was also examined using mixed-effects regression models (i.e., testing for genotype by time interactions).

Results

Thirty-nine of the 44 recruited subjects completed the study. Of the 39 subjects who completed, 33 were able to tolerate the highest dose of 20 mg by the end of the study.

When RBS-R-CRS and ABC-CV scores were examined across genotype groups at baseline, 5HTTLPR not SS participants were identified as having lower ABC-CV-IRR scores than the SS group ($p = 0.013$). No other baseline differences in ABC-CV-IRR or RBS-R CRS scores across HTR2A or 5HTTLPR genotype groups were identified (see Supplementary Tables 1 and 2) (see online supplementary material at <http://www.liebertonline.com/cap>).

TABLE 2. GENOTYPE ALLELE GROUPINGS BY DEMOGRAPHIC INFORMATION

	n (%)	HTR2A rs7997012			5-HTTLPR	
		AA	AG	GG	SS	Not SS
Gender						
Male	34 (77)	3	14	17	9	25
Female	10 (23)	4	4	2	2	8
Race/Ethnicity						
Caucasian/Non-Hispanic	26 (59)	6	12	8	5	21
African American	8 (18)	0	1	7	3	5
Asian	2 (5)	1	1	0	2	0
Caucasian/Hispanic	7 (16)	0	4	3	1	6
African American/Hispanic	1 (2)	0	0	1	0	1
Best estimate diagnosis						
Autism	35 (80)	5	13	17	8	27
ASD	9 (20)	2	5	2	3	6

5-HTTLPR, serotonin-transporter-linked polymorphic region; ASD, autism spectrum disorder; HTR2A, hydroxytryptamine receptor 2A; SS, short/short.

Results identified significant main effects of time for all models ($p < 0.0001$) indicating that when the study population was examined as a whole, IS and irritability symptoms significantly improved over the course of the 6 week treatment period (RBS-R-CRS baseline mean $\pm$ SD 20.24 ± 9.85 , end of study 12.20 ± 9.96 ; ABC-CV-IRR baseline 19.48 ± 9.23 , end of study 12.20 ± 8.16).

When treatment outcomes were examined as a function of 5HTTLPR groups (SS $n=11$ vs. not SS $n=33$) symptom change over 6 weeks did not differ (RBS-R-CRS time $\times$ 5HTTLPR interaction $p=0.273$; ABC-CV-IRR time $\times$ 5HTTLPR interaction $p=0.122$; see Figs. 1 and 2 respectively). A significant main effect of SS genotype was observed on ABC-CV-IRR scores ($p=0.048$), indicative of higher (worse) irritability scores in the SS group at baseline (which was maintained over the course of the study; see Fig. 2). In a post-hoc analysis that examined response over the course of the first 3 weeks of the study, a significant genotype by time interaction was identified ($p=0.009$) on the ABC-CV-IRR whereby the SS genotype group had greater reduction in irritability symptoms over this time frame, which may simply be the result of their increased irritability at baseline (see Fig. 2).

The improvement in RBS-R-CRS over time as a function of the HTR2A rs7997012 genotype groups was also similar among the three groups ($p=0.655$) (see Fig. 3). There were no significant differences in the rate of irritability improvement over time from week 0 to week 6 as a function of HTR2A genotype ($p=0.203$) (see Fig. 4).

The estimated difference in mean change and 95% confidence interval after 6 weeks of treatment for RBS-R-CRS and ABC-CV-IRR by 5HTTLPR and HTR2A rs7997012 genotype groups are shown in Table 3. Greater reductions in RBS-R-CRS (-2.60) and ABC-CV-IRR (-4.82) at week 6 were observed in the SS genotype

group compared with the not-SS group, but were not statistically significant (i.e., confidence interval included 0). A reduction in RBS-R-CRS for the HTR2A AG group was smaller than for the HTR2A AA group. Similarly, reductions in ABC-CV-IRR for the HTR2A GG and AG groups were smaller than for the HTR2A AA group.

Comparisons of dosing trajectory across genotype groups did not reveal any significant differences based on 5HTTLPR or HTR2A rs799012 groups (see Figs. 5 and 6).

Discussion

This study was designed to assess whether response to the SSRI escitalopram in the treatment of ASD was associated with selected genotypes in the SLC6A4 or the HTR2A genes. Furthermore, we examined whether these genetic variants predicted doses for effective treatment as well as tolerated dose.

In this study sample, our primary analyses did not identify significant differences in trajectory of response of RBS-R-measured compulsive, ritualistic, and sameness behaviors or ABC-CV-measured irritability based on serotonin transporter 5-HTTLPR or HTR2A receptor rs7997012 genotype groups. A post-hoc analysis identified a significant 5HTTLPR by ABC-CV-IRR interaction in the first 3 weeks of treatment. We did not identify a significant difference in the end dose among all genotypes.

Two previous studies (Sugie et al. 2005; Owley et al. 2010) have also investigated response to SSRIs in ASD as a function of genetic variation. These investigations only examined the 5HTTLPR. Our study is the first study to look at the response to SSRI in ASD as a function of both 5HTTLPR and HTR2A

FIG. 1. Mean Repetitive Behavior Scale Compulsive Behavior Subscale, and Ritualistic/Sameness Behavior Subscale Scores (RBS-R CRS) score as a function of 5-HTTLPR subgroups SS and not-SS over time, measured at baseline and then at the end of every week for 6 weeks. 5-HTTLPR, serotonin-transporter-linked polymorphic region; CRS, Compulsive Behavior and Ritualistic/Sameness Behavior Subscales combined total; RBS-R, repetitive behavior scale – revised; SS, short/short.

FIG. 2. Mean ABC-CV Irritability subscale scores by 5-HTTLPR subgroups SS and not-SS over time, measured at baseline and at the end of every week for 6 weeks. 5-HTTLPR, serotonin-transporter-linked polymorphic region; ABC-CV, Aberrant Behavior Checklist – Community Version; IRR, irritability; SS, short/short.

FIG. 3. Mean RBS-R CRS score by HTR2A genotypes GG, AG, and AA (0, 1, and 2 A alleles) over time, measured at baseline and at the end of every week for 6 weeks. CRS, Compulsive Behavior And Ritualistic/Sameness Behavior Subscales combined total; HTR2A, hydroxytryptamine receptor 2A; RBS-R, Repetitive Behavior Scale – Revised.

FIG. 4. Mean ABC-CV Irritability subscale scores by HTR2A genotypes GG, AG, and AA (0, 1, and 2 A alleles) over time, measured at baseline and at the end of every week for 6 weeks. ABC-CV, Aberrant Behavior Checklist – Community Version; HTR2A, hydroxytryptamine receptor 2A; IRR, irritability.

genotypes. Owley et al. (2010) observed smaller decreases (less response) in irritability on the ABC-CV in the 5HTTLPR SS group than in the SL and LL groups. Sugie et al. (2005) not only identified that subjects with SS genotypes had less response as measured by the CGI Severity scale, but also that the S allele was associated with significant improvement in language on the Behavioral Assessment Scale (BAS). Our findings did not identify these relationships with 5HTTLPR genotype groupings. The reasons for this are unclear. However, our study sample represented slightly older patients than those in the previous studies, and had a higher proportion of subjects of non-Western European ancestry than the Owley et al. (2010) study, and a higher proportion of subjects of Western European ancestry than Sugie et al.

(2005), who examined Japanese patients. Additionally, baseline irritability symptoms were lower in the 5HTTLPR not-SS group, which may have influenced the trajectory of response in that measure, and the magnitude of change that was observed over time.

Limitations

The results of this study need to be considered in light of its limitations. These include the small sample size, open-label nature of the pharmacological treatment, relatively broad age range assessed, ethnic heterogeneity, lack of a comprehensive assessment of systemic side effects, and the fact that our analyses were restricted to only one predefined variant in each gene. Although we required an RBS-R-CRS score of at least 5 for inclusion, greater symptom severity at baseline may be required to more clearly elucidate treatment effects. It is also possible that our requirement that patients be medication free before study entry resulted in a different population than those with symptoms requiring pharmacotherapy at study screening, who were excluded. Although our analyses did not identify effects of age, developmental status, or race on the outcomes, these as well as the other limitations mentioned may contribute to additional variability and reduced power and, therefore, chance for type-2 error and a reason for non-replication of prior findings. Future directions include integration of cytochrome p450 2C19 genotype (pharmacokinetic marker) with these pharmacodynamic markers.

Conclusions

In conclusion, symptom improvement was observed over the course of 6 weeks of open-label treatment with escitalopram in this open-label study. Differences in treatment outcomes and dosing as a function of genotype groups were not observed.

TABLE 3. ESTIMATED DIFFERENCE IN MEAN CHANGE AFTER 6 WEEKS OF TREATMENT

Outcome	Comparison group	Estimated difference in mean change at week 6	95% confidence interval	p value
CRS	SS vs Non-SS	–2.60	–7.22, 2.03	0.273
	HTR2A 0 vs 2	–1.39	–7.47, 4.70	0.655
	HTR2A 1 vs 2	0.64	–5.52, 6.81	0.838
IRR	SS vs Non-SS	–4.82	–10.90, 1.27	0.122
	HTR2A 0 vs 2	7.15	–0.67, 14.96	0.075
	HTR2A 1 vs 2	5.42	–2.49, 13.34	0.181

CSR, Repetitive Behavior Scale–Revised, Compulsive Behavior And Ritualistic/Sameness Behavior; IRR, Aberrant Behavior Checklist–Community Version Irritability Subscale Total; IRR, irritability; HTR2A, hydroxytryptamine receptor 2A; SS, short/short.

FIG. 5. Average escitalopram dose by 5-HTTLPR genotype SS and not-SS groups over time for 6 weeks. 5-HTTLPR, serotonin-transporter-linked polymorphic region; SS, short/short.

Clinical Significance

Prior studies have identified SLC6A4 and HTR2A associations with SSRI response in patients with depression, but these have not been demonstrated to have clinical utility. Pharmacogenetic studies in ASD are less common, but two previous investigations have

identified association between the 5HTTLPR and aspects of SSRI response. However, we did not observe evidence of a similar relationship in this specific ASD study population. The reasons for this are unclear, but it is possible that some pharmacogenetic associations may differ across study populations and diagnoses, or be dependent on other nuances of study design that influence these

FIG. 6. Average escitalopram dose by HTR2A genotypes GG, AG, and AA (0, 1, 2 A alleles) over time for 6 weeks. HTR2A, hydroxytryptamine receptor 2A.

relationships and require clarification. Considering the data across studies, it is unlikely that these variants will have clinical utility in predicting response to escitalopram in ASD.

Acknowledgments

We thank Kathleen Hennessy, Zengping Hao, and Kelley Moore for expert technical assistance.

Disclosures

Dr. Cook has been a consultant to Seaside Therapeutics, and served as a site investigator for a Seaside Therapeutics multisite clinical trial. Dr. Bishop serves as a scientific advisory board member for Physician's Choice Laboratory Services (PCLS). Dr. Owley is currently serving as a site investigator for a Forest Laboratories multisite clinical trial, as well as a Roche Pharmaceuticals multisite clinical trial. Dr. Najjar served as a site investigator for a Forest Laboratories multisite clinical trial, as well as a Seaside Therapeutics multisite clinical trial. Dr. Sweeney has served as a consultant to Bristol-Myers Squibb (BMS), Lilly, Roche, and Takeda. Drs. Gibbons, Hur, Jacob, Mosconi, and Mr. Guter have no conflicts of interests or financial ties to disclose.

References

- Aman MG, Singh NN, Stewart AW, Field CJ: The Aberrant Behavior Checklist: A behavior rating scale for the assessment of treatment effects. *Am J Ment Defic* 89:485–491, 1985.
- American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders. 4th ed., Text Revision. Washington, DC: American Psychiatric Association; 2000.
- Bodfish JW, Symons FJ, Lewis MH: The Repetitive Behavior Scale, Western Carolina Center Research Reports. Morganton, NC: Western Carolina Center; 1999.
- Cook EH, Rowlett R, Jaselskis C, Leventhal BL: Fluoxetine treatment of children and adults with autistic disorder and mental retardation. *J Am Acad Child Adolesc Psychiatry* 31:739–745, 1992.
- Cuccaro ML, Shao Y, Grubber J, Slifer M, Wolpert CM, Donnelly SL, Abramson RK, Ravan SA, Wright HH, DeLong G, Pericak-Vance MA: Factor analysis of restricted and repetitive behaviors in autism using the Autism Diagnostic Interview-R. *Child Psychiatry Hum Dev* 34:3–17, 2003.
- Elliott CD: Differential Ability Scales – Second edition (DAS-II). San Antonio: The Psychological Corporation; 2006.
- Gotham K, Pickles A, Lord C: Standardizing ADOS scores for a measure of severity in autism spectrum disorders. *J Autism Dev Disord* 39:693–705, 2009.
- Guy W: ECDEU Assessment Manual for Psychopharmacology. 2nd ed. Washington, DC: United States Government Printing Office; 1976.
- Hedeker D, Gibbons RD: Longitudinal Data Analysis. Hoboken, NJ: John Wiley & Sons; 2006.
- Hollander E, Soorya L, Chaplin W, Anagnostou E, Taylor BP, Ferretti CJ, Wasserman S, Swanson E, Settipani C: A double-blind placebo-controlled trial of fluoxetine for repetitive behaviors and global severity in adult autism spectrum disorders. *Am J Psychiatry* 169:292–299, 2012.
- Hus V, Gotham K, Lord C: Standardizing ADOS domain scores: separating severity of social affect and restricted and repetitive behaviors. *J Autism Dev Disord* 44:2400–2412, 2014.
- Hus V, Lord C: The Autism Diagnostic Observation Schedule, Module 4: Revised algorithm and standardized severity scores. *J Autism Dev Disord* 44:1996–2012, 2014.
- Kanner L: Autistic disturbances of affective contact. *Nerv Child* 2:217–250, 1943.
- King BH, Hollander E, Sikich L, McCracken JT, Scahill L, Bregman JD, Donnelly CL, Anagnostou E, Dukes K, Sullivan L, Hirtz D, Wagner A, Ritz L: Lack of efficacy of citalopram in children with autism spectrum disorders and high levels of repetitive behavior: Citalopram ineffective in children with autism. *Arch Gen Psychiatry* 66:583–590, 2009.
- Klumpp H, Fitzgerald DA, Cook E, Shankman SA, Angstadt M, Phan KL: Serotonin transporter gene alters insula activity to threat in social anxiety disorder. *NeuroReport* 25:926–931, 2014.
- Lam KS, Aman MG: The Repetitive Behavior Scale–Revised: Independent validation in individuals with autism spectrum disorders. *J Autism Dev Disord* 37:855–866, 2007.
- Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S: Autism Diagnostic Observation Schedule, Second Edition (ADOS–2) Manual (Part I): Modules 1–4. Torrance, CA: Western Psychological Services; 2012.
- McDougle CJ, Naylor ST, Cohen DJ, Volkmar FR, Heninger GR, Price LH: A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. *Arch Gen Psychiatry* 53:1001–1008, 1996.
- McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, Sorant AJ, Papanicolaou GJ, Laje G, Fava M, Trivedi MH, Wisniewski SR, Manji H: Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *Am J Hum Genet* 78:804–814, 2006.
- Mullen E: Mullen Scales of Early Learning. Circle Pines, MN: American Guidance Service; 1995.
- Owley T, Brune CW, Salt J, Walton L, Guter S, Ayuyao N, Gibbons RD, Leventhal BL, Cook EH: A pharmacogenetic study of escitalopram in autism spectrum disorders. *Autism Res* 3:1–7, 2010.
- Owley T, Walton L, Salt J, Guter SJ, Jr., Winneka M, Leventhal BL, Cook EH, Jr.: An open-label trial of escitalopram in pervasive developmental disorders. *J Am Acad Child Adolesc Psychiatry* 44:343–348, 2005.
- Risi S, Lord C, Gotham K, Corsello C, Chrysler C, Szatmari P, Cook EH, Jr., Leventhal BL, Pickles A: Combining information from multiple sources in the diagnosis of autism spectrum disorders. *J Am Acad Child Adolesc Psychiatry* 45:1094–1103, 2006.
- Rutter M, Le Couteur A, Lord C: Autism Diagnostic Interview, Revised (ADI-R). Los Angeles, CA: Western Psychological Services; 2003.
- Serretti A, Kato M, De Ronchi D, Kinoshita T: Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Mol Psychiatry* 12:247–257, 2007.
- Sugie Y, Sugie H, Fukuda T, Ito M, Sasada Y, Nakabayashi M, Fukushima K, Ohzeki T: Clinical efficacy of fluvoxamine and functional polymorphism in a serotonin transporter gene on childhood autism. *J Autism Dev Disord* 35:377–385, 2005.
- Szatmari P, Georgiades S, Bryson S, Zwaigenbaum L, Roberts W, Mahoney W, Goldberg J, Tuff L: Investigating the structure of the restricted, repetitive behaviours and interests domain of autism. *J Child Psychol Psychiatry* 47:582–590, 2006.
- Wechsler D: Wechsler Abbreviated Scale of Intelligence. San Antonio: The Psychological Corporation; 1999.

Address correspondence to:

Edwin H. Cook, MD

Institute for Juvenile Research

Department of Psychiatry

University of Illinois at Chicago

1747 W. Roosevelt Road, Rm 155

Chicago, IL 60608

E-mail: ecook@psych.uic.edu