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A Pharmacogenetic Study of Escitalopram in Autism Spectrum Disorders

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Scientific Abstract

Objective—To determine the effect of serotonin transporter polymorphism promoter region (5-HTTLPR) genotypic variation (low, intermediate, and high expression groups) on response to escitalopram treatment of children and adolescents with Autism Spectrum Disorders (ASDs).

Method—The study used a forced titration, open label design, with genotype blind until study completion. Participants were children and adolescents aged 4 to 17 years of age with a confirmed ASD (Autistic Disorder, Asperger's Disorder, or Pervasive Developmental Disorder, Not Otherwise Specified).

Results—There was an interaction between genotype group and time on the Aberrant Behavior Checklist Irritability Subscale (primary outcome variable) (linear MMLE = -4.84, Z = -2.89, SE = 1.67, p = 0.004). Examination of baseline to last-observation carried forward scores revealed that a genotype grouping based on a previous study of platelet 5-HT uptake revealed less response in the genotype group that had S/S genotype for 5-HTTLPR and did not have a diplotype in intron 1 previously shown to be associated with increased platelet 5-HT uptake.

Conclusion—This genotype-blind, prospective pharmacogenetic study found the group of subjects with associated with the lowest platelet 5-HT uptake from previous study had the smallest reduction in ABC-Irritability scores after open label treatment with escitalopram. Replication is necessary to confirm these findings.

Lay Abstract—Many children with Autism Spectrum Disorders have problems with anxiety, obsessions, compulsions, and insisting that things stay the same. When other interventions are not adequately helping the child deal with these difficulties, sometimes medication is considered a treatment option.

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Serotonin is inactivated when it is taken back into nerve cells by a protein called the serotonin transporter. Escitalopram blocks this protein. We wanted to know if variation in the gene that produces the protein target for escitalopram would be related to response to this treatment.

Keywords

autistic disorder; escitalopram; pharmacogenetics; open label; drug treatment

Introduction

The Autism Spectrum Disorders (ASDs) are a group of neurodevelopmental disorders characterized by qualitative impairments in reciprocal social interaction, language and communication, and the presence of restricted, repetitive, and stereotyped behaviors and interests (APA, 1994). It has been suggested that selective serotonin reuptake inhibitors (SSRIs) may be useful in the treatment of certain symptoms of subjects with ASDs (Posey, Erickson, Stigler, & McDougle, 2006) including repetitive behaviors (Hollander et al., 2005; McDougle, Epperson, Price, & Gelernter, 1998; McDougle et al., 1996), anxiety (Buchsbaum et al., 2001), irritability (Owley et al., 2005), aggression and self-injurious behavior (Hellings, Kelley, Gabrielli, Kilgore, & Shah, 1996), and more global behavior (Cook, Rowlett, Jaselskis, & Leventhal, 1992; DeLong, Ritch, & Burch, 2002; Namerow, Thomas, Bostic, Prince, & Monuteaux, 2003; Sugie et al., 2005). However, a recent large controlled trial study by King and colleagues (2009) did not find a reduction in repetitive behaviors by citalopram.

There continues to be some difficulties associated with the use of SSRIs for a substantial proportion of individuals. A substantial minority of children with ASDs will become activated (drug-induced insomnia, hyperactivity, and/or general disinhibition) on these medications, even at low doses (Cook et al., 1992; McDougle et al., 2000; Owley et al., 2005). Dosing is also complicated by the fact that there appears to be no relationship between weight and final dose, and only a weak correlation between age and final dose (Owley et al., 2005).

A pharmacogenetic study was conducted to determine if variation in the gene that codes for the primary protein target of SSRIs, the serotonin transporter, would be related to escitalopram response or final dose. A complex insertion/deletion/single nucleotide containing polymorphism in the promoter region of the transporter (5-HTTLPR), thought to be related to 5-HTT gene expression, was chosen as the primary candidate polymorphism (Heils et al., 1996; Hu et al., 2006).

Only one study has looked at the SSRI treatment as a function of 5-HTTLPR in ASDs. In a double-blind study of outcome using the SSRI fluvoxamine in a group of 18 children diagnosed with autism using DSM-IV (APA, 1994) criteria only, Sugie and colleagues (Sugie et al., 2005) found the L/L and S/L groups to have superior outcomes compared to subjects in the S/S group. With some exceptions, this finding was in keeping with the studies that had been done examining pharmacogenetic outcomes of SSRIs and 5-HTTLPR in major depression (Kirchheiner, Grunemann, & Schomig, 2006).

A previous study by our laboratory revealed that subjects homozygous for a TT diplotype in intron 1 of the serotonin transporter gene who had S/S or S/L genotypes had higher functional uptake more consistent subjects with the L/L genotype (Cross et al., 2007). More recently, a common functional A > G variant found predominately on the L 5HTTLPR allele was characterized (Hu et al., 2006). This L_G allele reduces messenger RNA levels to levels very similar that found in the S allele.

We have previously reported an open label study of escitalopram in subjects with ASDs (N=28), in which clinical data were collected independent of genotype (Owley et al., 2005). With the strong qualifier that pertains to open label trials in this population, those data indicated improvement of some symptoms associated with ASDs. In particular, the primary outcome variable, changes in the Irritability Subscale of the Aberrant Behavior Checklist-Community Version (ABC-CV), was reduced ($p < 0.001$) compared to baseline. The current manuscript extends this study to report on a total of 58 subjects with ASDs between the ages of 5 and 17, who received an SSRI, escitalopram, in a prospective, 10-week, forced titration design. The study was open-label in terms of medication, but all participants and investigators were blind to genotype until all clinical data had been collected. Our primary hypotheses were 1) genotype group would be related to response of ABC-Irritability scores after escitalopram, and 2) genotype group would be related to final dose with the lowest expressing genotype group having a lower final dose when compared with higher expressing genotype groups.

Method

Participants

Participants (N = 58) were recruited from the University of Chicago Developmental Disorders Clinic and the University of Illinois at Chicago Neurodevelopmental Psychopharmacology Clinic. An additional two participants were excluded from the analyses because we were unable to obtain a blood sample from them due to restlessness (n = 1) and dropout (n = 1). Recruitment criteria included: diagnosis of an autism spectrum disorder and a minimum score of 12 on the Aberrant Behavior Checklist-Community Version Irritability subscale (ABC-CV)(Aman, Singh, Stewart, & Field, 1985). Participants were excluded if sleep diaries (parent record of sleep and awake time during pre-medication baseline week) indicated that their awakening time was erratic (i.e. a range in time of awakening greater than 2 hours over 7 days) because an abrupt increase in sleep latency and early awakening were used as criteria to titrate dose throughout the study (described below). Participants were free of concomitant serious medical or psychiatric conditions, including seizures, as indicated by parent report of medical history and physical exam. No participant had been previously treated with either escitalopram or citalopram. All participants ceased psychoactive medication use one month prior to the study; participants previously on fluoxetine stopped medication 6 weeks prior to the study. Table 1 presents clinical characteristics of the sample.

Study design

This study was approved by the University of Chicago and University of Illinois at Chicago Institutional Review Boards. Informed consent was obtained from parents or other legal guardians of study participants. When developmentally appropriate, assent was also obtained from participants. Participants received a research diagnostic evaluation, which included administration of the Autism Diagnostic Interview-Revised (ADI-R)(Lord, Rutter, & Le Couteur, 1994), the Autism Diagnostic Observation Schedule (ADOS)(Lord et al., 2000), cognitive testing (all testing done by research-reliable psychologists), and a psychiatric evaluation (administered by T.O.). All but 4 participants met the ADI-R classification for autism. Three participants missed the cutoff for the Restricted and Repetitive Behavior domain and one participant missed the cutoff on the Communication domain by 1 point. Therefore, these 4 participants all met the proposed autism spectrum disorder (ASD2) criteria proposed by Risi and colleagues (Risi et al., 2006). Depending on the participant's age, language use, and developmental level, the appropriate cognitive test was selected to get a measure of verbal and non-verbal IQ. Cognitive tests included: Mullen Early Learning Scales, Differential Abilities Scales, Raven Colored Progressive Matrices,

Stanford Binet (5th edition), Wechsler Adult Intelligence Scale, Wechsler Intelligence Scale for Children (-III, -IV), and the Peabody Picture Vocabulary Test (-III, -IV). Best estimate clinical diagnosis according to DSM-IV-TR, presented in Table 1, was determined by the first author using all available data (T.O.).

This was a 10-week open label study. Participants were seen at the clinic by the first author (T.O.) at the start of the study (baseline), week 4, and week 10. The dosing method was forced titration, with a schedule of weekly increasing doses of escitalopram (2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg) unless specific criteria for downward titration due to side effects were met. Lack of tolerability requiring interruption of the forced titration was determined using two measures, a sleep diary and the ABC-CV. Specifically, significant change in sleep was operationalized as either 1) three consecutive days of an increase in time to get to sleep of one hour later than the average time to sleep during the baseline week or 2) three consecutive days of awakening of one hour earlier than the average time awakening during the baseline week. Either of these sleep problems would lead to a reduction of the dose to the highest previously tolerated dose. Significant increase in the ABC-CV Irritability or Hyperactivity Subscales (operationalized as an increase of greater than 10 points over the previous week on either scale) would also lead to a reduction of the dose to the highest previously tolerated dose. After a subject was dropped to the highest previously tolerated dose, the subject would remain on that dose for the remainder of the study.

Outcome measures

The ABC-CV was completed weekly by parents/caregivers to determine response and to help identify the onset of side effects. The ABC-CV is a questionnaire designed to help identify the effects of medication and other interventions in individuals with developmental disorders (Aman et al., 1985). The ABC-CV contains 58-items which are rated on a scale of 0 (“not at all a problem”) to 3 (“problem is severe in degree”) scale. Items contribute to one of five subscales (Irritability, Lethargy, Stereotypy, Hyperactivity, Inappropriate Speech) and an overall score. In previous clinical trials of autism spectrum disorders, the ABC-CV has been sensitive to drug effects (Jaselskis, Cook, & Fletcher, 1992; King et al., 2001). We chose change in ABC-CV Irritability subscale score as the primary outcome measure to analyze by genotype group across the 10-week open label trial because irritability has been effectively targeted in previous studies. Other outcome measures included study drop-out and final drug dose.

Data Analysis

DNA Analyses

Blood samples were genotyped after all participants had finished the protocol.

Genotyping—5-HTTLPR S/L was genotyped as described previously (Kim et al., 2002). Two SNP markers in SLC6A4, rs2020936 and rs2020937, were genotyped using TaqMan® Assays-by-Design. L_A/L_G was differentiated by MspI digestion after amplification of 5-HTTLPR, since L_G introduces an MspI restriction site.

Genotypes were grouped based on functional expression of 5-HTTLPR (Hu et al., 2006) as: low expression (S/S), intermediate expression (L_G/L_G, S/L_G, L_A/L_G, S/L_A), and high expression (L_A/L_A) for the primary analyses. Refined genotype groups incorporated a haplotype (rs2020936-rs2020937) in this region to refine the 5-HTTLPR expression groups. Subjects with the TT/TT diplotype were shifted into the high expression group regardless of 5-HTTLPR genotype because subjects with S/S or S/L genotype and TT/TT diplotype had

greater uptake rate than subjects with the L/L genotype in a previous study (Cross et al., 2007).

Analysis of ABC-CV Irritability Subscale Scores

To analyze whether genotype contributed to the medication response as measured by the ABC-CV over time, a mixed-effects polynomial (quadratic) regression model was used (Hedeker & Gibbons, 2006). The mixed-effects regression model (MRM) uses maximum marginal likelihood estimation (MMLE) and has a number of advantages over traditional mixed models like the repeated measures ANOVA (Hedeker & Gibbons, 2006). For instance, MRM does not require the assumption of compound symmetry (i.e. equal variances and covariances of the outcome measure over time) used in traditional mixed models (Laird & Ware, 1982). This is particularly important for medication trials in which it would not be expected for patients to show equal changes at each time point. MRM allows for missing data under quite general assumptions (Hedeker & Gibbons, 2006), which maximizes power and minimizes bias that may occur by excluding participants who dropped out. An alternative common in traditional mixed models is using the last observation carried forward (LOCF) for participants who dropout. Several recent reviews have highlighted reasons to be cautious about using LOCF, such as the invalid assumptions that subjects' data do not vary after dropout and that the imputed LOCF data points are equal to the observed data, in addition to challenging the notion that LOCF is the most conservative approach (Hamer & Simpson, 2009; Lavori, Brown, Duan, Gibbons, & Greenhouse, 2008; Siddique et al., 2008). In this study, MRM enabled us to analyze the total sample (N = 58) rather than those with data for the complete 10 week period (n = 45); excluding those who dropped out could mask an effect of genotype on response or lead to a false positive, particularly if dropout was not random. MRM was carried out using the MIXREG program (Hedeker & Gibbons, 2006).

A mixed-effects regression model was run with ABC Irritability subscale scores at 11 observations (baseline to week 10) nested within the probands. Model specification included random intercept and linear and quadratic time effects, and fixed-effects of genotype expression group, the interaction between genotype group and time (Group*TIME, Group*TIME²), ABC Hyperactivity subscale scores at baseline, proband age (months), and final dose (mg). The quadratic trend was included in the model to accommodate nonlinearity in the temporal response process and plateau in the genotype group effect over time. The focal analysis was whether the interaction between genotype group and time effects was significant, indicating that change in irritability scores differed by genotype group.

Analysis of Other Outcome Measures

Final drug dose was analyzed as the dependent variable of an univariate analysis of variance (ANOVA) with genotype group as the independent variable, and age (months) and weight (lbs) as covariates. The correlation between final dose, weight, and age was calculated using Pearson's r.

Results

Based on functional expression of 5-HTTLPR (Hu et al., 2006) genotypes were placed into 3 groups (Low: 9 S/S, Intermediate: 1 L_G/L_G, 2 S/L_G, 2 L_A/L_G, 24 S/L_A, High: 19 L_A/L_A). Genotype distribution (which excluded one proband with a rare genotype), was consistent with Hardy-Weinberg equilibrium ($\chi^2 = 5.20$, df = 3, p = .16). The groups did not differ significantly with respect to gender ($\chi^2 = 1.55$, df = 2, p = 0.46). All participants in the high expression group (n = 19) were Caucasian compared to 78% (n = 7) and 72% (n = 21) in the low and intermediate groups respectively ($\chi^2 = 6.20$, df = 2, p = .05). When the analyses

were restricted to Caucasian subjects, the pattern of results did not change. For the analyses of the refined genotype expression groups, 4 individuals with the S/S genotype, 2 individuals with the S/L_A genotype, and the proband with the rare genotype who had the TT/TT diplotype were put into the high expression group. Therefore, the refined groups included the following 5-HTTLPR genotypes (Low: 5 S/S, Intermediate: 1 L_G/L_G, 2 S/L_G, 2 L_A/L_G, 22 S/L_A, High: 4 S/S with TT/TT, 2 S/L_A with TT/TT, 1 rare genotype with TT/TT, 19 L_A/L_A).

Change in ABC Irritability Subscale Scores: Genotype Groups

The *a priori* hypothesis was that participants' expression of 5-HTTLPR as measured by genotype group would lead to a differential response on the ABC Irritability Subscale scores across time. Table 2 shows the MMLE from the model and their significance for the analysis of the change in ABC Irritability subscale scores and the post-hoc contrasting the 3 genotype groups.

There was no significant main effect of genotype group indicating that the groups were similar at baseline. The linear time trend showed a significant decrease in scores across time as seen in Figure 1. The quadratic time trend was also significant reflecting the initial decrease and subsequent plateau in the scores (Figure 1). The interaction between genotype group and time was significant for the linear and the quadratic trends indicating that the largest between group differences were observed earlier in time.

As seen in Figure 2, post-hoc examination of response from baseline to last visit (or last observation for those who dropped out) showed the least reduction in ABC-CV Irritability scores in the group of subjects with S/S genotype who did not have the intron 1 TT/TT diplotype.

Final Dose: Genotype Groups

Final dose did not differ significantly between groups (Low: $m = 12.22$, $sd = 7.01$; Intermediate: $m = 10.78$, $sd = 5.63$; High: $m = 12.37$, $sd = 6.69$). Age was a significant covariate, $F(1, 56) = 4.25$, $p = 0.04$; however, there was not a significant correlation between age and final dose for the whole group ($r = 0.23$, $p = 0.08$) or within genotype expression group ($ps > .10$). Weight was not a significant covariate, $F(1, 56) = 0.00$, $p = 0.99$, nor was it significantly correlated with final dose ($r = 0.04$, $p = 0.78$). When the intron 1 TT/TT diplotype group was treated as a separate group, the pattern of results remained the same.

Discussion

This study was designed to determine the possible effect of variation at the 5-HTTLPR promoter variant on treatment of children and adolescents with ASDs with the selective serotonin reuptake inhibitor, escitalopram.

Final dose did not differ significantly between the low, intermediate, and high expression genotype groups. With the exception of a subject who was an outlier in terms of worsened ABC-CV irritability score, participants with the low expression S/S genotype showed relatively little difference than the other two genotype groups. Although it was a secondary analysis, the refined genotype expression groups that included consideration of the TT/TT diplotype revealed the refined low expression group's response was poorer than the intermediate and high expression groups. This is consistent with previous research demonstrating that the presence of the intron 1 TT/TT diplotype leads subjects with S/S and S/L genotypes to have platelet 5HT uptake V_{max} more consistent with that of L/L genotype groups (Cross et al., 2007). It is unlikely that that diplotype is functional and more likely that the intron 1 haplotype is in linkage disequilibrium with a functional element increasing

expression of the serotonin transporter gene. One might expect that if genotype group influenced treatment response (i.e. reduction in ABC Irritability subscale scores) the least responsive group would end up on higher doses because they were insensitive to behavioral activation. This hypothesis is difficult to examine in our data set due to the small sample size of the least responsive group (i.e. low expression genotype group) which is a limitation to our study. However, the majority of subjects in all groups could not tolerate the maximum dose. Unfortunately, we did not formally study adverse events in this protocol.

The primary limitation in drawing conclusions from this study is the open-label design. There is a well-documented history of expectancy effects in this population, and we do not know what the rate of nonspecific or non-drug-attributable responses occurred in this sample. Some have suggested that there may be an association between 5-HTTLPR polymorphisms and response to multiple interventions; specifically, there is speculation that patients with the L/L genotype tend to have stronger expectancy responses to any treatment (for instance, in depression to light therapy, sleep deprivation, transcranial stimulation, or placebo) (Benedetti et al., 2003; Zanardi et al., 2007). The expectancy could, of course, be related to genetic variation of the parents, but we did not draw samples from parents in this study. The analysis was not robust to population stratification, although methods for control of population stratification in longitudinal designs have not yet been developed. An additional limitation is that the Children's Yale-Brown Obsessive Compulsive Scales modified for pervasive developmental disorders was not used in this study.

Finally, one must be cautious in extending the results of these data for those with Asperger's Disorder and Pervasive Developmental Disorder, NOS, as these groups represented a relatively small proportion of the study sample (10% and 29%, respectively) compared to the larger group with Autistic Disorder (60%).

While not definitive, this forced titration study found that groups with different haplotypes affecting expression of the serotonin transporter may differ in their response to escitalopram. Replication in a larger independent sample with a priori testing of the refined expression groups incorporating the intron 1 TT/TT diplotype is necessary to determine whether serotonin transporter genotype is related to response to escitalopram in ASD.

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Figure 1.

Symbols represent observed case means. LV represents the last visit score for all participants carrying the last observation forward for participants who dropped out. Values below the figure show the change in scores from baseline (week 0) to the last visit (LV) for participants who dropped out below the time at which the last rating occurred; bold scores indicate an improvement (i.e. reduction) in ABC Irritability scores. Note LV data were not used in the calculation of the observed means or the analyses.

Figure 2.

Baseline and final visit or last observation carried forward (LOCF) ABC Irritability subscale scores for participants who dropped out, separating individuals with the intron 1 TT/TT diplotype (4 Low - S/S, 2 Intermediate - S/L_A, 1 previously excluded) from their 5-HTTLPR genotype expression group.

Table 1**Demographic and Baseline Clinical Data**

Characteristics (N)	All (58) ^a	Low (9)	Intermediate (29)	High (19)
Gender (%)				
Female	10 (17)	1 (11)	4 (14)	5 (26)
Male	48 (83)	8 (89)	25 (86)	14 (74)
Age, mean $\pm$ SD, months	117 $\pm$ 31	124 $\pm$ 22	121 $\pm$ 34	106 $\pm$ 28
Age range, months	54–204	98–164	82–204	54–160
Race (%)				
African American	6 (10)	1 (11)	5 (17)	0
Asian	2 (3)	1 (11)	1 (3)	0
Caucasian	48 (83)	7 (78)	21 (72)	19 (100)
Hispanic	2 (3)	0	2 (7)	0
Best Estimate Diagnosis (%)				
Autistic disorder	35 (60)	6 (67)	20 (69)	8 (42)
Asperger's disorder	6 (10)	2 (22)	2 (7)	2 (11)
PDD- NOS	17 (29)	1 (11)	7 (24)	9 (47)
ADI-R, mean $\pm$ SD (range)				
Social interaction	23 $\pm$ 5 (11–30)	24 $\pm$ 4 (18–29)	24 $\pm$ 4 (14–30)	23 $\pm$ 5 (11–30)
Communication, verbal	17 $\pm$ 4 (7–26)	18 $\pm$ 5 (11–26)	17 $\pm$ 4 (10–23)	17 $\pm$ 4 (7–26)
Communication, nonverbal	13 $\pm$ 2 (10–15)	13 $\pm$ 1 (13–14)	12 $\pm$ 2 (10–15)	13 $\pm$ 2 (10–15)
Repetitive behavior	6 $\pm$ 3 (1–15)	6 $\pm$ 2 (2–9)	7 $\pm$ 3 (2–12)	6 $\pm$ 3 (1–15)
Abnormality of development	4 $\pm$ 1 (1–5)	4 $\pm$ 1 (2–5)	4 $\pm$ 1 (0–5)	4 $\pm$ 1 (1–5)
ADOS, mean $\pm$ SD				
Social interaction	9 $\pm$ 3 (4–14)	9 $\pm$ 3 (4–14)	10 $\pm$ 3 (4–14)	9 $\pm$ 3 (4–14)
Communication	5 $\pm$ 2 (2–10)	5 $\pm$ 2 (3–8)	5 $\pm$ 2 (2–10)	5 $\pm$ 2 (2–10)
Communication + social	15 $\pm$ 4 (7–23)	15 $\pm$ 5 (7–22)	15 $\pm$ 5 (7–23)	15 $\pm$ 4 (7–23)
Play	2 $\pm$ 1 (0–4)	2 $\pm$ 1 (0–4)	2 $\pm$ 1 (0–4)	2 $\pm$ 1 (0–4)
Stereotyped behavior/interests	3 $\pm$ 2 (0–6)	3 $\pm$ 2 (0–6)	3 $\pm$ 2 (0–6)	3 $\pm$ 2 (0–6)
Nonverbal IQ, mean $\pm$ SD	86 $\pm$ 34 (21–146)	63 $\pm$ 34 (21–103)	91 $\pm$ 33 (25–140)	86 $\pm$ 34 (21–146)
Verbal IQ, mean $\pm$ SD	76 $\pm$ 35 (11–141)	60 $\pm$ 36 (11–114)	75 $\pm$ 34 (14–141)	76 $\pm$ 35 (11–141)
ABC Irritability scale score	21 $\pm$ 6 (12–37)	21 $\pm$ 5 (14–30)	20 $\pm$ 6 (12–33)	21 $\pm$ 6 (12–37)
ABC Hyperactivity scale score	25 $\pm$ 11 (4–45)	22 $\pm$ 8 (10–36)	27 $\pm$ 10 (7–45)	25 $\pm$ 11 (4–45)

Note. PDD-NOS = Pervasive development disorder, Not Otherwise Specified; ADI-R = Autism Diagnostic Interview-Revised (Lord et al., 1994); ADOS = Autism Diagnostic Observation Schedule (Lord et al., 2000). One participant was blind and unable to complete a non-verbal IQ test; another participant failed to complete the non-verbal measure resulting in a total of 56 participants for this measure. Participants missing verbal IQ scores (n=5) were non-verbal and could not complete verbal IQ testing. ABC scores presented are from baseline.

^aOne participant with a rare genotype was excluded from the primary analyses (resulting in 57 total subjects for the Low, Intermediate, and High Expression groups).

Table 2

Effect of Genotype Expression Group on ABC-CV Irritability Subscale Scores over Time

	MMLE	Z	SE	p
Genotype group	0.74	0.66	1.11	0.51
Time (linear)	-6.70	-5.75	1.17	<.001
Time (quadratic)	1.04	2.29	0.45	0.02
Group x time (linear)	-4.84	-2.89	1.67	0.004
Group x time (quadratic)	2.01	3.09	0.65	0.002
ABC Hyperactivity	0.25	4.20	0.06	<0.001
Age	-0.01	-0.58	0.02	0.57
Final dose	0.17	0.74	0.11	0.46

MMLE (Maximum Marginal Likelihood Estimate) represents the estimated model parameters.