

Pharmacogenomics of and neural structural changes associated with risperidone in young autistic children

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I. Introduction. Autism is a highly heritable, behaviorally defined neurodevelopmental disorder. Primary symptoms include deficits in social development, communication, and repetitive or stereotypic behavior, but patients also present with aggression, irritability, and sometimes violence. One major current issue is the diagnostic criteria for Autistic Disorder: clusters of autistic symptoms exist with disparate etiologies, which often remain undefined. This issue consequently makes recommendations for pharmaceutical and behavioral therapy difficult. Further, the interaction between psychotropic drugs and neurodevelopment in infants and children has not been systematically studied, and the extrapolation of neural effects of treatment with these drugs from animal model studies is limited. In general, animal studies of psychotropic drugs have shown that persistent downregulation of a neurotransmitter system is associated with a blockade of that system, and a permanent upregulation of a neurotransmitter system results from stimulation of that system in early life (Vitiello 1995), suggesting that permanent neural modifications result from early exposure to psychotropic drugs.

Risperidone is an atypical antipsychotic approved by the Food and Drug Administration to treat autistic children and adolescents with such symptoms as aggression, temper tantrums, quickly changing moods, and deliberate self-injury with limited side effects (Aman 2005). Aman (2005) and McDougle (1998) found in double-blind placebo-controlled experimental designs that risperidone improves performance on such cognitive tasks as verbal learning and cancellation, and reduces such behaviors as irritability, disruptiveness, repetitiveness, aggression, anxiety or nervousness, depression and hyperactivity. Overall, risperidone decreased behavioral symptoms of autism (figure 1) and was well tolerated with no incidence of cardiac events, seizures, or extrapyramidal effects. At least 57% of autistic children are responders to risperidone treatment (McDougle 1998). The drug is a strong dopamine antagonist with high affinity for D2 receptors, as well as activity at 5HT2C, 5HT1A, and 5HT2A receptors, and is hypothesized to mainly affect the frontal-striatal systems.

Since there do not exist standard medications to treat the heterogeneous symptomology of autism, few pharmacogenetic studies examining therapeutic outcome of drug treatments have been performed in this patient population despite the great need for these investigations. Further, studies of other patient populations have found that chronic treatment with risperidone is associated with changes in volume of specific areas of the brain (Lang 2001). Moreover, decreases in metabolism of the ventral striatum, thalamus, and frontal cortex were observed after a single dose of risperidone, with decreases in frontal metabolism more widespread after six weeks of treatment (Liddle 2000). Thus, whether or not this drug will affect neural development during the early years of life is a concern, and a genetic test for therapeutic efficacy of this drug is necessary.

III. Open Question and Hypothesis. We are proposing to investigate the genetic variants of children with autism that respond behaviorally and cognitively to risperidone, and the neurodevelopmental effects of this treatment. We also ask whether changes in the volume of certain brain regions and fiber tracts correlate with improvement or decrement in behavior and cognition, and whether SNP variants can predict the neurodevelopmental response to risperidone. We propose to assess genetic variants using whole genome array single nucleotide polymorphisms (SNPs). Though we will be performing whole genome analyses, we hypothesize that variants of CYP2D6 (Leon 2007), 5HTR2A, DRD2, COMT (Yamanouchi 2003) will show differential response to risperidone treatment since the polymorphisms in these genes have been shown to influence this drug's responsiveness in patients with schizophrenia. Moreover, since few studies have examined the effect of risperidone on early brain development, we hypothesize that the integrity of the frontal-striatal white matter pathways in autistic children and the volume of the frontal lobe after treatment will be different from those with placebo, without treatment, and controls. Finally, we predict that these changes in brain structure due to risperidone will correlate with improvement in behavioral and cognitive symptoms, and that some SNP variants or combinations of variants may predict these changes in structure.

III. Experimental Approach. Subjects. 100 children aged 2-4 years from Children's Hospital diagnosed with Autistic Disorder according to the Autism Diagnostic Observation Schedule (ADOS), the Autism Diagnostic Inventory-Revised (ADI-R) and the Diagnostic and Statistical Manual of Mental Disorders text revision, 4th edition (DSM-IV-TR), will be entered into the study and will be randomly administered risperidone or placebo (lactose) by a pediatric neurologist (Dr Doris Trauner). 100 gender-matched individuals from the UC San Diego Infant Development Laboratory (director Dr Eric Courchesne) aged 2-4 years, 50 of which fall into the Autistic Disorder cutoff and 50 of which are typically developing, will also be recruited as our control groups. The autism group without placebo is necessary for isolation of factors such as behavioral treatment effects. Exclusion criteria include significant comorbid medical or psychiatric conditions, seizures, other medications, or a history of severe closed head trauma. Written informed consent from each participant and institutional review board approval will be obtained. Experimenters will be blind to the grouping of each subject.

(a) *Cognitive and behavioral Assessment.* The California Verbal Learning Task-Children's Version (VLT-C) and modified VLT-C (MVLT-C), a cancellation task of frontal attention, The Aberrant Behavior Checklist, the Y-BOCS (repetitive behavior), the Peabody Picture Vocabulary Test (language comprehension), the Preschool Language Scale (PLS-III; language development), the Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI), and the Ritvo-Freeman Real-life Rating Scale (number and frequency of autistic symptoms) will be administered to all subjects at time point one for baseline and three to six months later at time point two. Specifically, we hypothesize that these measures will improve significantly in the risperidone group, but will not change in the untreated children with autism. Trained psychometrists and psychologists in Dr. Trauner's lab will perform this portion of the study.

(b) *Structural MRI and Diffusion Tensor Imaging.* High-resolution MRI images will be obtained in the 1.5 Tesla scanner at the Radiology Imaging Laboratory using a T1-weighted three-dimensional fast spoiled echo gradient sequence. Subsequently, an axial multi-slice diffusion tensor imaging (DTI) scan will be used using a custom single-shot echo-planar imaging sequence with further eddy current correction capability (Zhou 1999). These measures will also be taken at time points one and two.

(c) *Genotyping and validation.* A blood sample will be obtained from each participant and DNA will be extracted three times as biological replicates. They will then be labeled and hybridized onto the Illumina Human1M-Duo chip. Subsequently, the microarrays will be scanned. The top 50 candidate SNPs will be validated using an independent platform using TaqMan assays (Applied Biosystems). This portion of the study will be performed in the laboratory of Dr. Xiong-Dong Fu and the UC San Diego Biogen Core.

Statistical Analysis. (a) A repeated measures within-between interaction analysis of variance (ANOVA) of a total sample size of 12 subjects in 3 groups yields power of 0.989 for an effect size of 0.8. An analysis of variance (ANOVA) will be performed for the cognitive and behavioral measures at time point one to ensure that there are no significant differences between groups using a posthoc Tukey test. Subsequently, a within-subject ANOVA before and after treatment will be performed with a threshold of $p < 0.05$ and Tukey correction for multiple comparisons to assess whether behavioral and cognitive measures shown to ameliorated after risperidone treatment replicate earlier findings.

(b) *DTI and MRI:* Axial diffusion weighted images will be aligned to remove distortion, and diffusion Eigen values and the principle diffusion eigenvector will be computed to calculate the fractional anisotropy (FA), mean diffusivity (ADC), and regional fiber coherence index. A color-coded FA map will be created for quantitative analysis, and fibers of interest identified from an atlas will be mapped onto FA images for each subject. For MRI measures, the images will be corrected for timing and motion, convolved with an isotropic Gaussian Kernel, normalized, segmented, and smoothed. Subsequently, with groups as between subject factor and regions as within subject factor, repeated measures analysis of variance (ANOVA) corrected for multiple comparisons will be used for each measure and region ($p < 0.05$). Gender, age, risperidone dosage, behavioral treatment type and frequency, and seizures will be entered as covariates in our analyses.

(c) *Pharmacogenomics.* In the whole genome analysis, candidate SNPs will be detected using significance-based ranking or clustering algorithm which takes into account the genomic distance between SNPs. The Fisher exact test will be used to compare genotype frequencies in the *a priori* and candidate SNPs between responders and nonresponders with corrections for multiple comparisons. The gene ontology program GoStat will be used to assess which categories of SNPs are over- or underrepresented in our candidate genes with $p < 0.05$ and correction for multiple comparisons.

(d) *Behavior-imaging-genetic associations.* A general linear model will be used to assess the SNP variants and combinations thereof that can best predict a change in brain structure after treatment with risperidone as well as a decrease in the frequency measures of behavioral and cognitive symptomology.

IV. Conclusion. The field of autism treatment research greatly requires investigations on the effect of antipsychotic medications on neurodevelopment, since these medications are prevalently prescribed to children with autism. It is also of vital importance to determine which subset of autistic patients will likely respond favorably using genetic tests, so that the risk of detrimental or unexpected side effects may be avoided by nonresponders if indeed changes in the trajectory of neurodevelopment occur with treatment. Since risperidone is shown and often prescribed to ameliorate some cognitive and behavioral symptoms of autism, it is an important pharmaceutical to examine in such pharmacogenetic and neurostructural investigations.

However, there are potential limitations with the interpretation of these experiments. Firstly, our sample size may be inadequate for statistical significance in our whole genome associations. The sample size required for significant findings in pharmacogenomic studies of drug response depend on such factors as power, level of statistical significance, frequency of response to the drug, number of individuals with a particular allele, and the minimum detectable effect (McCarthy and Hilfiker 2000). Moreover, a change in the volume of specific brain regions and integrity of the white matter tracts as measured by FA in the risperidone treated group may represent a beneficial or detrimental effect on neurodevelopment of treatment. Since all of our analyses will be correlational, it will be important to remember the limitations on the inferences we will be able to make. As with all clinical studies involving human subjects, we are aware of the ethical concerns regarding the placement of patients in the placebo group. In any case, these studies are vital to current and future patients with autism and to the medical field, and thus we are enthusiastic to begin our examination of these questions.

Figures

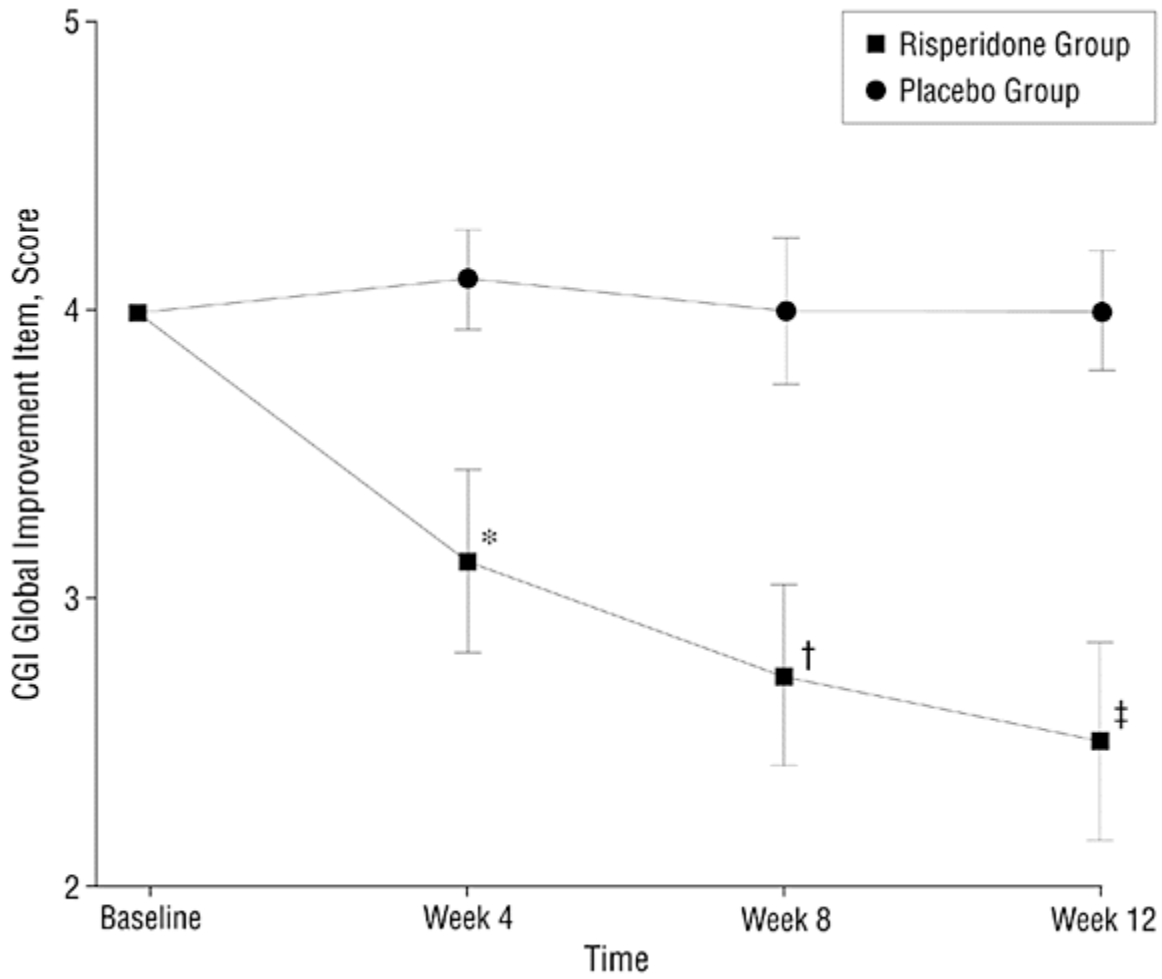


Figure 1. Improvement in CGI score in patients with autism or PDD-NOS treated with risperidone or placebo. (McDougle 1998)

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