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Gene expression in blood is associated with risperidone response in children with autism spectrum disorders

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Abstract

Children with autism spectrum disorders (ASD) often have severe behavioral problems. Not all children with these problems respond to atypical antipsychotic medications; therefore, we investigated whether peripheral blood gene expression before treatment with risperidone, an atypical antipsychotic, was associated with improvements in severe behavioral disturbances 8 weeks following risperidone treatment in 42 ASD subjects (age 112.7±51.2 months). Exon expression levels in blood prior to risperidone treatment were compared with pre-post risperidone change in Aberrant Behavior Checklist-Irritability (ABC-I) scores. Expression of exons within 5 genes was correlated with change in ABC-I scores across all risperidone-treated subjects: GBP6, RABL5, RNF213, NFKBID, and RNF40 (α<0.001). RNF40 is located at 16p11.2, a region implicated in autism and schizophrenia. Thus, these genes expressed prior to treatment were associated with subsequent clinical response. Future studies will be needed to confirm these results and determine whether this expression profile is associated with risperidone response in other disorders, or alternative antipsychotic response within ASD.

Keywords

autism; risperidone; gene expression; atypical antipsychotic

Introduction

Children with autism spectrum disorders (ASD) often have severe behavioral disturbances including aggression, self-injury and tantrums. Although not included in the diagnostic criteria, these behaviors can interfere with socialization, treatment, and education efforts, and pose enormous problems for parents, care-givers and educators.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary information is available at *The Pharmacogenomics Journal*'s website (excel file):

Supplemental Table S-1. Probes (n = 89) with expression that is significantly different between high responders and low responders, according to pre-post risperidone % change in Aberrant Behavior Checklist - Irritability (ABC-I % CHG).

Typical or atypical antipsychotic agents are often effective for decreasing severe behavioral symptoms associated with ASD and are beneficial for the treatment of marked anxiety, aggression, social-withdrawal, stereotypies and sleep disturbance in ASD. Though effective, concerns about troubling short term and long term side effects from antipsychotics lead to concern about their use in the treatment of ASD[1]. The atypical antipsychotic medications, which have effects on many serotonin (5-HT2A, 5-HT1A, 5-HT2C, and 5-HT6) receptors and dopamine (D1, D2, D3, D4) receptors, have begun to replace the typical antipsychotics due to their favorable side effect profile and their beneficial therapeutic effects in reducing symptoms of social withdrawal [2–6].

Although antipsychotic medications have limited effect on core symptoms of ASD such as social reciprocity[7], and few studies show clearly improved adaptive functioning[2], double blind, placebo controlled studies have demonstrated the effectiveness of the atypical antipsychotic risperidone in reducing irritability and aggression symptoms in children with autism[8–10]. In addition, the efficacy and side-effect profiles of risperidone used for ASD-associated symptoms are better than previously reported using the “typical” antipsychotic, haloperidol[7–9, 11].

Thus, the published short and long term trials and placebo-controlled trials of the efficacy of risperidone for the treatment of the severe behavioral problems in ASD children suggest the drug is effective and reasonably well tolerated. However, not all children respond, and there is a risk of serious side effects including increased lipids[12, 13]and diabetes[14–16]. Approaches that might help define those children who are most likely to respond to the drug would be useful for decision-making[1]. Therefore, this study investigated whether peripheral blood gene expression before treatment with risperidone, an atypical antipsychotic, was associated with improvements in severe behavioral disturbances 8 weeks following risperidone treatment in subjects with ASD.

Materials and Methods

Protocols were approved by the institutional review board at the University of California, Davis (UCD). Subjects were recruited from the UCD M.I.N.D. Institute. In addition to a DSM IV diagnostic interview and an Autism Diagnostic Observation Schedule (ADOS) consensus diagnosis of ASD (with use of the Autism Diagnostic Interview-Revised, ADI-R, if supplemental information for diagnosis required), all subjects had to have an initial Aberrant Behavior Checklist Irritability subscale (ABC-I) rating ≥ 18 (mean 25±6.7).

Exclusion criteria included bipolar disorder, schizophrenia, ASD of known genetic cause, nonverbal IQ < 55, seizures, fever, infection, metabolic disturbance or severe illness in the past year; antipsychotic use within 8 weeks of study entry; or inability of parents/care takers to give informed consent, travel to the visits, administer medication, and arrange for completion of rating scales. Other medications and treatments were permitted if started at least two months prior to initial screening and remained constant for the 8 week study duration. Subjects agreed not to start any new pharmacologic, dietary, behavioral or educational treatment during this study. The dosing schedule mirrored that used in the two recent positive trials of risperidone for treating severe behavioral problems in autism[8, 9]. Briefly, risperidone was started at 0.5 mg at bedtime for 4 days. If that dosage was tolerated and there were continued behavioral symptoms, the dose was increased to 1 mg at bedtime for an additional 4 days. If tolerated and indicated, 0.5 mg was added in the morning for a daily total of 1.5 mg.

Affymetrix GeneChip® Human Exon 1.0 ST Arrays were used to obtain gene expression values. Collection of peripheral blood samples and processing of arrays was completed

according to previously published protocols[17]. Raw data (Affymetrix.CEL files) was imported into Partek Genomics Suite 6.4 (Partek Inc., St. Louis, MO, USA). Probe summarization and probe-set normalization were performed using Robust Multi-Chip Average (RMA), which included background correction, quantile normalization, $\log_2$-transformation, and median polish probe set summarization.

All analyses used exon expression levels in blood prior to risperidone treatment (pre-risperidone expression levels) and pre-post risperidone change in ABC-I scores (ABC-I-%CHG), calculated as [(post-risperidone ABC-I score – pre-risperidone ABC-I score)/pre-risperidone ABC-I score]. Because improvement is reflected by a decrease in the ABC-I score, it is important to note that high responders are those demonstrating greater declines in ABC-I scores.

To initially detect the gene expression differences between subjects with the most pronounced response or lack of risperidone response, 17 subjects with the most extreme responses according to ABC-I-%CHG were identified. These subjects were grouped as high responders (ABC-I-%CHG = −95% to −71%; 9 subjects); or low responders (ABC-I-%-CHG = −29% to +6%; 8 subjects). Between-group gene expression profiles were compared for high vs. low responders using analysis of covariance (ANCOVA), controlling for the effects of age, gender and batch ($\alpha < 0.001$, fold change > |1.5|). Because analyses used pre-drug blood RNA, and because any effect of dosing on outcome measures would not change the nature of correlations between pre-drug RNA and outcome measures, dosing was not included as a covariate. Multivariate analysis (unsupervised hierarchical clustering) was applied to evaluate relationships between high and low responders determined by these probes.

Medication responses, including those to risperidone, result in a wide range of responses. The initial extremes comparison allowed identification of genes whose expression was significantly different between (in this case) high and low responders. We then sought to identify expression differences that might be associated with not only high and low responders, but the range of responses in between these extremes. Correlation analyses ($\alpha < 0.001$) using the probes identified in the extremes analysis were therefore performed to detect exons whose expression demonstrated a significant, linear relationship with ABC-I (coded as a continuous variable). Although an alternative approach was to do an omnibus correlation analysis, this typically yields large numbers of genes with significant correlations but ultimately low predictive power due to insufficient difference of expression at the extremes.

We considered gene ontology, pathway overrepresentation, and genomic co-regulation using the Database for Annotation, Visualization, and Integrated Discovery (DAVID, http://niaid.abcc.ncifcrf.gov/), supplemented with manual curation to consider additional functional overlaps.

Results

42 subjects with ASD (age 112.7±51.2 mos., 33 males; 24 Caucasian, 18 other) were included in analyses. In the initial extremes analysis, there were 89 exons identified with fold change > |1.5| and $p < 0.001$ (Supplementary Table S-1). These probes successfully separated high from low responders using unsupervised hierarchical cluster analysis (Figure 1a). Of these, expression of probes within 5 annotated genes was significantly correlated with ABC-I-%CHG across all 42 risperidone treated ASD subjects: GBP6, $r = 0.78$; RABL5, $r = 0.72$; RNF213, $r = -0.73$; NFKBID, $r = 0.75$; and RNF40, $r = -0.74$ ($p < 0.001$) (Figure 1b). Pathway analysis with these probes did not yield any significant findings.

Discussion

To examine whether pre-drug gene expression was associated with change in behavioral measures in ASD, this study used pre-risperidone peripheral blood gene expression values to identify associations with pre-post risperidone change in Aberrant Behavior Checklist – Irritability subscale scores. Of the five probes with pre-risperidone expression that best correlated with risperidone response across all 42 subjects, RNF40 was notable as the E3 ubiquitin-protein ligase that targets STX1 (syntaxin 1) for degradation by the ubiquitin-proteasome pathway.

STX1, synaptobrevin, and SNAP25 together comprise the SNARE complex. The SNARE complex is required to fuse vesicles to the presynaptic active zone[18]. Polymorphisms in SNAP25 have been associated with response to antipsychotics, including risperidone, in schizophrenia[19]. Our finding that RNF40 is associated with response to risperidone is particularly intriguing because STX1, in addition to its part in the SNARE complex, regulates expression of the serotonin transporter 5-HTT[20]. Thus the action of risperidone, in part, may depend on expression of RNF40 and its downstream effects on STX1 and possibly serotonin. Additionally, RNF40 is located at 16p11.2, a chromosome region implicated in both autism and schizophrenia[21–23].

Further, both RNF40 (Figure 1b) and RNF213 showed negative pre-risperidone expression correlations with behavioral improvement. That is, for these probes, higher initial expression was associated with greater response. Both these genes have RING (Really Interesting New Gene) domains. The RING domain contains a zinc finger binding site: a $Cys_3HisCys_4$ amino acid motif that binds two zinc cations[24]. This is notable because 5′-nucleotidase, an enzyme indicator of zinc status[25], is a modulator of the response to risperidone[26]. That is, a decrease in body zinc status while taking risperidone was strongly associated with greater behavioral improvement, while an increase in body zinc status while taking risperidone was associated with less behavioral improvement[26]. Our findings provide direction for further studies considering the relationships between expression of these RING-finger genes, polymorphisms and copy number variations in these genes and the relationship between zinc status and risperidone response.

While risperidone dosage would not affect pretreatment gene expression, it may have influenced which subjects showed the most improvement in ABC-I. Since the relative magnitude of this improvement was used as a selection factor in the extremes analysis, it may have impacted selection of genes for further exploration. However, because the dosage was initiated and increased based on uniform clinical assessment, this reflects real-world clinical response. Given that the dose was titrated based on behavioral symptoms and tolerability, the relationship between gene expression and adverse effects will need further study; however, this is beyond the scope of this paper and is the subject of additional work in progress in our lab.

This study is the first to suggest that gene expression in blood is associated with and may predict the behavioral response to risperidone use in ASD. Although two prior pharmacogenetic studies that examined genetic associations with risperidone response in schizophrenia and autism[27, 28] did not identify any of the genes in these expression profiles, the expression profiles we have identified may reflect convergent downstream biological mechanisms across multiple genetic backgrounds that are associated with behavioral response to risperidone in ASD. Future studies will be needed to confirm the results of this study by evaluating the efficacy of these markers in relation to prediction of response in a large, prospective setting. In addition, it will be necessary to determine whether this profile predicts risperidone response in schizophrenia or other disorders, or when used with

alternative antipsychotics for ASD. These studies may also include plasma levels of risperidone or its metabolites to strengthen relationships identified through expression analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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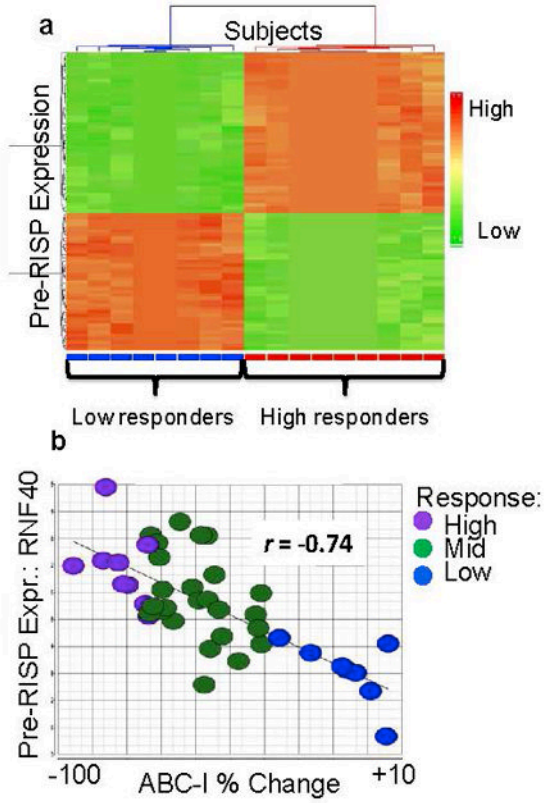

Figure 1.

(A) Unsupervised hierarchical cluster analysis of 89 probe sets expressed prior to risperidone treatment (Pre-risperidone, Y-axis) that were significantly different (ANCOVA, $p \leq 0.001$, fold change $\geq |1.5|$) between 9 high responders (red at the bottom) and 8 low responders (blue at the bottom) to risperidone using the Aberrant Behavior Checklist - Irritability (ABC-I) subscale. High expression is red and low is green. High responders had the greatest % change (decreases) in the ABC-I subscale and the low responders had the least changes in the ABC-I measured before and after 8 weeks of treatment with risperidone. (B) RNF40 gene expression prior to risperidone treatment (Pre-risperidone, Y-axis) versus the percent change in the ABC- I subscale (ABC-I % CHG, X-axis) measured before and after 8 weeks of risperidone treatment ($r = -0.74$, Pearson correlation, $p \leq 0.001$).