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

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Introduction

Children with autism spectrum disorders (ASDs) 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 before risperidone treatment were compared with pre–post risperidone change in Aberrant Behavior Checklist-Irritability (ABC-I) scores. Expression of exons within five genes was correlated with change in ABC-I scores across all risperidone-treated subjects: GBP6, RABL5, RNF213, NFKBID and RNF40 ($p < 0.001$). RNF40 is located at 16p11.2, a region implicated in autism and schizophrenia. Thus, these genes expressed before 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.

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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.2–6,11 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 lipids12,13 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. Subjects were recruited from the University of California Medical Investigation of Neurodevelopmental Disorders Institute. In addition to a DSM IV diagnostic interview and an autism diagnostic observation schedule consensus diagnosis of ASD (with use of the Autism Diagnostic Interview-Revised, if supplemental information for diagnosis required), all subjects had to have an initial Aberrant Behavior Checklist-Irritability (ABC-I) subscale rating ≥ 18 (mean 25 ± 6.7).

Exclusion criteria included bipolar disorder, schizophrenia, ASD of known genetic cause, non-verbal intelligence quotient < 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-2 months before initial screening and remained constant for the 8-week study duration. Subjects agreed not to start any new pharmacological, 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.5,9 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 (Affymetrix, Santa Clara, CA, USA) 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, St Louis, MO, USA). Probe summarization and probe set normalization were performed using robust multichip average, which included background correction, quantile normalization, log2 transformation and median polish probe set summarization.

All analyses used exon expression levels in blood before 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%; nine subjects), or low responders (ABC-I-%CHG = −29% to +6%; eight subjects). Between-group gene expression profiles were compared for high versus low responders using analysis of covariance, controlling for the effects of age, gender and batch (z < 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 (z < 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 because of 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 (http://niaid.abcc.ncifcrf.gov/), supplemented with manual curation to consider additional functional overlaps.
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Results

A total of 42 subjects with ASD (age 112.7 ± 51.2 months, 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 five annotated genes was significantly correlated with ABC-I-% CHG across all 42 risperidone treated ASD subjects: GBP6, r = 0.78; RABLS, r = 0.72; RNF213, r = −0.73; NFKB1D, 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 ABC-I 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 syntaxin 1 for degradation by the ubiquitin–proteasome pathway.

Syntaxin 1, synaptobrevin and SNAP25 together comprise the SNARE complex. The SNARE complex is required to fuse vesicles to the presynaptic active zone. Polymorphisms in SNAP25 have been associated with response to antipsychotics, including risperidone, in schizophrenia. Our finding that RNF40 is associated with response to risperidone is particularly intriguing because syntaxin 1, in addition to its part in the SNARE complex, regulates expression of the serotonin transporter 5-HTT. Thus, the action of risperidone, in part, may depend on expression of RNF40 and its downstream effects on syntaxin 1 and possibly serotonin. In addition, RNF40 is located at 16p11.2, a chromosome region implicated in both autism and schizophrenia.

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(His)Cys4 amino acid motif that binds two zinc cations. This is notable because S'-nucleotidase, an enzyme indicator of zinc status, is a modulator of the response to risperidone. That is, a decrease in body zinc status while taking risperidone was strongly associated with greater behavioral improvement, whereas an increase in body zinc status while taking risperidone was associated with less behavioral improvement. 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.

Although risperidone dosage would not affect pretreatment gene expression, it may have influenced which subjects showed the most improvement in ABC-I. As 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 laboratory.

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 previous pharmacogenetic studies that examined genetic associations with risperidone response in schizophrenia and autism 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.

Conflict of interest

The authors declare no conflict of interest.

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References

1. des Portes V, Hagerman RJ, Hendren RL. Pharmacotherapy. In: Ozonoff S, Rogers SJ, Hendren RL (eds). Autism Spectrum Disorders: A Research Review for Practitioners. American Psychiatric Association Publishing: Washington, DC, 2003, pp 161–186.
2. Williams SK, Scabill L, Vitiello B, Aman MG, Arnold LE, McDougle CJ et al. Risperidone and adaptive behavior in children with autism. J Am Acad Child Adolesc Psychiatry 2006; 45: 431–439.
3. Bantick RA, Deakin JF, Grasby PM. The 5-HT1A receptor in schizophrenia: a promising target for novel atypical neuroleptics? J Psychopharmacol 2001; 15: 37–46.
4. Ichikawa J, Meltzer HY. Relationship between dopaminergic and serotonergic neuronal activity in the frontal cortex and the action of typical and atypical antipsychotic drugs. Eur Arch Psychiatry Clin Neurosci 1999; 249(Suppl 4): 90–98.
5. Swann AC. Neuroreceptor mechanisms of aggression and its treatment. J Clin Psychiatry 2003; 64(Suppl 4): 26–35.
6. Tarazi FJ, Zhang K, Baldessarini RJ. Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: implications for antipsychotic drug treatment. J Pharmacol Exp Ther 2001; 297: 711–717.
7. McDougle CJ, Holloway J, Scabill L, Koenig K, Aman MG, McGough JJ et al. Risperidone for the core symptom domains of autism: results from the study by the autism network of the research units on pediatric psychopharmacology. Am J Psychiatry 2005; 162: 1142–1148.
8. McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG et al. Risperidone in children with autism and serious behavioral problems. N Engl J Med 2002; 347: 314–321.
9. Shea S, Turgay A, Carroll A, Schulz M, Orlik H, Smith I et al. Risperidone in the treatment of disruptive behavioral symptoms in children with autistic and other pervasive developmental disorders. Pediatrics 2004; 114: E634–E641.
10. Pandina GJ, Bossie CA, Zhu Y, Flanders S. The aberrant behavior checklist: use in clinical trials of pediatric autism. J Child Adolescent Psychopharmacol 2006; 16: 661–662.
11. Posey DJ, McDougle CJ. The pharmacotherapy of target symptoms associated with autistic disorder and other pervasive developmental disorders. Harv Rev Psychiatry 2000; 8: 45–63.
12. Su KP, Wu PL, Pariante CM. A cross-over study on safety of lipid profiles associated with olanzapine and risperidone. Eur Neuropsychopharmacol 2005; 15: S463–S464.
13. Danielyan A, Kovatch RA. Management options for bipolar disorder in children and adolescents. Paediatr Drugs 2005; 7: 277–294.
14. Bottai T, Quintin P, Perrin E. Antipsychotics and the risk of diabetes: a general data review. Eur Psychiatry 2005; 20(Suppl 4): S349–S357.
15. McKee JR, Bodfish JW, Mahoney SL, Heeth WL, Ball MP. Metabolic effects associated with atypical antipsychotic treatment in the developmentally disabled. J Clin Psychiatry 2003; 66: 1161–1168.
16. Erickson CA, Stigler KA, Posey DJ, McDougle CJ. Risperidone in pervasive developmental disorders. Expert Rev Neurother 2005; 5: 713–719.
17. Stamova B, Xu H, Jickling G, Bushnell C, Tian Y, Ander BP et al. Gene expression profiling of blood for the prediction of ischemic stroke. Stroke 2010; 41: 2171–2177.
18. Sollner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 1993; 75: 409–418.
19. Muller DJ, Klempan TA, De Luca V, Sicard T, Volavka J, Czobor P et al. The SNAP-25 gene may be associated with clinical response and weight gain in antipsychotic treatment of schizophrenia. Neuropsi Lett 2005; 379: 81–89.
20. Haase J, Killian AM, Magnani F, Williams C. Regulation of the serotonin transporter by interacting proteins. Biochem Soc Trans 2001; 29(Part 6): 722–728.
21. Hanson E, Nasir RH, Fong A, Lian A, Hundley R, Shen Y et al. Cognitive and behavioral characterization of 16p11.2 deletion syndrome. J Dev Behav Pediatr 2010; 31: 649–657.
22. Shen Y, Dies KA, Holm IA, Bridgemohan C, Sobeih MM, Caronna EB et al. Clinical genetic testing for patients with autism spectrum disorders. Pediatrics 2010; 125: e727–e735.
23. Vassos E, Collier DA, Holden S, Patch C, Rujescu D, St Clair D et al. Penetrance for copy number variants associated with schizophrenia. Hum Mol Genet 2010; 19: 3477–3481.
24. Borden KL, Freemont PS. The RING finger domain: a recent example of a sequence-structure family. Curr Opin Struct Biol 1996; 6: 395–401.
25. Sunderman Jr FW. The clinical biochemistry of S-nucleotidase. Ann Clin Lab Sci 1990; 20: 123–139.
26. Arnold LE, Farmer C, Kraemer HC, Davies M, Witwer A, Chuang S et al. Moderators, mediators, and other predictors of risperidone response in children with autistic disorder and irritability. J Child Adolesc Psychopharmacol 2010; 20: 83–93.
27. Correia CT, Almeida JP, Santos PE, Sequeira AF, Marques CE, Miguel TS et al. Pharmacogenetics of risperidone therapy in autism: association analysis of eight candidate genes with drug efficacy and adverse drug reactions. Pharmacogenomics J 2010; 10: 418–430.
28. Ikeda M, Tomita Y, Mouri A, Koga M, Okochi T, Yoshimura R et al. Identification of novel candidate genes for treatment response to risperidone and susceptibility for schizophrenia: integrated analyses among pharmacogenomics, mouse expression, and genetic case-control association approaches. Biol Psychiatry 2010; 67: 263–269.

Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)