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Gene expression changes in children with autism

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Abstract

The objective of this study was to identify gene expression differences in blood differences in children with autism (AU) and autism spectrum disorder (ASD) compared to general population controls. Transcriptional profiles were compared with age- and gender-matched, typically developing children from the general population (GP). The AU group was subdivided based on a history of developmental regression (A–R) or a history of early onset (A–E without regression). Total RNA from blood was processed on human Affymetrix microarrays. Thirty-five children with AU (17 with early onset autism and 18 with autism with regression) and 14 ASD children (who did not meet criteria for AU) were compared to 12 GP children. Unpaired *t* tests (corrected for multiple comparisons with a false discovery rate of 0.05) detected a number of genes that were regulated more than 1.5-fold for AU versus GP ($n=55$ genes), for A–E versus GP ($n=140$ genes), for A–R versus GP ($n=20$ genes), and for A–R versus A–E ($n=494$ genes). No genes were significantly regulated for ASD versus GP. There were 11 genes shared between the comparisons of all autism subgroups to GP (AU, A–E, and A–R versus GP) and these genes were all expressed in natural killer cells and many belonged to the KEGG natural killer cytotoxicity pathway ($p=0.02$). A subset of these genes ($n=7$) was tested with qRT-PCR and all genes were found to be differentially expressed ($p<0.05$). We conclude that the gene expression data support emerging evidence for abnormalities in peripheral blood leukocytes in autism that could represent a genetic and/or environmental predisposition to the disorder.

Keywords: Autism; Gene expression; NK cells; Microaarays

Autism is a severe neurodevelopmental disorder characterized by social and communication deficits and ritualistic or repetitive behaviors that appear by age 3. Many etiologies have been suggested for this complex syndrome. Though it is associated with a high degree of heritability, specific genes have yet to be associated with autism. Various other factors have been implicated, including immunological [1–6], neurological [7–9], and environmental [10–13].

Several genomic scans utilizing different cohorts have been performed to identify genes associated with autism or potential susceptibility regions in the genome [14]. These studies have yielded few definitive and reproducible gene associations, in part due to the heterogeneity of the syndrome. It is becoming clear that autism is a complex disorder resulting from the collective actions of multiple genes [15]. Recently, several groups have proposed segregating subjects with autism into subtypes or endophenotypes

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that have measured heritable biological traits, to obtain more homogeneous study populations. This approach has shown promise in identifying significant linkage on chromosome 7 using age of first word as an autism endophenotype [16,17]. Despite these extensive efforts, definitive genetic association or genes that contribute to autism susceptibility have yet to be identified, suggesting the need for an alternative strategy [18].

Genomics, the comprehensive study of genes and their functions, offers an alternative approach to studying autism because of the ability to study global changes in gene expression. To date, the application of microarray technology to neurological diseases has been limited. In a study of autism, postmortem brain tissue from individuals with autism was utilized to show that the glutamate neurotransmitter system is altered in patients with autism [19]. However, the availability of postmortem brain tissues is limited, and the quality of RNA from these tissues can be poor and can potentially erode the robustness of this approach. Therefore, the genomic profiling of peripheral blood cells has emerged as an alternative that is used as a proxy to represent the neural transcriptome.

Gene expression profiling of white blood cells using microarrays has been applied to malignant and immune disorders, including leukemia, lymphoma, systemic lupus erythematosis, rheumatoid arthritis, and many others [20–25]. These studies have helped to identify important diagnostic and prognostic markers as well as potential therapeutic targets. Proof-of-principle blood genomic studies of neurological diseases have been performed in animals [26]. Subsequent studies have demonstrated characteristic blood genomic patterns for acute ischemic stroke [26–28], migraine headache [28,29], Tourette syndrome [28,30], renal cell carcinoma [31], multiple sclerosis [32], benzene exposure [33], trauma [34], and neurogenetic disorders including neurofibromatosis type I, tuberous sclerosis type II, and Down syndrome [35,36]. This approach has been applied to bipolar disorder, for which XBP1 was identified as a genetic risk factor using gene expression profiling of lymphoblastoid cell lines from two sets of discordant twins [37]. These data provide significant support that gene expression analysis derived from circulating blood cells can be utilized to diagnose genetic disorders, identify the genetic and environmental components of the disorders, and help understand the underlying biology.

With regard to autism, studies utilizing cell lines derived from children with autism have shown the potential of this approach. In one study, monozygotic twins with autism of disparate severities were analyzed with gene expression profiling and found to harbor gene expression differences that correlated with the severity of autism and language impairment [38]. In a second report, genomic studies of lymphoblastoid cells differentiated children with autism from typically developing children based upon differences in expression of dopamine- and serotonin-related genes [39]. Further, gene expression profiling differentiated cell lines derived from children with autism and isodicentric chromosome 15 abnormalities from cell lines derived from typically developing children [40]. These data strongly support the idea that genomic profiling of cells in peripheral blood could be particularly promising for providing mechanistic insights and surrogate markers in autism.

Here we report for the first time using genomic profiling of whole blood several novel observations. (1) There are gene expression differences between children with autism and typically developing children. (2) There are gene expression differences between subtypes of autism: autism with regression and early onset autism without regression. (3) A small group of genes expressed predominantly in natural killer (NK) cells is associated with autism.

Results

Microarray data

Genes were identified as being significantly regulated between each group and subgroup using the following criteria: the unpaired t tests corrected for multiple comparisons had a false discovery rate (FDR) of 0.05 (5% false positives) or better; and the fold changes in the regulated genes were 1.5 or more. The combination of the FDR and fold change was used to help ensure the identified genes were likely to be biologically significant. The data sets were then stratified according to diagnostic groups (AU, autism; A-E, early onset autism; A-R, autism with regression; ASD, autism spectrum disorder; and GP, general population). The AU group contained both the A-E and the A-R groups. For the ASD group compared to the AU group and GP group, no genes were identified at the $p < 0.05$ level, respectively. Therefore these ASD comparisons were not utilized for further analysis.

A significant number of genes were identified in the AU groups compared to the GP group. In the AU group compared to the GP group, 55 genes were identified as differentially expressed (unpaired t test, FDR $q < 0.05$, fold change > 1.5) (Supplemental Table 1). For the comparison of A-E to GP group, 140 genes were identified as differentially expressed (Supplemental Table 2). In the A–R group, 20 genes were identified as differentially expressed in comparison to GP (Supplemental Table 3). A comparison of the genes regulated in the A-E and A-R subgroups showed 494 genes (Supplemental Table 4).

In Fig. 1, the three gene lists from the above analysis (AU vs GP, A-E vs GP, and A-R vs GP) are compared to identify a small group of genes shared among the three groups. A total of 12 probe

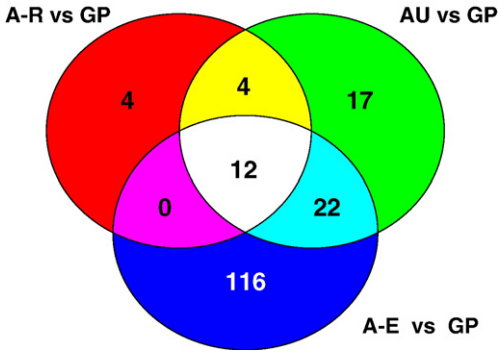

Fig. 1. Venn diagram. Numbers of probe sets significantly regulated for comparisons of the three autism subgroups versus GP (unpaired t test, FDR > 0.05, and fold change > 1.5). This diagram shows the overlap in the numbers of genes for these three comparisons. There were 12 probe sets representing 11 genes for the comparisons of AU vs GP, A-R vs GP, and A-E vs GP.