Available online at www.sciencedirect.com
ScienceDirect
NeuroToxicology 27 (2006) 671–684
NeuroToxicology

Review

# Autism and environmental genomics

M.R. Herbert [a,*], J.P. Russo [b], S. Yang [c], J. Roohi [d], M. Blaxill [e], S.G. Kahler [f], L. Cremer [g], E. Hatchwell [d]

[a] *Pediatric Neurology, Massachusetts General Hospital, Harvard Medical School, USA*
[b] *Computer Science and Systems, Wentworth Institute of Technology, USA*
[c] *Bioinformatics, Boston University, USA*
[d] *Cold Spring Harbor Laboratory, USA*
[e] *Safe Minds, USA*
[f] *Department of Pediatrics, University of Arkansas for Medical Sciences, USA*
[g] *Neurology, Massachusetts General Hospital, USA*

Received 15 January 2006; accepted 21 March 2006
Available online 28 March 2006

**Abstract**

Autism spectrum disorders (ASD) are defined by behavior and diagnosed by clinical history and observation but have no biomarkers and are presumably, etiologically and biologically heterogeneous. Given brain abnormalities and high monozygotic concordance, ASDs have been framed as neurobiologically based and highly genetic, which has shaped the research agenda and in particular criteria for choosing candidate ASD genes. Genetic studies to date have not uncovered genes of strong effect, but a move toward ''genetic complexity'' at the neurobiological level may not suffice, as evidence of systemic abnormalities (e.g. gastrointestinal and immune), increasing rates and less than 100% monozygotic concordance support a more inclusive reframing of autism as a multisystem disorder with genetic influence and environmental contributors. We review this evidence and also use a bioinformatic approach to explore the possibility that ''environmentally responsive genes'' not specifically associated with the nervous system, but potentially associated with systemic changes in autism, have not hitherto received sufficient attention in autism genetics investigations. We overlapped genes from NIEHS Environmental Genome Project, the Comparative Toxicogenomics Database, and the SeattleSNPs database of genes relevant to the human immune and inflammatory response with linkage regions identified in published autism genome scans. We identified 135 genes in overlap regions, of which 56 had never previously been studied in relation to autism and 47 had functional SNPs (in coding regions). Both our review and the bioinformatics exercise support the expansion of criteria for evaluating the relevance of genes to autism risk to include genes related to systemic impact and environmental responsiveness. This review also suggests the utility of environmental genomic resources in highlighting the potential relevance of particular genes within linkage regions. Environmental responsiveness and systems impacts consistent with system-wide findings in autism are thus supported as important considerations in identifying the numerous and complex modes of gene-environment interaction in autism.


*Keywords:* Autism; Genetics; Environmental genomics; Toxicogenomics; Systems biology; Bioinformatics; Genomics; Immune

**Contents**



* Corresponding author at: Pediatric Neurology/Center for Morphometric Analysis, Massachusetts General Hospital, 149 13th Street, Room 6012, Charlestown, MA 02129, USA. Tel.: +1 617 724 5920/617 726 2066 (page); fax: +1 617 812 6334.
*E-mail address:* mherbert1@partners.org (M.R. Herbert).

0161-813X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2006.03.017

## 1. Introduction

Autism is a behaviorally defined syndrome that is diagnosed on the basis of clinical history (American Psychiatric Association, 1994) but for which there are no known biomarkers. Diagnostic criteria include presence before the age of three of language impairment, social reciprocity deficits and a tendency to engage in repetitive or ritualistic behavior—to manifest a desire for sameness (American Psychiatric Association, 1994). Autism has been considered a brain-based, highly genetic disorder, and has often been presumed to be based upon abnormal brain developmental events in utero. Nervous system malfunction certainly underlies autism's defining core behavioral features. A strong genetic contribution is suggested by a much higher concordance among monozygotic than dizygotic twins as well as a recurrence rate within families many times higher than the background rate in the population at large (Santangelo and Tsatsanis, 2005). A prenatal onset has been inferred from certain neuroanatomical findings in postmortem brain samples (Kemper and Bauman, 1998; Rodier et al., 1996), and has been considered consistent with the strongly genetic nature of the disorder. At the current time, however, although there are suggestive findings in genetics and neurobiology, the syndrome has many features that are not well described or understood (Gillberg and Coleman, 2000), and we do not yet have a firm grasp on the underlying mechanisms or developmental processes driving the autism phenotype. Moreover, autism is considered to be heterogeneous etiologically, biologically and phenotypically: it is known that the autism syndrome is commonly found in a number of biologically distinct genetic syndromes such as Fragile X and tuberous sclerosis, and it is presumed that ''idiopathic'' autism (comprising 85–95% of autism cases) is substantially heterogeneous as well.

From the vantage point of genetics, some researchers have moved toward implementing emerging strategies for identifying genetic contributors to complex disorders (Tabor et al., 2002; Veenstra-Vanderweele et al., 2004). However, many epidemiological and pathophysiological considerations suggest that the formulation of autism centered upon genetic, neurobiological and prenatal timing factors should be expanded to encompass additional features including systemic abnormalities and multisystem involvement as well as environmental and postnatal influences.

### 1.1. Epidemiology—increasing rates

Epidemiological studies of autism are revealing much higher rates in recent years than those reported prior to 1990. Several decades ago autism was considered a rare disorder occurring in 3–4/10,000 individuals, while current rate estimates range between 1/500 and 1/166 (Blaxill, 2004b; Byrd et al., 2002; Fombonne, 2003; Newschaffer et al., 2005; Scott et al., 2002; Yeargin-Allsopp et al., 2003). While it has been suggested that this increase may be largely due to increased awareness or what has been called ''diagnostic substitution'' (Blaxill, 2004a; Croen et al., 2002; Gurney et al., 2003), and while these numbers are therefore not universally considered to be reflections of true increases, arguments for the increases being artifactual have not been definitively substantiated, and doubt has been expressed in a number of publications that apparent increases can be fully explained away (Croen, 2003; Newschaffer et al., 2005; Rutter, 2005). Epidemiological studies have not to date supported the identification of any one environmental factor as causal (Lawler et al., 2004), but there is evidence of environmental influence (Chess, 1971, 1977; Ingram et al., 2000; Palmer et al., 2005; Stromland et al., 1994; Williams et al., 2001), while selective vulnerability (Hornig et al., 2004; Pletnikov et al., 2002) and the combined, synergistic or parallel effects of multiple environmental factors have not been systematically evaluated or excluded (Kreiling et al., 2005).

### 1.2. Clinical history—postnatal changes

Although reports of autistic ''regression'' are scattered through the literature, in recent years retrospective and now prospective studies have confirmed the existence of a subgroup that loses previously acquired milestones in language, social and behavioral development and develops the autistic behavioral profile – typically between the first and second birthday – after presenting as essentially normal in at least some cases, or at worst as minimally impaired (Lord et al., 2004; Luyster et al., 2005; Richler et al., in press; Werner and Dawson, 2005). Various trajectories of worsening abnormality are potentially consistent with gene–environment interaction and not just with the unfolding of innate programming. In addition, a growing number of studies have documented unusually rapid enlargement of head size after birth, with the postnatal development of frank macrocephaly in as many as 20% of individuals with autism during early childhood (Courchesne et al., 2003; Dementieva et al., 2005; Herbert, 2005b; Lainhart et al., 1997; Redcay and Courchesne, 2005).

### 1.3. Clinical history—multisystem involvement

Although autism's diagnostic criteria are strictly behavioral, there are a variety of comorbidities that in many cases

accompany the behavioral features. While some of these comorbidities involve the central nervous system (e.g. epilepsy, sleep and anxiety disorders), others (such as recurrent infection and a range of gastrointestinal disturbances) appear to be rooted elsewhere in the organism (Ashwood and Van de Water, 2004a; Herbert, 2005a; Horvath and Perman, 2002; Jass, 2005; Jyonouchi et al., 2005; Torrente et al., 2002; Torres et al., 2001).

### 1.4. Pathophysiology—features of chronic illness

Although autism has been considered a static encephalopathy whose abnormalities are based upon hard-wired structural or genetically based neurochemical changes, indicators of chronic neuroinflammation and oxidative stress have been identified in autistic brains (Perry et al., 2005; Vargas et al., 2005), and markers of inflammation and oxidative stress have also been identified in blood and urine of autistic subjects (Ashwood and Van de Water, 2004a,b; Chauhan et al., 2004; Dalton et al., 2003; Herbert, 2005a; James et al., 2004; Ming et al., 2005). Such changes are indicative of ongoing disease not previously suspected, suggesting that autism may be at least in some cases a component of a chronic illness with metabolic encephalopathy either in addition to or instead of inborn and "hard-wired" changes. Moreover there is often substantial *intra*-individual variability to the nature and severity of the features that occurs over short as well as medium and long time intervals; this has not been systematically investigated, but may point to metabolic or environmental modulators of function that are labile, and some that may even be related to treatment targets.

### 1.5. Evidence of improvement and recovery

Not all children diagnosed with autism retain that diagnosis throughout their lives. In some cases the improvement or recovery may be spontaneous, while in others it appears to be associated with treatments, that could include behavioral or biomedical interventions, either alone or in combination. Autism improvement and recovery are poorly studied, much like autistic regression was poorly studied until recently, although there are some academic articles (Dawson and Zanolli, 2003; Fein et al., 2005; Mundy and Crowson, 1997; Kelley et al., in press) in addition to abundant anecdotal reports (Edelson, 2003). Some recovery case reports credit treatment of underlying or accompanying metabolic derangements including inborn errors of metabolism. One obstacle to investigation of such phenomena has been the belief that autism is incurable, so that individuals who improve or recover "did not really have autism." However there is nothing in the definition of autism that constrains either pathophysiology or disease course. Phenomena of improvement or recovery are arguably plausible when contributions of brain plasticity, environmental insult and presence of features of chronic disease – all of which involve changeability – are taken into account. Moreover, the means by which treatment response and recovery are achieved may point toward environmentally responsive disease mechanisms, where the response to "environment" may include improvement as well as deterioration.

Each of these more recently recognized features of autism gives grounds for an expansion of the formulation of the disorder in the direction of a multisystem condition deriving from gene–environment interactions and epigenetic processes (Herbert, 2005a).

- Although increasing rates have been attributed to greater awareness, environmental factors cannot be excluded.
- Although postnatal changes have been construed as the unfolding of events with much earlier onset, they could also be a consequence of postnatal or ongoing cumulative influences.
- Although multisystem comorbidities and chronic disease features have been regarded as coincidental and unrelated to the autism diagnosis, they could also be manifestations of common underlying mechanisms.

From this vantage point, genetic contributors to autism may be associated with a broader array of mechanisms beyond those directly implicating the central nervous system. In particular, they may confer increased vulnerability to environmental factors, and they involve other organ systems such as the immune or gastrointestinal systems, two systems that are on the frontlines of the organism's interaction with the environment. Overall, this expanded conception of the nature of and potential contributors to autism provides a basis for responding to the medical and public health challenges posed by autism.

Several published studies point toward genetic contributions to mechanisms that may increase susceptibility to environmental triggers for autism. These include an association of abnormal thiol metabolism (important in metabolism of xenobiotics) with an increase in frequency of some common polymorphisms in related pathways that confer an increased vulnerability to oxidative stress (James et al., 2005, 2006), paraoxonase gene variants associated with autism sample cohorts in the United States (but not in Italy) conferring vulnerability in the setting of in utero organophosphate exposure to neuronal migration disorders associated with Reelin (D'Amelio et al., 2005), and atypical patterns of transmission for some metal metabolism genes in autism (Serajee et al., 2004). In animal models relevant to autism, altered genetic background regarding immune vulnerability has been shown to impact susceptibility to environmental perturbation (Hornig et al., 2004; Pletnikov et al., 2002). In addition, the MeCP2 gene, associated with Rett syndrome, has broader relevance than the central nervous system; and methylation, one of its functions, appears to be a modulator of gene–environment interactions (Abdolmaleky et al., 2004). An increased excitation/inhibition ratio, that could contribute to the "cortical noise" that may be causative at the brain level in autism, has been proposed to underlie many varieties of autism (Rubenstein and Merzenich, 2003). This increased ratio could be a consequence of various combinations of genetic and environmental factors. For example, impaired GABA functioning (less inhibition) (Ma et al., 2005), an mGluR receptor

abnormality (more excitation) (Bear et al., 2004)) or a neuroligin mutation (more excitation) (Cline, 2005) in combination with exposure to excitatory neurotoxins such as PCBs (Gafni et al., 2004; Kenet et al., 2005; Kim and Pessah, 2004) or mercury (Albrecht and Matyja, 1996; Juarez et al., 2002) would increase this ratio (Mutkus et al., 2005). These studies suggest that gene–environment interactions and genes with systemic functions are both relevant to autism.

For the most part, autism genome scans have limited their choice of candidate genes to those with direct central nervous system relevance, such as serotonin transporter and GABA receptor genes (Muhle et al., 2004; Santangelo and Tsatsanis, 2005; Yonan et al., 2003). But if brain structure or function changes could occur in parallel with other more systemic impacts, or if brain changes could occur downstream of other disease features so that impact on the brain was secondary, this would mean that many potentially relevant genes may have hitherto received insufficient attention. A careful characterization of molecular, cellular and physiological levels of the autism phenotype is probably the optimal guide to identifying genetic contributors to autism in various subgroups, and these processes and their biomarkers need much greater attention in the overall autism research program. This is because these levels in the hierarchy of organismic functioning are more proximal to gene functions than are behaviors. However, environmental genomics and bioinformatics may also have a useful contribution to make in expanding the scope of autism genetic investigations, although findings derived from these tools require further laboratory and clinical evaluation.

Several gene databases have been developed that are directly oriented toward identifying genes with relevance to environmental responsiveness. Variability in responsiveness to environmental influences may be related to disease vulnerability. In one such database, the Environmental Genome Project (Kaiser, 1997; NIEHS, 2004; Olden et al., 2001; Wilson and Olden, 2004), such environmentally relevant variability was sought in genes with functions related to cell cycle, cell division, cell signaling, cell structure, DNA repair, gene expression, homestasis, metabolism, immune and inflammatory response, hormone metabolism, nutrition, oxidative metabolism and stress, membrane pumps and/or drug resistance, and signal transduction (Olden and Wilson, 2000). Further databases addressing environmental genomics include the Comparative Toxicogenomics Database (CTD) (Mattingly et al., 2003, 2004; Waters et al., 2003) and the SeattleSNPs database (oriented toward identifying SNPS in candidate genes and pathways underlying the human inflammatory response) (Program for Genomic Applications, 2006). Genes associated with responses to environmental factors including toxins and pro-inflammatory agents may well be important in autism, given phenotypic and epidemiological features reviewed above. Because these environmentally relevant bioinformatic resources have apparently not been used to inform autism genetics or genomics research to date, we supported our review by investigating how many genes from these databases that fall within previously identified autism linkage regions and that could have relevance for autism have hitherto been neglected in autism genetics research.

## 2. Methods

Genes from three environmentally relevant genomic databases were identified that overlapped with autism linkage regions, and neighboring genes also in autism linkage regions were also identified. The sequence of steps in our method is detailed below and illustrated in Fig. 1.

(1) An Excel spreadsheet was prepared containing data from all autism genome scans to date (Table 1). Autism linkage regions contained approximately 5300 genes.
(2) Utilizing MySQL, overlap was established between autism linkage regions and genes in the following web-based environmentally related genomic databases:

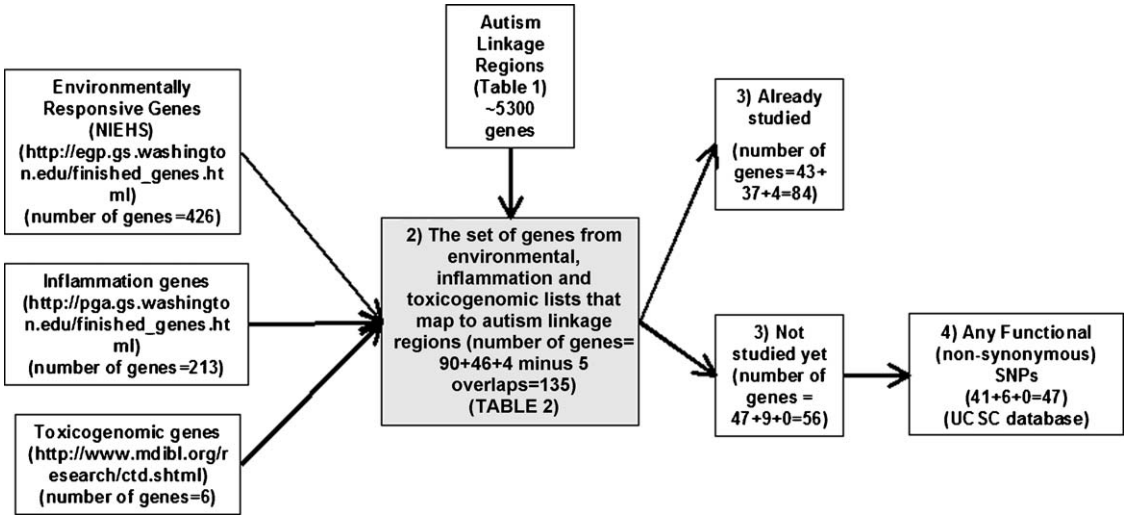


Fig. 1. Bioinformatics methodology. The flow chart illustrates the steps discussed in Section 2 by which we identified overlaps between three environmentally relevant genomics databases and autism genetic linkage regions. Indicated within the flowchart are the steps in the process that generated Tables 1 and 2.

Table 1
Autism genetic linkage regions

| Chromosome | Position | Peak position (centimorgans) | Physical location (Mb) | Marker (at or near) | Scan group | MLS (multipoint LOD score) | Sib pair families | Summary score |
|---|---|---|---|---|---|---|---|---|
| 1 | 1p13 | 149 | 113 | D1S1675 | Risch/Stanford | 2.15 | 90 | 2.15 |
| | 1p13 | 149 | | D1S1675 | Auranen | 2.63+? | 28 | 2.63? |
| | 1q21–22 | 164 | 154 | D1S1653 | Auranen | 2.63 | 19 | 2.63 |
| | 1q | 274 | | D1S2842 | Buxbaum/Seaver | 1.17 | 35 | 1.17 |
| 2 | 2p | 8 | | D2S319 | Buxbaum/Seaver | 1.2 | 35 | 1.2 |
| | 2p12 | 111 | 76 | D2S1351 | IMGSAC-2 | 1.6 | 152 | 1.6 |
| | 2q | 198 | | D2S116 | Shao/CAT | 1.3 | 99 | 1.3 |
| | 2q31 | 206 | 175 | D2S2188 | IMGSAC-2 | 3.74 | 152 | 3.74 |
| | 2q31 | 186 | 183 | D2S364 | Buxbaum/Seaver | 1.96 | 95 | 1.96 |
| 3 | 3p25 | 32 | 11 | D3S3680 | Shao/CAT | 1.51 | 99 | 1.51 |
| | 3q26 | 191 | 180 | D3S3715/D3S3037 | Auranen | 4.81 | 28 | 4.81 |
| 4 | 4p16 | 4.8 | 3.5 | D4S412 | IMGSAC-1 | 1.55 | 99 | 1.55 |
| | 4q21 | 94 | 85 | D4S2361/D4S2909 | Yonan/AGRE | 1.72 | 345 | 1.72 |
| 5 | 5p | 12 | | D5S406 | Buxbaum/Seaver | 1.21 | 345 | 1.21 |
| | 5p13 | 58 | 40 | D5S2494 | Yonan/AGRE | 2.54 | 345 | 2.54 |
| | 5p | 59 | | D5S2494 | Liu/AGRE | 2.01 | 110 | 2.01 |
| 6 | 6q13 | 132.8 | 70 | D6S283 | Philippe/PARIS | 2.23 | 51 | 2.23 |
| 7 | 7p | NA | | D7S2564 | Risch/Stanford | 1.01 | 90 | 1.01 |
| | 7q21 | 104 | 91 | D7S1813 | Barrett/CLSA | 1.6 | 75 | 1.6 |
| | 7q22 | 119.6 | 100 | D7S477 | IMGSAC-2 | 3.2 | 152 | 3.2 |
| | 7q | 123 | | D7S523 | Liu/AGRE | 0.4 | 160 | 1.02 |
| | 7q31–35 | (near 128) | | D7S2527/640 | Ashley-Koch | 2.01 | 76 | 2.01 |
| | 7q | 128 | | D7S495 | Shao/CAT | 1.66 | 99 | 1.66 |
| | 7q32 | 144.7 | 128 | D7S530 | IMGSAC-1 | 2.53 | 99 | 2.53 |
| | 7q | 165 | | D7S483 | Liu/AGRE | 1.5 | 160 | 2.13 |
| | 7q36 | 170 | 153 | D7S1824 | Auranen | 3.66? | 36 | 3.66? |
| 8 | 8 | 39 | | D8S261 | IMGSAC-2 | 1.12 | 152 | 1.12 |
| | 8q24 | 132 | 125 | D8S1832 | Yonan/AGRE | 1.5 | 345 | 1.6 |
| | 8q | 134 | | D8S1179 | Liu/AGRE | 1.71 | 110 | 1.71 |
| 9 | 9 | 141.87 | | D9S1826 | IMGSAC-2 | 1.46 | 152 | 1.46 |
| | 9 | 162 | | D9S158 | Buxbaum/Seaver | 1.66 | 35 | 1.66 |
| 10 | 10 | 51.9 | | D10S197 | IMGSAC-1 | 1.36 | 99 | 1.36 |
| | 10 | 53.66 | | D10S197 | IMGSAC-2 | 1.08 | 152 | 1.08 |
| | 10 | 64.3 | | D10S208 | IMGSAC-2 | 1.43 | 152 | 1.43 |
| | 10 | 116.6 | | D10S201 | IMGSAC-2 | 1.22 | 152 | 1.22 |
| 11 | 11p13 | 46 | 34 | D11S1392/D11S1993 | Yonan/AGRE | 2.24 | 345 | 2.24 |
| 12 | | | | | | | | |
| 13 | 13q12 | 19 | 30 | D13S217/12229 | Barrett/CLSA | 2.3 | 75 | 2.3 |
| | 13q22 | 55 | 73 | D13S800 | Barrett/CLSA | 2.3 | 75 | 2.3 |
| 14 | | | | | | | | |
| 15 | 15q | 41.1 | | D15S118 | Philippe/PARIS | 1.1 | 51 | 1.1 |
| | 15q | 43 | | D15S269 | Shao/CAT | 0.96 | 99 | 0.96 |
| 16 | 16p13 | 17.3 | 10 | D16S407 | IMGSAC-1 | 1.51 | 99 | 1.51 |
| | 16p13 | 23 | 12 | D16S3102 | IMGSAC-2 | 2.93 | 152 | 2.93 |
| | 16p | 28 | | D16S2619 | Liu/AGRE | [∼1.5] | 110 | [1.91] |
| 17 | 17p | NA | | D17S1876 | Risch/Stanford | 1.21 | 90 | 1.21 |
| | 17q11 | 45.37 | 28 | HTTINT2 | IMGSAC-2 | 2.34 | 152 | 2.34 |
| 18 | 17q11 | 52 | 29 | D17S1800 | Yonan/AGRE | 2.83 | 345 | 2.83 |
| | 18q | | | D18S878 | Risch/Stanford | 1 | 90 | 1 |
| 19 | 19p | 24.1 | | D19S226 | Philippe/PARIS | 1.37 | 51 | 1.37 |
| | 19p | 48 | | D19S49 | IMGSAC-1 | 0.99 | 99 | 1.11 |
| | 19p | 52/[35] | | D19S433 | Liu/AGRE | [3.36] | 110 | [3.36] |
| 20 | 19 | 59 | | D19S425 | Shao/CAT | 1.21 | 99 | 1.21 |
| 21 | | | | | | | | |
| 22 | 22 | 5 | | D22S264 | IMGSAC-1 | 1.39 | 99 | 1.39 |
| X | Xq21 | 60 | 94 | DXS6789 | Shao/CAT | 2.54 | 99 | 2.54 |
| | Xq | 82/[139] | | DXS1047 | Liu/AGRE | [2.27] | 110 | [2.27] |

Linkage regions identified in genome scans of autism thus far are shown, including references to studies. Genes from environmentally relevant genomics databases were overlapped onto these linkage regions.

(a) Environmental Genome Project: http://egp.gs.washington.edu/finished_genes.html
(b) Inflammatory genes http://pga.gs.washington.edu/finished_genes.html
(c) Toxicogenomics http://www.mdibl.org/research/ctd.shtml

(3) Pubmatrix (http://pubmatrix.grc.nia.nih.gov/) was used to identify genes that had not been previously studied in relation to autism.
(4) The Golden pathway database (http://genome.ucsc.edu/index.html) was used to identify functional SNPs (i.e. non-synonymous coding change), and genes that have functional SNPS.

## 3. Results

Autism linkage regions, shown in Table 1 with references, contained approximately 5300 genes. The overlaps of each database with autism linkage regions is illustrated in Fig. 2. There were 135 genes from all three databases that overlapped with autism linkage regions. These are listed in Table 2, and the database(s) containing each of these genes is indicated. Of these 135 genes, 56 had not previously been studied in autism at the time we performed our analyses (late spring, 2005). Also we indicate the 47 of these genes that have functional SNPs.

## 4. Discussion

Our review of recent autism research suggests that while autism has been framed as a neurobiological disorder with strong genetic determination, it now needs a more inclusive framing as a systemic genetically influenced disorder with significant environmental contributors. This argument is supported by epidemiological, clinical, pathophysiological and neurobiological evidence. Previous autism genetics investigators and reviewers have stated a bias toward restricting autism candidate genes to those explicitly linked to the central nervous system (e.g. Muhle et al., 2004; Yonan et al., 2003). Our bioinformatics exercise, by finding that 56 of the 135 genes in overlap regions had never been considered in autism, supports the notion that the more narrow framing of autism as a gene-brain disorder has been biasing the examination of genetic and genomic data in autism, by overlooking genes of possible relevance. While asserting the validity of relating these genes to autism will require further investigation, these findings are sufficient to support the argument that we derive from a review of autism research in the introduction, that there are further genes of interest and potential relevance that can rise to attention when considerations of gene–environment interaction and multisystem involvement in autism become more prominent.

We are aware of a number of shortcomings in the bioinformatics part of our project. The most fundamental issue is that bioinformatics in itself only suggests possibilities but does not validate them. However our argument is not dependent upon whether or not the environmentally responsive genes we have found within autism linkage regions will ultimately be valid as autism genes. The most important component of our findings is simply that many of these genes have not previously been studied at all, and the implication is that they have probably been passed over simply because they did not seem interesting in a neurobiologically based model that assumes a predominant or exclusively genetic determination with little or no environmental contribution. We believe that our findings adequately support the claim that these genes may merit some consideration as *potentially* relevant to autism (which of course does not exclude the relevance of other genes).

In addition, while it might be of interest if a disproportionate number of environmentally responsive genes were found within autism linkage regions, it does not undermine our argument here if this is not the case, since our central point is at the level of individual genes – i.e., that many individual environmentally responsive and/or systemic genes have been overlooked – and not that there is a broad pattern of predominance of environmentally responsive genes in autism. We identified 135 out of 645 genes (21%) from all three databases as overlapping with linkage regions containing approximately 5300 genes, which in turn are 21% of the approximately 25,000 genes in the human genome. However, at best only weak conclusions can be drawn from such ratios, since the number of

| Intersections between databases | |
|---|---|
| AL ^ E | 90 |
| AL ^ C | 4 |
| AL ^ I | 46 |
| Total | 140 |
| | |
| Overlaps between Intersections | |
| (AL ^ E) ^ (AL ^ C) | 2 |
| (AL ^ E) ^ (AL ^ I) | 3 |
| (AL ^ C) ^ (AL ^ I) | 0 |
| Total overlap between databases | 5 |
| | |
| Net Total Genes | 135 |

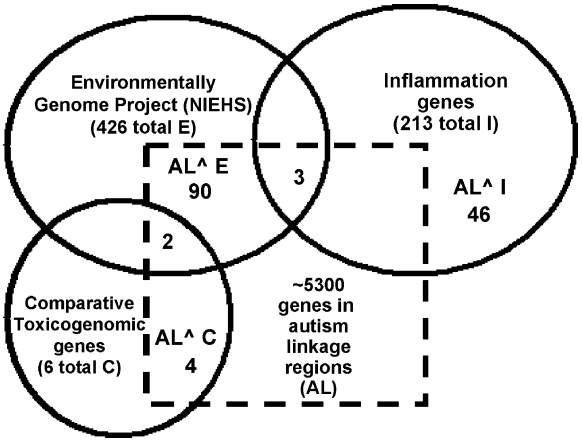


Fig. 2. Overlap of environmental genome databases with autism linkage regions. The number of genes in each of the three databases that intersects ("^") with autism linkage regions is illustrated in the Venn diagram and in the table. We also indicate the five genes that are in more than one of the databases.

Table 2
Genes from environmentally relevant genomics databases are listed

| Gene name | Gene ID | Location | SNP | No SNP | New | Database | Gene function |
|---|---|---|---|---|---|---|---|
| ABP1 | 26 | 7q34–q36 | x | | x | E | Amiloride binding protein 1 (amine oxidase (copper-containing)) |
| ALOX5AP | 241 | 13q12 | | x | x | I | Arachidonate 5-lipoxygenase-activating protein |
| AREG | 374 | 4q13–q21 | | x | x | E | Amphiregulin (schwannoma-derived growth factor) |
| BAK1 | 578 | 6p21.3 | x | | x | E | BCL2-antagonist/killer 1 |
| BLVRB | 645 | 19q13.1–q13.2 | x | | x | E | Biliverdin reductase B (flavin reductase (NADPH)) |
| CAPN6 | 827 | Xq23 | x | | x | E | Calpain 6 |
| CAPNS1 | 826 | 19q13.13 | x | | x | E | Calpain, small subunit 1 |
| CCND3 | 896 | 6p21 | x | | x | E | Cyclin D3 |
| CCNI | 10983 | 4q21.22 | x | | x | E | Cyclin I |
| CDC5L | 988 | 6p21 | x | | x | E | CDC5 cell division cycle 5-like (*S. pombe*) |
| CDK6 | 1021 | 7q21–q22 | | x | x | E | Cyclin-dependent kinase 6 |
| DCLRE1B | 64858 | 1p13.1 | x | | x | E | DNA cross-link repair 1B (PSO2 homolog, *S. cerevisiae*) |
| EDNRA | 1909 | 4q31.22 | | x | x | E | Endothelin receptor type A |
| ERCC4 | 2072 | 16p13.3–p13.11 | x | | x | E | Excision repair cross-complementing rodent repair deficiency, complementation group 4 |
| FANCF | 2188 | 11p15 | x | | x | E | Fanconi anemia, complementation group F |
| FGA | 2243 | 4q28 | x | | x | I | Fibrinogen, A alpha polypeptide |
| FGF9 | 2254 | 13q11–q12 | x | | x | E | Fibroblast growth factor 9 (glia-activating factor) |
| FMO1 | 2326 | 1q23–q25 | x | | x | E | Flavin containing monooxygenase 1 |
| FMO2 | 2327 | 1q23–q25 | x | | x | E | Flavin containing monooxygenase 2 |
| FMO3 | 2328 | 1q23–q25 | x | | x | E | Flavin containing monooxygenase 3 |
| FMO4 | 2329 | 1q23–q25 | x | | x | E | Flavin containing monooxygenase 4 |
| FMO5 | 2330 | 1q21.1 | x | | x | E | Flavin containing monooxygenase 5 |
| GLP1R | 2740 | 6p21 | x | | x | E | Glucagon-like peptide 1 receptor |
| GTF2H4 | 2968 | 6p21.3 | x | | x | E | General transcription factor IIH, polypeptide 4 (52 kDa subunit) |
| HMOX2 | 3163 | 16p13.3 | x | | x | E | Heme oxygenase (decycling) 2 |
| HSU24186 | 29935 | Xq21.33 | x | | x | E | Replication protein A complex 34 kDa subunit homolog Rpa4 |
| IGF2 | 3481 | 11p15.5 | | x | x | I | Insulin-like growth factor 2 (somatomedin A) |
| IL13RA2 | 3598 | Xq13.1–q28 | x | | x | I | Interleukin 13 receptor, alpha 2 |
| KLK1 | 3816 | 19q13.3 | x | | x | I | Kallikrein 1, renal/pancreas/salivary |
| LIG1 | 3978 | 19q13.2–q13.3 | x | | x | E | Ligase I, DNA, ATP-dependent |
| MGST3 | 4259 | 1q23 | x | | x | E | Microsomal glutathione S-transferase 3 |
| MSH5 | 4439 | 6p21.3 | x | | x | E | mutS homolog 5 (*E. coli*) |
| NFKBIB | 4793 | 19q13.1 | x | | x | I | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta |
| NFKBIE | 4794 | 6p21.1 | | x | x | I | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon |
| PKLR | 5313 | 1q21 | x | | x | E | Pyruvate kinase, liver and RBC |
| PKMYT1 | 9088 | 16p13.3 | x | | x | E | Membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase |
| PLA2G4C | 8605 | 19q13.3 | x | | x | E | Phospholipase A2, group IVC (cytosolic, calcium-independent) |
| PLAUR | 5329 | 19q13 | x | | x | I | Plasminogen activator, urokinase receptor |
| PNKP | 11284 | 19q13.3–q13.4 | x | | x | E | Polynucleotide kinase 3′-phosphate |
| POLD1 | 5424 | 19q13.3 | x | | x | E | Polymerase (DNA directed), delta 1, catalytic subunit (125 kDa) |
| POLH | 5429 | 6p21.1 | x | | x | E | Polymerase (DNA directed), eta |
| PPARD | 5467 | 6p21.2–p21.1 | | x | x | E | Peroxisome proliferative activated receptor, delta |
| PPID | 5481 | 4q31.3 | x | | x | E | Peptidylprolyl isomerase D (cyclophilin D) |
| RAD21 | 5885 | 8q24 | x | | x | E | RAD21 homolog (*S. pombe*) |
| REV3L | 5980 | 6q21 | x | | x | E | REV3-like, catalytic subunit of DNA polymerase zeta (yeast) |
| SEI1 | 29950 | 19q13.1–q13.2 | x | | x | E | CDK4-binding protein p34SEI1 |

Table 2 (*Continued*)

| Gene name | Gene ID | Location | SNP | No SNP | New | Database | Gene function |
|---|---|---|---|---|---|---|---|
| SFTPB | 6439 | 2p12–p11.2 | x | | x | I | Surfactant, pulmonary-associated protein B |
| SNN | 8303 | 16p13 | x | | x | E | Stannin |
| TAF11 | 6882 | 6p21.31 | x | | x | E | TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28 kDa |
| TAF4 | 6874 | 20q13.33 | | x | x | E | TAF4 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 135 kDa |
| TAF6 | 6878 | 7q22.1 | x | | x | E | TAF6 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 80 kDa |
| TNFRSF11B | 4982 | 8q24 | x | | x | E | Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) |
| TNFRSF17 | 608 | 16p13.1 | x | | x | E | Tumor necrosis factor receptor superfamily, member 17 |
| XPC | 7508 | 3p25 | | x | x | E | Xeroderma pigmentosum, complementation group C |
| XRCC2 | 7516 | 7q36.1 | x | | x | E | X-ray repair complementing defective repair in Chinese hamster cells 2 |
| ZFP36 | 7538 | 19q13.1 | x | | x | E | Zinc finger protein 36, C3H type, homolog (mouse) |
| ABCB1 | 5243 | 7q21.1 | | | | E | ATP-binding cassette, sub-family B (MDR/TAP), member 1 |
| ABCB11 | 8647 | 2q24 | | | | C | ATP-binding cassette, sub-family B (MDR/TAP), member 11 |
| ABCC1 | 4363 | 16p13.1 | | | | C | ATP-binding cassette, sub-family C (CFTR/MRP), member 1 |
| ACHE | 43 | 7q22 | | | | I | Acetylcholinesterase (YT blood group) |
| AHR | 196 | 7p15 | | | | E C | Aryl hydrocarbon receptor |
| AKT2 | 208 | 19q13.1–q13.2 | | | | E | V-AKT murine thymoma viral oncogene homolog 2 |
| ARNT | 405 | 1q21 | | | | E C | Aryl hydrocarbon receptor nuclear translocator |
| BAX | 581 | 19q13.3–q13.4 | | | | E | BCL2-associated X protein |
| BF | 629 | 6p21.3 | | | | I | B-factor, properdin |
| BRCA2 | 675 | 13q12.3 | | | | E | Breast cancer 2, early onset |
| C2 | 717 | 6p21.3 | | | | I | Complement component 2 |
| CAT | 847 | 11p13 | | | | I | Catalase |
| CCL2 | 1231 | 3p21 | | | | I | Chemokine (C–C motif) receptor 2 |
| CCNG2 | 901 | 4q21.22 | | | | E | Cyclin G2 |
| CDK4 | 1019 | 12q14 | | | | E | Cyclin-dependent kinase 4 |
| CDK5R1 | 8851 | 17q12 | | | | E | Cyclin-dependent kinase 5, regulatory subunit 1 (p35) |
| CDKN1A | 1026 | 6p21.2 | | | | E | Cyclin-dependent kinase inhibitor 1A (p21, Cip1) |
| CEBPB | 1051 | 20q13.1 | | | | I | CCAAT/enhancer binding protein (C/EBP), beta |
| CKM | 1158 | 19q13.2–q13.3 | | | | I | Creatine kinase, muscle |
| CRP | 1401 | 1q21–q23 | | | | I | C-reactive protein, pentraxin-related |
| EDN3 | 1908 | 20q13.2–q13.3 | | | | E | Endothelin 3 |
| EDNRB | 1910 | 13q22 | | | | E | Endothelin receptor type B |

Table 2 (*Continued*)

| Gene name | Gene ID | Location | SNP | No SNP | New | Database | Gene function |
|---|---|---|---|---|---|---|---|
| ERCC1 | 2067 | 19q13.2–q13.3 | | | | E | Excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence) |
| ERCC2 | 2068 | 19q13.3 | | | | E | Excision repair cross-complementing rodent repair deficiency, complementation group 2 (xeroderma pigmentosum D) |
| F5 | 2153 | 1q23 | | | | I | Coagulation factor V (proaccelerin, labile factor) |
| F9 | 2158 | Xq27.1–q27.2 | | | | | Coagulation factor IX (plasma thromboplastic component, Christmas disease, hemophilia B) |
| FGB | 2244 | 4q28 | | | | I | Fibrinogen, B beta polypeptide |
| FGF10 | 2255 | 5p13–p12 | | | | E | Fibroblast growth factor 10 |
| FGFR3 | 2261 | 4p16.3 | | | | E | Fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism) |
| FGG | 2266 | 4q28 | | | | I | Fibrinogen, gamma polypeptide |
| FOSB | 2354 | 19q13.32 | | | | E | FBJ murine osteosarcoma viral oncogene homolog B |
| FUT1 | 2523 | 19q13.3 | | | | I | Fucosyltransferase 1 (galactoside 2-alpha-L-fucosyltransferase) |
| FUT2 | 2524 | 19q13.3 | | | | I | Fucosyltransferase 2 (secretor status included) |
| GAD1 | 2571 | 2q31 | | | | E | Glutamate decarboxylase 1 (brain, 67 kDa) |
| GPI | 2821 | 19q13.1 | | | | E | Glucose phosphate isomerase |
| GTF2H1 | 2965 | 11p15.1–p14 | | | | E | General transcription factor IIH, polypeptide 1 (62 kDa subunit) |
| HGF | 3082 | 7q21.1 | | | | E | Hepatocyte growth factor (hepapoietin A; scatter factor) |
| IFNG | 3458 | 12q14 | | | | I | Interferon, gamma |
| IGF2AS | 51214 | 11p15.5 | | | | I | Insulin-like growth factor 2, antisense |
| IL11 | 3589 | 19q13.3–q13.4 | | | | I | Interleukin 11 |
| IL7R | 3575 | 5p13 | | | | I | Interleukin 7 receptor |
| LTA | 4049 | 6p21.3 | | | | E I | Lymphotoxin alpha (TNF superfamily, member 1) |
| LTB | 4050 | 6p21.3 | | | | E I | Lymphotoxin beta (TNF superfamily, member 3) |
| LU | 4059 | 19q13.2 | | | | I | Lutheran blood group (Auberger b antigen included) |
| MDM2 | 4193 | 12q14.3–q15 | | | | E | Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse) |
| MMP9 | 4318 | 20q11.2–q13.1 | | | | I | Matrix metalloproteinase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase) |
| MPG | 4350 | 16p13.3 | | | | E | *N*-Methylpurine-DNA glycosylase |
| MUC1 | 4582 | 1q21 | | | | E | Mucin 1, transmembrane |
| MYC | 4609 | 8q24.12–q24.13 | | | | E | v-myc myelocytomatosis viral oncogene homolog (avian) |
| NF1 | 4763 | 17q11.2 | | | | E | Neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease) |
| NOS3 | 4846 | 7q36 | | | | | Nitric oxide synthase 3 (endothelial cell) |
| NRAS | 4893 | 1p13.2 | | | | E | Neurblastoma RAS viral (v-ras) oncogene homolog |
| NTHL1 | 4913 | 16p13.3 | | | | E | nth endonuclease III-like 1 (E.coli) |

Table 2 (*Continued*)

| Gene name | Gene ID | Location | SNP | No SNP | New | Database | Gene function |
|---|---|---|---|---|---|---|---|
| ORC3L | 5000 | 6q14.3–q16.1 | | | | E | Origin recognition complex, subunit 4-like (yeast) |
| PCK1 | 5105 | 20q13.31 | | | | E | Phosphoenolpyruvate carboxykinase 1 (soluble) |
| PLTP | 5360 | 20q12–q13.1 | | | | I | Phospholipid transfer protein |
| PON1 | 5444 | 7q21.3 | | | | I | Paraoxonase 1 |
| PON2 | 5445 | 7q21.3 | | | | I | Paraoxonase 2 |
| PON3 | 5446 | 7q21.3 | | | | I | Paraoxonase 3 |
| PPARG | 5468 | 3p25 | | | | I | Peroxisome proliferative activated receptor, gamma |
| RAF1 | 5894 | 3p25 | | | | E | v-raf-1 murine leukemia viral oncogene homolog 1 |
| RAG1 | 5896 | 11p13 | | | | E | Recombination activating gene 1 |
| SELE | 6401 | 1q22–q25 | | | | I | Selectin E (endothelial adhesion molecule 1) |
| SELL | 6402 | 1q23–q25 | | | | I | Selectin L (lymphocyte adhesion molecule 1) |
| SELP | 6403 | 1q22–q25 | | | | I | Selectin P (granule membrane protein 140 kDa, antigen CD62) |
| SERPINE1 | 5054 | 7q21.3–q22 | | | | I | Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 |
| SHH | 6469 | 7q36 | | | | E | Sonic hedgehog homolog (*Drosophila*) |
| SPRR1A | 6698 | 1q21–q22 | | | | E | Small proline-rich protein 1A |
| SPRR1B | 6699 | 1q21–q22 | | | | E | Small proline-rich protein 1B (cornifin) |
| STAT1 | 6772 | 2q32.2 | | | | E | Signal transducer and activator of transcription 1, 91 kDa |
| STAT4 | 6775 | 2q32.2–q32.3 | | | | I | Signal transducer and activator of transcription 4 |
| TAF10 | 6881 | 11p15.3 | | | | E | TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30 kDa |
| TFPI | 7035 | 2q31–q32.1 | | | | I | Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor) |
| TNF | 7124 | 6p21.3 | | | | E I | Tumor necrosis factor (TNF superfamily, member 2) |
| TNFRSF5 | 958 | 20q12–q13.2 | | | | E | Tumor necrosis factor receptor superfamily, member 5 |
| USF1 | 7391 | 1q22–q23 | | | | I | Upstream transcription factor 1 |
| VTN | 7448 | 17q11 | | | | I | Vitronectin (serum spreading factor, somatomedin B, complement S-protein) |
| WT1 | 7490 | 11p13 | | | | E | Wilms tumor 1 |
| XRCC1 | 7515 | 19q13.2 | | | | E | X-ray repair complementing defective repair in Chinese hamster cells 1 |

Columns are included to indicate which database(s) contained each gene, whether the gene has been not previously been studied in autism research (''new''), and whether the gene contains functional SNPs.

''environmentally responsive'' genes is highly likely to change, and the number of genes in the human genome has not been determined with precision. We also make no claim that there are *more* environmentally responsive genes in autism than in other neurobehavioral disorders, as such comparisons are beyond the scope of our paper.

A further concern is the unequal strength and power of the results of the genome scans that were included in the linkage regions we overlapped with genes from the three databases. A previous bioinformatics study by Yonan et al. used more stringent criteria for inclusion of linkage regions from genomewide linkage studies (Yonan et al., 2003). While it would be more conservative to use the same constraints, our motivations for the present bioinformatics exploration were somewhat different and so we decided that a more inclusive list might yield more suggestive results. A key consideration in our decision was the problem of heterogeneity in autism. Since autism is a syndrome of behaviors that almost certainly rests upon a heterogeneous set of biological underpinnings, the heterogeneity of cohorts studied may have contributed to

variability in genome scan findings. From this vantage point, we can justify being more inclusive regarding autism linkage regions by pointing out that this heterogeneity is probably present at the level of genetics both within and across populations studied, and that genes of weak effect at the level of larger populations may be more relevant in some subgroups although this is not demonstrable using the current methods.

Biological heterogeneity among subjects who share common behavioral features raises the question of what final common pathways might be involved, and at what level the commonalities may lie—what factors are necessary and/or sufficient to create this behavioral syndrome. It is conceivable that multiple genetic mechanisms may impact on a smaller number of molecular, cellular and metabolic pathways, altering brain conditions in a fashion or a number of fashions sufficient to produce autism. In this regard we have already mentioned the many possible combinatorial interactions of genetic and environmental factors that could contribute to cortical noise, e.g. through increasing the excitation/inhibition ratio (Rubenstein and Merzenich, 2003). Given such heterogeneity, it may be that various sets of genes, in combination with environmental factors and in epistatic combination with each other, may be sufficient to lead to autism, while few or none of these genes and factors may be *necessary* or present in all cases.

A further consideration is that given our limited understanding of the biological mechanisms underlying the autism behavioral phenotype, we cannot exclude the possibility that the alterations in signaling and connectivity (e.g. neurotransmitters) most proximally associated with observable atypical behaviors may themselves be networked with or downstream of alterations associated with genetically modulated environmentally responsive vulnerabilities.

Identifying genetic susceptibility may be complicated. Some of the genes that confer susceptibility to autism may be fairly common. This could lead to a model of complex genetic influence where many genes can interact to produce a specific disease or phenotype but each would have only a modest contribution (McCarthy, 2002; Reich and Lander, 2001). Such genes might fall outside of autism linkage regions and would not therefore be identified by a bioinformatics approach such as ours. The Environmental Genome Project is oriented toward studying common polymorphisms in alleles associated with low penetrance, where issues of phenotypic expression may not be straightforward (Olden and Guthrie, 2001). In such settings, the vulnerability created might be more generic, leading to the specificity of autism or other diseases only in the setting of other features of genetic background as well as timing of environmental exposures (Becker, 2001, 2004; Becker et al., 2003; Chanock and Wacholder, 2002). Vulnerability might also be related more to haplotypes than to individual genes (Wilson and Olden, 2004). In psychiatric genetics, multivariate analysis has shown that differing traits may be associated with different ratios of functionally related groups of genes rather than with specific genes (Comings et al., 2000), and it is possible that this type of analysis when it incorporates environmentally responsive genes may note involvement of some of them in autism. In such combinatorial frameworks, the contribution of susceptibility genes might be necessary or at least contributory but not sufficient.

There could also be a contribution from maternal factors that affect the *in utero* environment (Dalton et al., 2003; Patterson, 2002). This idea is developed in a model of ''teratogenic alleles'' that discusses how the interaction of maternal genes (e.g. related to folate/homocysteine pathways and immune/inflammatory mechanisms) may modulate the likelihood of disease in the offspring (Johnson, 2003). Other factors add further complication to the identification of genetic contributors to autism. Epigenetically altered gene expression could play a significant role that would not be detectible through linkage or association studies (Jaenisch and Bird, 2003; Roux-Rouquie, 2000). Disease susceptibility might be affected not by specific genetic differences but by alterations in complex networks of gene expression (Featherstone and Broadie, 2002; Jeong et al., 2000; Schadt et al., 2005; Vidal, 2005), with subnetworks rather than individual genes or haplotypes serving as biomarkers (Holme et al., 2003; Nikolsky et al., 2005).

The considerations we review above regarding various approaches to complexity of genetics and gene–environment interactions will pose a range of methodological challenges. Our bioinformatics methodology of identifying the overlap between environmental genome databases and autism linkage regions is only a starting point. Common genetic variants, as well as epigenetic factors and modulation by maternal genetic background, may contribute to autism or other common complex disorders but may either not involve specific genes or may involve genes that are outside of the linkage regions identified in genome scans. If this is true, they would thus would not be included in the genes we have identified through overlap between autism linkage regions and environmental genomic databases. However, the general point can still be maintained that criteria used for assessing the relevance of genetic mechanisms should be framed more broadly.

## 5. Conclusion

We have reviewed a range of literature supporting a reframing of autism as a systemic genetically influenced condition with environmental contributors that affects the brain. Our accompanying bioinformatics exploration of autism environmental genomics has identified many environmentally responsive genes in autism linkage regions that have not previously been considered of relevance to autism, presumably at least in part because of a bias toward direct nervous system impact of genetic mechanisms. While it is true that more intensive genetics investigations would be needed to gain clarity about these genetic contributions, it is also likely that efforts to identify a subset of these genes as candidate genes for further investigation would be helpfully informed and facilitated by more careful characterization of relevant phenotypic features (Jones et al., 2005), particularly changes in environmentally responsive molecular, cellular and chemical systems and pathways and their biomarkers. This phenotypic information has not been a major focus in autism research to

date, but it may prove crucial in focusing attention in genetics research.

For many clinical and scientific reasons, the focus of autism research needs to be expanded to include the possibility that the brain may be altered by environmental factors, or that the brain may be altered downstream of environmentally modulated changes in other systems. This expansion needs to occur in every level of autism research, including genetics and genomics. Our review and bioinformatics exercise reveal that without this orientation we could be leaving important stones unturned, and it also demonstrates that relevant but hitherto untapped environmental genomic resources are already available that could facilitate incorporation of environmental considerations into autism research. Identification of genetic vulnerabilities and of contributing environmental factors will likely be important for understanding disease mechanisms, for targeting these mechanisms with treatments to reduce the level of disease burden, and for developing prevention strategies.

## Acknowledgements

This work was supported by funds from the Cure Autism Now Foundation, the Cody Center for Autism and Developmental Disorders, and the Bernard Fund for Autism Research.

## References

Abdolmaleky HM, Smith CL, Faraone SV, Shafa R, Stone W, Glatt SJ, et al. Methylomics in psychiatry: modulation of gene–environment interactions may be through DNA methylation. Am J Med Genet B Neuropsychiatr Genet 2004;127(1):51–9.

Albrecht J, Matyja E. Glutamate: a potential mediator of inorganic mercury neurotoxicity. Metab Brain Dis 1996;11(2):175–84.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM IV). 4th ed. Washington, DC: APA; 1994.

Ashwood P, Van de Water J. A review of autism and the immune response. Clin Dev Immunol 2004a;11(2):165–74.

Ashwood P, Van de Water J. Is autism an autoimmune disease? Autoimmun Rev 2004b;3(7–8):557–62.

Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. Trends Neurosci 2004;27(7):370–7.

Becker K, Friedlin B, Simon RM. Comparative genomics of autism, tourette syndrome and autoimmune/inflammatory disorders; 2003. http://www.grc.nia.nih.gov/branches/rrb/dna/pubs/cgoatad.pdf.

Becker KG. The common genetic hypothesis of autoimmune/inflammatory disease. Curr Opin Allergy Clin Immunol 2001;1(5):399–405.

Becker KG. The common variants/multiple disease hypothesis of common complex genetic disorders. Med Hypotheses 2004;62(2):309–17.

Blaxill MF. Study fails to establish diagnostic substitution as a factor in increased rate of autism. Pharmacotherapy 2004a;24(6):812–3 author reply 813–5.

Blaxill MF. What's going on? The question of time trends in autism Publ Health Rep 2004b;119(6):536–51.

Byrd RS, Sage AC, Keyzer J, Shefelbine R, Gee K, Enders K, et al. Report to the legislature on the principal findings from the epidemiology of autism in California: a comprehensive pilot study. Davis, CA: MIND Institute and California Department of Developmental Services; 2002. http://www.dds.ca.gov/autism/pdf/study_final.pdf.

Chanock S, Wacholder S. One gene and one outcome? No way Trends Mol Med 2002;8(6):266–9.

Chauhan A, Chauhan V, Brown WT, Cohen I. Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferring–the antioxidant proteins. Life Sci 2004;75(21):2539–49.

Chess S. Autism in children with congenital rubella. J Autism Child Schizophr 1971;1(1):33–47.

Chess S. Follow-up report on autism in congenital rubella. J Autism Child Schizophr 1977;7(1):69–81.

Cline H. Synaptogenesis: a balancing act between excitation and inhibition. Curr Biol 2005;15(6):R203–5.

Comings DE, Gade-Andavolu R, Gonzalez N, Wu S, Muhleman D, Blake H, et al. A multivariate analysis of 59 candidate genes in personality traits: the temperament and character inventory. Clin Genet 2000;58(5):375–85.

Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. JAMA 2003;290(3):337–44.

Croen LA, Grether JK, Hoogstrate J, Selvin S. The changing prevalence of autism in California. J Autism Dev Disord 2002;32(3):207–15.

Croen, LA, Grether, JK. Response: a response to Blaxill, Baskin and Spitzer on Croen et al. (2002), "The changing prevalence of autism in California." J Autism Dev Disord 2003;33:227.

D'Amelio M, Ricci I, Sacco R, Liu X, D'Agruma L, Muscarella LA, et al. Paraoxonase gene variants are associated with autism in North America, but not in Italy: possible regional specificity in gene–environment interactions. Mol Psychiatr 2005.

Dalton P, Deacon R, Blamire A, Pike M, McKinlay I, Stein J, et al. Maternal neuronal antibodies associated with autism and a language disorder. Ann Neurol 2003;53(4):533–7.

Dawson G, Zanolli K. Early intervention and brain plasticity in autism. Novartis Found Symp 2003;251:266–74 discussion 274–80, 281–97.

Dementieva YA, Vance DD, Donnelly SL, Elston LA, Wolpert CM, Ravan SA, et al. Accelerated head growth in early development of individuals with autism. Pediatr Neurol 2005;32(2):102–8.

Edelson SM. Treating autism: parent stories of hope and success. San Diego: Autism Research Institute; 2003.

Featherstone DE, Broadie K. Wrestling with pleiotropy: genomic and topological analysis of the yeast gene expression network. Bioessays 2002;24(3):267–74.

Fein D, Dixon P, Paul J, Levin H. Pervasive developmental disorder can evolve into ADHD: case illustrations. J Autism Dev Disord 2005;35:525–34.

Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. J Autism Dev Disord 2003;33(4):365–82.

Gafni J, Wong PW, Pessah IN. Non-coplanar 2,2′,3,5′,6-pentachlorobiphenyl (PCB 95) amplifies ionotropic glutamate receptor signaling in embryonic cerebellar granule neurons by a mechanism involving ryanodine receptors. Toxicol Sci 2004;77(1):72–82.

Gillberg C, Coleman M. The biology of the autistic syndromes (clinics in developmental medicine). Cambridge, UK: Cambridge University Press; 2000.

Gurney JG, Fritz MS, Ness KK, Sievers P, Newschaffer CJ, Shapiro EG. Analysis of prevalence trends of autism spectrum disorder in Minnesota. Arch Pediatr Adolesc Med 2003;157(7):622–7.

Herbert MR. Autism: a brain disorder or a disorder that affects the brain? Clin Neuropsychiatr 2005a;2(6):354–79.

Herbert MR. Large brains in autism: the challenge of pervasive abnormality. Neuroscientist 2005b;11(5):417–40.

Holme P, Huss M, Jeong H. Subnetwork hierarchies of biochemical pathways. Bioinformatics 2003;19(4):532–8.

Hornig M, Chian D, Lipkin WI. Neurotoxic effects of postnatal thimerosal are mouse strain dependent. Mol Psychiatr 2004;9(9):833–45.

Horvath K, Perman JA. Autistic disorder and gastrointestinal disease. Curr Opin Pediatr 2002;14(5):583–7.

Ingram JL, Peckham SM, Tisdale B, Rodier PM. Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. Neurotoxicol Teratol 2000;22(3):319–24.

Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33 Suppl:245–54.

James S, Melnyk S, Jernigan S. Low plasma methionine, cysteine, and glutathione levels are associated with increased frequency of common polymorphisms affecting methylation and glutathione pathways in children with autism. FASEB Exp Biol 2005;19(4):A51–2 Abstract # 59.14.

James SJ, Melnyk S, Jernigan S, Cleves M, Halsted C, Wong D, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet 2006; in press.

James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr 2004;80(6):1611–7.

Jass JR. The intestinal lesion of autistic spectrum disorder. Eur J Gastroenterol Hepatol 2005;17(8):821–2.

Jeong H, Tombor B, Albert R, Oltvai ZN, Barabasi AL. The large-scale organization of metabolic networks. Nature 2000;407(6804):651–4.

Johnson WG. Teratogenic alleles and neurodevelopmental disorders. Bioessays 2003;25(5):464–77.

Jones R, Pembrey M, Golding J, Herrick D. The search for genotype/phenotype associations and the phenome scan. Paediatr Perinat Epidemiol 2005;19(4): 264–75.

Juarez BI, Martinez ML, Montante M, Dufour L, Garcia E, Jimenez-Capdeville ME. Methylmercury increases glutamate extracellular levels in frontal cortex of awake rats. Neurotoxicol Teratol 2002;24(6):767–71.

Jyonouchi H, Geng L, Ruby A, Reddy C, Zimmerman-Bier B. Evaluation of an association between gastrointestinal symptoms and cytokine production against common dietary proteins in children with autism spectrum disorders. J Pediatr 2005;146(5):605–10.

Kaiser J. Environment institute lays plans for gene hunt. Science 1997;278(5338):569–70.

Kelley E, Fein D, Naigles L. Language functioning in optimal outcome children with a history of autistic spectrum disorders. J Autism Dev Disord; in press.

Kemper TL, Bauman M. Neuropathology of infantile autism. J Neuropathol Exp Neurol 1998;57(7):645–52.

Kenet T, Pessah I, Merzenich M. The influence of environmental factors on critical period plasticity in rats auditory cortex. Soc Neurosci Abstr 2005; 919.5.

Kim K, Pessah I. GABAa receptor deficiency significantly potentiates excitotoxicity of noncoplanar PCB 170 in the acute hippocampal slice preparation. In: IMFAR 2004. 2004 Abstract S2.6.5.

Kreiling J, Stephens R, Reinisch C. A mixture of environmental contaminants increases cAMP-dependent protein kinase in *Spisula* embryos. Environ Toxicol Pharmacol 2005;19:9–18.

Lainhart JE, Piven J, Wzorek M, Landa R, Santangelo SL, Coon H, et al. Macrocephaly in children and adults with autism. J Am Acad Child Adolesc Psychiatr 1997;36(2):282–90.

Lawler CP, Croen LA, Grether JK, Van de Water J. Identifying environmental contributions to autism: provocative clues and false leads. Ment Retard Dev Disabil Res Rev 2004;10(4):292–302.

Lord C, Shulman C, DiLavore P. Regression and word loss in autistic spectrum disorders. J Child Psychol Psychiatr 2004;45(5):936–55.

Luyster R, Richler J, Risi S, Hsu WL, Dawson G, Bernier R, et al. Early regression in social communication in autism spectrum disorders: a CPEA study. Dev Neuropsychol 2005;27(3):311–36.

Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, et al. Identification of significant association and gene–gene interaction of GABA receptor subunit genes in autism. Am J Hum Genet 2005;77(3): 377–88.

Mattingly CJ, Colby GT, Forrest JN, Boyer JL. The Comparative Toxicogenomics Database (CTD). Environ Health Perspect 2003;111(6):793–5.

Mattingly CJ, Colby GT, Rosenstein MC, Forrest JN Jr, Boyer JL. Promoting comparative molecular studies in environmental health research: an overview of the Comparative Toxicogenomics Database (CTD). Pharmacogenomics J 2004;4(1):5–8.

McCarthy MI. Susceptibility gene discovery for common metabolic and endocrine traits. J Mol Endocrinol 2002;28(1):1–17.

Ming X, Stein TP, Brimacombe M, Johnson WG, Lambert GH, Wagner GC. Increased excretion of a lipid peroxidation biomarker in autism. Prostaglandins Leukot Essent Fatty Acids 2005;73(5):379–84.

Muhle R, Trentacoste SV, Rapin I. The genetics of autism. Pediatrics 2004; 113(5):e472–86.

Mundy P, Crowson M. Joint attention and early social communication: implications for research on intervention with autism. J Autism Dev Disord 1997; 27(6):653–76.

Mutkus L, Aschner JL, Syversen T, Aschner M. Methylmercury alters the in vitro uptake of glutamate in GLAST- and GLT-1-transfected mutant CHO-K1 cells. Biol Trace Elem Res 2005;107(3):231–46.

Newschaffer CJ, Falb MD, Gurney JG. National autism prevalence trends from United States special education data. Pediatrics 2005;115(3):e277–82.

NIEHS. Environmental Genome Project, National Institute of Environmental Health Sciences; 2004. http://www.niehs.nih.gov/envgenom/.

Nikolsky Y, Ekins S, Nikolskaya T, Bugrim A. A novel method for generation of signature networks as biomarkers from complex high throughput data. Toxicol Lett 2005;158(1):20–9.

Olden K, Guthrie J. Genomics: implications for toxicology. Mutat Res 2001;473(1):3–10.

Olden K, Guthrie J, Newton S. A bold new direction for environmental health research. Am J Publ Health 2001;91(12):1964–7.

Olden K, Wilson S. Environmental health and genomics: visions and implications. Nat Rev Genet 2000;1(2):149–53.

Palmer RF, Blanchard S, Stein Z, Mandell D, Miller C. Environmental mercury release, special education rates, and autism disorder: an ecological study of Texas. Health Place 2005.

Patterson PH. Maternal infection: window on neuroimmune interactions in fetal brain development and mental illness. Curr Opin Neurobiol 2002;12(1): 115–1158.

Perry G, Nunomura A, Harris P, Siedlak S, Smith M, Salomon R. Is autism a disease of oxidative stress?In: Oxidative Stress in Autism Symposium. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, 16 June; 2005;15.

Pletnikov MV, Rubin SA, Vogel MW, Moran TH, Carbone KM. Effects of genetic background on neonatal Borna disease virus infection-induced neurodevelopmental damage. II. Neurochemical alterations and responses to pharmacological treatments. Brain Res 2002;944(1–2):108–23.

Program for Genomic Applications. SeattleSNPs; 2006: http://pga.gs.washington.edu/finished_genes.html.

Redcay E, Courchesne E. When is the brain enlarged in autism? A meta-analysis of all brain size reports Biol Psychiatr 2005;58(1):1–9.

Reich DE, Lander ES. On the allelic spectrum of human disease. Trends Genet 2001;17(9):502–10.

Richler J, Luyster R, Risi S, Hsu W, Dawson G, Bernier R, et al. 2006. Is there a 'regressive phenotype' of autism spectrum disorder associated with the measles–mumps–rubella vaccine? A CPEA study. J Autism Dev Disord; in press.

Rodier PM, Ingram JL, Tisdale B, Nelson S, Romano J. Embryological origins for autism: developmental anomalies of the cranial nerve motor nuclei. J Comp Neurol 1996;370:247–61.

Roux-Rouquie M. Genetic and epigenetic regulation schemes: need for an alternative paradigm. Mol Genet Metab 2000;71(1–2):1–9.

Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003;2(5):255–67.

Rutter M. Incidence of autism spectrum disorders: changes over time and their meaning. Acta Paediatr 2005;94(1):2–15.

Santangelo SL, Tsatsanis K. What is known about autism: genes, brain, and behavior. Am J Pharmacogenomics 2005;5(2):71–92.

Schadt EE, Lamb J, Yang X, Zhu J, Edwards S, Guhathakurta D, et al. An integrative genomics approach to infer causal associations between gene expression and disease. Nat Genet 2005;37(7):710–7.

Scott FJ, Baron-Cohen S, Bolton P, Brayne C. Brief report: prevalence of autism spectrum conditions in children aged 5–11 years in Cambridgeshire, UK. Autism 2002;6(3):231–7.

Serajee FJ, Nabi R, Zhong H, Huq M. Polymorphisms in xenobiotic metabolism genes and autism. J Child Neurol 2004;19(6):413–7.

Stromland K, Nordin V, Miller M, Akerstrom B, Gillberg C. Autism in thalidomide embryopathy: a population study. Dev Med Child Neurol 1994;36(4):351–6.

Tabor HK, Risch NJ, Myers RM. Opinion: candidate-gene approaches for studying complex genetic traits: practical considerations. Nat Rev Genet 2002;3(5):391–7.

Torrente F, Ashwood P, Day R, Machado N, Furlano RI, Anthony A, et al. Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Mol Psychiatr 2002;7(4) 334, 375–82.

Torres AR, Maciulis A, Odell D. The association of MHC genes with autism. Front Biosci 2001;6:D936–43.

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 2005;57(1):67–81.

Veenstra-Vanderweele J, Christian SL, Cook EH Jr. Autism as a paradigmatic complex genetic disorder. Annu Rev Genomics Hum Genet 2004;5:379–405.

Vidal M. Interactome modeling. FEBS Lett 2005;579(8):1834–8.

Waters MD, Olden K, Tennant RW. Toxicogenomic approach for assessing toxicant-related disease. Mutat Res 2003;544(2–3):415–24.

Werner E, Dawson G. Validation of the phenomenon of autistic regression using home videotapes. Arch Gen Psychiatr 2005;62(8):889–95.

Williams G, King J, Cunningham M, Stephan M, Kerr B, Hersh JH. Fetal valproate syndrome and autism: additional evidence of an association. Dev Med Child Neurol 2001;43(3):202–6.

Wilson SH, Olden K. The environmental genome project: phase I and beyond. Mol Interv 2004;4(3):147–56.

Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. JAMA 2003;289(1): 49–55.

Yonan AL, Palmer AA, Smith KC, Feldman I, Lee HK, Yonan JM, et al. Bioinformatic analysis of autism positional candidate genes using biological databases and computational gene network prediction. Genes Brain Behav 2003;2(5):303–20.