The Evolving Role and Nature of Gene Mutations in the Neuropathology of Autism Spectrum Disorders
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Abstract
Recent research increasingly suggests that autism spectrum disorders (ASDs) can be caused by genetic factors. ASDs are one of the fastest growing neurodevelopmental disorders, encompassing a spectrum of disorders marked by difficulties with social interaction, communication (verbal and non-verbal), and unusual, repetitive behaviors. The etiology, or specific factors that cause a disorder, are relatively uncertain for ASDs. Consequently, viable treatment options for ASDs have received greater attention among autism researchers – in particular, the neurological consequences of genetic mutations found in people with ASDs. In the past year, the literature has presented many novel treatments to address this promising neurobiological etiology of ASDs. The current trajectory of autism research, supported by wealth of studies connecting genetic mutations in neural substrates to the core symptoms of ASDs, suggests a greater appreciation for and understanding of the genetic complexity that underlies ASDs. Additionally, results of ASD twin studies have encouraged consideration of environmental factors that may act as triggers for gene mutations associated with ASDs. Genetic and environmental factors are increasingly accepted as joint contributors to the etiology of ASDs, rather than isolated factors strictly regulated by nature or nurture, respectively. While there has been substantial progress on the genetic-neurobiological front of ASD research in the past decade, there is a burgeoning avenue of genetic-environmental ASD research. With impending changes to the definition of ASDs in the newest edition of the Diagnostic and Statistical Manual (5th edition) in May 2013, it is likely that these multifactorial etiologies of ASDs will receive even greater attention in the field.

Keywords: autism spectrum disorders; etiology; de novo mutations; copy number variations; epigenetics

Introduction
Autism Spectrum Disorders
Autism Spectrum Disorders (ASDs) are a group of neurodevelopmental disorders with a markedly growing prevalence: more children are diagnosed with ASDs each year in the United States than are diagnosed with AIDS, cancer, and diabetes combined (Autism Speaks, 2012). ASDs affect 1 in 88 children in the United States and are four times more prevalent in males than they are in females - 1 in 54 boys affected compared with 1 in 252 girls affected (MMWR, 2008). Prevalence estimates of ASDs have increased significantly in recent years, from 1 in 150 in the year 2000 to 1 in 88 in the year 2008 (see Figure 1), yet it is unclear whether this is a true increase or merely a reflection of expanded diagnostic criteria (Fombonne, 2009).

Regardless, the measured increase has encouraged researchers to investigate possible etiologies of these disorders, paying particular attention to genetics given the sex difference in prevalence statistics.

The core deficits of ASDs include delayed speech development and communication skills (verbal and non-verbal), impaired social interaction, and restricted, repetitive behaviors (APA, 1994). ‘Autism’ first received its own classification in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 1994)

Copyright: © 2013 The Trustees of Columbia University, Columbia University Libraries, some rights reserved, Sorrento, et al. Received 1/1/2013. Accepted 2/1/2013. Published 1/1/13
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and Statistical Manual of Mental Disorders, third edition (DSM-3) in 1980 (APA, 1980). When the DSM-4 was updated in 1994, “autistic disorder” was subsumed under a new umbrella category of “Pervasive Developmental Disorders” (PDD), which included four other disorders: Asperger’s disorder, childhood-onset disintegrative disorder (CODD), Rett syndrome, and PDD-Not Otherwise Specified (PDD-NOS). Although each of these conditions shared some features, the DSM-4 attempted to differentiate these different conditions with respect to age of onset, symptom severity, prognosis, and associated features (APA, 1994). Even though these disorders are now generally known as ASDs, they were never labeled this way in the DSM-4. Efforts have been made to officially list these disorders under the single name ‘Autism Spectrum Disorder’ in the newest edition of the DSM-5, due for release in May 2013. This revision is mainly influenced by inconsistent diagnoses in time and among physicians, which may be minimized by consolidating ASDs into ‘ASD’. I hypothesize that the semantic changes in diagnostic criteria will clarify the controversy between actual ASD prevalence versus over-diagnosis from broad diagnostic criteria, especially when data reflecting identified prevalence rates under the more narrow DSM-5 definition of ASD are available (Tchaconas & Adesman, 2013).

The diagnostic criteria for “ASD” in the DSM-5 will reflect several major changes in the specific criteria that qualify a diagnosis (see Figure 2).

**Figure 1:** Identified Prevalence of Autism Spectrum Disorders, 2000 – 2008. Figure 1 is adapted from the Center for Disease Control (CDC)’s Morbidity and Mortality Weekly Reports from 2000-2008. (http://www.cdc.gov/ncbddd/autism/imagesautism_data_graphic2012.jpg)

A. Persistent deficits in social communication and social interaction across contexts, not accounted for by general developmental delays, and manifest by all 3 of the following:

1. Deficits in social-emotional reciprocity; ranging from abnormal social approach and failure of normal back and forth conversation through reduced sharing of interests, emotions, and affect and response to total lack of initiation of social interaction.

2. Deficits in nonverbal communicative behaviors used for social interaction; ranging from poorly integrated- verbal and nonverbal communication, through abnormalities in eye contact and body-language, or deficits in understanding and use of nonverbal communication, to total lack of facial expression or gestures.

3. Deficits in developing and maintaining relationships, appropriate to developmental level (beyond those with caregivers); ranging from difficulties adjusting behavior to suit different social contexts through difficulties in sharing imaginative play and in making friends to an apparent absence of interest in people.
Figure 2: DSM-5 Proposed Revisions of Diagnostic Criteria for Autism Spectrum Disorders (ASDs). Figure 2 is original (featured in Tchaconas & Adesman, 2013), and the information is adapted from the ASD criteria in American Psychiatric Association’s DSM-5 Development (APA 2011). (http://www.dsm5.org/ProposedRevision/Pages/proposedrevision.aspx?rid=94)

The three domains previously defining ASDs in the DSM-4 are consolidated into two domains in the DSM-5: “social and & communication deficits” and “fixed interests & repetitive behaviors” (APA, 2011). Social and communication deficits are combined to improve diagnostic specificity without compromising sensitivity because they are considered clinically inseparable domains. Furthermore, numerical severity levels will be added to enhance diagnostic specificity (see Figure 3).
Figure 3: DSM-5 Proposed Revisions of Severity Level Specification for Autism Spectrum Disorders (ASDs). Figure 3 is original (featured in Tchaconas & Adesman, 2013), and the information is adapted from the ASD criteria in American Psychiatric Association’s DSM-5 Development (APA 2011). ([http://www.dsm5.org/ProposedRevision/Pages/proposedrevision.aspx?rid=94](http://www.dsm5.org/ProposedRevision/Pages/proposedrevision.aspx?rid=94))

Data analyses suggest that these DSM-5 core-deficit criteria will yield the highest cross-diagnostic consistency yet for people with autism (Jabr, 2012).

As displayed in Figure 2, DSM-5 ASD diagnostic criteria will now require children to exhibit all three symptoms in social communication and interaction (A) and two of four symptoms in repetitive behavior domain (B); the symptoms must present in early childhood (C) and limit everyday functioning (D). Though it is unclear how these changes will impact autism prevalence rates, the most thorough case analysis of the effect of the revised criteria on patients currently diagnosed with ASDs suggests that 91% of these patients will retain their diagnosis under these criteria (Huerta et al. 2012). The actual diagnostic efficacy of these revisions will play an integral role in the success of etiological research, as diagnostic criteria dictate the core symptoms of autism around which researchers will design their future studies.

Until recently, the core symptoms of ASDs were largely untreatable, as most research focused on alleviating the disruptive behavioral symptoms rather than on targeting the core deficits. In recognizing the neurobiological component of the disorder, current research into genetic factors influencing neurodevelopment has created a promising model for ASD brain pathology. The extensive neurogenetic autism research over the past few years has supported the development of ASD treatments that can target the unique neural circuitry that results from ASD neuropathology. Since genes code for proteins and proteins are responsible for specific functions in their area(s) of expression, dysfunctional genes responsible for neurological function in patients with and ASD are of particular interest in the neurobiological front of autism research. This review highlights the current advances in ASD etiological research, with a focus on the well-established neurobiological advances and our growing knowledge of environmental contributions, and how these advances are shaping future research efforts in light of the evolving definition of ASD.
The Genetic Basis of ASDs

Interest in the genetic heritability of ASDs originated with the first twin study reporting a significant difference between monozygotic and dizygotic twins (Folstein & Rutter, 1977); subsequent studies reported even higher monozygotic concordance rates and nearly 0% dizygotic concordance (Szatmari, 2011). In the past decade, genetic susceptibility to ASDs has continued to be a prime focus among researchers, with studies reporting ASD concordance rates as high as 90% in monozygotic twins and as low as 10% in dizygotic twins (Mendelsohn & Schaefer, 2008). These polarized concordance rates have implied a strong genetic component to ASDs, attributing almost all variances in phenotypic expression to heritable factors (Szatmari, 2011). This pattern of inheritance is exhibited in the single-gene disruption known as Fragile X syndrome, the most common inherited cause of ASDs and intellectual disability (AAP, 2012), attributed to mutation on the FMR1 gene (Henderson et al., 2012). However, nearly two years ago the results of the largest ASD twin study, the California Autism Twin Study, found ASD concordance rates to be much lower than previously reported (Hallmayer et al., 2011). Based on the total of 192 twin pairs born between 1987 and 2004, with at least one twin affected by either autism or a milder ASD, ASD was 55% attributable to the twins’ shared environmental factors, a much greater percentage than previously predicted (Hallmayer et al., 2011). The California twin population was ethnically, demographically, and socioeconomically diverse, with a fairly large sample size, providing convincing evidence for the environmental contribution to ASD etiologies.

Despite these findings, the genetic component of ASDs cannot be simply rejected; instead, the data revise the genetic explanation of ASDs to include environmental factors that work with, or possibly even trigger, gene mutations linked to ASDs. The new twin study evidence by revealing that ASD concordance rates for dizygotic twins were previously underestimated, has thus shifted the field’s focus toward potential prenatal and postnatal environmental triggers for gene mutations (Stoltenberg et al., 2010). Although ASD etiologies were once divided into separate genetic or environmental etiologies, there is now a growing acceptance of epigenetics—the theory that environmental factors influence gene expression to produce mutations in a non-Mendelian manner. It is believed that this combined etiological hypothesis can explain the presence of these non-heritable, de novo gene mutations increasingly observed in ASD populations (Sanders et al., 2012; Gilman et al., 2011; Sebat et al., 2007).

De novo gene mutations and copy number variations

Most known mutations without an identified genetic etiology attributed to ASDs are de novo gene mutations: rare genetic variations that are found in neither of the individual’s parents. The risk for de novo mutations has been associated with increased parental age, one of many environmental factors that may account for the spontaneous appearance of these mutations (Sanders et al., 2012). It also appears that the more de novo mutations found in the same gene, the greater the chance that these are ASD risk-associated mutations (see Figure 4).
Figure 4: Identification of multiple de novo mutations in the same gene reliably distinguishes risk-associated mutations. Figure 4 is adapted from Sanders et al., 2012. (a) reflects a simulation experiment modeling the probability of observing two independent nonsense/splice site de novo mutations in a single brain-expressed gene among unrelated subjects. (b) is the application of the simulation described in (a) to predict the number of genes found to carry two or more nonsense/splice site de novo mutations for a sample of varied size (indicated on the x-axis). (c) is a repeat of the simulation in (a) for non-synonymous de novo mutations. Three or more independent non-synonymous de novo mutations in a brain-expressed gene offer significant evidence for association with ASD, though this sample is limited by its size and heterogeneity models.

A common type of de novo mutation involves copy number variations (CNVs), or an abnormal number of copies of a DNA segment (i.e. a deletion or duplication of a gene) that often causes dysfunction of the gene’s normal protein. Fernandez et al.’s initial implication of a CNV on the contactin4 gene has led to research linking other contact in family candidate genes vital for the functioning of the central nervous system (i.e. CNTNAP2) to small populations with ASDs (Bakkaloglu, 2008). Gilman and colleagues’ analysis of de novo variants suggests that ASD is a disorder of ‘perturbed synaptogenesis’, based on their model of a large biological network of genes affected by rare de novo CNVs in ASD, known as NETBAG, which contains genes primarily related to synapse development, axon targeting, and neuron motility (Gilman et al., 2011). Their model supports that theory that networks underlying complex human phenotypes – i.e., ASD - can be identified by a network-based functional analysis of rare genetic variants (see Figure 5).
De novo mutations appear to produce the brain pathologies and core symptoms of ASDs in some cases. Given that new gene mutations are found each year in people with ASDs, there are likely hundreds of genes linked to ASDs that have yet to be found. Accordingly, it may be more effective to identify the neural circuits, or networks of genes, involved in functions often impaired in the brains of those with ASDs. The identification of ASD-specific neural circuits, coupled with neuroimaging of people with ASDs performing specific tasks representative of the disorder's core deficits, can perhaps enable the mapping of ASD neuropathology in the brain. In the coming years, it will also be crucial to determine whether de novo gene mutations versus inherited gene mutations implicated in ASD etiology induce identical or distinct brain pathologies.

While treatment-based genetic studies are limited by the presently sparse representation of mutations in small ASD subpopulations, investigation of these genes and their proper functions opens a valuable pathway for understanding the complex mechanisms that propagate ASDs. This has led to the concurrent growth of ASD neuroimaging studies as a means of analyzing the effects of
ASD-related gene mutations in their respective neural substrates.

**Neuroimaging reveals the neuropathology of ASDs**

Among the increasingly sophisticated neuroimaging technologies available today, functional magnetic resonance imaging (fMRI) is an effective research tool for isolating and studying frequently disrupted neural systems connected to ASDs (AAP, 2012). fMRI is a technique that measures brain activity by detecting changes in blood oxygenation and blood flow which is a proxy for neural activity. It is a more helpful technique than the previously used neuroimaging techniques. It is thought that brain abnormalities found consistently among patients with ASDs are a product of underlying genetic mutations that influence the expression of key proteins in the brain, and thus result in inefficient neural circuitry, especially with neuronal migration and cortical organization (Sowell & Bookheimer, 2012). Current studies are using neuroimaging to isolate these problem regions in the brains of ASD patients as opposed to the brains of normal controls, noting differences in gene expression, brain architecture, and behaviors linked to ASDs (Anagnostou & Taylor, 2011, Wolff et al., 2012). For example, language skills in children with autism who exhibit language impairment was compared to the language skills in age-matched controls by presenting these children with recordings of their parents talking to them while measuring each child’s brain activity during the task (Lai et al., 2011). Differential activation in autistic versus control subjects upon passive speech stimulation was localized to the superior temporal gyrus (STG), a brain region that regulates language; this observed differential activation could potentially implicate fMRI as an objective detector of language impairment in children with autism, and perhaps a general ASD diagnostic test (Lai et al., 2011).

In addition to fMRI, recent studies using diffusion tensor imaging (DTI, a functional neuroimaging technique that measures water diffusion within a tissue) have identified a distinct white-matter fiber tract maturation pattern discernible in high-risk infants who eventually develop an ASD or associated symptoms (Wolff et al., 2012). The results indicate that abnormal development of the white-matter pathways in infant brains may precede ASD symptom onset in the first year of life and can potentially be used as an early detection test for ASDs from 6 to 24 months. Clinicians, who currently lack a medical test to diagnose autism, hope that identification of ASD brain biomarkers will soon allow for earlier, more accurate ASD-risk detection and that neuroimaging will evolve to eventually provide an objective diagnostic test. While careful analysis of the structural and volumetric measures from brain MRIs has yet to identify a consistent pattern of early brain development in children with ASDs, functional neuroimaging techniques have used these volume abnormalities to uncover atypical structural connectivity in the ASD brain (Anagnostou & Taylor, 2011). Therefore, functional neuroimaging techniques have emerged as highly informative neuroimaging tools for ASD etiological research that will ultimately be used to relate imaging findings to behavior and the underlying genes, and the expression of these genes, which produce these neurobiological abnormalities.

Collectively, these neuroimaging studies suggest that a critical, preclinical period exists during which anatomical brain abnormalities that are found in neurodevelopmental disorders such as ASDs, do manifest before the associated behavioral symptoms. Responding to these ASD biomarkers with early behavioral interventions, once they can be more clearly defined, could presumably minimize ASD severity and improve the child’s prognosis. Though it must be further explored in the future, identifying biomarkers for ASDs during the critical neurodevelopmental period would be an invaluable tool for early detection and preventive interventions that could minimize the disorder’s severity.
Neuropharmacological Interventions

Genetic investigations of ASDs have led researchers to develop experimental medical treatments that target these genetic abnormalities in the brain. The neurobiology research-centered pharmaceutical company Seaside Therapeutics recently developed arbaclofen, an investigational drug for patients with Fragile X syndrome that has functioned as a disease-modifying drug in preclinical mice models (Henderson et al., 2012) due to significant improvements in social interaction and engagement observed in clinical trials (Berry-Kravis et al., 2012). The causative mutation of Fragile X syndrome is in the FMR1 gene and involves a loss of the protein that regulates synaptic protein synthesis, which disrupts the function of the inhibitory neurotransmitter GABA. The drug targets this mutation via postnatal activation of the GABAB receptors. The drug increases inhibitory neurotransmitter function which can reverse the pathological synaptic overstimulation caused by fragile X syndrome. Arbaclofen was applied in vitro to the hippocampus of mice lacking the FMR1 protein, and was found to reduce the severity of Fragile X. The positive results in these mice models led to ongoing human clinical trials this last year which showed, the potential of being the first viable drug to treat the core symptoms of Fragile X in humans. In phase 2 of the clinical trials, researchers observed statistically significant improvements in social avoidance, a core symptom of fragile X syndrome (Berry-Kravis et al., 2012). Given that arbaclofen was well tolerated by humans in this double-blind, placebo-controlled, clinical trial, it has the potential, with further testing, to become the first FDA-approved medicine to treat core deficits of fragile X syndrome. By logical extension, if arbaclofen can improve the social functioning of children with comorbid ASD and Fragile X, it may also be effective in other ASD populations or lead to the development of other effective medications.

Enzyme replacement therapy is another experimental treatment that has advanced to the level of clinical trials in the U.S. The treatment targets children with autism who present biomarkers suggesting enzyme deficiencies. These enzyme deficiencies then result in an inability to digest protein, thereby compromising amino acid production. A proprietary high-protease enzyme replacement formulation (CM-AT) has just been reported to be significantly more effective than a placebo in treating core and noncore symptoms of children aged 3–8 years with autism in a randomized, double-blind, Phase III clinical trial (Curemark, 2011, 2012).

Another potential therapeutic approach focuses on patients with autism, intellectual disability, and seizures because of a defect of branched chain amino acid (BCAA) metabolism. Patients with this rare mutation have decreased levels of BCAAs. Novarino et al. (2008) used a genetically engineered ‘knockout mice’ model to demonstrate that these abnormal brain amino acid profiles and neurobehavioral deficits can be corrected with dietary supplementation, or the addition of foods rich in the depleted amino acids into a patient’s diet. The researchers also used skin samples from patients with this gene defect and converted them into neural stem cells; these neural cells functioned normally in the presence of an environment rich in the depleted amino acids, suggesting that this one very rare form of autism, at least, may be treatable (Novarino et al., 2012).

Certain medications already on the market may be helpful in treating children with irritability related to an ASD. A recent pilot study indicates that oral administration of N-acetylcysteine (NAC), an antioxidant and a glutamatergic modulator, displayed efficacy in treating disruptive symptoms in children with autistic disorder (Hardan et al., 2012). Glutamatergic dysfunction and redox imbalance are potential links to irritability in some forms of ASD, originating from genetic disruptions (Hardan et al., 2012). This 12-week double-blind, randomized, placebo-controlled pilot investigation found that NAC was helpful in managing irritability and was relatively well
tolerated. Although there were benefits from NAC treatment, the study is limited by its small sample size and narrow age range, so it should be repeated with a larger population and a broader age range to assess the feasibility of using NAC as a routine treatment option for disruptive behaviors from autistic disorder and related disorders of repetition and compulsion (i.e. obsessive compulsive disorder).

Similarly, preliminary results of a large-scale, placebo-controlled, double-blind study suggest that nasally-administered oxytocin, a naturally occurring substance produced in the brain to regulate social abilities, improves the social-communicative core deficits of ASDs in children and adolescents, aged 7 to 18 years. fMRI detected increased brain activity in regions known to process social information following oxytocin treatment, behaviorally translating to heightened social interaction (Peart, 2012). As before, additional data demonstrating sustained core symptom improvements over longer time periods following administration of these therapeutics are needed to determine whether such interventions are safe and effective for use in individuals with ASDs.

<table>
<thead>
<tr>
<th>Substance Name</th>
<th>Disorder Targeted</th>
<th>Therapeutic Mechanism</th>
<th>Stage in Clinical Trials</th>
<th>Relevant Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arbaclofen</td>
<td>Fragile-X</td>
<td>Increase inhibitory neurotransmitter (GABA) activity that is disrupted by an FMR1 gene mutation.</td>
<td>Completed Phase II</td>
<td>Henderson et al., 2012; Berry-Kravis et al., 2012</td>
</tr>
<tr>
<td>High-protease enzyme replacement formulation (CM-AT)</td>
<td>ASD</td>
<td>Correcting enzyme deficiencies that compromise amino acid production.</td>
<td>Completed Phase III</td>
<td>Curemark, 2011, 2012</td>
</tr>
<tr>
<td>Branched chain amino acid (BCAA) metabolism enrichment</td>
<td>Rare form of autism with intellectual disability and seizures, induced by defect in BCAA metabolism.</td>
<td>Dietary supplementation with foods rich in the depleted amino acids.</td>
<td>N/A – pilot study</td>
<td>Novarino et al., 2012</td>
</tr>
<tr>
<td>N-acetylcysteine (NAC)</td>
<td>Autistic Disorder (specifically, its disruptive symptoms)</td>
<td>Alleviating glutamatergic dysfunction and redox imbalance from genetic disruptions.</td>
<td>N/A – pilot study</td>
<td>Hardan et al., 2012</td>
</tr>
<tr>
<td>Oxytocin (nasally-administered)</td>
<td>ASD (specifically, the social-communicative core symptoms)</td>
<td>Increases brain activity in regions processing social information.</td>
<td>N/A – ongoing preliminary investigation</td>
<td>Preliminary results from Ilanit Gordon and Kevin Pelphrey 2012; Peart, 2012</td>
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**Figure 6:** Summary of Potential ASD Neuropharmacological Interventions. Figure 6 is an original figure.

**Future Directions**

Results of genetic research of ASDs over the past decade suggest that mutations in hundreds of genes vital to the brain’s growth and circuitry may underlie the development of ASDs. The wealth of studies indicating that gene mutations cause some ASD cases points to the growing body of literature supporting a genetic basis of ASD neuropathology. Accordingly, drug development studies will continue to search for ways to correct aberrant protein functioning resulting from the plethora of gene mutations.
already positively connected to ASD neuropathology. Specifically, researchers are currently working to identify commonly disrupted molecular pathways in the brains of patients with ASDs, as these findings can lead to highly specific treatments. Regardless of whether the exact cause of ASDs remains unknown, treatments targeting these disrupted neurobiological pathways can still correct the core deficits of ASDs, assuming that such disruptions are an etiology rather than a consequence of ASDs.

Furthermore, environmental factors that are increasingly believed to trigger ASD-related gene mutations will continue to be explored, especially in the case of de novo mutations that cannot be explained by typical Mendelian inheritance patterns, and the new data reporting higher concordance rates among dizygotic twins with ASDs. Twin studies in the future will likely analyze twins’ shared environment prior to the second year of life, when signs of ASDs typically manifest themselves (Stoltenberg et al., 2010). More ASD-related gene mutations will undoubtedly be discovered in the coming years; the challenge for scientists will be to consolidate the scattered mutations observed into specific ASD etiologies, and to connect them to their potential environmental triggers. In this regard, it is likely that many ASDs will ultimately be classified as multifactorial genetic disorders.

References
Neuron 70 898-907.