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Autism Spectrum Disorder: Genomics and Epigenomics

Autism spectrum disorder (ASD) refers to a group of similarly-named disorders that all include the same behavioral clinical manifestations, namely: impaired ability to socialize, impaired language and communication skills, and tendency towards abnormally repetitive behavior. ASD is a highly heritable disease, but like most behavioral conditions the search for a specific causal genetic mutation has proven elusive. The difficulty of searching for clinically relevant genetic and epigenetic loci is compounded by the lack of specific quantifiable criteria available to diagnose the disease, as well as the similarity of ASD to other related but distinct conditions.

Diagnosis and Symptoms

Prior to the release of the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders 5th edition* (DSM-5) in 2013, the conditions falling under the ASD umbrella were referred to as a group of separate but related diseases known as pervasive developmental disorders – these included autism, Asperger syndrome, Pervasive developmental disorder not otherwise specified (PDD-NOS), Rett syndrome, and childhood disintegrative disorder. Subsequent advancement in the understanding of these diseases, such as the discovery of *MECP2* mutations as the primary cause of Rett syndrome (which stands to this day as the only behavioral disorder with a concretely defined genetic cause), as well as insight into the similarity between diagnoses of different autism-related disorders, prompted the reorganization of the diagnostic criteria of these behavioral diseases. As of the DSM-5, autism, Asperger syndrome, and PDD-NOS have been redefined as three different points on a continuous spectrum collectively referred to as ASD, while Rett syndrome and childhood disintegrative disorder continue to be diagnosed as distinct disorders. Accordingly, the diagnostic criteria for each of these three conditions have changed significantly; Asperger syndrome, for example, is now no longer

given as a clinical diagnosis – patients who would have formerly been diagnosed with the syndrome are now referred to as having ASD with a certain degree of severity. The rationale behind the change in diagnostic criteria in the DSM-5 lies in the high degree of similarity of symptoms between patients with different autism spectrum disorders – rather than treating the conditions as separate but similar, the conditions are more accurately represented as different dimensions of severity of the same disease.

The symptoms in question can be summarized as a combination of social impairment, deficiencies in communication, and repetitive behaviors. The disease can manifest itself as early as up to 1 year of age – autistic infants will dislike being held or cuddled, and become irritated or panicked when not left alone. Other standard signs that not only appear early on in development but are characteristic of the disease as a whole include a marked failure to initiate eye contact and frequent staring into space. Infants who develop the syndrome will often exhibit sleep problems, and will display the repetitiveness of certain behaviors, such as rocking back and forth, that are more fully realized later in the onset of the disease.

The classical behavioral problems that are typical of ASD start in years two to three. Central to the social impairment that autistic children exhibit at this age include the inability to read into the intentions of other people. Afflicted children will often avoid social interaction altogether, and will show a lack of interest in other people; parents for example will be treated as objects to climb over or to be used as a tool to pick up a desired object. In addition, children will refuse to make any eye contact whatsoever and will often fail to respond to their given names. The degree to which afflicted children stop responding to external social cues is such that deafness is often mistakenly diagnosed in autistic children.

Language deterioration will develop as well – children will begin to learn to speak normally, but as time passes will become more distant and often will stop speaking entirely – this change can be either gradual or immediate depending on the child. This deficit in expressive language is accompanied by impaired receptive language, i.e. the ability to comprehend and respond to speech.

Autistic children commonly exhibit echolalia, the automatic tendency to repeat words or phrases spoken by others; pronoun reversal, or the misapplication of pronouns in speech; and abnormal speech inflections and intonations. Patients will have difficulty sustaining conversation, particularly in the aspects of taking turns while talking and allowing the other party to introduce topics of conversation.

Another classical symptom of autistic children is the tendency towards repetitive behavior, a key characteristic of ASDs. Autistic toddlers commonly exhibit repetitive motor activities that range from simple (licking, twirling strings, idiosyncratic movement of fingers) to more complex patting, twirling or rubbing motions; often these movements will be repeated for as long as several hours. The cause of this behavior is unclear, but the consensus is that these repetitive movements serve to relieve stress or calm the child, tying into the more general theme of the need for rigidness, routine, sameness, and familiarity in the lives of autistic patients. This is also reflected in the play patterns of autistic children: rather than engaging in the more typical imaginative games or make-believe play that a normal child would, the autistic child will favor repetitive, nonspontaneous activities such as sorting possessions or throwing objects.

At three to four years, autistic children exhibit varying degrees of improvement in language skills and socializing: about 75% of autistic children will require continuous parental, scholastic, and societal support, whereas 25% will begin to talk and communicate by the age of six or seven and will start to integrate at varying degrees of success into the school population – however, social impairment will generally continue for most autistic children. Estimates of the number of children that recover mostly or completely from ASD hover around 5% or more, depending on the stringency of criteria used to determine what constitutes recovery. As indicated by Farley et al., who report a study showing that only 12% of a group of autistic adults between the ages of 22 and 46 lived independently and 56% lived with their parents, the majority of autistic patients continue to suffer from the symptom throughout their lifetimes (Farley et al., 2009).

Physical manifestations of the disease appear in a significant portion of autistic patients. 25%

of ASD patients suffer from seizures (Kim et al., 2006). 15-20% of patients exhibit a significant degree of generalized dysmorphology – the significance of dysmorphology is evaluated by the Autism Dysmorphology Measure (ADM), an assessment of 12 body areas, including height, nose size, hand size, and others (Miles et al., 2008). The most common of these dysmorphologies are micro- and macrocephaly, which occur in 5-15% and 30% of autistic children respectively. Interestingly, microcephaly alone is highly predicative of poor outcome of the disease (Miles et al., 2005). The prominence of these physical phenotypes has prompted the classification of autism as complex or essential. Complex autism, defined as autism accompanied by dysmorphology and/or microcephaly, occurs in about 20% to 30% of patients and is accompanied by a generally poorer prognosis than essential autism, defined as autism in the absence of physical abnormalities.

ASD patients exhibit a host of other miscellaneous symptoms and impairments. Hypersensitivity to sound and touch is common. Interestingly, autistic patients will sometimes exhibit exaggerated reactions to light touches or sharp but harmless noises such as the whistle of a vacuum cleaner, but will ignore more serious or painful stimuli such as burns or cuts. Along these lines, autistic patients are known to have a diminished or, in some cases, nonexistent sense of self-preservation; death from drowning is common among autistic patients. ASD patients will commonly exhibit a general lack of motor coordination and development. Hypotonia, or the underdevelopment of muscle mass due to lack of activity, is frequently observed as well.

Genetic Causes of Autism

The pathology of autism is complex and not fully understood. Environmental factors have been traditionally implicated – exposure to air pollution, teratogens, certain infectious diseases, solvents, and a cohort of other stimuli have been linked to the onset of autism with varying levels of legitimacy (Lyall et al., 2014). Recent appearances of ASD in the media have largely concerned the alleged link between autism and vaccine usage, stemming from Andrew Wakefield's 1998 paper published in *The Lancet* that has since been retracted on the basis of fraudulent claims. However, the

>90% heritability of the disease (Monaco and Bailey 2001) strongly suggests a significant genetic component to the disease. The genetic variations that cause autism fall into three major categories: chromosomal abnormalities, copy number variants, and single gene disorders that include ASD as a symptom.

About 5% of all autism patients display chromosomal aneuploidy – as high as 7% of Trisomy 21 patients, the chromosomal duplication that causes Down Syndrome, also have ASD (Kent et al., 1999). An additional 3-5% of autistic patients display chromosomal abnormalities, verified using molecular imaging techniques such as fluorescent in situ hybridization (FISH). As ASD patients display chromosomal abnormalities in every chromosome, work remains to be done to figure out which abnormalities directly contribute to the ASD phenotype. Jacquemont et al. report that a duplication in 15q11-q13 and deletions in 2q37 and 22q13.3 are frequent abnormalities in ASD patients (Jacquemont et al., 2006). The 15q11-q13 region is of special interest and is referred to as the critical region of Prader-Willi syndrome, a congenital disease resulting in a variety of developmental defects such as short stature and cognitive disabilities. Deletion of this region of chromosome 11 in the paternal chromosome results in Prader-Willi syndrome, while duplication of the same region in the maternal chromosome results in one of the most highly penetrant chromosomal causes of autism (Hogart et al., 2010). Increasing number of replicates of the 15q11-q13 region correspond with increasingly severe phenotypes, with a greater occurrence of hypotonia, seizures, and microcephaly in patients with four copies (Dykens et al., 2004). Interestingly, duplication in the paternal allele results in little to no phenotype, suggesting the importance of genomic imprinting on the effect of potentially harmful chromosomal aberrations in this region of chromosome 11.

A metaanalysis of existing reviews and studies of chromosomal abnormalities conducted by Vorstman et al. reveal the possibility of additional novel cytogenic mutations that frequently occur in autistic patients – risk regions identified by this study include not only those mentioned by Jacquemont et al., but also abnormalities in additional chromosomes such as 5p15 and Xp22.2-p22.3 (Vorstman et

al., 2006). More recently a database of chromosomal structural variation associated with ASD is currently being maintained at the Autism Chromosome Rearrangement Database (http://projects.tcag.ca/autism/) (Marshall et al., 2008). However, despite the evidence presented by these studies, no concrete causal linkages can be drawn between any chromosomal abnormality and the onset of ASD; causal relationships can only be inferred by analyzing frequency of occurrence. That being said, the presence of gross chromosomal abnomalities and aneuplodies in a percentage of ASD patients and – crucially – the high rate of correlation between unbalanced chromosomal abnormalities and complex (associated with dysmorphology) autism, is highly suggestive of the causative power of chromosomal aberrations with regards to ASD.

Copy number variants (CNVs) are the most common genetic mutations associated with autism with 10-20% of patients exhibiting clinically relevant CNV muations. As opposed to the previously discussed larger scale chromosomal abnormalities that are visible via cytogenic techniques, CNVs are submicroscopic and are only detectable via the appropriate molecular biology techniques. The primary diagnostic technique used for the evaluation and identification of CNVs in autistic patients is array comparative genome hybridization (aCGH). CGH is a technique that involves the use of FISH to attach fluorescent probes to the isolated and denatured metaphase spreads of two separate genomes, which are then compared using computer sofware to identify any dissimilar regions. Varying intensity between the two samples indicates a difference in the CNV at that region. Array-based CGH techniques utilize DNA microarrays for increased resolution. This procedure represents a powerful and time-efficient method for the identification of CNV regions in a given region, and is only limited by its inability to detect balanced chromosomal abnormalities, e.g. inversions or translocations.

A wide variety of studies have been performed utilizing aCGH to identify possible clinically relevant CNVs. These studies have revealed *de novo* genetic causes for an additional 7-10% of patients with autism of unknown causes (Sebat et al., 2007). Jacquemont et al. use a 1-Mb array to identify clinically relevant CNVs in 27.5% of patients with complex autism – crucially, these patients were

karyotypically normal as revealed by cytogenic studies, reinforcing the importance for more precise techniques such as aCGH (Jacquemont et al., 2006). Glessner et al. report an aCGH study utilizing 550,000 SNP markers on a cohort of 859 ASD patients and 1409 healthy children (Glessner et al., 2005). This study revealed several *de novo* genes that contained CNVs that were statistically significant with regards to autism, including neuronal cell-adhesion molecules (*NLGN1* and *ASTN2*), as well as genes involved in the ubiquitin pathway (*UBE3A*, *PARK*2). Similarly to genome-wide association studies, which identify SNPs that are associated with certain diseases, then use the identified SNPs to detect possible genes of interest, these aCGH studies exhibit vast potential for the discovery of new sources of clinically relevant genetic variations. However, care must be taken again to separate causation from mere correlation: Weiss et al. report the deletion or duplication of 16p11.2 in 1% of autism patients, verified by Marshall et al. (Weiss et al., 2008; Marshall et al., 2008). However, this mutation is also found at a rate of 1.5% in children with more generalized developmental and language delays, suggesting that 16p11.2 duplication may not be specific for ASD. In addition, 16p11.2 mutation has been observed in a number of other disorders, including schizophrenia and bipolar disorder (McCarthy et al., 2009). 15q13.3 deletion is another mutation that seems to bear clinical relevance to ASD, but is also more generally implicated in epilepsy and intellectual disability (Sharp et al., 2008).

Several specific single-gene Mendelian diseases have been discovered to cause a host of symptoms that are highly similar to ASD. The most famous culprit is Fragile X Syndrome, a highly frequent inherited disease for mental disability resulting from the expansion of greater than 200 CGG repeats in the *FMR1* gene on the X chromosome. Nearly half of all Fragile X patients exhibit some level of autistic behavior; one study showed that 30% of Fragile X patients met clinical criteria for ASD (Harris et al., 2008). Moreover, patients with premutations (CGG repeats in the 55-200 range) are found to be highly correlated with ASD: Farzin et al. report that 71% of patients with premutations were found to meet ASD diagnostic criteria. The functional link between the CGG CNV mutation and

the onset of ASD is unclear; Handa et al. suggest that untranslated CGG repeat transcripts are not only toxic to neuronal cells but also affect the expression level of certain genes, including caspase-8 and neurotensin (Handa et al., 2005). These genes are involved in neuronal connectivity, further providing clues towards the molecular mechanisms pushing Fragile X patients towards the ASD phenotype. Another disease of note that shares profound similarities with ASD is Rett Syndrome, a condition that prior to 2013 was grouped with ASD under the umbrella category of pervasive developmental disorders. Rett Syndrome's genetic etiology was identified as an X-linked mutation in *MECP2*, and results in patients with phenotypes that largely overlap with autism. Similarities include the progressive loss of language proficiency as well as the onset of abnormal repetitive motions. Rett Syndrome is distinguished from ASD via certain specific criteria unique to the disease, namely gait disturbance and a decreasing rate of head growth (Young et al., 2008). The similarity of phenotype and the variability of MeCP2 expression in ASD patients indicates a linkage between the two diseases, but no causal linkage has been concretely established. Evidence has been found that MeCP2 deficiency also reduces the expression of *UBE3A* and *GABRB3*, two genes that have been implicated in ASD (Samaco et al., 2005). A host of other Mendelian diseases have been shown to correlate with ASD, including Duchenne muscular dystrophy, Angelman syndrome, Cohen syndrome, and Williams syndrome.

Large-scale genome-wide association studies performed in the past decade have reached the consensus that there is no single gene defect that is the major contributor to ASD. Instead, research efforts have resulted in the collation of a cohort of different candidate genes that are implicated in the onset of ASD, using methods such as aCGH and GWAS studies to identify genes of interest. A database of clinically relevant genes is curated at https://gene.sfari.org/. As might be expected for a behavioral disease, the gene ontology terms associate with ASD are largely related to neuronal development, regulation, and gene expression. Neuroligin 2 and 4 are synapse formation proteins, coded by *NLGN3* and *NLGN4X* respectively, that are implicated in ASD (Lintas & Persico, 2009).

Neurodevelopmental genes such as *HOXA1* and *HOXB1* have been implicated in autism as well. Other genes involved in neuronal activity regulation, as well as coding genes for certain neurotransmitter receptors (such as *SLC6A4,* the coding gene for serotonin transporter), have also been associated with ASD (Page et al., 2009).

Despite extensive research concerning the genetic origins of autism, a significant amount of autism cases remain unexplained or of unknown cause. Epigenomics offer an alternative and intriguing explanation for the variability observed with genetic risk factors of autism. Epigenetics refers to variations in traits that are caused by mechanisms extrinsic to the DNA sequence; common epigenetic modifications include DNA methylation of CpG islands and histone modifications (acetylation and methylation). Evidence for the involvement of epigenomics in the onset of behavioral diseases is already abundant and strongly intimates the larger underlying role of epigenetic modifications in the pathology of diseases such as ASD. The copy number variation implicated in Fragile X syndrome involves the methylation of CGG repeats found in the 5' untranslated region of the gene *FMR1*, resulting in the silencing of the gene and the onset of the disease. Similarly, MeCP2 is a protein involved in the "reading" of epigenetic marks and is the primary gene implicated in Rett syndrome. More significantly, 79% of ASD patients show reduced levels of MeCP2 and subsequently higher levels of methylation of the *MECP2* promoter in males (Nagarajan et al., 2006). Recent development in next-generation high-throughput DNA methylome sequencing promises to reveal greater insight into the variation of epigenetic modification between different genomes and cell types (Zemach et al., 2010).

Perspective

There is little doubt that genetics are heavily implicated in the onset of ASD. However, determining the genetic basis of ASD is muddied by the countless number of genetic loci that have been implicated in contributing to the disease, or at the very least ASD-like symptoms. This conundrum highlights the deeper, underlying issue of the vagaries of ASD diagnosis: the fact that

diagnosis of the disease itself is so nebulously defined contributes to the countless – and growing – number of genomic defects associated with the disease. The DSM-5 outlines a set of behavioral criteria that serve as the primary means of identifying ASR, but the fundamentally subjective nature of the application of these criteria, and the similarity of symptoms of autism to other related diseases such as Rett syndrome, may hamper the efforts of genetic and epigenetic screens by contributing to a possibly insurmountable level of unwanted variation. The association of ASD with such a wide variety of genes and diseases may be a consequence of the lack of a clear definition of the condition itself. A common analogy used to describe genome-wide association studies is that of a man trying to find a key in the dark (the genetic basis of a disease) and utilizing various sources of light to aid his search (utilizing SNP data to pinpoint possible clinically relevant genetic mutations). In the case of behavioral diseases such as ASD, the key in this case is either hard to recognize or not easily distinguishable from objects surrounding it, rendering any usage of additional light sources less effective. Nevertheless, ASD research has revealed a number of critical loci that are highly correlated with symptoms of ASD, providing valuable genetic information for clinical and scientific usage.

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