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Pathogenesis of autism: a patchwork of genetic causes

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Abstract

Autism spectrum disorders (ASDs) are relatively infrequent but are devastating developmental conditions characterized by marked deficiencies in social, communicative and other behavioral domains. It has been known for a substantial period of time that these disorders are genetic in nature. However, elucidating the specific mechanisms of these disorders has been difficult. A major reason for such difficulty is the recognized genetic heterogeneity of ASDs. Specifically, many genetic mechanisms related to structural variations in the genome have been reported as possible genetic causes of these disorders. This review briefly exemplifies these genetic mechanisms, presents a concise overview of the evidence for the genetic basis of ASDs and provides an appraisal of the specific structural genetic variants thought to contribute to the pathogenesis of these complex disorders.

Keywords

common genetic variants; copy number variation; familial risk; heritability; rare genetic variants

The disorders forming the cluster of so-called autism spectrum disorders (ASDs) are neurodevelopmental conditions characterized primarily by severely disturbed patterns of reciprocal social interaction, but are further differentiated by a number of other features, such as atypical or deficient communication skills and stereotypical/repetitive behaviors (Box 1) [1]. When considered collectively across many studies, the prevalence of ASDs is estimated at approximately 0.004, or less than 0.5% in the general population [2]. These estimates are conservative and possibly reflect the lower boundary of the frequency of ASDs in the general population [3]. Distinguished both by the severity of their core deficits and their rarity, ASDs have been the focus of many clinical and scientific inquiries during the last 20–30 years. These years have been very productive in describing the manifested clinical facets of ASDs [4], developing ASD-specific and -sensitive diagnostic approaches (Box 2) [5,6], and gaining insights into the complex etiology of this cluster of neurodevelopmental disorders [7].

One of the main realizations gained from these etiological investigations into ASDs is that this cluster of disorders is predominantly genetic in nature [8,9]. However, along with this observation has come the apprehension of the remarkable genetic heterogeneity and complexity associated with the pathogenesis of ASDs. Given that ASDs are behaviorally defined and, thus, are similar to each other behaviorally, the tremendous amount of genetic variation that has

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been encountered in studies of these disorders is truly remarkable. The working hypothesis of ASDs today is that their genetic texture is related to no single common cause, but to a variety of variable genetic causes, which might act independently, in combination with each other or in combination with the environment. The consensus in the field [10] is that there are multiple pathways resulting in ASDs and that they are all characterized by the presence of genetic risk factors (i.e., there is no evidence of any purely 'environmental' ASDs). These genetic risk factors appear to be extremely diverse in terms of their frequency, magnitude, mechanism of action, sensitivity to environmental effects and ASD-related specificity.

For example, etiologically, some cases of ASDs have been associated with rare (or very infrequent) causes [10]. This means that there are a relatively limited number of occurrences of ASDs that can be explained by such uncommon causes, yet, for these cases, the explanatory power of these rare variants is high, although their ASD-related specificity might be relatively low. It is possible that these rare or infrequent genetic causes are a part of the common genetic pathways central to human developments, such as corticogenesis [11], brain connectivity [12] and synaptic function [13]. It is possible as well that these pathways may be disrupted in multiple ways, that is, each type of a disruption might be infrequent, but, collectively, these disruptions form a common mechanism prevalent in ASDs [14]. However, these possibilities are far from being well documented and, at this point, they remain hypothetical [1].

There is also growing evidence that the core facets of ASDs (i.e., their social, communication and repetitive/restrictive behavioral manifestations) appear to correlate only moderately behaviorally and genetically [15]. In other words, there are accumulating data that suggest that each of these facets of ASDs might be controlled only partially (if at all) by overlapping constellations of genes [16]. Contrary to the mechanics of the impact of rare variants, the genetic variation contributing to ASD facets could be common, representing the lower tails of the distribution of the indicators of these facets in the general population. The reasoning here is that ASDs arise from large constellations of common risk variants that, when below a particular quantitative threshold, may constitute a source of individual differences in disorder-free samples, but, over a particular quantitative threshold, might then form the genetic foundation for the emergence of ASDs [15].

Box 1. Criteria for autism spectrum disorder

The autism spectrum disorder (ASD) cluster typically includes autism disorder, pervasive developmental disorder and Asperger's syndrome. The diagnosis of ASDs requires the presence of specific impairments in each of three core domains before 3 years of age. Impairments in the social domain include marked deficiencies in nonverbal communication (e.g., eye-to-eye gaze, facial expression and body language), lack of developmentally appropriate skills for establishing and maintaining peer relationships, lack of socially appropriate spontaneous drive for inclusion and lack of social-emotional reciprocity. Impairments in the communicative domain include delays in the development of spoken language and other means of human interactions, inappropriate, unnatural, stereotyped or restricted use of language, and lack of skills pertaining to play initiation or engagement. The presence of restricted or stereotyped behaviors are indicated by preoccupations with one or more stereotyped and restricted areas of interest, unbendable observance of nonfunctional routines or rituals, recurring motor mannerisms, and fixation on parts of objects. Across all ASDs, affected males are observed at a higher frequency than females (4:1), but the ratio approaches equality when only severe cases of ASDs are considered.

The literature offers a basis for hypothesizing that rare mutations tend to be more deleterious and generate more severe phenotypes in multiple ASD-related domains, whereas common mutations tend to be less detrimental and associated with less severe and more ASD facet-

specific phenotypes [10]. It has also been observed that various types of ASD-related genetic mechanisms are vulnerable to environmental impacts, both generating risk and protection to varying degrees. Such variable environmental sensitivity might possibly explain the role and presence of comorbid conditions in ASDs (e.g., immune dysfunction; see following sections for more discussion).

This tension between the abundant convincing evidence that ASDs are genetic in nature and the failure thus far to identify any specific genetic mechanisms that consistently explain not only selected but whole categories of ASD cases has caused researchers working in the field of ASDs to focus on different approaches, hoping to elicit plausible elements of the genetic machinery that, when broken, form the risk factors for the emergence of ASDs. Here I briefly sample from these various approaches, outlining the exciting work that is unfolding within the attempts to understand the roles of, first, structural variation in the genome and the associations between this variation either across the whole genome or across particular gene candidates (or gene families or gene pathways) and the emergence of ASDs; and second, functional characteristics of the genome and the connections between these characteristics and the manifestation of ASDs. The overarching assumption of this brief commentary is that the pathogenesis of ASDs is complex and most likely involves multiple mechanisms. This assumption underscores the true nature of the diverse landscape of genetic, genomic and epigenomic findings in studies of ASDs. Thus, it is very likely that some of the relevant findings (for a comprehensive review, refer to [1]) might not sustain the pressure of replication and interpretation. Future research into this pieced together picture of the genetic bases of ASDs, will hopefully clarify the genetic texture of ASDs and separate true and misleading findings.

ASDs & the nonspecific genetic evidence

The first appreciation of the role of genetic factors in the pathogenesis of ASD came from biometric investigations of families of probands with ASDs. These investigations revealed substantially elevated, compared with the population-based, risk ratios for relatives of individuals with ASDs, for both twins [17–19] and siblings [20]. Of significance, was the fact that ASD-associated, although not clinically diagnosable, behavioral features, such as slight cognitive, language and motor impairments, also appeared to be occurring in families of ASD probands at levels higher than those in the general population [21–23]. Finally, there was a large body of evidence indicating a remarkable amount of individual differences in the general population on the traits that are deficient in ASDs indicating a substantial role of genes in generating these differences [24–27].

Findings from early family and twin studies of ASDs allowed researchers to justify the initiation of searches for the regions in the human genome that, based on statistical evidence, should harbor genes governing the transmission of genetic liability factor for the manifestation of ASDs. Initially these whole-genome investigations were limited to linkage studies only, engaging siblings affected with ASDs. At the start, such studies were characterized by modest sample sizes but, due in part to the collaborative efforts of many investigators around the world, sample sizes grew considerably. Such linkage studies generated a network of results implicating, collectively, multiple regions of the genome that appeared to contain genes of relevance to ASDs (for details of this work, see meta-analysis [28] and recent reviews [1]). However, there is no single region of the genome and no single study that has generated unequivocal evidence toward the identification of a strong candidate gene (i.e., a discovery path that is characteristic of many other neuropsychiatric disorders such as Alzheimer's disorders or cystic fibrosis). Currently, the field entertains approximately a dozen or more distinct regions in the genome (e.g., 1q, 2q, 3q, 4q, 5p, 7q, 9p, 9q, 11p, 11q, 14q, 15q, 17q, 19p, 21q, 22q and Xq) that, based on the available statistical evidence, might be harboring one or more ASD-associated genes; this evidence is used directly (i.e., by means of regional fine-

mapping) and indirectly (i.e., as a confirmational evidence for other studies) in the ongoing research into the genetics of ASDs.

In summary, there is a substantial body of research, conducted both with biometric and genetic linkage methodologies, implicating the genome in the etiology of ASDs. However, translating this general convergence of evidence into an understanding of the specifics of the genetic machinery involved has been frustratingly difficult. Some of the sources of this frustration are discussed below.

ASD & candidate genes

The evidence implicating specific genetic variants in the pathogenesis of ASDs has been generated from studies involving various genetic methodologies (e.g., cytogenetic and statistical techniques) and designs (e.g., case-control and family studies). Based on the results of these studies, multiple hypotheses of the cellular deficiencies underlying the manifestation of ASDs have been developed. These hypotheses assume roles for both rare and common genetic variants in ASDs. Many of these variants are now listed in the AutDB [201], a publicly available web portal for the ongoing collection, manual annotation and visualization of genes linked to the disorder [29].

As mentioned above, the emergence of ASDs has been shown to be associated with the impact of rare variants in a number of genes. In an attempt to summarize the common denominator of these genes, researchers have proposed clustering them by function.

One group of such genes are those encoding synaptic proteins [1]. Specifically, the literature contains evidence of the involvement of the genes coding for proteins that are from or related to the families of neuroligins [30–32] and neurexins [33–36]. Both of these types of proteins – the former being presynaptic and the latter being postsynaptic – are crucial for ensuring the precise organization of the neuronal terminals needed for neural connections. There is also evidence that ASDs are associated with rare alterations in a member of the family of shank proteins [37,38]. These are scaffolding adaptors whose function is, in part, to integrate neurotransmitter receptors into the cortical cytoskeleton at postsynaptic densities; of note, at least some shank proteins interact with neuroligins [39].

A second group of genes that has been implicated in ASDs are genes involved in short- and long-range signaling. Specifically, there is evidence that excitatory (e.g., glutamatergic) neurotransmission may be disrupted [40,41] in ASDs. Nonetheless, there is also a string of data pointing to deficiencies in the inhibitory synapses in ASDs [42]; these data are in concert with the presence of seizures in a subgroup of patients with ASDs [43]. Similarly, there is a substantial body of evidence implicating disrupted serotonin-based signaling in this disorder [44]. Also, of interest is a cluster of genes whose proteins are related to hormonal signaling; variants in a number of such genes, most notably genes implicated in bonding behaviors in animals (e.g., oxytocin and prolactin), have been associated with ASDs [16]. Moreover, research also points out the disruption of calcium signaling in ASDs [45].

Box 2. Assessment devices used for autism spectrum disorder diagnosis

Major advances in the field of autism spectrum disorders (ASDs) have been reached with the introduction of standardized clinical instruments used for ASD diagnosis (i.e., Autism Diagnostic Interview [ADI] and Autism Diagnostic Observational Schedule [ADOS]). The ADI is typically presented to the parents of probands with ASDs. The administration of the ADI results in three subscores (ADI social, ADI communication and ADI stereotypical behaviors). The ADOS is administered to probands themselves and the schedule's

administration results in four subscores (ADOS social, ADOS communication, ADOS stereotypical behaviors and ADOS imaginative skills). The threshold-based combination of ADI and ADOS scores, as well as consensus-based clinical judgments, are used in clinical and research work involving ASDs.

A third large group of candidate genes thought to be involved with the pathogenesis of ASDs involves genes whose products have been found in the regions of the brain indicated to be abnormal in ASDs or malfunctioning in ASDs, and/or which are known to be involved with the manifestations of behaviors deficient in ASDs [46]. Specifically, these pertain to the structural characteristics of the cortex and its frontal and anterior temporal regions [47,48], hemispheres [49] and cerebellum [50] in ASDs, as well as disrupted connectivity between the various regions of the brain [51]. Functional evidence brings the research focus to brain regions that are hypothesized to be involved in the networks supporting such behaviors, such as joint attention and early social reciprocity [52].

The final group of molecules to be mentioned here is the group that affects cortical neuron growth and differentiation. This line of inquiry is directly related to the observation that approximately 20% of individuals with ASDs have macrocephaly (e.g., a disproportionally, to the rest of the body, large head at two or more standard deviations above the population mean [53]). Correspondingly, genes regulating neuronal number, migration and differentiation form an important group of genes to consider in the pathogenesis of ASDs. Among these genes are FGF genes, whose signaling regulates the number of excitatory neurons and the maturation of the cerebral cortex [11]. This group also includes genes involved in the regulation of GABAergic neuron migration [54–56] and the semaphoring/neuropilin system [11]. It also includes MET tyrosine kinase, a gene product that regulates the migration of GABA interneurons [57], and PTEN, a gene product that downregulates the PI3K pathway and is involved in cell-cycle regulation [58,59]. Of note is the observation that the PI3K pathway has been reported to enhance precursor cell proliferation and survival, and is hypothesized to be regulated, at least in part, by FGF receptor signaling [60].

Thus, there are numerous candidate genes, and even groups of candidate genes, that have been identified as possibly contributing to the genetic bases of ASDs. There appears to be a wealth of data in this literature. Nonetheless, it should be made clear that most of this evidence is viewed as interesting and promising, but not conclusive.

ASDs & dosage effects

The discovery of extensive copy-number variation (CNV) [61,62] began yet another page in the inquiry of the genetic bases of ASDs. CNV is a source of interindividual disparity in the human genome resulting from the duplication, deletion or rearrangement of bits of DNA ranging from 1 kb to multiple megabases in length [63]. Whereas microscopic (e.g., larger than 5 Mb) chromosomal rearrangements have been known to be a source of genetic variation since the late 1950s, the detection of submicroscopic rearrangements is a direct outcome of advances in technology with regard to the level of resolution in genomic studies. There is a long history of research at the microscopic level of chromosomal abnormalities in ASDs; one of the most common abnormalities identified in individuals with ASDs is a maternal duplication on 15q11–13 [64]. Recently, much attention has also been given to deletions/duplications of chromosomal regions 15q13.3 [65] and 16p11.2 [34]. CNVs are quantitative structural variants in the genome that can stretch over none or one or more genes. They arise from losses (deletions) and/or gains (duplications) of genetic material at a locus of variable length [66]. Thus, CNVs are typically defined as regions of altered gene dosage, whereas chromosomal rearrangements are typically defined as structural changes with no alterations in gene dosage. They are observed at numbers much larger than initially suspected, and it is assumed that many of them affect gene function

[67]. There is now evidence that the presence of CNVs indicates a genomic imbalance; this imbalance appears to be reflective of human evolution [68], and might have benign or clinical significance. Although initially CNVs were detected in samples of healthy individuals [61, 62], there is a growing number of literature associating CNVs with various clinical phenotypes [69,70]. One such association is with ASDs [35,71,72].

These newly accumulating data on the role of CNVs in ASDs indicate that both inherited (i.e., present in parents of individuals with ASDs) and *de novo* (i.e., arising only in individuals with ASDs themselves) CNVs appear to contribute to the genetic vulnerability for this disorder. These data are contextualized by well-known evidence implicating various karyotypic and submicroscopic CNV abnormalities in the pathogenesis of ASDs [1]. With regard to the role of CNVs in ASDs, relevant findings are presented along the following lines [70].

Copy-number variations in individuals with ASDs are observed at substantially higher rates than in typically developing individuals [71]. There appear to be ASD-specific CNVs, both heritable and *de novo* [73]. In addition, there appear to be family-specific heritable CNVs that are transmitted in families with ASDs, and up to 40% of such CNVs are present in both probands and asymptomatic parents [73–75]. The proportion of *de novo* CNVs differed in probands depending on whether they did (i.e., 2–3% of *de novo* CNVs) or did not (i.e., 7–10% of *de novo* CNVs) have other relatives with ASDs [71,75]. It is also notable that the rate of *de novo* CNVs was particularly low in ASD families with shared ancestry [72]. Probands with ASDs can carry multiple *de novo* mutations [73,75]. Unrelated individuals with ASDs were reported to have CNVs at the same locus; some of these co-incidental CNVs were inherited, whereas others were *de novo* [70]. A large proportion (27%) of families with syndromic ASDs (i.e., families of probands with ASDs observed in the context of a recognized genetic syndrome, such as fragile X syndrome) carry *de novo* CNVs [76]. It is also notable that there is a disproportionately frequent occurrence of CNVs within genes that have been reported to be involved in neural signal transmission and transduction [35,72,73,77].

In summary, it appears that the degree and nature of the genomic imbalance bestowed by CNVs differentiates individuals with ASDs from typically developing individuals. This is clearly a promising lead, but it needs to be developed further both through differentiating ASD-specific patterns of CNVs from those of other developmental disorders and through understanding the specifics of the genomic machinery behind CNVs in ASDs.

ASDs & other disorders

Individuals with ASDs often exhibit additional disorders, including seizures, gastrointestinal and neurological symptoms, and immune abnormalities. Here I briefly present the links between ASDs and immune abnormalities; these links exemplify yet another aspect of ASD manifestations, the one related to somatic health disturbances in ASDs.

The association between immunological deficiencies and ASDs has been present in the literature for some time [78], although the etiology of this connection is not clear. Early reports contain evidence of a high frequency of autoimmune diseases among family members of probands with ASDs [79] when compared with control families [80,81]. Similarly, when compared with groups of typically developing individuals, probands with ASDs demonstrate elevations in indicators of chronic neuroinflammation in the brain [82–85], blood and urine [86–89]. Searching for the mechanisms underlying these differences, researchers have registered, in probands with ASDs, abnormal cellular immune responses [90–94], especially in the brain tissue [95] and other autoimmune abnormalities [96,97]. In addition, there have been ASD studies investigating allelic association and genetic linkage in the region of the MHC; however, the results of these studies are mixed, with some pointing to the presence of

the association [98,99] and others excluding linkage [100]. There is also some evidence of the involvement of innate neuroimmune genetic mechanisms in the foundation of ASDs [101].

In summary, the literature on the presence of immune problems in probands with ASDs and their families contains a critical mass of information sufficient to generate a hypothesis regarding the involvement of autoimmune genes in the pathogenesis of ASDs. However, at present, views of possible immune dysfunction in ASDs range from proposals that it may contribute to manifestations of the disorder in some patients [86] to hypotheses that neuroimmunopathogenic responses play a fundamental role in ASDs [102]. It is also important to note that studies indicate that innate rather than adaptive neuroimmune responses contribute to the pathogenesis of ASDs [103]. One active hypothesis in the field is that, given the complex model of inheritance in ASDs, one possible etiopathic mechanism might involve an immunologic insult to the CNS in individuals with a susceptible genetic background [101].

Conclusion

To conclude, what is known regarding the genetic bases of ASDs now suggests that multiple types of genetic mechanisms appear to be involved in the development and manifestations of this disorder. These mechanisms vary in their observed frequencies (rare vs common), their mode of transmission (Mendelian vs complex oligogenic), the number of genes involved (single vs multiple), their origin (familial vs *de novo*), the degree of vulnerability to environmental influences (sensitivity and susceptibility to environmental risk and protective factors) and, perhaps, many other dimensions of heterogeneity. All in all, the journey toward understanding the genetic mechanisms of ASDs has been both exciting and frustrating; the field, at this point, possesses much more knowledge with regard to the etiology of ASDs, but, as before, it has not yet uncovered any specific genetic mechanisms that are understood well enough to develop and implement therapeutic interventions.

Future perspective

Owing to the particularly asocial nature of the manifestation of ASDs – the inability to relate to others, whether family members or strangers – this cluster of disorders is particularly debilitating for parents of probands with ASDs. Today, as a result of successful campaigns for public awareness and allocation of financial support, this cluster of disorders has the unprecedented focus of researchers. Thus, progress into the understanding of the genetic nature of ASDs will continue, and it is likely that this progress will unfold along the lines discussed in this review.

Specifically, given that rare *de novo* and heritable mutations account for at least 10–20% of the cases with ASDs [1], although each of them represents less than 1% of cases, researchers will continue exploring these rare mutations in the hope that they will unveil some common genetic pathways related to ASDs.

Of note, the very infrequent nature of these rare mutations opens a hypothetical possibility that other, perhaps more common, genetic variants interact with these mutations, somehow modulating, either positively or negatively, the impact of the rare variants. Thus, further explorations into the common mutations associated with ASDs are also very important. Given what we currently know regarding the genetic texture of ASDs, it is most likely these common variants that are associated with the three core deficits are responsible for the behavioral diversity in the manifestation of ASDs. Understanding this diversity will enhance our knowledge of the pathogenesis of ASDs and, possibly, lead to facet-specific therapeutic interventions.

Finally, forming a discernment with regard to the role of rare and common genetic variants in the etiology of ASDs has a potential to teach us the importance (or lack of such) of the boundaries between various disorders. It is possible that the impact of rare variants is nonspecific and characterized by a general liability to developmental abnormalities, whereas the impact of common variants differentiate these abnormalities into what we consider to be specific developmental disorders.

Executive summary

- Autism spectrum disorders (ASDs) form a group of severe developmental conditions, characterized by deficiencies in social, communicative and behavioral domains of functioning.
- Early descriptive presentations of cases of ASDs anticipated the role of genetic factors in the etiology of these disorders. These hypotheses were investigated in twin and family studies of probands with ASDs; these disorders are now viewed as developmental disorders with the highest degree of genetic control. However, these nonspecific indicators of the presence of genetic control have been difficult to convert into a clear account of the genetic causality of ASDs.
- The currently dominating view of the genetic bases of ASDs acknowledges the high degree of genetic heterogeneity of these disorders that manifests itself by recruiting both rare and common genetic variants, as well as heritable and de novo genetic mechanisms.
- Although the specifics of the contributions of all these genetic mechanisms are unclear, it is possible that the impact of the rare variants is more severe and less specific, whereas the impact of the common variants is more defining and related to specific facets of ASDs.

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