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Abstract

Autism spectrum disorder (ASD) is a broad, heterogeneous neurodevelopmental disorder encompassing a range of presentation and severity. The common characteristics include communication deficits, impaired social skills, dependency on routine, sensitivity to environmental change, and stereotyped behavior (DSM-5, 2013). When ASD is accompanied by a host of other symptoms it is often referred to as syndromic autism. Syndromic autism is usually severe and can usually be traced to deletions or duplications on a specific gene. These monogenic disorders are by definition easier to diagnose and are good candidates for study since specific biological markers can be assessed and tracked. There have been many discoveries about monogenic autism which help provide insight into the mechanisms of disease. Nonsyndromic autism (also called idiopathic autism) is defined by the absence of additional symptoms or underlying syndromes. Although nonsyndromic autism is far more common and often manifests in a milder form of the disorder, the genes and proteins involved are much more difficult to nail down, and therapeutics are harder to discover. Family and twin studies have provided evidence that the majority of cases are due to common genetic variation (Gaugler et al., 2014).

Shifting the focus to syndromic autism has been a critical step in understanding the disease process that underlies the different manifestations of ASD. This is the key to solving the complex web of common genetic variants implicated in nonsyndromic autism.

Keywords: Autism, ASD, monogenic, syndromic, common variants, loss of function.

Introduction

Autism Spectrum Disorder (ASD) has been historically classified into two basic categories, syndromic and nonsyndromic. In nonsyndromic autism, communication and social impairment, accompanied by stereotyped behaviors represent the main symptoms observed. Syndromic ASDs are disorders wherein the autistic presentation is just one part of a broader neurological syndrome. In Phelan-McDermid Syndrome, for example, which is caused by mutations in the SHANK3 gene, the autistic phenotype is accompanied by developmental delay, intellectual disabilities, seizures, and hypotonia (Phelan et al., 1992). Another example is tuberous sclerosis complex, caused by mutations in either TSC1 or TSC2 (European Chromosome 16 Tuberous Sclerosis Consortium, 1993), wherein ASD is featured along with many other developmental and physical symptoms (Slegtenhorst et al., 1997). Many other chromosomal deletion and duplication syndromes include ASD as a feature of the disease, including Smith Magenis syndrome, Wolf-Hirschhorn syndrome, and Rett syndrome (Laje et al., 2010, Fisch et al., 2008, Hu et al., 2018).

Autism was first described in 1943 (Kanner, 1943). Even then it was assumed to be caused by an inborn (i.e genetic) disturbance. Great scientific and technological strides have been made since then and we are just beginning to uncover the molecular pathways involved. At this point, most ASD diagnoses are still made based on behavior (Lord et al., 2012), and most available therapies target symptoms and not underlying pathophysiology.

The extreme range of ASD manifestation adds a level of complexity when trying to understand its biology. Its very name indicates that there is a continuum of clinical phenotypes that make up what would seem more appropriate to call a category of neurodevelopmental disorders that are similar in symptomatology. On a genetic level, autism becomes even more complicated, as hundreds of diverse genes are found to have an association with autism susceptibility (Berg & Geshwind, 2012).

Dramatic advances over the last few years have led to an unraveling of the mystery that is ASD. The complete picture is not yet clear, but we are beginning to see coherence and cohesion within the wide range of variants and presentations and this has led to a reevaluation of the way we currently classify ASD. The newer classification will likely stratify ASDs by which biological pathway is affected. This will enable a more targeted approach to therapy and treatment, which will focus on the restoration of underlying biological issues and not just symptom control.

Methods

To gain a better perspective on the current direction of autism study, the available literature on Touro Online Library was searched using the search term "monogenic autism". Many review articles from the last 10 years were read and their information was synthesized to form a coherent overview of the latest thinking on ASD. The focus has been to highlight the differences between syndromic and nonsyndromic autism, as well as the areas in which they are beginning to converge.

Autism Etiology

The causes of autism were presumed to be genetic since it was first described (Kanner, 1943). Although there has been lots of speculation about environmental causes of autism, most of this has been thoroughly debunked (e.g. Wakefield's vaccine hypothesis). Environmental causes for ASD have been implicated only in a small percentage of cases (Newschaffer et al., 2007).

Genetic factors have been demonstrated to cause autism in many of the monogenic syndromes in which autism is commonly featured, such as Rett, ADNP, Phelan-McDermid,

and Fragile X. Additionally, epidemiological evidence from family and twin studies make it clear that genetics plays a large role in etiology. Nearly 20% of babies with an affected older sibling will develop ASD (Ozonoff et al., 2011). Twin studies show that monozygotic twins share a higher concordance rate of diagnosis for autism as compared with dizygotic twins (Hallmayer et al., 2011).

Although the case for genetic etiology of ASD is quite convincing, it has been very difficult to identify which genes account for the majority of incidence. One of the major problems with studying ASD is that its heterogeneity creates small sample sizes within each manifestation of autism along the spectrum. Much larger sample sizes are needed to locate the responsible genetic variants.

Autism Sequencing Consortium

One of the main advances in autism over the last decade has come about due to the cooperation of many different autism research centers under one umbrella group called the Autism Sequencing Consortium (ASC). The goal of ASC is to increase the power of genetic analysis by pooling resources and samples from research centers around the world. A large sample size is obtained through the consortium, allowing for meta-analysis of tens of thousands of samples. This increases the power of genome-wide association studies (GWAS) and more can be extrapolated from the data.

By February 2020, the ASC had identified 102 genes associated with ASD, of which, 31 are novel risk genes never previously identified. These genes are quite diverse and run the gamut of different cell functions. However, further analysis showed lots of connections between the biological pathways mediated by these genes (Satterstrom et al., 2020).

Understanding the Genetics of Autism

When thinking about genetic mutations there are two key classifications. The first is the frequency of the allele, namely if it is common, low frequency, or rare. The second is the potency of its effect; whether it is penetrant or if the effect is modest. Generally, the more penetrant a mutation is for a syndrome like ASD, the less frequent it is due to natural selection. Namely, someone carrying such a gene is far less likely to reproduce and thus the allele will become less common. Additionally, a variant that is rare and has a mild ASD causing effect is not part of the discussion since the sample size would have to be enormous to pick up such a rare and subtle ASD contributor.

Thus the current autism discourse is focused on the linear progression of genetic variation; there are the most common variants, which tend to have smaller effect sizes, while the rarer are highly penetrant and the likelihood of an autism diagnosis is almost certain (Manolio et al., 2009, Figure 1). Of the 102 ASD risk genes discovered by the ASC, it is clear that some have a larger impact than others. For example, there is an 80% chance of ASD in those with a SHANK3 mutation (Soorya et al., 2013), the gene deletion that causes Phelan-McDermid syndrome (Nesslinger et al., 1994). Meanwhile, there are many gene variants among the 102 genes which are prevalent in the population and this is because they only marginally predispose a person to ASD. Each of these common genetic variants individually only represents a small effect size in producing an autistic phenotype. Enough of these variations en masse seems to push the patient over a figurative threshold. Thus these common variants combine to increase the possibility of ASD (Gaugler et al., 2014).

The Focus of Autism Research

The challenge upon identifying these mutations is in mapping the variation to specific biological changes and understanding their outcome. This step is crucial in creating a model for that particular mutation. Once a model is made, the mechanics in which that gene is involved can be analyzed, which will help identify the pathogenic pathways. Eventually, experiments can be designed to try to counteract the effects created, leading to a potential treatment.

It is extremely difficult to measure the biological changes they create with common genetic variants since the effect size is often infinitesimal ((Manolio et al., 2009). Consequently, the path toward understanding and discovering treatments for idiopathic autism runs through monogenic syndromic autism (Ziats, et al., 2021). These rarer variants have major effects and are highly penetrant. Figuring out the biological conditions created by these variations is straightforward. Therefore, the potential for designing biological models–both cell and animal–is very high.

Thus, the current thinking on autism research is a move to personalized medicine. Narrowing the focus to one or several of the monogenic ASDs allow researchers to create models, discover phenotypes, and establish baseline biomarkers, all to discover treatments that can work for that particular autistic presentation. In doing so, not only can this provide relief for patients who struggle with some of the most severe forms of autism, but the hope is that these studies can help uncover the mystery of idiopathic autism, as well.

The discovery of a new drug for one small subset of ASD can help further the science in autism more broadly. Firstly, the new drug can be tested on the wider autistic population in the hope that its efficacy might not be limited to just that subset. Secondly, drug discovery

often proves or provides clarity to a particular theory of disease. For example, if we find a compound that helps to normalize biomarkers in a certain disease phenotype, this can lead to the confirmation of a previous disease hypothesis or the formation of a new one. All of these findings may offer clues for pathophysiological pathways in other disorders on the autism spectrum in addition to the one being studied.

Modeling Autism Spectrum Disorder Pathophysiology

Despite all the progress made in identifying genes and fashioning animal models, there is a lot more to uncover in ASD. We can potentially create many different models for all of the different genetic mutations but it may not be necessary. After all, the various mutations all lead to similar symptomatology, namely, the autistic phenotype. This seems to imply convergence of biological pathways at some point to create a common result (Geschwind, 2008). This is another angle that autism researchers are probing.

Systems biology is put to excellent use in connecting the various genes that have been associated with risk for autism. It has been repeatedly demonstrated that the autism implicated genes are part of networks that involve gene expression and synaptic modeling (Voineagu, et al., 2011, Gilman, et al., 2011). Different cutting edge techniques were used in these studies that all show how these genes are grouped together in performing neurodevelopmental work, particularly, in the prenatal and neonatal period. This also demonstrates a relationship among ASD risk genes that is statistically significant as well as gives evidence of their function (Chen et al, 2015).

Finally, as the power of genome-wide association studies increases, the more we can connect common genetic variants to the rare ones. From a molecular perspective, polygenic autism is more similar to monogenic autism than previously thought. This is because risk genes for syndromic and nonsyndromic converge in several different cellular pathways (De Rubeis et al., 2014). Mastering these pathways will help us to classify and treat autistic disorders in groups with the same affected pathway.

Drug Discovery for Monogenic Autism

The next step would be to create human neurons derived from patient-induced pluripotent stem cells and develop tests to measure their protein expression and function. All of these studies brought into a systems biology framework will help piece together multiple levels of anatomical, physiological, and genomic data. This will enhance the ability to test the functional, spatial, and temporal convergence of ASD genes (Dolmetsch & Geschwind, 2011).

The potential for the discovery of an effective treatment for monogenic autism is high. The reason for this is because many of the mutations discovered through GWAS are loss of function (LoF) de novo (arising in the germ cell of a parent) mutations (De Rubeis et al., 2014). The fact that autism is a result of a heterozygous LoF allele is an optimistic finding. It implies that the problem can be corrected if we can find a way to boost the remaining (i.e. functioning) allele. If it is overexpressed sufficiently, it may fully compensate for the mutated allele.

Drug Discovery is a multi-step process and takes years to go from promising compounds to clinical trials. It starts with isolating peripheral blood mononuclear cells (PBMCs) derived from syndromic ASD patients and their unaffected siblings or parents. These PBMCs are given reagents that regulate gene expression and dedifferentiate them back to pluripotency. In this state, they are called IPSCs (induced pluripotent stem cells). Then, using a retrovirus, the IPSCs are infected with a plasmid that contains certain genes which induce neuron differentiation (Yang et al., 2011).

After the induced neuronal (iN) cells are created, the goal is to find a method of distinguishing the neurons obtained from the proband vs the immediate relative which can be employed in a high-throughput assay. That way, a normal and abnormal phenotype can be defined in terms of this disorder. One potential test is to see if conductance can be altered between the two phenotypes given certain conditions. This can lead to many hundreds or even thousands of prospective drugs being tested simultaneously to see which has the potential to restore the iN cells to their normal phenotype.

If compounds that restore patient-derived iN cells to a healthier phenotype are identified, they can be studied further, used on animal models, and ultimately, a clinical trial can be conducted on actual patients. Using these steps has the potential to identify treatments that are aimed not merely toward symptom relief but the treatment of underlying biology. Additionally, the drug can be tried on other ASD variants and may shed light on the broader ASD pathophysiology.

Conclusion: Rethinking Autism Classification

Over the last decade, autism research has grown in leaps and bounds. Its heterogeneity and wide variations in phenotype make it difficult to determine etiology. However, through the use of an array of new technologies which can map the genome down to the base pair, much information has come out regarding particular genes that contribute to autism predisposition.

The wide variation in ASD phenotypes and the different genes and pathways would seem to fly in the face

of a biological convergence, yet we have encountered evidence from many studies that indicate how ASD etiologies can be organized by the intermediate pathway in which it plays a part. Perhaps as more research is done and treatments are discovered, it will become clear that ASD is a very particular manifestation that can result from many different genes that share common pathways. There is a great deal of evidence that shows convergence on intermediate biological levels which corroborate a "many genes, common pathways" hypothesis (Geschwind, 2008, Chen et al., 2015).

Therefore, a shift of focus toward the rare variants that cause syndromic autism has begun to garner results. This is because these variants represent low-hanging fruit in that they are really easy to detect and study. Normal and knockout phenotypes can be described, created in models, studied, and tested. This avenue of research can also benefit nonsyndromic autism research by helping define certain parameters and mechanisms of autism pathology.

While the historical classification between syndromic and nonsyndromic autism has been useful, it seems likely that this classification scheme will come to an end. As we start to see some convergence between syndromic and idiopathic autism on the level of molecular pathways, the various disorders will start being categorized–and treated–based on their individual pathogenic routes.

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