Review

A Review of the Neurobiological Basis of Autism

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ABSTRACT

This manuscript reviews ongoing developments in autism research that defines it as a behavioral phenotype with many known neurobiological and molecular causes. Strides in neuroimaging, histopathology, neurophysiology, genetics and metabolic disorders are discussed that support the understanding of autism as a pervasive abnormality of neural systems with particular dysfunction of neuronal connections that impacts the development of socialization, communication and behavior. The aim of this review is to provide insight into the current knowledge of the complex pathophysiology of autism.

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KEY WORDS: *Autism, Seizures, Neurobiological basis, connectivity*

INTRODUCTION

Autism, as defined by the Diagnostics and Statistics Manual of Mental Disorders, 4th ed. (DSM-IV), is a disorder consisting of variable degrees of abnormal social reciprocity and interaction, communicative intent, and repetitive, stereotyped patterns of behavior, interests and activities with onset before three years of age. Autism has been recognized as a biologically and molecularly based complex neuro-developmental disorder that appears to be heritable involving multiple genes and demonstrates varied phenotypes with heterogenous etiology.[1] Increasingly researchers refer to "the autisms" rather than a single autism phenotype.[2] The reported prevalence of autism has increased to an estimated rate of 10-17% per year. It does not appear to have correlation with gender, race, ethnicity or socio-economic level. The neurobiological findings have led to the current understanding of autism as a disorder of neural systems and connections, mainly affecting local intra-cortical connections, cortico-cortical connections and cortico-subcortical connections.

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MACROCEPHALY AND INCREASE IN BRAIN VOLUME

Children with autism have a larger head circumference, with frank macrocephaly occurring in about 20%. MRI Voxel Brain Morphometry (VBM) studies have reported increase in total brain volume. This volume increase has been documented to begin between ages 2 to 4 years, the earliest age of clinical recognition and persists into childhood but not adolescence. The tissues contributing to this increase are total cerebral white and total cortical gray matter.[3,4] The onset of brain overgrowth coincides with the onset of signs and symptoms of autism, indicating that this critical period of overgrowth is part of a pathologic process that disrupts the development of normal brain structure and function. It occurs during a period of synapse formation, pruning and myelination that takes place during early postnatal periods. Synaptic pathology and abnormal connectivity appears to be a common denominator in autism etiologies and may have an important role in the increased prevalence of seizures in the autistic population. We will discuss below studies related to epilepsy, molecular and genetic studies, histopathologic studies, imaging studies and findings of abnormal metabolism in mitochondrial disorders as they relate to autism.

EPILEPSY

The relationship between autism and epilepsy has been recognized since the 1960's.[5] This distinct relationship gives insight into the neurobiological basis of autism. Seizures are common, occurring in 20-30% of patients diagnosed with the more symptomatic subset of individuals with autism as compared to 0.5-1% of the general population.[6] The risk of epilepsy in autism appears to be associated with the degree of intellectual disability and the severity of autistic symptoms.[7] The relationship between epilepsy, epileptiform discharges without clinical seizures and cognitive language and behavioral symptoms is not clearly understood, however it is likely due to a common underlying pathology. Subclinical epileptiform abnormalities are seen in 6.1-31% of patients

with autism.[8] It was suggested that the dysfunction of the specific neuronal network accounting for the behavioral syndrome of autism is due to a wide variety of insults to the developing brain and these insults affect more than one single neuronal network.[9] Environmental factors and prenatal exposures are explored in recent immunological studies supporting a fetal brain antibody in those with regressive autism[10] as well as history of maternal infection and medications that contribute to the autism phenotype.[11] Although antibodies towards brain proteins in children were reported to be associated with lower adaptive and cognitive function as well as core behaviors associated with autism, it is unclear whether these antibodies have direct pathologic significance, or if they are merely a response to previous injury.[10]

Certain seizure syndromes are often associated with cognitive and behavioral impairments. The presence of seizures and not just epileptiform abnormalities has been associated with more difficult behavior in autistic patients.[12] Additionally epilepsy has been noted to be significantly more frequent in autistic children with a history of regression.[13] Of great importance is that children with epilepsy appear to be at the greatest risk of developing autism when seizures start at around age 2 years or earlier. Early onset seizures are known to affect synaptic reorganization that likely hinders normal cognitive and behavioral development.[5] In autistic patients, a bi-modal distribution of seizures has been described with a peak before five years of age and an additional peak after 10 years. While the early peak during early development is likely due to significant insults to the developing brain, the second peak is known to occur in patients with the more moderate to severe mental retardation. This is perhaps due to a more progressive disease representing a disorder of energy metabolism such as a mitochondrial disease.[5] There is significant overlap in the development of Tuberous Sclerosis, Rett syndrome, epilepsy and the presence of autistic features which leads to the idea that genetic models can give a framework from which an etiology for autism can be deduced.

GENETIC TESTING

Family studies have shown that there is a 50-100 fold increase in the rate of autism in first degree relatives of autistic children. Although at least one autism linked abnormality has been found on almost every chromosome, the most significant correlations appear to be associated with chromosomes X, 2,3, 7, 15, 17 and 22.[1] A chromosomal abnormality seen in Prader-Willi Sydrome and Angelman Syndrome is reported in more than 1% of autistic individuals, and involves the proximal long arm of chromosome 15 (15q11-q13). Patients with Angelman syndrome typically have a happy, excitable demeanor with frequent smiling and laughter, a short attention span, and hand flapping movements. They present with global developmental delay, hypotonia, wide-based ataxic gait, seizures and spasticity. These patients lack appropriate social and language reciprocity and therefore often meet the criteria for autism. It is associated with a loss of maternally expressed ubiquitin protein ligase gene on a 15q deletion. A specific gene (SHANK3) known to encode a synaptic protein critical for brain development has been identified due to deletions of 22q13 which causes developmental disabilities, absent or delayed speech or other autistic behaviors. Findings of autistic features in macrocephalic patients with mutations in the PTEN tumor suppressor gene on chromosome 10 have been recently reported.[14]

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder that affects neural crest cells and a mutation in the NF1 gene on chromosome 17 results in the development of tumors or neurofibromas on nerve tissue. This neurocutaneous disorder has been associated with autistic features. The NF1 gene product, neurofibromin, regulates activation of the Ras intracellular signaling pathway in Schwann cells. It was suggested that *NF1*-dependent signaling cascades in neurons, fibroblasts, as well as Schwann cells, are required for normal myelination.[15]

Another syndrome associated with autism is Fragile X disorder. It is the most common known inherited genetic cause of mental retardation. It accounts for about one half of cases of X-linked mental retardation and is the most common cause of genetic mental impairment after trisomy 21. It is caused by a mutation in the FMR1 gene. The abnormality is caused by a trinucleotide (CGG) repeat expansion of greater than 200 repeats. Its phenotype includes intellectual disability, macrocephaly, large pinnae, large testicles, hypotonia and joint hyperextensibility. While the yield of DNA testing has a mean of 3-4%, 30-50% of patients with Fragile X demonstrate autistic features.[16] Since there are significant similarities between the autism that has been associated with Fragile X syndrome and idiopathic forms of autism, it is thought that the autism of the Fragile X syndrome may represent a paradigm for the study of possible common molecular pathways that lead to all forms of autism.[17]

One of the five pervasive developmental disorders, Rett syndrome is present in females who demonstrate autistic-like regression, microcephaly, seizures and hand-wringing stereotypies. This diagnosis is confirmed with DNA testing finding a mutation in the X-linked MECP2 gene. Newer studies have found that although previously thought to be lethal in males, this mutation can be found in males with intellectual disability.[18] Tuberous Sclerosis is another neurocutaneous disorder that is characterized by hypopigmented macules, fibroangiomata, kidney lesions, CNS hamartomas, seizures, intellectual disability, and very commonly autistic and/ or ADHD-like behaviors. Mutations are seen at 9q and 16p. De novo mutations contribute to 70% of Tuberous Sclerosis cases. The discovery of the mTOR signaling pathway has contributed to further understanding of the neurobiological basis of autism in that mutations seen in Tuberous Sclerosis, Fragile X syndrome, Neurofibromatosis and autism associated with PTEN mutations alter this

cascade which disrupts the regulation of numerous cellular processes in the developing and mature brain.[14]

Autism is thought to be due to the culmination of mutations in multiple genetic categories of function that includes genes that regulate expression of other genes, alter actin cytoskeleton dynamics, affect synapse formation, operate as components of second messenger systems, influence neuronal migration, guide neuronal connectivity and inhibit or stimulate synaptic connections.[17] For example, certain genetic studies supported by mouse models have proposed mutations in the Neurexin 1-alpha gene, a gene that helps neurons form brain synapses. Deletions in the Neurexin 1-alpha gene were identified in patients with autism or schizophrenia. This was further supported when mouse models that were Neurexin 1-alpha deficient displayed behaviors that included increased grooming behaviors and an impairment in nesting abilities, a phenotype that begins to approximate impairments seen in human subjects with autism.[19] Various discoveries in the genetic and molecular basis of autism indicate that rather than a single gene disorder, it is likely mutations influencing multiple genetic pathways that results in the phenotype of autism. This alteration in normal connectivity and circuitry likely aid in maldevelopment of socialization, communication and behavior with more severe symptomatology in those with seizures as evidenced by genetic disorders such as Tuberous Sclerosis and Rett syndrome.

NEUROIMAGING

Both functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) have supported the conceptual basis of autism as a disorder of aberrant connectivity. The discovery and study of mirror neurons through fMRI technique has been monumental in the understanding of autism. These were first discovered in the ventral premotor cortex of monkeys which fired not only while performing actions but also while observing the same actions performed by others. The mirror neuron system has been implicated in the underlying mechanism for social cognition and appears to provide the anatomic basis for understanding the actions and intentions of others.[20] Activity of mirror neurons has been found in the premotor cortex, supplementary motor area, primary somatosensory cortex and inferior parietal cortex. Most consistently reported to be activated during imitation, actions observation and intention understanding is the pars opercularis in the inferior frontal gyrus. During the imitation of emotional expressions, bilateral activation of striate and extra striate cortices, primary motor and premotor regions, limbic structures, the cerebellum and pars opercularis has been seen. It was also found that the greater the activity in the pars opercularis in children during imitation of facial expression as determined by fMRI, the higher the child's level of functioning as determined by ADOS (autism diagnostic observation schedule) and ADI-R (autism diagnostic interview-revised) testing.[20]

One of the most important cognitive skills for social interaction is the ability to attribute mental states to self and others referred to as "theory of mind" or the ability to look at a situation from another person's perspective. Evidence from fMRI studies investigating theory of mind tasks in control subjects has shown activation in a network consisting of the amygdala, the medial prefrontal cortex, the cingulate cortex, extra-striate cortex and the temporo-parietal junction[2]

Additionally, the brain areas that have been implicated in the three areas of core behaviors noted in children with autism have been identified. Regions implicated in social behavior include the frontal lobe, superior temporal cortex, parietal cortex and the amygdala.[2] Language function has been identified in the inferior frontal gyrus (Broca's area), supplementary motor cortex and superior temporal sulcus (Wernicke's area). Repetitive behaviors have been found to originate in the orbitofrontal cortex and the caudate nucleus.[2] (Table 1.) Patients with autism fail to activate the amygdala normally when processing emotional facial and eye expressions. Also, the fusiform face area is activated less in subjects with autism compared with control subjects during face perception tasks in fMRI studies.[21] A recent study determined that there was decreased neural synchronization in sleeping autistic patients using fMRI techniques. This disruption of neural synchronization during sleep may be generated by abnormal anatomical connectivity, synaptic function, local neural network or abnormal excitation/inhibition balance. These abnormalities in interhemispheric synchronization were seen significantly in the inferior frontal gyrus and superior temporal gyrus which was not seen in either controls or patients with language delay.[22]

Table 1. Brain Regions implicated in the three defining features of autism.

Autistic Feature	**Brain Regions**
Social Interaction	Frontal Lobe Superior Temporal Cortex Parietal Cortex Amygdala
Language Function	Inferior Frontal Gyrus (Broca's Area) Supplementary Motor Cortex Superior Temporal Sulcus (Wernicke's Area)
Repetitive Behaviors	Orbitofrontal Cortex Caudate Nucleus

While many techniques including fMRI, magnetoencephalography and PET (positron emission tomography) scanning have elucidated functional connectivity differences between the autistic and typically developing populations, structural connectivity between gray matter areas associated with intermediating white matter tracts are best evaluated with DTI. DTI is an MRI based technique that measures the

directional diffusion profile of water molecules which manifests the axonal architecture of the brain at the micrometer level. Fractional anisotropy and mean diffusivity provide an index for the integrity of neural tissue. DTI has been shown to be a sensitive measure of white matter maturation. Abnormalities in myelination, axonal number, diameter and orientation can all lead to changes in fractional anisotropy and apparent diffusion coefficient.[23,24] While several imaging studies discuss abnormal neural connectivity between grey matter areas, many DTI studies have shown decreased fractional anisotropy and thus directionality of the white matter microstructural architecture in the frontal white matter, temporal white matter, corpus callosum, superior temporal gyrus and cerebellum in the autism population.[25]

HISTOPATHOLOGY

Minicolumns are composed of radially oriented arrays of pyramidal neurons (layers II-VI), interneurons (layers I-VI) axons and dendrites. Minicolumns have been hypothesized to be the smallest radial unit of information processing in the cortex. They organize neurons in cortical space. Minicolumn formation has been associated with early stages of cortical development when post-mitotic neurons migrate in linear arrays along radial glial scaffolding. Therefore, changes based on circuitry may affect this unit of cortical structure.[26] As the brain develops there is an almost 1,000 fold increase in cortical surface which likely stems from an increase in number of ontogenetic cell columns. Although in autism while an increase in processing units occur in the developing brain, normal selective pruning may not occur. Therefore they may experience a chronic state of over-arousal and exhibit behaviors in order to diminish this arousal. Within the first year of life, there is a dramatic increase in dendritic growth and by three years of age, the minicolumns are spaced farther apart with a lower cell density. In autism, minicolumns have been reported to be increased in number and narrower in width with reduced neuropil space with smaller neuron cell bodies and nucleoli. These abnormalities have been observed bilaterally in the primary somatosensory cortex, primary motor cortex, dorsolateral prefrontal cortex, primary visual cortex, middle temporal gyrus and superior temporal gyrus. This narrowing of the minicolumns which was related to a reduction in neuropil space occupied by unmyelinated projections of gamma-aminobutyric acid (GABA) interneurons led to the hypotheses of a deficit of cortical inhibition, giving rise to increased seizure susceptibility. This phenomenon is postulated to explain the increased prevalence of seizures, sensory sensitivities and bias towards low level perceptual processing.[27]

MITOCHONDRIAL DISORDERS

Due to the critical role the mitochondria have in muscle, brain and nerve tissue, mitochondrial dysfunction results in disorders that have characteristics of poor growth and muscle coordination, weakness and often neurodegeneration and neuropathies. The mitochondria converts food molecules into adenosine triphosphate (ATP) giving the cell energy for routine functions. Disorders in mitochondrial function are caused by mutations in mitochondrial DNA or nuclear genes that code for mitochondrial components. Increasingly, mitochondrial disorders are being associated with autism spectrum disorders. Mitochondrial dysfunction has also been seen more commonly in childhood epilepsy including those associated with autism.[28] While an elevated lactate level in children with confirmed autism was found in 20.3%, mitochondrial respiratory chain dysfunction as determined by muscle biopsy was seen in rates of approximately 7.2%.[29] A study comparing lymphocytic mitochondria of autistic children to controls found that there were abnormalities in low PDHC (Pyruvate Dehydrogenase Complex) activity accompanied by low lactate to pyruvate ratios, impaired complex I alone or in combination with other complexes, enhanced mitochondrial rate of hydrogen peroxide production and mtDNA over-replication and/or deletions.[30] While a causal role cannot be determined, it is conceivable that mitochondrial dysfunction may propagate brain dysfunction given the high energy demand of brain tissue.[29]

In summary, new developments continue to characterize autism as a neurobiologically based disorder of aberrant connectivity. Observations are supported where the more severe disorders of connectivity occur in those with seizures. Whether the more affected individuals have more disrupted circuitry as a result of genetic susceptibility, environmental factors or abnormal energy metabolism or epileptiform discharges propagate the dysfunction of the synaptic connections remains unclear. The quest to unearth the multifaceted and multilayered etiology and phenotype of autism lies in the wealth of present and future studies and perhaps will afford us with the mechanism of a hidden final common pathway resulting in this disorder.

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