# Autism BrainNet

## A Collaboration Between Medical Examiners, Pathologists, Researchers, and Families to Advance the Understanding and Treatment of Autism Spectrum Disorder

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• ***Context.***—**Autism spectrum disorder is a neurodevelopmental condition that affects over 1% of the population worldwide. Developing effective preventions and treatments for autism will depend on understanding the neuropathology of the disorder. While evidence from magnetic resonance imaging indicates altered development of the autistic brain, it lacks the resolution needed to identify the cellular and molecular underpinnings of the disorder. Postmortem studies of human brain tissue currently represent the only viable option to pursuing these critical studies. Historically, the availability of autism brain tissue has been extremely limited.**

***Objective.***—**To overcome this limitation, Autism BrainNet, funded by the Simons Foundation, was formed as a network of brain collection sites that work in a coordinated fashion to develop a library of human postmortem brain tissues for distribution to researchers worldwide. Autism BrainNet has collection sites (or Nodes) in California, Texas, and Massachusetts; affiliated, international Nodes are located in Oxford, England and Montreal, Quebec, Canada.**

***Data Sources.***—**Pubmed, Autism BrainNet.**

***Conclusions.***—**Because the death of autistic individuals is often because of an accident, drowning, suicide, or sudden unexpected death in epilepsy, they often are seen in a medical examiner's or coroner's office. Yet, autism is rarely considered when evaluating the cause of death. Advances in our understanding of chronic traumatic encephalopathy have occurred because medical examiners and neuropathologists questioned whether a pathologic change might exist in individuals who played contact sports and later developed severe behavioral problems. This article highlights the potential for equally significant breakthroughs in autism arising from the proactive efforts of medical examiners, pathologists, and coroners in partnership with Autism BrainNet.**

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Autism Spectrum Disorder (ASD) affects 1 of 59 people in the United States, and likely worldwide, with 4 times as many males diagnosed compared with females.[1] According to the *Diagnostic and Statistical Manual,* the core diagnostic symptoms of autism are as follows: (1) persistently impaired social communication and interaction, and (2) restricted, repetitive patterns of behavior, interests, or activities.

These symptoms arise early in development and may manifest as problems with social conversation, understanding nuances of language, or engaging with peers in social activities. Challenges in nonverbal communication are also seen including problems in the use or interpretation of body language (gesturing) and facial expressions. Restricted and repetitive patterns of behavior can include self-stimulatory (stimming) behaviors, fixated interest on objects (eg, toys, cars), insistence on sameness, difficulty with changing routine, and hypersensitivity or hyposensitivity to light or sound.[2]

Typically, the diagnosis can be made by 24 months of age, but sometimes not until 36 months or later,[3,4] although earlier detection and diagnosis is sought for early treatment intervention. There is a wide range of symptom severities.

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However, the autism diagnosis is also dependent on significant clinical impairment, such that symptoms interfere with daily functions. Of note, individuals with autism often have comorbid medical, neurologic, and psychiatric symptoms, including seizures sometimes resulting in sudden unexpected death in epilepsy,[5] gastrointestinal symptoms,[6] anxiety, depression sometimes resulting in suicide, and sleep problems.[7] Individuals with autism may also demonstrate varying deficits in language and cognitive ability, ranging from profound disability to savant skills.[8] Previous to the most recent version of the *Diagnostic and Statistical Manual*, there were different subtypes of autism, including Asperger syndrome and Pervasive Developmental Disorder Not Otherwise Specified. In 2013, these subtypes were collapsed into 1 diagnosis "Autism Spectrum Disorder" (ASD).[8]

Because a diagnosis of ASD requires specialized clinical training, many pediatricians and pediatric and adult neurologists and psychiatrists taking care of these individuals for other conditions (eg, epilepsy or depression) will often use more generic labels, such as developmental delay or neurodevelopmental disorder. It is therefore important to be aware of alternative labels when considering whether a death might have arisen in connection with a case of autism. There is a higher mortality rate in autism compared with those without this diagnosis (further details below), and the causes of death differ based on sex and the presence or absence of intellectual disability.[9] Postmortem diagnosis of autism through interview of a parent or other caregiver has been validated[10] and is now in common use by Autism BrainNet.

## WHAT IS KNOWN ABOUT THE NEUROPATHOLOGY OF ASD?

As realized now for other behavioral disorders, such as chronic traumatic encephalopathy,[11] the goal of eventually achieving a comprehensive understanding of the neuropathology and neurobiology of autism will require contributions of the frontline efforts of medical examiners and pathologists who see many of the autism postmortem cases. The neuropathology of autism spectrum disorder is a rapidly evolving area of research.[12–14] The first substantial papers using postmortem brain tissue from individuals with ASD were published in the mid-1980s[15]; however, the paucity of available brains has slowed progress.[16] Much of our knowledge since has come from magnetic resonance imaging studies (reviewed in Ref. 17). Recent studies reveal increased extra-axial cerebrospinal fluid volumes[18,19] in children at risk for autism. A subset (~15%) of individuals with ASD have enlarged heads (macrocephaly) and brains (megalencephaly),[20,21] including ASD, because of some rare genetic disorders that magnify PI3K-AKT-mTOR signaling pathway, such as phosphatase and tensin homolog (*PTEN*) heterozygosity[22] and heterozygous mutations of the gene regulatory chromodomain helicase DNA binding protein 8 (*CHD8*).[23] Studies have also reported defects in brain development that implicate in utero insults to the fetal brain. Cortical dysplasia is found in cases of idiopathic ASD[24–26] and in tuberous sclerosis with *TSC1* and *TSC2* gene mutations where the dysplasia correlates with the severity of autistic traits.[27] Decreased numbers of cerebellar Purkinje neurons (with relative preservation of the inferior olive but also basket cells) suggests an early but post-developmental mechanism found in a subset of idiopathic cases of ASD.[28–32] There have also been reports of reduced neuronal size and local number, particularly of pyramidal neurons, in several neocortical areas, including the fusiform face area, the anterior insula, Broca's area, the posteroinferior occipital gyrus, and the anterior cingulate cortex, in cases of idiopathic autism.[13,33–36] This has also been observed to some degree in cases of ASD associated with maternal 15q11-13 duplication, dup15q syndrome.[33] Of note, postmortem brains from dup15q syndrome (a relatively frequent and strongly penetrant genetic cause of ASD[37–39]) also share many of the changes in gene expression and DNA methylation found in idiopathic ASD.[40,41]

Regions of the brain associated with social and emotional cognition, such as the amygdala and prefrontal cortices, are also implicated in the neuropathology of ASD. A number of magnetic resonance imaging studies find these brain regions are, in general, larger in children with ASD and do not undergo the same growth trajectory that occurs in neurotypical development.[42–44] Although magnetic resonance imaging studies provide a macroscopic view of when and where the brain may be developing differently in people with ASD, postmortem brain tissue is required to understand the underlying cellular and molecular mechanisms. For example, a recent study of 52 human postmortem brains found that the enlarged amygdala in children with ASD may be because of an excess number of neurons.[12] In contrast, adults with ASD have fewer neurons, spines, and oligodendrocytes in the amygdala indicating potential degenerative processes.[12,45,46] In addition, functional magnetic resonance imaging studies have pointed to impairment in face recognition and decreased activity in the fusiform face area in patients with ASD, which correlates to findings of abnormal pyramidal neuron numbers and volumes, specifically in this cortical region.[36,47] In addition, empathy deficits in ASD patients have been correlated to dysfunction of the anteroinferior insular cortex, a region in which neuron type-specific alterations are known to occur in ASD.[35,48]

Approximately 10% to 15% of autism cases carry genetic defects that are considered to play a major causal role in the behavioral disorder. The rapidly increasing number of autism-associated genetic defects include the following: (1) large cytogenetic abnormalities, such as extranumerary isodicentric chromosome 15q (idic[15]); (2) minute deletions or duplications of regions of the genome called copy number variations; and (3) single nucleotide mutations within the exome or coding sequences of specific genes.[49] Genetic analysis of the autism postmortem brain cases has already begun and is ongoing.[50] Of note, no single genetic defect accounts for more than 1% of the autism cases. The large number of genetic changes in ASD has suggested to some that the use of behavioral measurements to diagnose autism may capture a heterogenous group of neurodevelopmental disorders.

Despite this clear genetic heterogeneity, there is some evidence of convergence of these genetic defects into specific molecular pathways. These pathways include those underlying gene regulation and chromatin modification (eg, CHD8) and, more directly, the pathways that determine how neuronal circuits of the brain function, including genes encoding synaptic proteins (eg, neurexin, glutamate receptor delta subunit, or SHANK family members).

Beyond these rarer genetic forms of autism, there is building evidence of a neuroimmune form of autism based on the findings of ongoing neuroinflammation in a majority of autism postmortem brains.[51] These discoveries were

made possible using the limited human autism and matching control postmortem brain tissues collected, in part, from medical examiners and pathology departments across the country. This neuroinflammatory phenotype within the brain is reflected in increased levels of cytokine and chemokine proteins and reactive astroglial and microglial transcripts.[51–54] Recent studies using advanced single-cell sequencing techniques in postmortem autism brain specimens have revealed transcriptional changes that are most dramatic in upper cortical layer excitatory neurons along with evidence of an activated microglial response.[55] A significant number of the postmortem autism donors have epilepsy and have died of sudden unexpected death in epilepsy. Sudden unexpected death in epilepsy is a fatal complication of epilepsy defined as the sudden and unexpected, nontraumatic and nondrowning death of a person with epilepsy, without anatomic or toxicologic causes of death detected upon completion of the postmortem examination. One potential explanation for the high incidence of an inflammatory state in the autism brain is seizures, yet seizures do not correlate with the changes identified by single-cell sequencing[55] or the T-cell infiltrates recently discovered in the ASD postmortem brain (see below).[56]

The driver of this inflammatory state present in a majority of autism brain remains undefined, but recent studies suggest a subset of autism cases may be caused by autoimmune disease. Approximately 5% to 10% of cases of autism have been associated with maternal antibodies directed at the fetal brain[57,58] and a larger proportion of autism cases may have self-directed antibodies[57,59] implicating the humoral immune system (B cells). While performing neuropathologic examinations of the postmortem brains from individuals with autism collected into Autism BrainNet, increased numbers of perivascular CD8-rich T-lymphocyte cuffs were observed in approximately 65% of autism cases relative to control cases (Figure 1, A–L). In addition, a unique pathology, astrocyte membranous debris, was found in the Virchow-Robin perivascular and subarachnoid cerebrospinal fluid spaces, which resemble the blebs generated when cytotoxic T-cells attack tumor cells (Figure 2, A–O).[56] Interestingly, the quantity of these GFAP+ astrocyte blebs (Figure 2, D–I) correlated to the number of lymphocytes across the autism cases (Figure 3). While still under investigation, these findings suggest the possibility that a lymphocyte-based adaptive T-cell immune response might drive the inflammatory state of the brain in autism. These exciting new findings represent possible new therapeutic targets that are not yet a part of the standard treatment for ASD and could positively impact the lives of many individuals and their families. Further studies of the human postmortem brain in ASD are essential to justify and motivate the expensive clinical trials that will need to be performed to advance the standard of treatment in autism based on these new and exciting findings.

## WHAT IS KNOWN ABOUT THE CAUSES OF DEATH IN ASD?

A 2016 study in Sweden on the premature mortality in ASD showed that individuals with this diagnosis have a 2.5-fold higher risk of premature death as compared with the general population.[9] Other studies have confirmed this increased standardized mortality ratio among individuals with ASD in different population groups.[60–64] Interestingly, in preparation for writing this article, we reviewed the database held by the Office of the State Medical Examiner in New Jersey and found that of 593 840 reported deaths between 1989 and 2017, only 22 cases had "Autism" included in the cause of death. Of these 22 cases, the majority were white (16 cases) and male (21 cases), with an age range of 13 to 70 years (mean age of 34.5 years). Included in the cause of death of these individuals were conditions that are seen at a higher rate in people with a diagnosis of ASD, such as seizures and drowning. In other cases, autism was simply added as a contributory cause without the condition actually leading to the person's demise, as was the case of a 70-year old whose proximate cause of death was listed as "Stroke due to atherosclerosis" (unpublished observations). When completing the death certificate, the physician should consider any potentially lethal conditions of increased risk in a case of autism (see below) in order to accurately complete the death certification for these cases.

Medical examiners face the challenge of establishing a direct link between autism and the underlying cause of death. In general, physicians would not have an issue adding autism as a contributory factor to the cause of death when, for example, it is classified as "Sudden Unexpected Death in Epilepsy" given the strong correlation between the 2 conditions. Arguably, even the deficits of ASD (eg, poor social skills, insensitivity to pain) can lead to diagnostic delays and jeopardize optimal health care, exacerbating potentially treatable conditions, such as pneumonia that could end with a fatal outcome. It is the position of this group that in light of the increased mortality rate in patients with ASD, a careful case review should be conducted when completing the death certificate in order to identify any association between the cause of death and autism. The main causes of premature death in ASD includes the following.

### Epilepsy

Epilepsy co-exists with autism in 20% of children and is particularly frequent in those with intellectual disabilities. A strong correlation exists between deaths associated with epilepsy and ASD.[65] Recent studies have demonstrated multiple genomic variants that may account for the co-existence of these conditions.[66] Increased awareness and further research in this area may lead to the development of novel therapeutic interventions to reduce the high incidence of premature death related to the seizures in ASD. Seizures account for 7% to 30% of deaths in individuals with ASD, including sudden unexpected death in epilepsy, intractable seizures, and cardiac/respiratory arrest as a consequence of seizures.[62,67,68]

### Suicide

Depression is more commonly diagnosed in persons with ASD who have preserved language fluency and average or above intellectual functioning. Persons with this particular ASD profile often present with co-existing psychiatric conditions.[69] Importantly, individuals (children and adults; females > males) with autism have a much higher incidence of suicidal ideation and attempts as compared with the general population.[9,70–72]

### Accidental Deaths/Wandering/Elopement

Accidental deaths including drowning and suffocation are more frequent in ASD as compared with the general

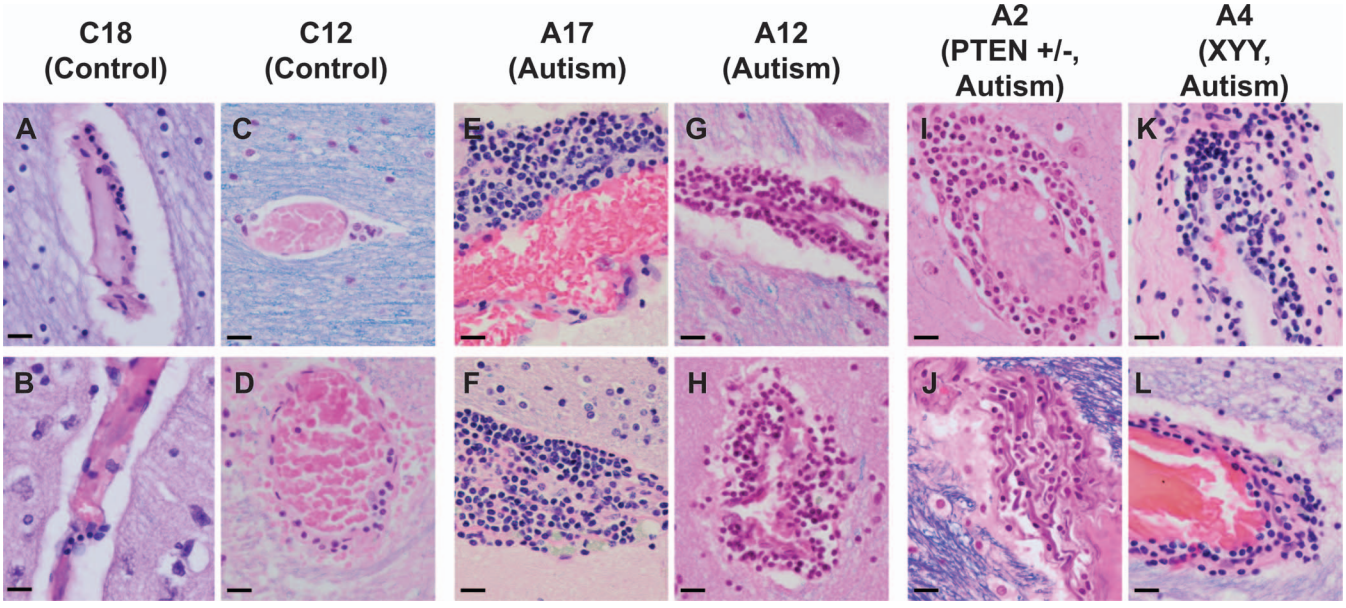


**Figure 1.** *Expanded lymphocytic cuffs were found in approximately 65% of autism spectrum disorder cases. Representative images of blood vessels in the brain parenchyma of 2 control patients (A–D) and 4 patients carrying an autism diagnosis (E–H) (including genetic conditions PTEN heterozygous mutations of phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase,* PTEN *[I and J], and an extra Y chromosome, XYY [K and L]) show increased abundance of perivascular lymphocytes in the autism cases. H&E + LFB, hematoxylin and eosin/luxol fast blue (myelin) stain. All scale bars represent 40 μm. All available H&E + LFB–stained slides were reviewed with a standard bright field microscope, and photographs were taken using a ×60 objective (×600 total magnification). Adapted from Distasio et al.* Annals of Neurology, *2019 with permission by John Wiley & Sons, Inc.*

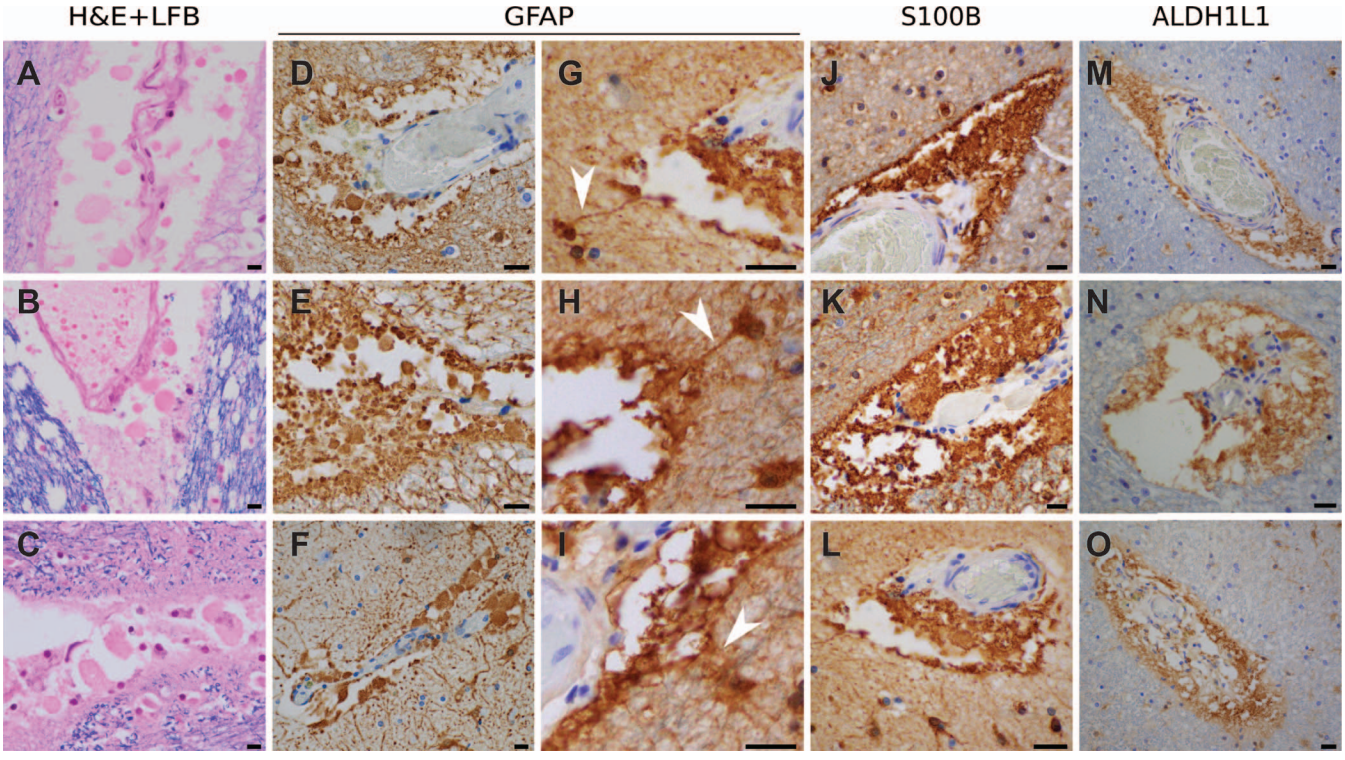


**Figure 2.** *Astrocyte blebs form from the glia limitans at the cerebrospinal fluid–brain barrier in autism spectrum disorder. Left, Round and uniformly eosinophilic membranous blebs of varying sizes were identified in the perivascular Virchow-Robin spaces of autism brains (A–C). H&E + LFB, hematoxylin and eosin/luxol fast blue (myelin) stain (A–C). Middle, glial fibrillary acidic protein (GFAP) immunohistochemistry with high magnification view of astrocyte foot processes (white arrows) extending to glia limitans where blebs are forming (D–I). Right, S100B, astrocyte marker immunohistochemistry (J–L). Right, Aldehyde Dehydrogenase 1 Family Member L1 (ALDH1L1), a cytosolic 10-formyltetrahydrofolate dehydrogenase, immunohistochemistry labeling astrocytes (M–O). All scale bars represent 40 μm. Photographed using a ×40 objective (×400 total magnification). Adapted from Distasio et al.* Annals of Neurology, *2019 with permission by John Wiley & Sons, Inc.*

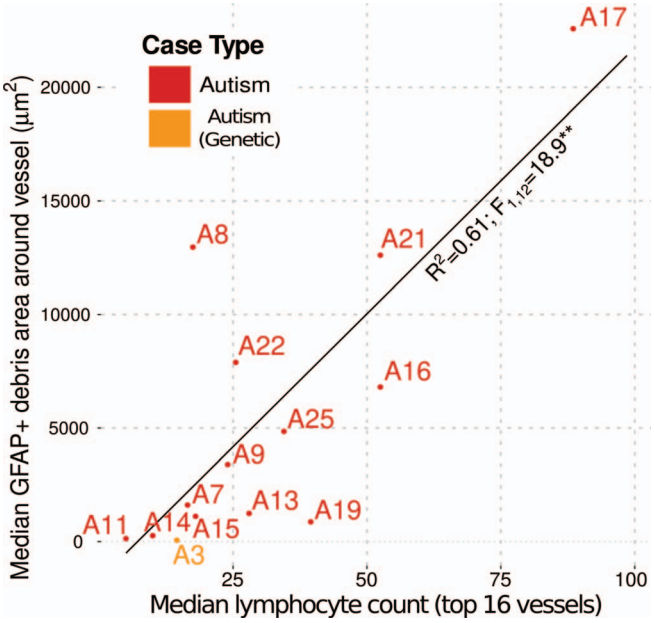


**Figure 3.** *The quantity of perivascular astrocyte debris correlates to perivascular lymphocytes in autism spectrum disorder. A plot of perivascular GFAP+ debris against perivascular lymphocyte counts (top 16 vessels) for each case shows the correlation between astrocyte-derived debris and lymphocyte numbers.* **P $<$ .001 *for linear regression; the rank-order correlation has a Kendall's tau of 0.51 (*P = .01*). Adapted from Distasio et al.* Annals of Neurology, *2019 with permission by John Wiley & Sons, Inc.*

population.[61,73] Poor social communication is thought to predispose ASD individuals to an increased risk of accidental death. Roughly half of children with ASD attempt to elope from their safe environment at a rate nearly 4 times higher than their unaffected siblings.[74–76] Accidental drowning accounts for 70% to 90% of total US deaths reported in children with ASD ages 14 and younger subsequent to wandering/elopement.[73,74] More than one-third of ASD children who wander/elope are never or rarely able to communicate their name, address, or phone number. Two-thirds of parents of elopers reported that their missing children had a "close call" with a traffic injury or drowning.[74]

### Other

Excluding seizures, suicides, and accidents, the cause of death in the remaining ASD group mirrors the heterogenous medical conditions seen in the general population, including cardiac and cancer-related deaths.

Given the wide symptomatic variation of ASD, the presence of co-morbidities, and the possible side effects of medications, determining the exact cause of death in ASD can be a challenge for the physician completing the death certificate. In the forensic setting, medical examiners and coroners may not be aware of the diagnosis of ASD. Scene investigators should question the family about the possibility of autism in cases with death related to the mechanisms listed above. The authors suspect that many cases of ASD have been missed in the past because of the lack of awareness of these presenting features. Because death certificates are the principal source of statistical data, it would be beneficial if physicians (including medical examiners) familiarized themselves with autism and any associated co-morbidities/risks to accurately capture this information.

## WHAT IS AUTISM BRAINNET AND WHY WAS IT ESTABLISHED?

Researchers studying other clinical disorders such as Parkinson disease, Alzheimer disease, and schizophrenia have demonstrated substantial success in collecting brain donations with a confederated model of regional collection sites distributed across the country. The Stanley Brain Collection, focused on schizophrenia and bipolar disorder, was an inspiration for Autism BrainNet. When the Stanley Brain Collection began in 1994, there was a severe shortage of brain tissue available for research on these major psychiatric diseases. In a decade and a half, the Stanley Brain Collection has successfully collected more than 600 brains into their network, resulting in research publications employing a wide range of methodologies and examining a diverse array of brain regions.[77]

For Autism BrainNet, a plan was drafted in 2009 that proposed the implementation of a confederated network model for the optimal acquisition, preparation, and distribution of postmortem brains to support autism research. In short, this model was composed of a series of university-based collection sites around the country. A key element of the model was that although brain tissue would be collected regionally at several sites, all acquired tissue would contribute to a general pool for use by the autism research community. The rationale for having several regional collection sites was 2-fold. First, it was thought that families would be more inclined to make a donation to an institution that had local name recognition; second, collecting cases locally could reduce the interval from death to brain processing.

To insure standardized regulatory management across all Nodes, Autism BrainNet engaged the services of Western institutional review board to provide in-depth expertise and capacity for multisite coordination. Before launching these operations, the Western institutional review board conducted an initial review of Autism BrainNet's standard operating procedures, including clinical processes (consenting and postmortem characterization of donated brain tissue). Upon satisfying the Western institutional review board's requirements, effective June 19th, 2014, the Western institutional review board provided a certificate of approval for Autism BrainNet's national consent for postmortem tissue donation, and consent for donor family clinical documentation, clinical questionnaires, and protocol. Once the Node sites were selected and institutional review board oversight was established, Autism BrainNet was formally launched at the International Meeting for Autism Research in May of 2014. The overall structure of Autism BrainNet is illustrated in Figure 4.

Each of the nodes is staffed with personnel who are highly trained in the preparation and storage of donated brains. Using standard operating procedures that are consistent across all nodes, these staff prepare 1 hemisphere as unfixed, fresh-frozen coronal slabs while the contralateral hemisphere is fixed and is used for neuroanatomic studies and a clinical neuropathology report when required. The fixed half is alternated or directed to the side with clinical, radiologic, or gross evidence of brain disease. A number of meetings took place, with the assistance of several knowledgeable consultants, to establish the standard

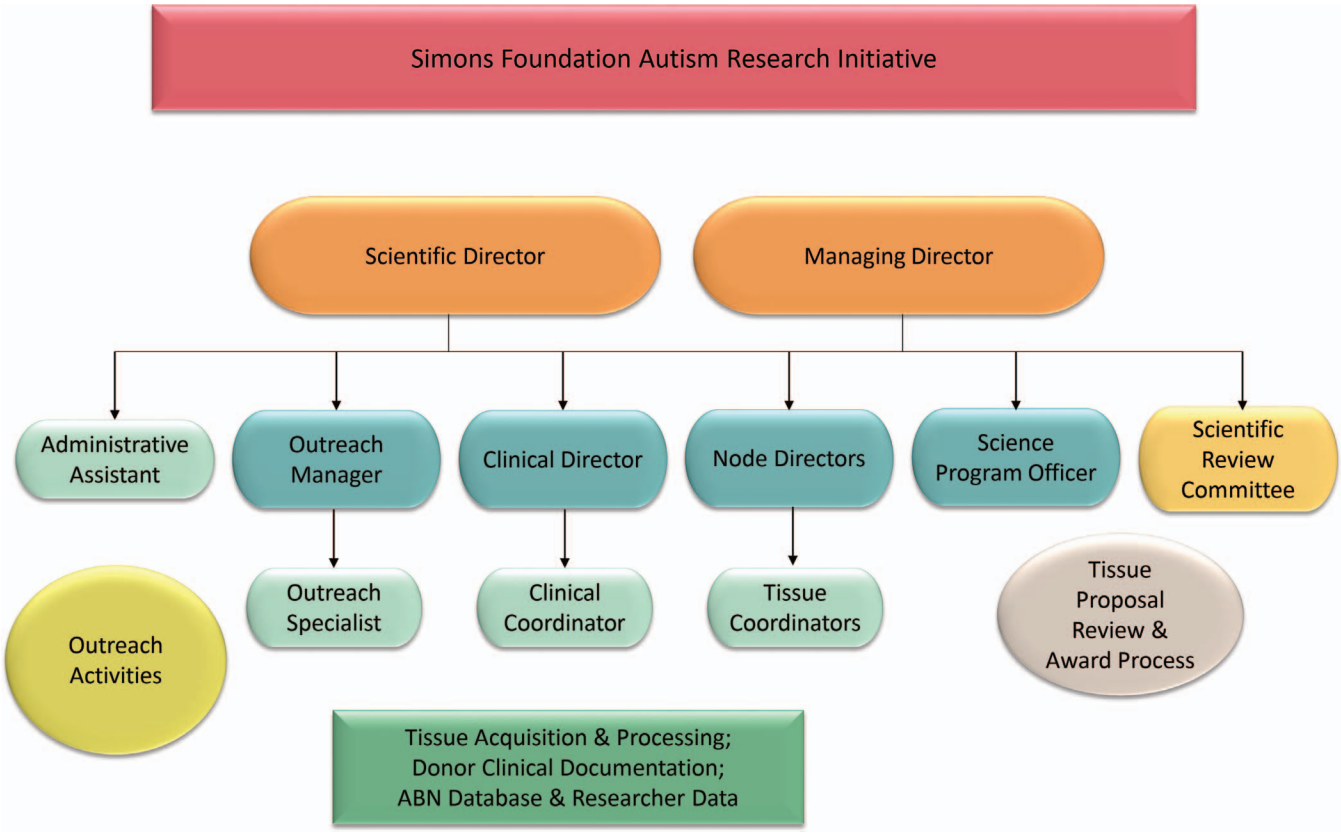


**Figure 4.** *Overall organizational structure of Autism BrainNet (ABN).*

operating procedures for preparation of the brain tissue. This is an evolving process, with the goal of establishing procedures that will allow distribution of samples from each brain to as many researchers as possible. By collating the experimental results (eg, genetics, neuropathology, immunology, neurobiology) from each case and individual samples into a central data repository, it is anticipated that the ASD cases can be subclassified into more uniform sets, possibly with shared etiologic mechanisms, enriching the value of each donation over time.

### Successful Collaborations with Medical Examiners Offices and Pathology Autopsy Services

Over the past several years, Autism BrainNet has developed successful partnerships with medical examiner offices around the country. Each of these partnerships is uniquely configured based upon the demands, state statutes, culture, and interests of the physicians and staff at each medical examiner's office. For example, in 1 medical examiner's office, Autism BrainNet provides an embedded diener who conducts brain removal after identifying and consenting potential donors. Another medical examiner's office designates an Autism BrainNet supported staff person to review daily field investigation reports to identify potential donors; this individual then conducts the consent when appropriate. A third example involves a medical examiner who alerts Autism BrainNet about potential donors by calling the 24/7 donor hotline. In this example, Autism BrainNet clinical staff do the consenting and the medical examiner procures the brain and uses packaging sent to the medical examiner's office and courier services arranged by the nearest Node tissue coordinator where the brain will be sent. Any medical examiner, pathologist, diener, or scene investigator can support Autism BrainNet by emailing or faxing a daily list of cases for review (following Health Insurance Portability and Accountability Act regulations), or by calling the hotline regarding a specific donation. It is very important to watch for both autism (any age) and control cases (<50 years of age); the collection of autism cases is growing while the number of control cases is beginning to fall behind. The National Association of Medical Examiners supports the efforts of individual medical examiners by providing Autism BrainNet's contact information on its website.

The benefits of partnering with Autism BrainNet include the following: (1) reimbursement for time, materials, and other costs associated with brain recovery; (2) a free gross examination or full clinical neuropathology report by an expert clinical neuropathologist at Harvard Medical School, including gross and microscopic examinations and clinical diagnoses with references, includes standard hematoxylin and eosin and luxol fast blue myelin stain (demyelinating diseases), as well as other special and immunohistochemical stains for infectious organisms (bacteria, fungus, spirochetes, and specific central nervous system viruses), T-lymphocytes and B-lymphocytes (autoimmune or viral encephalitis), reactive astrogliosis and microgliosis, neoplasms, β-amyloid and tau proteins (typical of chronic traumatic encephalopathy, chronic traumatic encephalopathy ,and other neurodegenerative diseases), and neuronal abnormalities (cortical dysplasia or heterotopias, ischemic/hypoxic injuries or strokes, changes typical of mesial temporal lobe epilepsy). Molecular diagnostic tests are not routinely performed during the neuropathology workup for

these cases; (3) recovery protocols, shipping materials and courier services all coordinated by Autism BrainNet; and (4) next of kin is provided with access to the Donor Aftercare Program provided by Autism BrainNet. Materials for shipping brain donations as well as protocols for tissue preparation are available on request. Shipping materials have been sent to several medical examiner's offices in anticipation of future donations.

## Developing a Strategy for Increasing Collaboration of Autism BrainNet with Medical Examiners Offices and Pathology Autopsy Services

While Autism BrainNet has developed successful relationships with a few medical examiners and pathology departments across the country, there remains a shortage of brain tissue available for research in autism. Increasing the number of partnerships with medical examiners, coroners, and pathology departments is key in developing this precious resource for autism and related neurodevelopmental research. Autism BrainNet aims to better understand ways to promote these collaborations and to overcome the limitations and obstacles medical examiners may face in providing tissues for research.

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