



# CONTRIBUTIONS OF POST-MORTEM TISSUE TO THE STUDY OF DEVELOPMENTAL DISORDERS

20th Anniversary of the NICHD Brain and Tissue Bank for Developmental Disorders

July 16–17, 2012

Neuroscience Center Building • 6001 Executive Boulevard • Rockville, MD 20852

## PRESENTATION ABSTRACTS

### Challenges for a Brain and Tissue Bank

#### Presenter

**H. Ronald Zielke, Ph.D.**

The Eunice Kennedy Shriver NICHD Brain and Tissue Bank for Developmental Disorders, University of Maryland

#### Abstract

The discussion of tissue donation at a very vulnerable time in the life of a family is compounded when the death is sudden and the deceased is a child. It is critical to have an educational program designed to reach families with members affected by developmental disorders. Equally important is a program to educate and to elicit participation of the first legal/medical personnel that come in contact with the family, namely, medical examiners. Medical examiners also are the primary, if not sole, source of control cases. Adherence to strict ethical rules overseen by Institutional Review Boards protects the rights of the families as well as those of medical examiners. In addition to collecting tissue for current research needs, the NICHD Brain and Tissue Bank must also anticipate future research interests. Research with human postmortem tissue is one critical component that complements research conducted with both the living patient and animal models of human disorders. The goal is to hasten the advancement of cures or treatments for a wide spectrum of developmental disorders.

## Developmental Cortical Patterning Abnormalities and Pathological Age-Related Changes in Autism

### Presenter

**Eric Courchesne, M.D.**

Children's Hospital Health Center

### Abstract

Although the neurobiology of autism has been studied for more than two decades, the majority of studies have examined brain anatomy 10 or more years after the onset of clinical symptoms. The early neural defects that cause autism remain unknown, but their signature is likely to be most evident during the first years of life when clinical symptoms are emerging. This lecture presents identification of neural, genomic, and brain imaging abnormalities that underlie the development of autism during the first years of life and then discusses autism as a lifespan disorder.

## Epigenome Mapping in Developing and Diseased Prefrontal Cortex. A Post-Mortem Study Across the Life Span of the Human Brain

### Presenter

**Schahram Akbarian, M.D., Ph.D.**

Mount Sinai School of Medicine

### Abstract

Neurons residing in the human cerebral cortex permanently exit from the cell cycle during the second trimester of prenatal development, but little is known about changes in neuronal chromatin during the subsequent periods of maturation and aging or potential alterations in neuropsychiatric disease. Here, we explore the genome-wide distribution of histone H3-trimethyl-lysine 4 (H3K4me3), an epigenetic mark associated with transcriptional regulation, in neuronal chromatin from the prefrontal cortex across the life span and in a cohort of subjects diagnosed with autism and other neurodevelopmental disease. We present evidence for a highly regulated, preprogrammed remodeling of histone methylation landscapes in immature prefrontal cortex neurons that continues at least into the early childhood years, involving hundreds of loci and a distinct set of transcription factors. Changes during subsequent decades of life were comparatively minor. Furthermore, histone methylation alterations in prefrontal neurons of 16 diseased subjects (autism) were highly variable. As a group, loci with disease-associated H3K4me3 alterations showed a significant, two- to threefold enrichment for genes implicated in cognition and social behaviors and heritable risk of neurodevelopmental disease. We estimate that less than 3% of altogether 711 loci affected in our cohort of 16 autism subjects were related to a copy number variant in cis. Taken together, these findings highlight the epigenetic vulnerability of the immature prefrontal cortex and point to significant overlap between the genetic and epigenetic risk architecture of autism spectrum disorders.

### Acknowledgements

We thank the Autism Tissue Program (Dr. J. Pickett), the Brain and Tissue Bank for Developmental Disorders in Baltimore, and the Harvard Brain Tissues Resource Center (Dr. F. M. Benes) for providing postmortem brain specimens.

## Fragile X Mental Retardation Protein (FMRP) and Gamma-Amino Butyric Acid (GABA) Signaling in Autism

### Presenter

**S. Hossein Fatemi, M.D., Ph.D.**  
University of Minnesota

### Abstract

Fragile X syndrome is caused by loss of function of the fragile X mental retardation-1 gene (*FMR1*) and shares multiple phenotypes with autism. Gamma-amino butyric acid is the primary inhibitory neurotransmitter in the brain. We have identified reduced mRNA and protein for a number of GABA(A) and GABA(B) receptors in subjects with autism. In the BA9, there is significantly reduced mRNA and protein for GABR $\alpha$ 4, GABR $\alpha$ 5, and GABR $\beta$ 1, while in the cerebellum there is significant reduction of mRNA and protein for GABRR1. We have found reduced expression of the protein product of *FMR1* (FMRP) in the vermis and BA9 of subjects with autism. Loss of FMRP has been shown to reduce expression of GABA(A) receptors in animal models and may be responsible for the changes in GABA receptor expression. Because FMRP regulates the translation of approximately 4% of all genes in the brain, we also measured protein levels for downstream molecules mGluR5, GABR $\beta$ 3, and GFAP in the cerebellar vermis. We found significantly increased dimerized and total mGluR5 in the cerebellar vermis of children with autism; significantly reduced expression of GABR $\beta$ 3 in the cerebellar vermis of adults with autism; and significantly increased expression of GFAP in the cerebellar vermis of both adults and children with autism. These results may open new means of therapeutic intervention for the treatment of autism, including drugs that mitigate the loss of FMRP expression or allosteric modulators of mGluR5 function.

### References

- Fatemi SH, Folsom TD, Kneeland RE, Liesch SB, 2011. Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both Fragile X mental retardation protein and GABAA receptor beta 3 in adults with autism. *Anat Rec (Hoboken)* 294:1635-1645.
- Fatemi SH, Folsom TD, 2011. Dysregulation of fragile X mental retardation protein and metabotropic glutamate receptor 5 in superior frontal cortex of individuals with autism: a postmortem brain study. *Mol Autism* 2:6.

## Human Brain Development and Risk for Schizophrenia

### Presenter

**Joel Kleinman, M.D., Ph.D.**

National Institute of Mental Health, National Institutes of Health

### Abstract

Postmortem human brains may be particularly useful for identifying alternate transcripts in genes that are crucial in human brain development and risk for schizophrenia (Kleinman et al., *Biol Psych*, 2011). A number of studies have found that genetic variation that increases risk for schizophrenia is associated with transcripts that are preferentially expressed in fetal human brain. These include the following genes: KCNH2 (Huffaker et al., *Nat Med*, 2009), DISC1 (Nakata et al., *PNAS*, 2009), GAD1 (Hyde et al., *J Neurosci*, 2011), and NRG3 (Kao et al., *PNAS*, 2010). With this in mind we have created an application that includes data on genetic variation (650,000 SNPS) and expression (49,000 probes in 20,000 genes) across the human lifespan (from Week 14 in the fetus to 80 years of age) in postmortem prefrontal cortex from 269 normal controls (Colantuoni et al., *Nature*, 2011). More than 10,000 significant associations ( $10^{-8}$  and  $10^{-79}$ ) between genetic variation/SNPS and expression have been demonstrated with this database/application. Postmortem human brains are also quite useful in examining epigenetic mechanisms (Numata et al., *AJHG*, 2012).

## The Importance of ASD Phenotyping in Post-Mortem Research

### Presenter

**Audrey Thurm, Ph.D.**

National Institute of Mental Health, National Institutes of Health

### Abstract

This talk will focus on reporting of ASD phenotyping in prior post-mortem studies. In addition, the talk will outline methods that may be utilized in the future to provide systematic, consistent phenotyping methodology in reports of post-mortem studies.

## Mapping Gene Expression and Connections in the Central Nervous System (CNS): Tools and Data from the Allen Institute for Brain Science

### Presenter

**Allan Jones, Ph.D.**

Allen Institute for Brain Science

### Abstract

The Allen Institute for Brain Science is a nonprofit research organization dedicated to providing tools and data for the larger research community. Since 2003, the Allen Institute has created a suite of large-scale data efforts along with a web portal to view and analyze the data. These efforts include gene expression atlases of the developing and adult mouse brain and spinal cord, developing and adult human and nonhuman primate gene expression studies, and more recent efforts on connectivity atlases of the mouse brain. The presentation will cover an overview of the Allen Institute, its current projects and infrastructure, a few data highlights, and a look at future directions.

## Mitochondrial Disorders

### Presenter

**Michio Hirano, M.D.**

Columbia University Medical Center

### Abstract

Mitochondrial diseases are clinically and genetically heterogeneous disorders. Caused by defects in mitochondria, the powerhouses of the cell, these diseases are characterized by a variety of symptoms, including muscle weakness, seizures, mental retardation, dementia, hearing loss, blindness, strokes, diabetes, and premature death. Some affect only skeletal muscle but most are multisystemic, often affecting the brain and muscle (encephalomyopathies). Because of the range of symptoms and the frequent involvement of multiple organs, mitochondrial diseases are often challenging to diagnose. Even when accurately diagnosed, they pose an even more formidable challenge to treat because there are very few therapies. Nevertheless, it is important for clinicians to properly identify mitochondrial diseases because the correct diagnosis is important for prognosis, providing genetic counseling, and guiding treatments. In addition to their clinical complexity, mitochondrial diseases are genetically diverse due to their dual genomic origins: nuclear DNA (nDNA) and maternally inherited mitochondrial DNA (mtDNA). Thus, mitochondrial diseases can be inherited maternally as well as via autosomal dominant, autosomal recessive, or X-linked patterns. Patients with mtDNA mutations often harbor mixed populations of mutant and normal mitochondrial genomes in each cell, a concept known as heteroplasmy. The degree of heteroplasmy and distribution of mutant mtDNA in different cells (mitotic segregation) influences the clinical phenotype. Thus, a single mtDNA mutation can cause diverse clinical phenotypes depending upon the level of heteroplasmy and tissue distribution of the mtDNA mutation. Adding to the complexity of mitochondrial disease are greater than 1,000 nDNA genes required for diverse mitochondrial functions. Mutations in more than 100 nDNA genes are known to cause mitochondrial diseases and with advances in whole-exome sequencing, many more autosomal mitochondrials will be identified in the near future.

## Overview of the National Institute of Child Health and Development (NICHD) Brain and Tissue Bank and this Workshop

### Presenter

**H. Ronald Zielke, Ph.D.**

The Eunice Kennedy Shriver NICHD Brain and Tissue Bank for Developmental Disorders, University of Maryland

### Author(s)

H. Ronald Zielke, Ph.D. and Kathleen M. Currey, M.D.

### Abstract

NICHD established two brain and tissue banks in 1991 and 1992 that focused on the collection of postmortem tissue to accelerate research on developmental disorders. Support groups were strong advocates of this effort. In 2004 the two tissue banks were consolidated at the University of Maryland, Baltimore. With the active participation of dozens of support groups, hundreds of pathologists, and multiple medical examiners, postmortem tissue was collected worldwide from more than 3,500 donors. Several hundred different disorders are represented. Medical records were obtained to verify the diagnosis. The tissue is stored at -80oC and/or in formalin and is analyzed for tissue and RNA quality. To date nearly 32,000 tissue samples have been distributed to 866 researchers in 23 countries. More than 500 full-length scientific papers have been published that are based in part on tissue received from the NICHD Brain and Tissue Bank for Developmental Disorders. About 100 of these papers have addressed the biological basis for autism spectrum disorders (ASD). The Bank recently established a national program to elicit the assistance of medical examiners outside of the state of Maryland to identify potential ASD donors. Currently, 80% of the ASD donors in the Bank were identified by medical examiners. This workshop is designed to present data and to gather insights in a variety of areas that are the mission of the Bank: Developmental Changes and Diseases, Autism Spectrum Disorders, and Additional Developmental Disorders. These findings will affirm the importance of tissue donations that families make when a family member dies. It will shorten the time until a cure/treatment is found for the many ailments affecting our children.

## Post-Mortem Brain Resources are Critical for Understanding the Gene-Environment- Epigenetic Interface in Autism-Spectrum Disorders

### Presenter

**Janine M. LaSalle, Ph.D.**

University of California School of Medicine

### Author(s)

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### Abstract

Autism is an increasingly common disorder of complex etiology, affected by multiple genetic and environmental influences. Epigenetic mechanisms act at the interface of genetic and environmental risk factors in autism. Methylation of CpG dinucleotides and methyl-specific binding proteins are part of an epigenetic pathway essential for parental imprinting and chromatin dynamics during normal brain development. Persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs) that bioaccumulate in lipid-rich tissues are of concern as developmental neurotoxicants. The relationship between POP levels and DNA methylation patterns in individuals with and without neurodevelopmental disorders has not been previously investigated.

Autism has several phenotypic features in common with the neurodevelopmental disorders with altered epigenetic pathways. Rett syndrome (RTT) is an X-linked pervasive developmental disorder caused by mutations in *MECP2*, which encodes methyl-CpG-binding protein 2 (MeCP2). Prader-Willi (PWS), Angelman (AS), and 15q duplication syndromes are imprinted disorders caused by paternal or maternal 15q11-13 deficiency or duplication, respectively. Copy number variants within 15q11-13 are also associated with a spectrum of neurodevelopmental disorders, including autism, schizophrenia, and epilepsy. Epigenetic alteration in autism human brain samples have included reduced MeCP2 due to increased *MECP2* promoter methylation in males, reduced 15q11-13 GABA<sub>A</sub> receptor subunit *GABRB3* due to reduced homologous pairing and loss of biallelic expression, and altered global levels of DNA methylation. Epigenetic differences also exist within brain samples with maternal chromosome 15 duplication syndrome, with gene expression and DNA methylation patterns not predicted from additional maternal copies.

Human brain samples are required for further understanding the complex interaction between genetics, environmental toxins, and epigenetics in 15q11-13 because 1) many

of the neurologically relevant genes within 15q11-13 are expressed exclusively in brain, 2) there are distinct tissue-specific patterns of DNA methylation and parental imprinting not observed in blood, and 3) because brain is lipid-rich, POPs accumulate in brain and can be measured and correlated with genetic and epigenetic alterations in postmortem brain.

In this study, a total of 107 human frozen post-mortem brain samples were analyzed for 8 NDL PCBs and 7 PBDEs by GC-micro electron capture detector and GC/MS using negative chemical ionization. Human brain samples were grouped as neurotypical controls, neurodevelopmental disorders with known genetic basis (genetic ND, including Down, Rett, Prader-Willi, Angelman, and 15q11-q13 duplication syndromes), and autism of unknown etiology (idiopathic ASD). Unexpectedly, PCB 95 was significantly higher in genetic ND, but not idiopathic ASD, compared to NC.

Interestingly, the samples with detectable PCB 95 levels were almost exclusively those with maternal 15q11-q13 duplication (Dup15q) or deletion in PWS. When sorted by birth year, Dup15q samples represented five out of six of genetic ND samples born after the 1976 PCB ban exhibiting detectable PCB 95 levels. Dup15q was the strongest predictor of PCB 95 exposure over age, gender, or year of birth. Dup15q brain showed lower levels of repetitive DNA methylation measured by LINE-1 pyrosequencing. These results demonstrate a novel paradigm by which a specific POP may predispose to genetic copy number variation of 15q11-q13 as a consequence of DNA hypomethylation.

## Results of Application of New Methods of Tissue Handling, Distribution, and Sharing for Research on Autism

### Presenter

**Jerzy Wegiel, V.M.D., Ph.D.**

New York State Institute for Basic Research

### Author(s)

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### Abstract

To test the hypothesis that clinical phenotype of autism is associated with selective delayed neuronal development (neuronal immaturity), the volume of neuron soma and nucleus was estimated in 18 brain structures and their 19 subdivisions (nuclei, layers, and sectors) in 13 autistic and 14 control subjects 4 to 64 years of age. A significantly lower volume of neuron soma detected in 92% of structures examined, including all 18 brain structures and 16 of 19 of their anatomical subdivisions in 4-8 year old children, indicates a global delay of neuron growth and maturation. However, a very severe volume deficit in 17% of structures, severe in 44%, moderate in 22% and mild in 17% of brain structures indicate that neuronal growth in brain structures and networks is desynchronized. Delayed and desynchronized neuron growth in 92% examined brain structures and their subdivisions demonstrates that alterations are not restricted to structures functionally related to narrow scope of diagnostic features of autism including social and communication deficits, and restricted repetitive and stereotyped patterns of behavior. The detected pattern reflects global developmental encephalopathy that may contribute not only to diagnostic signs of autism but also to intellectual deficit, anxiety, aggressive and/or self-abusive behavior, depression, and other clinical disorders observed in autism. The accelerated growth of neurons reduces developmental deficit from an average 20% in 4-8 year old subjects to 7% in >8 year old, but correction of neuron volume to control level is observed in only 3 brain structures of adult autistic subjects. These changes reveal a dynamic nature of delayed and desynchronized brain development and maturation. The most severe delay in 4-8 year old autistic children indicates that structural abnormalities develop before the 4th year of life, and that they define brain neuropathology and dysfunction for life.

## Studies of Neurologic Disease in Hereditary DNA Repair Disorders: Xeroderma Pigmentosum, Ataxia Telangiectasia, and Cockayne Syndrome

### Presenter

**P.J. Brooks, Ph.D.**

National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health

### Abstract

Neurologic disease is observed in patients from several different hereditary DNA repair diseases. In this talk, I will discuss work with three such diseases, xeroderma pigmentosum, ataxia telangiectasia, and Cockayne syndrome, using tissue samples obtained from the University of Maryland Brain Bank. Specifically, I will discuss how these samples have been used to test the specificity of antibody staining and determine the subcellular localization of proteins that are mutated in different diseases, identify novel cellular mechanisms of disease pathogenesis, and test hypotheses regarding disease pathophysiology.

## The Study of Down Syndrome Using Post-Mortem Tissue

### Presenter

**Jonathan Pevsner, Ph.D.**  
Johns Hopkins Medicine

### Abstract

Down syndrome, occurring in 220,000 births per year worldwide, is the most common genetic cause of cognitive impairment. It has been known since 1959 that Down syndrome results from trisomy of chromosome 21 (TS21). Down syndrome is the most common autosomal aneuploidy compatible with postnatal survival, with a frequency of occurrence of 1:800 live births. This frequency rises dramatically with increasing maternal age. More than 80% of trisomy 21 conceptuses die in utero, making TS21 a common cause of human pregnancy failure. A major unresolved question is the mechanism by which trisomy of chromosome 21 results in a wide range of symptoms, including some that are universal (craniofacial abnormalities, changes in brain morphology, cognitive impairment) and variable occurrence of dozens of other symptoms. We and others have shown that RNA transcripts assigned to chromosome 21 genes are up-regulated in postmortem tissues (cerebrum, cerebellum, heart) as well as cell lines (astrocytes derived from fetal brain and lymphoblastoid cell lines derived from children and adults). In addition to primary gene dosage effects, secondary (downstream) effects on disomic genes are likely to have a major role in aneuploidies in general and Down syndrome in particular. However, the nature and extent of such effects in trisomy 21 are controversial. This presentation will emphasize the essential role of postmortem tissue in defining the genetic and phenotypic effects of trisomy, including studies of transcription, proteomics, and epigenetics.