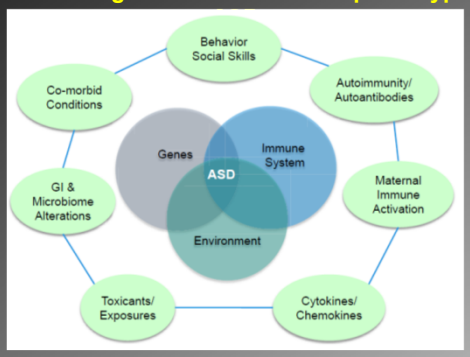
Aluminum, Alzheimer's disease (AD) and autism spectrum disorder (ASD)

Walter J. Lukiw, BS, MS, PhD
Professor of Neurology, Neuroscience and Ophthalmology
Bollinger Professor of Alzheimer's disease (AD)
Neuroscience Center & Departments of Neurology and Ophthalmology, Louisiana State University, New Orleans, LA 70112 USA

Complex relationships and multifactorial etiology lead to heterogeneous and variable phenotypes in



Lauren Matelski and Judy Van de Water, Risk factors in autism: Thinking outside the brain, *Journal of Autoimmunity*, 67 (2016) 1-7

Aluminum, Alzheimer's disease (AD) and autism spectrum disorder (ASD)

Lukiw WJ, Alexandrov PN, Grigorenko AI, Andreeva TV, Rogaev EI, Bhattacharjee S, Clement C, Zhao Y, Jones BM, Sethi-Dua P, Li YY, Cui CG, Sambamurti K, Percy ME, Pogue AI

Neurology, Neuroscience & Ophthalmology, Louisiana State University Health Science Center, New Orleans LA USA; LSU Neuroscience Center, Louisiana State University Health Sciences Center, New Orleans LA USA; University of Miami School of Medicine, Miami FL USA, Bioinformatics and Health Information Management, LA Technical University, Ruston LA USA; Medical University of South Carolina SC USA; Neurogenetics, University of Toronto, Toronto ON CANADA; University of Massachusetts, Worcester MA USA; Vavilov Institute of General Genetics, Moscow, RUSSIA; wlukiw@lsuhsc.edu

Autism One Meeting – Chicago IL USA
25-29 May 2016

Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

Earth's Crust

Element Approximate % by weight

Oxygen 46.6
Silicon 27.7
Aluminum 8.1
Iron 5.0
Calcium 3.6
Sodium 2.8
Potassium 2.6
Magnesium 2.1
All others 1.5

Other major sources of Aluminum

Food, water, beverages, medicines, vaccines, dust, industrial and mining exposure

Common Elements Important in Living Organisms

Element	Percent in Earth/Percent in Human Body
Hydrogen	0.14/9.5
Helium	Trace/Trace
Carbon	0.03/18.5
Nitrogen	Trace/3.3
Oxygen	47/65
Sodium	2.8/0.2
Magnesium	2.1/0.1
Phosphorus	0.07/1
Sulfur	0.03/0.3
Chlorine	0.01/0.2
Potassium	2.6/0.4
Calcium	3.6/1.5
Iron	5/Trace

Earth's Crust

Element Approximate % by weight

Oxygen 46.6
Silicon 27.7
Aluminum 8.1
Iron 5.0
Calcium 3.6
Sodium 2.8
Potassium 2.6
Magnesium 2.1
All others 1.5

Common Elements Important in Living Organisms

Element	Percent in Earth/Percent in Human Body
Hydrogen	0.14/9.5
Helium	Trace/Trace
Carbon	0.03/18.5
Nitrogen	Trace/3.3
Oxygen	47/65
Sodium	2.8/0.2
Magnesium	2.1/0.1
Phosphorus	0.07/1
Sulfur	0.03/0.3
Chlorine	0.01/0.2
Potassium	2.6/0.4
Calcium	3.6/1.5
Iron	5/Trace

Other major sources of Aluminum

Food, water, beverages, medicines, vaccines, dust, industrial and mining exposure

ABILITY OF METAL SULFATES TO INTERACT WITH BIOMOLECULES

[nanomolar concentrations; physiologically relevant]

Aluminum is an extremely high charge density cation

ionic charge 3^+
ionic radius 0.05 nm
ionic $Z^2/r = 18$

Al >>>>>Fe >> Zn >> Cu >>>> Mg

22 May 2015

Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

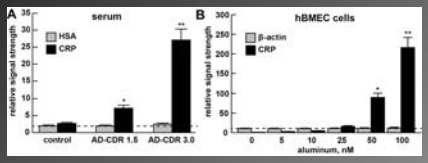
- ALUMINUM VACCINE ADJUVANTS AND ALZHEIMER'S DISEASE
- ALUMINUM FED TRANSGENIC MURINE MODELS FOR ASD (TgASD) and BIOCEHEMICAL , GENETIC AND BEHAVIORAL OUTCOMES

ADDITIONAL EVIDENCE THAT ALUMINUM IS INVOLVED IN NEUROLOGICAL DISEASES OF THE BRAIN (AD) AND RETINA (AMD)

blood samples	CDR	age (yr)
C1	0	58.4
C2	0	65.2
C3	0	77.0
C4	0	62.3
C5	0	71.2
C6	0	66.0
C7	0	62.2
C8	0	67.0
control mean +/- 1 SD	0	66.2+/-5.8
A1	1.5	60.8
A2	1.0	57.8
A3	2.0	66.8
A4	1.5	66.9
A5	1.5	62.8
A6	1.5	73.4
AD Group 1 mean +/- 1 SD	1.5	64.8+/-5.5
A7	3.0	54.7
A8	3.0	69.4
A9	3.0	67.1
A10	3.0	66.3
A11	3.0	77.9
A12	3.0	65.2
AD Group 2 mean +/- 1	3.0	66.8+/- 7.5

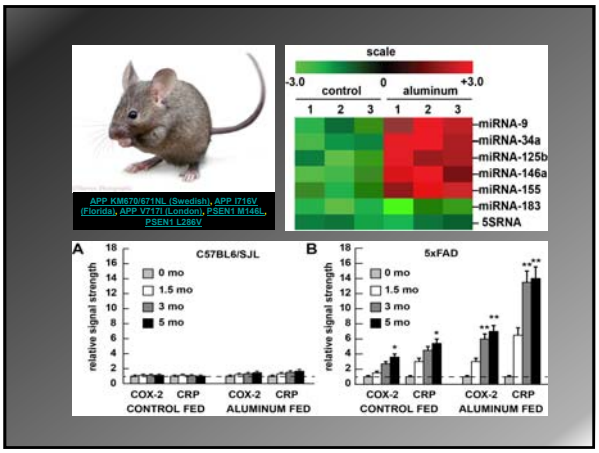
Alexandrov PN, Kruck TP, Lukiw WJ. Nanomolar aluminum induces expression of the inflammatory systemic biomarker C-reactive protein (CRP) in human brain microvessel endothelial cells (hBMECs). J Inorg Biochem. 2015 Nov;152:210-3. doi:10.1016/j.jinorgbio.2015.07.013. Epub 2015 Aug 1. PubMed PMID: 26265215.

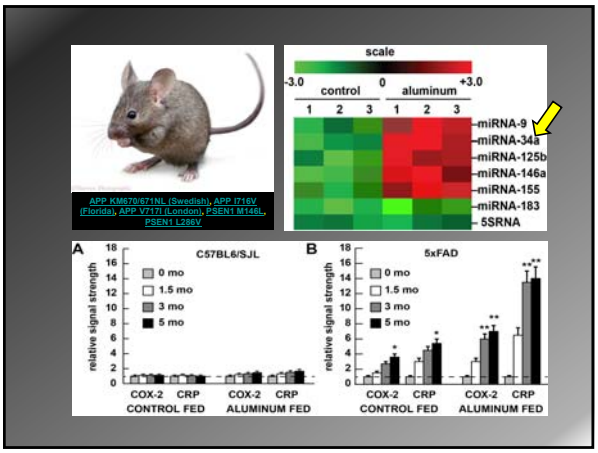
ADDITIONAL EVIDENCE THAT ALUMINUM IS INVOLVED IN NEUROLOGICAL DISEASES OF THE BRAIN (AD) AND RETINA (AMD)



Alexandrov PN, Kruck TP, Lukiw WJ. Nanomolar aluminum induces expression of the inflammatory systemic biomarker C-reactive protein (CRP) in human brain microvessel endothelial cells (hBMECs). J Inorg Biochem. 2015 Nov;152:210-3. doi:10.1016/j.jinorgbio.2015.07.013. Epub 2015 Aug 1. PubMed PMID: 26265215.

1: Pogue AI, Lukiw WJ. Aluminum, the genetic apparatus of the human CNS and Alzheimer's disease (AD). Morphologie. 2016 Mar 8. pii: S1286-0115(16)00024-2. doi: 10.1016/j.morpho.2016.01.001. [Epub ahead of print] PubMed PMID: 26969391.



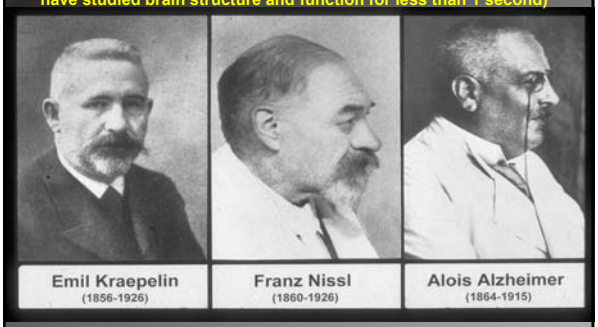


Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

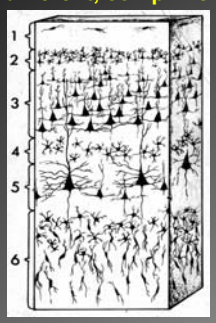
EVOLVING CONCEPTS OF HUMAN BRAIN FUNCTION

(100 years ago; on the 24 hr clock system of human evolution, humans have studied brain structure and function for less than 1 second)

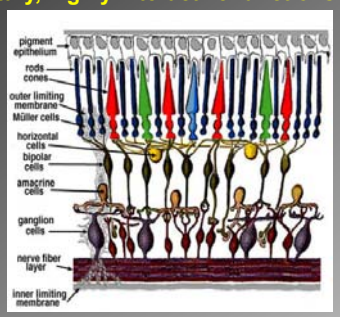


Notable contemporaries: Camillo Golgi 1844-1926; Maxwell Bielschowsky 1845-1928; Sigmund Freud 1856-1939; Santiago Ramon y Cajal 1852-1934; Korbinian Brodman 1868-1918)

Both the human association neocortex and the retina are composed of multiple cellular layers each with different, complimentary, highly interactive functions

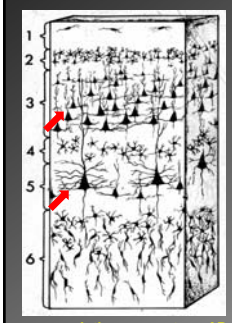


association neocortex - control
~1 square foot of neocortex

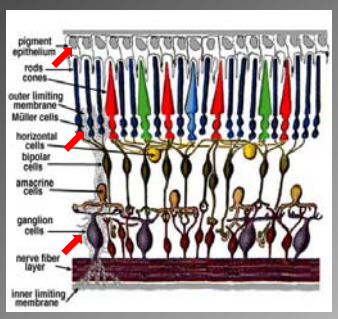


human retina – control
~1 square inch of retina

Both AD and AMD are highly focused disruptions of the association neocortex and the retina

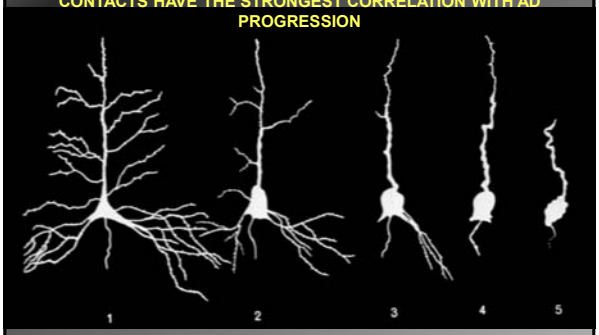


association neocortex - AD



retina - AMD

DISTORTED CYTOARCHITECTURE AND LOSS OF NEURITES/SYNAPTIC CONTACTS HAVE THE STRONGEST CORRELATION WITH AD PROGRESSION



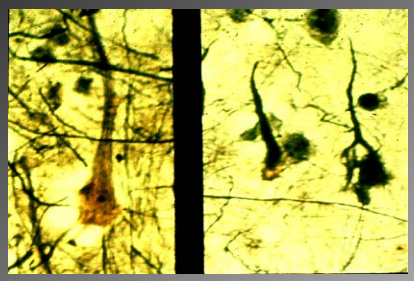
Early → Moderate → Severe

AD Progression

NISSL/GOLGI - SILVER STAINING

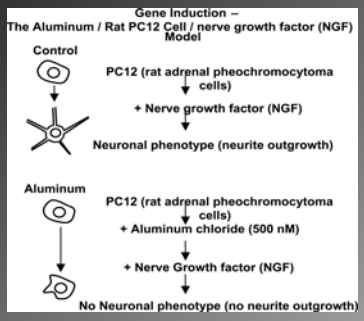
RABBIT BRAIN CONTROL

RABBIT BRAIN Al3+ treated



Lukiw WJ, McLachlan DRC (1985) unpublished

PC12 CELL NEUROTOXICITY EXPERIMENTS



Lukiw et al., Lancet. 1989; McLachlan et al., Neurobiol Aging. 1992; Pogue AI and Lukiw, J Inorg Biochem. 2010

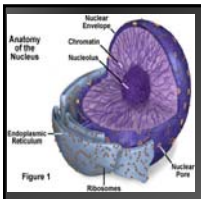
Aluminum is implicated in Alzheimer's disease (AD); N=9 major reasons

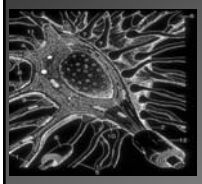
- aluminum increased in AD brain (active regions)
- aluminum affects the same messenger RNAs (mRNAs) as in AD
- aluminum affects the same microRNAs (miRNAs) as in AD
- aluminum recapitulates inflammatory signaling and changes in the innate-immune response as is seen in AD
- aluminum induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB as is seen in AD
- aluminum induces amyloidogenesis as is seen in AD
- aluminum up-regulates proinflammatory genes and down-regulates brain essential genes (i.e. synaptic proteins) as is seen in AD
- aluminum in drinking water increases AD risk
- chelators that remove aluminum from the brain improves the outlook for AD patients

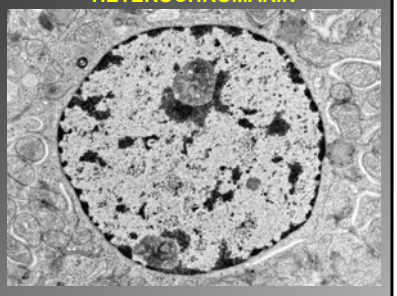
Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

HUMAN BRAIN NEURONAL NUCLEI – ACTIVE EUCHROMATIN AND INACTIVE HETEROCHROMARIN

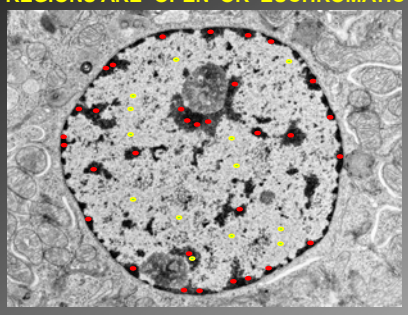






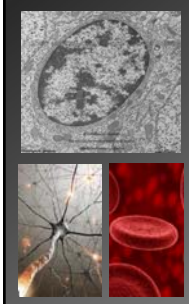
Walker PR, LeBlanc J, Sikorska M. Effects of aluminum and other cations on the structure of brain and liver chromatin. Biochemistry. 1989 May 2;28(9):3911-5; Lukiw WJ. Evidence supporting a biological role for aluminum in chromatin compaction and epigenetics. J Inorg Biochem. 104:1010-1012 (2010); Walsh B, Bazan NG, Gordon W, Lai B, Vogt S, Lukiw WJ; manuscript in preparation (2015)

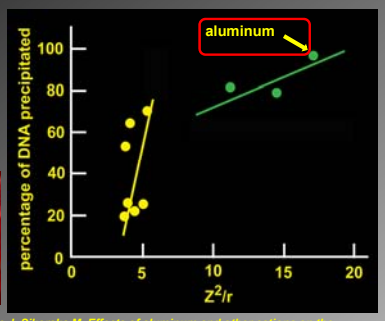
HUMAN BRAIN NEURONAL NUCLEI – ACTIVE GENE REGIONS ARE 'OPEN' OR 'EUCHROMATIC'



- *gene not expressed (hemoglobin; GFAP)*
- *gene expressed (synapsin, NFL, VAMP)*

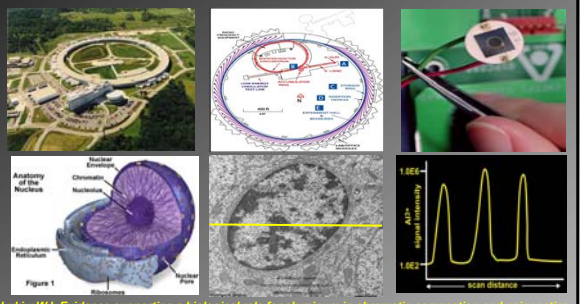
The ability of aluminum to compact chromatin from active euchromatin to inactive heterochromatin is second to none; an important biological function for aluminum





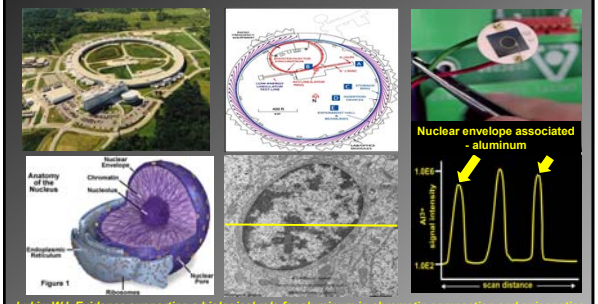
Walker PR, LeBlanc J, Sikorska M. Effects of aluminum and other cations on the structure of brain and liver chromatin. Biochemistry. 1989 May 2;28(9):3911-5; Lukiw W.J. Evidence supporting a biological role for aluminum in chromatin compaction and epigenetics. J Inorg Biochem. 104:1010-1012 (2010); Walsh B, Bazan NG, Gordon W, Lai B, Vogt S, Lukiw WJ; manuscript in preparation (2015)

Aluminum analysis (X-ray fluorescence microscopy; XFM) of human brain nuclei using high brilliance X-ray beamline XFM-2-ID-E of the Advanced Photon Source (APS); 1.4 km diameter ring; US Department of Energy, Argonne National Laboratory, University of Chicago; 0.1-0.4 um beam diameter, 10 Kev; raster scan of 3x3 mm silicon nitride windows; using an energy dispersive germanium detector (Ultra-LEGe, Canberra Instruments, Meriden CT); ~4 million separate analyses of 16 elements in a 4-day experiment; CONCLUSION - aluminum highly enriched in heterochromatin; virtually absent from euchromatin



Lukiw WJ. Evidence supporting a biological role for aluminum in chromatin compaction and epigenetics. J Inorg Biochem. 104:1010-1012 (2010); Walsh B, Bazan NG, Gordon W, Lai B, Vogt S, Lukiw WJ; manuscript in preparation (2015)

Aluminum analysis (X-ray fluorescence microscopy; XFM) of human brain nuclei using high brilliance X-ray beamline XFM-2-ID-E of the Advanced Photon Source (APS); 1.4 km diameter ring; US Department of Energy, Argonne National Laboratory, University of Chicago; 0.1-0.4 um beam diameter, 10 Kev; raster scan of 3x3 mm silicon nitride windows; using an energy dispersive germanium detector (Ultra-LEGe, Canberra Instruments, Meriden CT); ~4 million separate analyses of 16 elements in a 4-day experiment; CONCLUSION - aluminum highly enriched in heterochromatin; virtually absent from euchromatin



Lukiw WJ. Evidence supporting a biological role for aluminum in chromatin compaction and epigenetics. J Inorg Biochem. 104:1010-1012 (2010); Walsh B, Bazan NG, Gordon W, Lai B, Vogt S, Lukiw WJ; manuscript in preparation (2015)

Conclusions for this section

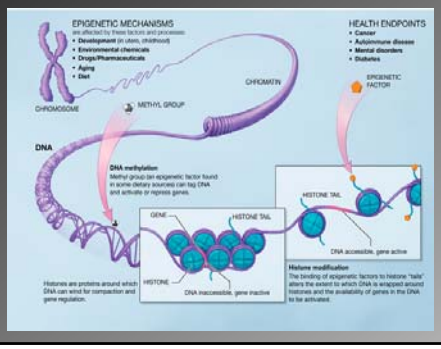
- aluminum is involved in chromatin compaction;
- the ability of aluminum to compact chromatin from active euchromatin to inactive heterochromatin is second to none;
- an important biological function for aluminum is to modulate the state of compaction of chromatin and hence the global regulation of gene expression;
- non-homeostatic aluminum-mediated compaction of euchromatin to heterochromatin is an important pathological effect of aluminum in eukaryotic brain cells

Lukiw WJ. Evidence supporting a biological role for aluminum in chromatin compaction and epigenetics. J Inorg Biochem. 104:1010-1012 (2010); Walsh B, Bazan NG, Gordon W, Lai B, Vogt S, Lukiw WJ; manuscript in preparation (2015)

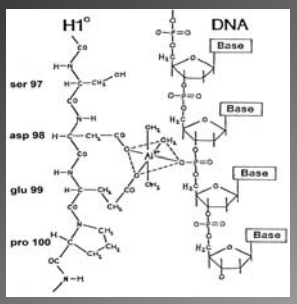
Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

Gene Expression – the process by which DNA signals are converted into functional proteins: the first step is 'transcription'

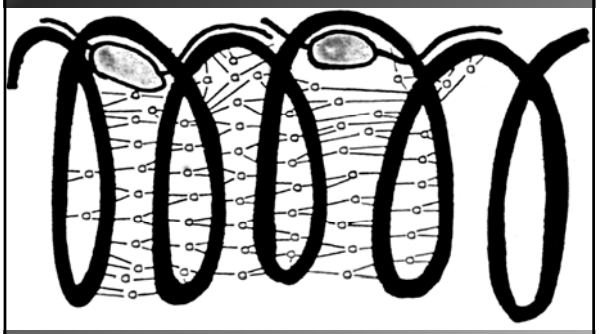


Aluminum crosslinking of chromosomal protein and DNA



Lukiw WJ, Kruck TP, McLachlan DRC Aluminium and the nucleus of nerve cells. Lancet 1:781-783 (1989); Lukiw WJ Chromatin structure, gene expression and nuclear aluminum in Alzheimer's disease (AD) PhD thesis (1992)

Aluminum crosslinks DNA



Lukiw WJ, Kruck TP, McLachlan DRC Aluminium and the nucleus of nerve cells. Lancet 1:781-783 (1989); Lukiw WJ Chromatin structure, gene expression and nuclear aluminum in Alzheimer's disease (AD) PhD thesis (1992)

ABILITY OF METAL SULFATES TO REPRESS GENE TRANSCRIPTION BY RNA POLYMERASE II (RNAP II)

[nanomolar concentrations; physiologically relevant]

[Al+Fe] >> Al >> Fe > Zn > Cu >>>> Mg

22 May 2015

ABILITY OF METAL SULFATES TO REPRESS GENE TRANSCRIPTION BY RNA POLYMERASE II

[nanomolar concentrations; physiologically relevant]

[Al+Fe] > Al >> Fe > Zn > Cu >>>> Mg

AS in AD, ~66% OF ALL TRANSCRIPTS DOWN-REGULATED [REPRESSION]

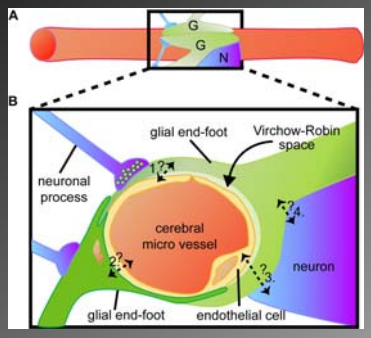
AND ~33 % OF ALL TRANSCRIPTS UP-REGULATED [ACTIVATION]

A FEATURE COMMON TO METAL ION TOXICITY (Al + Fe) AND ALZHEIMER'S DISEASE

THIS APPEARS TO INVOLVES DIFFERENTIAL GENE REGULATION MECHANISMS

22 May 2015

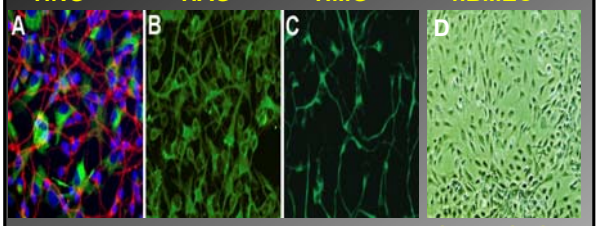
ESSENTIAL COMPONENTS OF THE HUMAN NEUROVASCULAR UNIT (NVU)



The highly vascularized human brain contains about 400 km of 5 µM diameter cerebrovascular (cerebral micro vessels) tubes that supply the brain with oxygen, glucose and other nutritive support

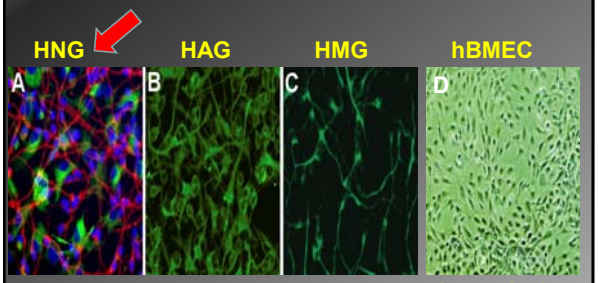
Zhao Y, Cui JG and Lukiw WJ, Molecular Neurobiology, 34:181-192 (2007)

HUMAN PRIMARY BRAIN CELL MODELS



human neuronal-glial cell co-culture | human astroglial cells | human microglial cells | human brain microvascular endothelial cells

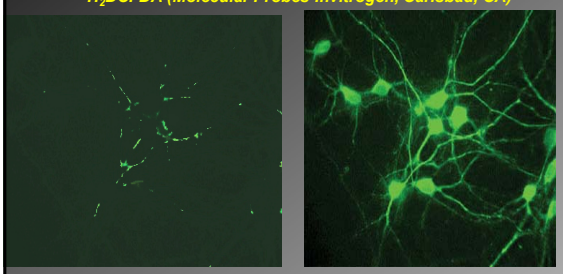
HUMAN PRIMARY BRAIN CELL MODELS



human neuronal-glial cell co-culture | human astroglial cells | human microglial cells | human brain microvascular endothelial cells

INDUCTION OF ROS IN HUMAN NEURONAL-GLIAL CELLS

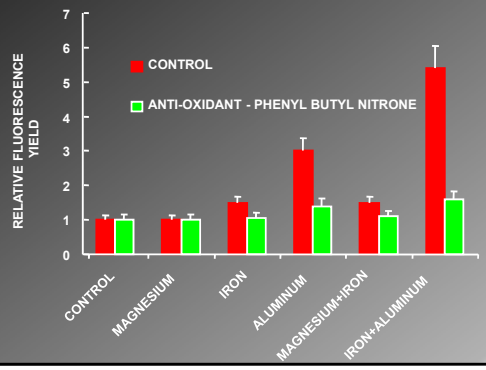
ROS-sensitive green fluorescent reporter (λ_{max}=525 nm) such as H_2DCFDA (Molecular Probes-Invitrogen, Carlsbad, CA)



CONTROL

STRESSED BY HYPOXIA, IL-1β, Aβ PEPTIDES, NEUROTOXIC METAL (Al3+)

METAL-INDUCED OXIDATIVE STRESS IN HUMAN NEURAL CELLS – ROS ASSAY



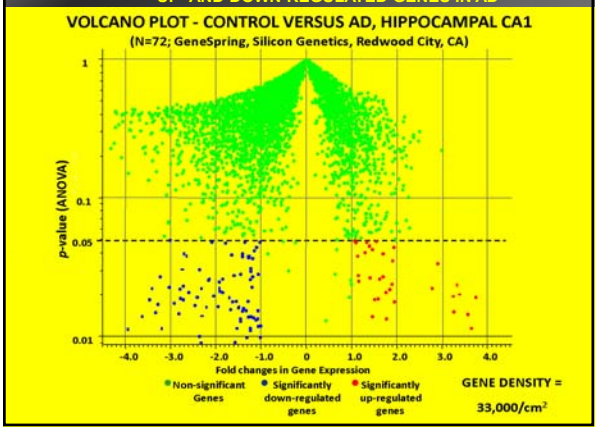
Gene expression profiling of Alzheimer's disease neocortex and hippocampus (mRNA)

1. Colangelo V et al., Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. J Neurosci Res. 70:462-73 (2001)

2. Loring JF et al., A gene expression profile of Alzheimer's disease. DNA Cell Biol. 20:683-95 (2002)

3. Arisi I et al., Gene expression biomarkers in the brains of Alzheimer's disease: mining of microarray data by logic classification and feature selection. J Alzheimers Dis. 24:721-38 (2011)

UP- AND DOWN-REGULATED GENES IN AD



Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

STUDIES OF HUMAN BRAIN mRNA COMPLEXITY Messenger RNA ANALYSIS

- A HIGHLY LABILE, TRANSITORY MOLECULE (HALF-LIVES IN MINUTES-HOURS)
- TRANSMITS ESSENTIAL GENETIC INFORMATION FROM DNA TO PROTEIN
- RNA POPULATIONS ARE A REFLECTION OF THE PHYSIOLOGICAL STATUS OF THE CELL (HEALTH, DISEASE, STRESS, DEVELOPMENT, AGING)

Tuesday 27 February 2007

CENTRAL DOGMA OF MOLECULAR BIOLOGY

DNA → RNA → PROTEIN

Friday 22 May 2015

CENTRAL DOGMA OF MOLECULAR BIOLOGY

DNA → RNA → PROTEIN

DNA	RNA	PROTEIN
years	minutes, hours	years
(12,000 yr)	(1 min to 12 hr)	(10,000 yr)

Friday 22 May 2015

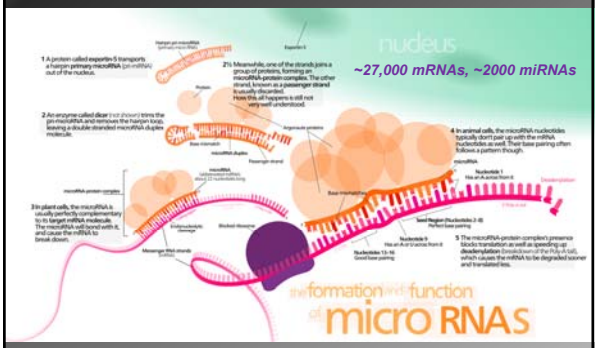
GENETICS-EPIGENETICS OF ALZHEIMER'S DISEASE (AD) AND AGE-RELATED MACULAR DEGENERATION (AMD)

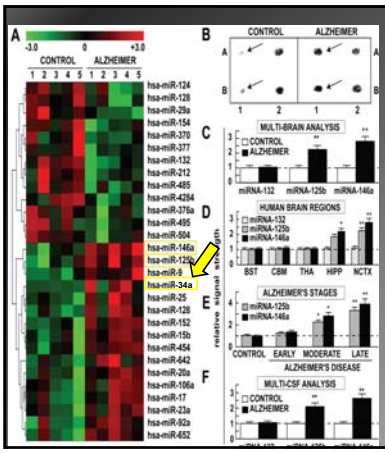
micro RNA ↑ messenger RNA ↓ (miRNA↑, mRNA↓)

up-regulation of specific micro RNA (miRNA), and down-regulation of messenger RNA (mRNA) complexity appears to be a very active genetic process in the aging human brain and retina

Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466:835-840 (2010).

miRNA – GENETIC MECHANISM



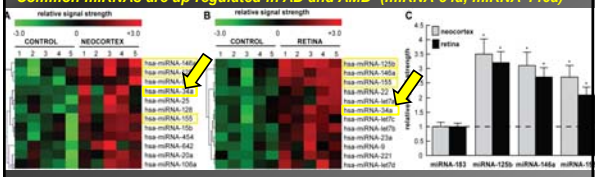


specific miRNA up-regulation in AD but not in age-matched controls Brodmann area A22; superior temporal neocortex (N=153)

- *aging human and AD brain*
- *stressed mouse and human brain cells (neuronal, astroglial, microglial, endothelial)*
- *Tg2576, 5xFAD (amyloid over-expressing) brain and retina*
- *age-related macular degeneration (AMD)*
- *human prion disease (CJD, GSS)*

Dua P, Zhao Y, Li YY, Pogue AI, Alexandrov PN, Eicken C, Lukiw WJ Louisiana State University Neuroscience Center; New Orleans LA, USA; Moscow State University, Moscow, Russia; University of Texas, Houston TX, USA; LC Sciences, Houston TX, USA; in press, 2015)

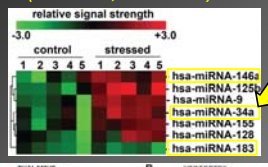
Common miRNAs are up-regulated in AD and AMD (miRNA-34a, miRNA-146a)

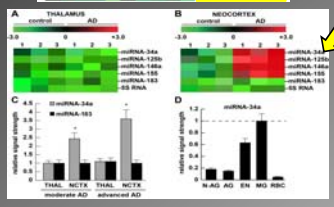


*Color-coded cluster analysis of significantly up-regulated miRNAs in (A) in the neocortex of AD versus age-matched controls and (B) in the retina of AMD versus age-matched controls; relative expression levels for miRNA- 125b, miRNA-146a and miRNA-155 the brain neocortex and retina are shown in (C) relative to levels for an unchanging control miRNA-183 which was set to 1.0 and marked by a dashed horizontal line. In this selective sampling, all control (N=5) and AD (N=5) neocortical samples were obtained from the superior temporal neocortex (Brodmann area A22). Retinal samples including controls (N=5) and AMD (N=5) included whole retina; all control, AD and AMD samples had post-mortem intervals (PMI; death to brain freezing interval) of 2 h or less. Controls were age-matched to moderate-to-late stages of AD or AMD; increases in specific miRNAs increased as disease stage advanced; further details on the pathology of these samples have been recently published. There were no significant differences in age, PMI, or RNA yield or quality between either the brain or retinal groups. Of the 12 different homo sapien micro-RNAs (hsa-miRNAs) shown, miRNA-146a, miRNA-125b and miRNA- 155 exhibited the greatest up-regulation compared with age-matched controls (p<0.01, ANOVA); in some samples miRNA-9 also showed a trend for up-regulation; miRNA-9 can also target the CFH mRNA 3'-UTR. We cannot exclude the participation of other human brain-enriched miRNAs or other small non-coding RNAs (sncRNAs) which may additionally contribute to the neuropathological mechanisms which define the AD or AMD process; *p<0.01 (ANOVA);*

Lukiw WJ, Surjyadipta B, Dua P, Alexandrov PN. Common micro RNAs (miRNAs) target complement factor H (CFH) regulation in Alzheimer's disease (AD) and in age-related macular degeneration (AMD). Int J Biochem Mol Biol. 3:105-16 (2012).

Common miRNAs are up-regulated in stressed human brain primary cells (miRNA-34a, miRNA-146a)





Lukiw WJ, Bhattacharjee S, Dua P, Alexandrov PN. Common micro RNAs (miRNAs) target complement factor H (CFH) regulation in Alzheimer's disease (AD) and in age-related macular degeneration (AMD). Int J Biochem Mol Biol. 3:105-16 (2015); Bhattacharjee S, Zhao Y, Lukiw WJ J. Biol. Chem in preparation (2015).

Transgenic animal models of Alzheimer's disease (Tg-AD; N=54)

Tg-AD model	Strain*	age of SP onset[1]	Tg-AD promoter/transgene[2]	SPD[3]	CNS[4]	synaptic pathology[5]	retinal pathology[6]	source
Tg2576	C57B6J X SJL F1 hybrid**	10	hamster prion promoter/human APP695 cDNA with K670N/M671L	++++	++	+++	++	[Hsiao et al., 1996; Phillipson et al., 2012]
PSAPP	C57BL6/D2AF*	6	Tg2576 x PSEN1M146L	++	+	-	-	[Holcomb et al., 1998]
3xTg-AD	hybrid 129/C57BL6*	6	Thy1 promoter/APP695-Swedish/Tau isoform 4R0N(P301L mutation)/ PSEN1M146L	++	+++	+	+	[Oddo et al., 2003; Kumar-Singh et al., 2005]
5xFAD	C57BL6/SJL**	2	Thy1 promoter/B6SJL-Tg(APPSwFlLon,PSEN1M146L*L286V)6799Vas/J	++++	++++	++++	++++	[Oakley et al., 2006]

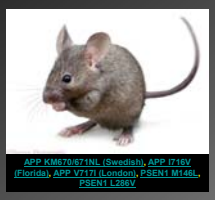
Alexandrov PN, Pogue AI, Bhattacharjee S, Lukiw WJ. Retinal amyloid peptides and complement factor H in transgenic models of Alzheimer's disease (AD) Neuroreport – Molecular Neuroscience 22:623-627 (2011)

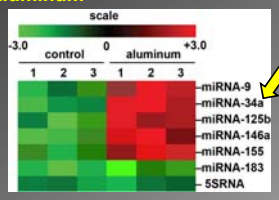
Transgenic animal models of Alzheimer's disease (Tg-AD)

Tg-AD model	Strain*	age of SP onset[1]	Tg-AD promoter/transgene[2]	SPD[3]	CNS[4]	synaptic pathology[5]	retinal pathology[6]	source
Tg2576	C57B6J X SJL F1 hybrid**	10	hamster prion promoter/human APP695 cDNA with K670N/M671L	++++	++	+++	++	[Hsiao et al., 1996; Phillipson et al., 2012]
PSAPP	C57BL6/D2AF*	6	Tg2576 x PSEN1M146L	++	+	-	-	[Holcomb et al., 1998]
3xTg-AD	hybrid 129/C57BL6*	6	Thy1 promoter/APP695-Swedish/Tau isoform 4R0N(P301L mutation)/ PSEN1M146L	++	+++	+	+	[Oddo et al., 2003; Kumar-Singh et al., 2005]
5xFAD	C57BL6/SJL**	2	Thy1 promoter/B6SJL-Tg(APPSwFlLon,PSEN1M146L*L286V)6799Vas/J	++++	++++	++++	++++	[Oakley et al., 2006]

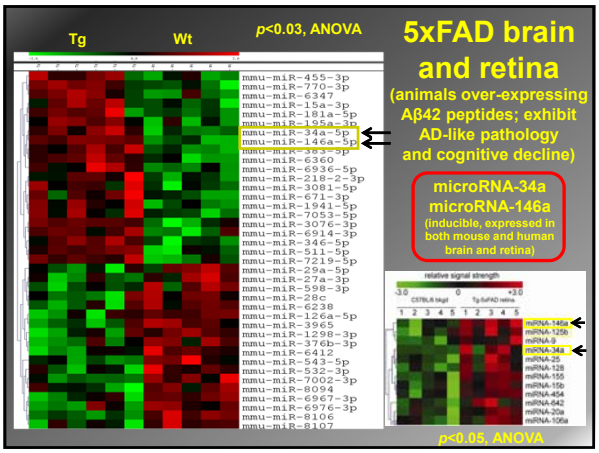
Alexandrov PN, Pogue AI, Bhattacharjee S, Lukiw WJ. Retinal amyloid peptides and complement factor H in transgenic models of Alzheimer's disease (AD). Neuroreport – Molecular Neuroscience 22:623-627 (2011)

Specific microRNAs (miRNAs) over-expressed in AD brain are also over-expressed in 5xFAD mice fed diets enriched in aluminum



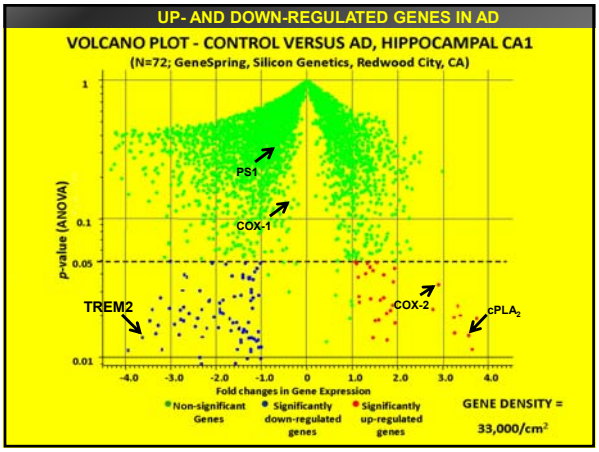


Array-based cluster analysis of miRNA abundance in aluminum-fed 5xFAD mice vs. controls. In these experiments the brain (cortex) of 3month-old 5xFAD mice fed aluminum-enriched diets (Al lactate; 10 mg/gm food for 3 months) were analyzed for miRNA speciation compared to age-matched controls (receiving standard diets); the NF-kB-sensitive miRNAs miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a and miRNA-155 were significantly up-regulated compared to 2 unchanging control RNAs miRNA-18s and 5SRNA; Bhattacharjee S, Zhao Y, Hill JM, Percy ME, Lukiw WJ. Aluminum and its potential contribution to Alzheimer's disease (AD). Front Aging Neuroscience 6:62-66 (2014).



Sequence of homo sapiens microRNA-34a-1 (hsa-miR-34a-1) gene and 5' regulatory region (total = 560 base pairs) at human chromosome 1p24 (chr 1p24); NF-kB strongly induces transcription from many genes including miRNAs and miRNA-34a

```
  1 tattgtagac tatggtttta aaaacgtggg catttctgag tctattcata ttattttccc

 61 attttagtca actgttttgc ccaaatcttg aaagagtttt ctctgatata ctcgtgattg

121 tatgtatgtg cgtaattgta tatgcgcccc cagatactgc gtatgtgtgt atgtatgtat
                                NF-κB
181 taggcttaaa tggaatctca attttgtgaa ggaaaggagc ttagagaaga aataccatac
                                                    NF-κB
241 cacctgtttg ttgcatctta gttatgaacc tcgaacagaa attgcctgtc /ttcttgttt
                          RNA Pol II                        miRNA-34a-1
301 tgctttgttt tgtctcaaga aagaaaacat tgttgcgctc ctctcagtcc ctgagaccct

361 aacttgtgat gtttaccgtt taaatccacg ggttaggctc ttgggagctg cgagtcgtgc

421 ttttgcatcc tggaaatttg gtggaacttt attctttaaa gcaaaaacaa aagaaaagaa

481 agtttgtctg aagtgattga gtatacctct gaggttttca ttgttagatg ggatcaggtg

541 accagagagt ggcagctcct
```



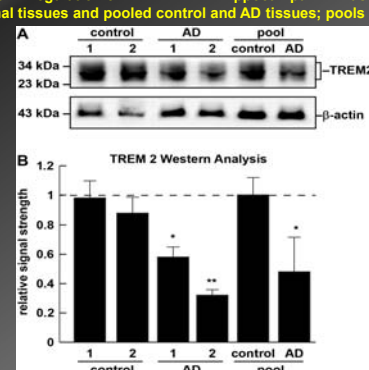
Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

miRNA-34a and TREM2

microRNA-34a↑
TREM2 messenger RNA ↓

Down-regulation of TREM2 in AD hippocampal CA1 using individual brain or retinal tissues and pooled control and AD tissues; pools N=9 control and N=9 AD



miRNA-34a ⇧
TREM2 ⇩

*p<0.05; **p<0.01; ANOVA

Zhao et al., Regulation of TREM2 expression by an NF-κB-sensitive miRNA-34a. Neuroreport (2013); Bhattacharjee, Zhao, Jones, Lockwood, Lukiw, manuscript submitted (2015).

2 AD CASES – and 1 case of Balints syndrome (brain and retina in the same specimen available; all females)

- *Balints syndrome (88 yr old female) – AD that moves posterially in the brain - damage in the temporal lobe-occipital lobe circuits – inflammatory degeneration and amyloid pathology in the limbic system, primary visual cortex (PVC) and retina*
- *advanced AD – amyloid inflammatory degeneration and amyloid pathology in the limbic system, primary visual cortex (PVC) and retina*

advanced AD inflammatory degeneration and amyloid pathology in the limbic system, primary visual cortex (PVC), thalamus (LGN) and retina

- *TREM2 expression down in the temporal lobe neocrtex and retina*

Conclusion: in all 3 AD/Balints syndrome cases where the eye and brain were both available at autopsy, the entire neocortical-PVC-LGN-retinal axis showed signs of inflammatory degeneration, amyloid pathology and TREM2 deficits

Pathologists: Drs J. Deck and DRC McLachlan, University of Toronto

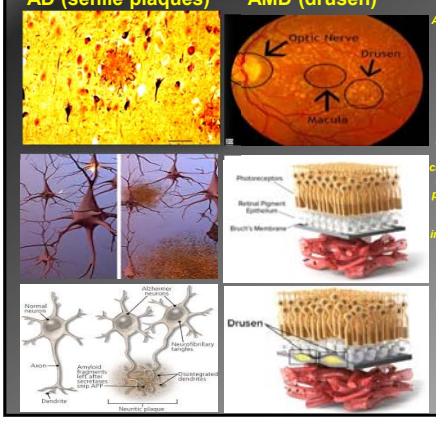
Brain neocortex and retinal TREM2 – data from the same brain

AGE-MATCHED CONTROL and AD CASES:

CONTROL = 79.4 yr; AD1 = 78.9 yr; AD2 = 81.1 yr; all samples female, Caucasian, the diagnosis of AD was both pre- and post-mortem confirmed - family history, genotyping (BACE1, PSEN1), ApoE4, CDR, MMSE; SP and NFT counts had no significant difference; no significant differences in brain weight, PMI <2.1 hrs; TREM2 decreased in both brain and retina

Deck JD, McLachlan DRC, Lukiw WJ, unpublished 2015

AD (senile plaques) **AMD (drusen)**

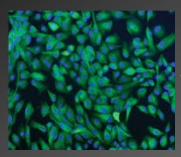


AD and AMD drusen share at least 40 common components; common amyloid peptides (Aβ37, Aβ39, Aβ40, Aβ41, Aβ42, Aβ43), sAPPα, vitronectin, neprilysin, common complement proteins (CFH, C3, C3b, C5), neurotoxic metals (Al, Cu, Zn), oxidized lipids, cholesterol, 24S-OH cholesterol, PS1, PS2, BACE, α-secretase, sortilin, TSPAN proteins (TSPAN8, TSPAN12), CRP, S100A9; each amyloid type is intensely insoluble, immunogenic, amyloidogenic, pro-inflammatory; their deposition is age-related

Prusiner S. and Glenner GG. Cell 35:349-358 (1983); Glenner GG. Cell 52:307-308 (1988); Selkoe D. Journal of Alzheimer's Disease 9:163-168 (2006); Gehrs KM et al., Ann Med. 38:450-471 (2006); Masters CL et al., Arch Ophthalmol. 247:1213-1221 (2009

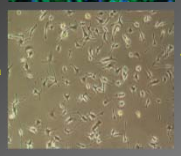
phagocytotic cells of the human retina and neocortex

H-RPE



+Aβ42 peptides = apoptosis, H-RPE cell death

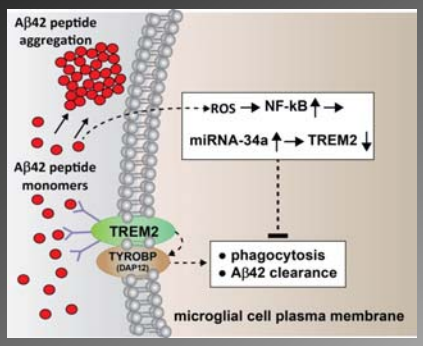
C8B4
microglia



+Aβ42 peptides = phagocytosis, homeostasis

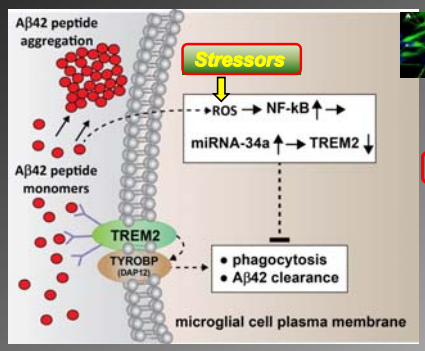
Cell sources: H-RPE - human primary retinal pigment epithelial (RPE) Cells; Lonza Cat No 194987, Lonza Cell Systems, Allendale NJ, USA; C8B4 murine microglia (ATCC; Manassas VA, USA); Aβ42 peptides are primarily in the monomeric and dimeric forms

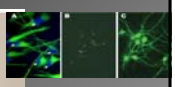
the induction of ROS is a particularly strong inducer of NF-kB and an NF-kB-miRNA-34a mediated down-regulation of TREM2 and ultimately the ability to sense and clear amyloid



miRNA-34a ⇧
TREM2 ⇩

the induction of ROS is a particularly strong inducer of NF-kB and an NF-kB-miRNA-34a mediated down-regulation of TREM2 and ultimately the ability to sense and clear amyloid



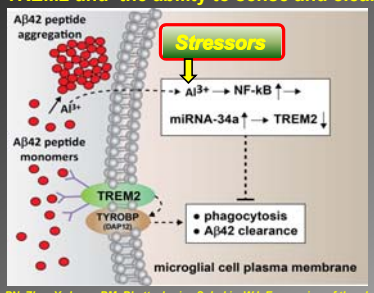


miRNA-34a ⇧
TREM2 ⇩

Stressors

Aβ42
IL-1β
IL-6
TNFα
hypoxia
UV
aluminum
viruses

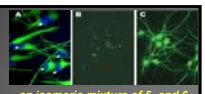
aluminum contributes to amyloidogenesis: (i) by aggregating amyloid monomers into higher order structures; (ii) while triggering an NF-kB-miRNA-34a mediated down-regulation of TREM2 and the ability to sense and clear amyloid



miRNA-34a ↑
TREM2 ↓

Stressors
Aβ42
IL-1β
IL-6
TNFα
hypoxia
UV
aluminum
viruses

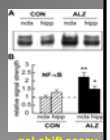
Alexandrov PN, Zhao Y, Jones BM, Bhattacharjee S, Lukiw WJ. Expression of the phagocytosis-essential protein TREM2 is down-regulated by an aluminum-induced miRNA-34a in a murine microglial cell line. J Inorg Biochem. 128:267-9 (2013); Zhao Y, Hill JM, Bhattacharjee S, Percy ME, Pogue AI, Lukiw WJ. Aluminum-induced amyloidogenesis and impairment in the clearance of amyloid peptides from the CNS in Alzheimer's disease. Front Neurol 5:157-170 (2014)



an isomeric mixture of 5- and 6-carboxy-2',7'-dichlorofluorescein diacetate (dicarboxy-DCFDA)

Stressors
Aβ42
IL-1β
TNFα
hypoxia
UV
virus (HSV1)
aluminum

ROS ↑
NF-kB ↑
miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, miRNA-155
CFH ↓
TREM2 ↓
inflammation
amyloidogenesis



gel-shift assay or NF-kB-p65 antibodies

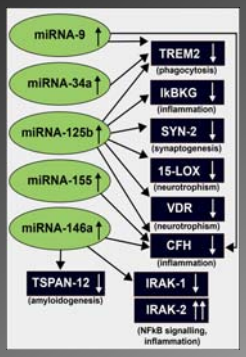
Zhao et al., Regulation of TREM2 expression by an NF-κB-sensitive miRNA-34a. Neuroreport (2013); Zhao Y, Jones BM, Bhattacharjee S, Lukiw WJ; manuscript in preparation; (2013)

Stressors
Aβ42
IL-1β
TNFα
hypoxia
UV
aluminum

ROS ↑ — PBN, curcumin
NF-kB ↑ — PDTC, CAY10512, CAPE
miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, miRNA-155 ↑ — anti-miRNA, AM, antagomirs
CFH ↓
TREM2 ↓
inflammation
amyloidogenesis

Lukiw WJ. Antagonism of NF-κB-up-regulated micro RNAs (miRNAs) in sporadic Alzheimer's disease (AD) – anti-NF-κB versus anti-miRNA strategies, Frontiers in Genetics (2013)

Disrupted miRNA-mRNA signaling in Alzheimer's disease (AD)

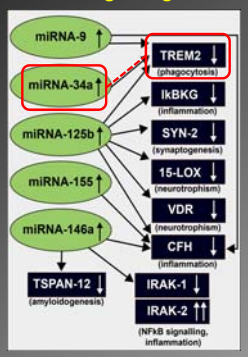


miRNA-mRNA signals in

- *aging human and AD brain*
- *stressed mouse and human brain cells (neuronal, astroglial, microglial, endothelial)*
- *5xFAD (amyloid over-expressing) brain and retina*
- *age-related macular degeneration (AMD)*
- *human prion disease (CJD, GSS)*

Zhao Y, Bhattacharjee S, Jones BM, Hill J, Dua P, Lukiw WJ. Regulation of neurotropic signaling by the inducible, NF-kB-sensitive miRNA-125b in Alzheimer's disease (AD) and in primary human neuronal-glial (HNG) cells. Mol Neurobiol. 50:97-106 (2014)

Disrupted miRNA-mRNA signaling in Alzheimer's disease (AD)

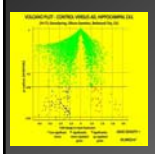


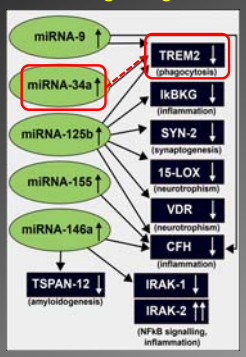
miRNA-mRNA signals in

- *aging human and AD brain*
- *stressed mouse and human brain cells (neuronal, astroglial, microglial, endothelial)*
- *5xFAD (amyloid over-expressing) brain and retina*
- *age-related macular degeneration (AMD)*
- *human prion disease (CJD, GSS)*

Zhao Y, Bhattacharjee S, Jones BM, Hill J, Dua P, Lukiw WJ. Regulation of neurotropic signaling by the inducible, NF-kB-sensitive miRNA-125b in Alzheimer's disease (AD) and in primary human neuronal-glial (HNG) cells. Mol Neurobiol. 50:97-106 (2014)

Disrupted miRNA-mRNA signaling in Alzheimer's disease (AD)





miRNA-mRNA signals in

- *aging human and AD brain*
- *stressed mouse and human brain cells (neuronal, astroglial, microglial, endothelial)*
- *5xFAD (amyloid over-expressing) brain and retina*
- *age-related macular degeneration (AMD)*
- *human prion disease (CJD, GSS)*

Zhao Y, Bhattacharjee S, Jones BM, Hill J, Dua P, Lukiw WJ. Regulation of neurotropic signaling by the inducible, NF-kB-sensitive miRNA-125b in Alzheimer's disease (AD) and in primary human neuronal-glial (HNG) cells. Mol Neurobiol. 50:97-106 (2014)

Topics to be covered in this lecture

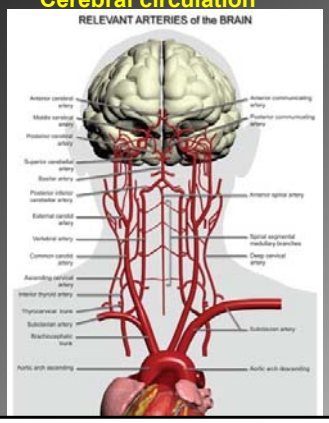
- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

How does aluminum access the neuronal nuclei of the brain neocortex and hippocampus?

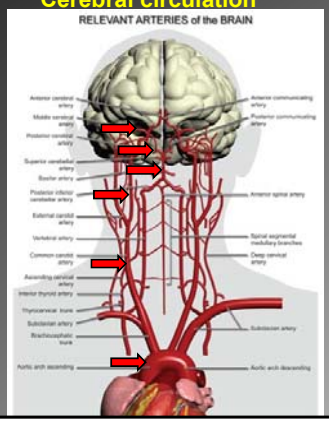
- pathways through the systemic circulation
- one existing study in human arteries
- *Minami T, Tohno S, Utsumi M, Moriwake Y, Yamada M, Tohni Y. Selective accumulation of aluminum in five human arteries. Biological Trace Element Research 79:29-38 (2001)*

The brain is very highly vascularized; 4,000 km of blood vessels in the average adult human brain; blood volume: 5 liters, circulates through the brain once every 1-1.5 minutes; [SV * HR * minutes = BV; time = BV/(SV*HR); time = 5L / (60ml*60) = 1 - 1.5 minutes]; brain receives first blood from the cardiopulmonary circulation that is 20-25% of the total

Cerebral circulation



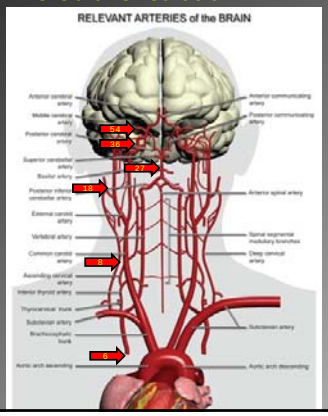
Cerebral circulation



Studies in Human Blood Vessels

- excise blood vessels from 1 hr post-mortem interval (PMI) brains or brain biopsy
- perfuse vessels with ultrapure saline
- ash in platinum vessels, heat and hydrolyze with ultrapure nitric acid
- analyze for trace metals using inductively coupled plasma emission (ICPE) or electrothermal atomic absorption (EAA)
- only Mg, Hg and Al levels were investigated further

Cerebral circulation



aluminum (sulfate) affinity for various cell types (in culture)

- hepatocyte (1 +/- 1 ug/g)
- microglial (human) (1.5 +/- 1.5 ug/g)
- astroglial (rat) (2.1 +/- 1.5 ug/g)
- astroglial (human) (6.1 +/- 2 ug/g)
- endothelial (HUVEC) (11 +/- 5 ug/g)
- endothelial (hBMEC) (36 +/- 8 ug/g)
- neuron (rat, interneuron) (15 +/- 5 ug/g)
- neuron (human, interneuron) (58 +/- 12 ug/g)
- neuron (pyramidal) (104 +/- 18 ug/g; 10-25x)

aluminum (sulfate) affinity for various cell types (in culture)

- hepatocyte (1 +/- 1 ug/g)
- microglial (human) (1.5 +/- 1.5 ug/g)
- astroglial (rat) (2.5 +/- 1.5 ug/g)
- astroglial (human) (6.1 +/- 2 ug/g)
- endothelial (HUVEC) (11 +/- 5 ug/g)
- endothelial (hBMEC) (36 +/- 8 ug/g)
- neuron (rat, interneuron) (15 +/- 5 ug/g)
- neuron (human, interneuron) (58 +/- 12 ug/g)
- neuron (pyramidal) (104 +/- 18 ug/g; 10-25x)

aluminum (sulfate) affinity for various cell types (in culture)

- hepatocyte (1 +/- 1 ug/g)
- microglial (human) (1.5 +/- 1.5 ug/g)
- astroglial (rat) (2.5 +/- 1.5 ug/g)
- astroglial (human) (6.1 +/- 2 ug/g)
- endothelial (HUVEC) (11 +/- 5 ug/g)
- endothelial (hBMEC) (36 +/- 8 ug/g)
- neuron (rat, interneuron) (15 +/- 5 ug/g)
- neuron (human, interneuron) (58 +/- 12 ug/g)
- neuron (pyramidal) (104 +/- 18 ug/g; 10-25x)

Topics to be covered in this lecture

- aluminum is abundant in our environment
- aluminum is implicated in autism spectrum disorders (ASD)
- aluminum is implicated in Alzheimer's disease (AD)
- aluminum is attracted to the genetic material in the nucleus
- aluminum binds to biological phosphates, induces reactive oxygen species (ROS) and activates the 'activator' transcription factor NF-kB
- NF-kB up-regulated micro RNA (miRNA) and down-regulates many messenger RNAs (mRNAs) and hence gene expression
- aluminum, TREM2 and amyloidogenesis (the buildup of amyloid in the central nervous system, CNS)
- aluminum enters the brain through the circulation (vasculature)
- mobilization of aluminum into the biosphere

Nuclear energy will power world's largest aluminum smelter; now being built in Russia's Saratov region, complete with two 950 MW pressurized water reactors linked to a 2.3 million tons per year aluminum smelter; RUSAL 12% world aluminum production; www-world-nuclear-news.org; www-rusal.ru

- it takes about 2 tons of alumina and 17000 kWhr of electricity to produce 1 ton of pure aluminum
- this facility alone will produce 525,000 pounds per hour; 8750 pounds per minute; 146 pounds per second; 24/7
- fiber reinforced aluminum (FRA), a mixture of carbon fiber and aluminum, is the most recent advance in aluminum structural products

fission

U-235 + n ===> Ba-141 + Kr-92 + 3n + 170 MeV

⬇ *steam turbine*

$2(Al_2O_3)$ + electricity -> $4Al^{3+} + 3O_2$

Pogue AI, Lukiw WJ. The mobilization of aluminum into the biosphere. Front Neurol. 2014 Dec 8;5:262. doi: 10.3389/fneur.2014.00262.

Moblization of aluminum into the biosphere

- *in the last 60 minutes our civilization has produced another ~6,100 tons (12,200,000 pounds) of 99.9% pure aluminum*
- *together our laboratories in the US, Canada and Russia have shown that very much less than 1/100 billionth of that amount is extremely genotoxic to human brain and CNS gene structure and function*

Pogue AI, Lukiw WJ. The mobilization of aluminum into the biosphere. Front Neurol. 2014 Dec 8;5:262. doi: 10.3389/fneur.2014.00262.

Conclusions-1

- aluminum is ubiquitous in our diet and our environment;
- aluminum is involved in chromatin compaction; the ability of aluminum to compact chromatin from active euchromatin to inactive heterochromatin is second to none;
- a biological function for aluminum may be to compact chromatin (DNA plus protein) into higher order structures; in diseases such as AD this system may get out of control and down-regulate the expression of many brain essential genes;

Lukiw WJ. Evidence supporting a biological role for aluminum in chromatin compaction and epigenetics. J Inorg Biochem. 104:1010-1012 (2010); Walsh B, Bazan NG, Gordon W, Lai B, Vogt S, Lukiw WJ; manuscript in preparation (2015)

Conclusions-2

- many aspects of aluminum treated primary brain cells and/or transgenic animals (5xFAD) recapitulate what is seen in AD, and especially in aged AD patients;
- specific vascular cell types (i.e endothelial cells) serving the human brain may act as a conduit to attract and accumulate aluminum in specific regions of the brain;
- the basilar arteries, middle cerebral artery (MCA) and the posterior cerebral artery (PCA) which serves the hippocampus contain the highest values of Al in both post-mortem tissues and in aluminum-treated hBMEC cells;

Conclusions-3

- molecular carriers for aluminum in the blood, serum and other blood components require further investigation; the role of aluminum transport and aluminum carriers in the systemic circulation is not well understood;
- aluminum interactions with neurobiology is exceedingly complex and more research needs to be undertaken to further clarify its role in the aging CNS;
- the continuing mobilization of aluminum into the environment is of serious and continuing healthcare concern

Conclusions for this section

- aluminum is involved in chromatin compaction;
- the ability of aluminum to compact chromatin from active euchromatin to inactive heterochromatin is second to none;
- an important biological function for aluminum is the state of compaction of chromatin and hence the global regulation of gene expression
- non-homeostatic aluminum-mediated compaction of euchromatin to heterochromatin is an important pathological effect of aluminum in eukaryotic brain cells

Lukiw WJ. Evidence supporting a biological role for aluminum in chromatin compaction and epigenetics. J Inorg Biochem. 104:1010-1012 (2010); Walsh B, Bazan NG, Gordon W, Lai B, Vogt S, Lukiw WJ; manuscript in preparation (2015)

Conclusions

- aluminum has an important function in selective chromatin compaction;
- pathological amounts of aluminum probably shift normally euchromatic gene regions into dense, inactive heterochromatin regions;
- an important biological function for aluminum is the global regulation of gene expression;
- aluminum promotes amyloidogenesis and senile plaque formation by at least two interdependent mechanisms;
  directly, by inducing Aβ42 peptide aggregation, and
  indirectly, by a miRNA-34a-mediated inhibition of TREM2 and the ability of natural TREM-2-mediated Aβ42 clearance mechanisms

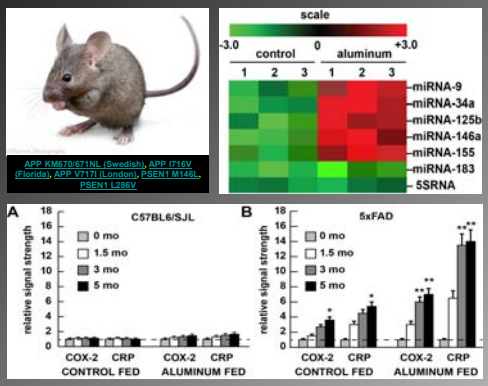
Lukiw WJ. Evidence supporting a biological role for aluminum in chromatin compaction and epigenetics. J Inorg Biochem. 104:1010-1012 (2010); Walsh B, Bazan NG, Gordon W, Lai B, Vogt S, Lukiw WJ; manuscript in preparation (2015)

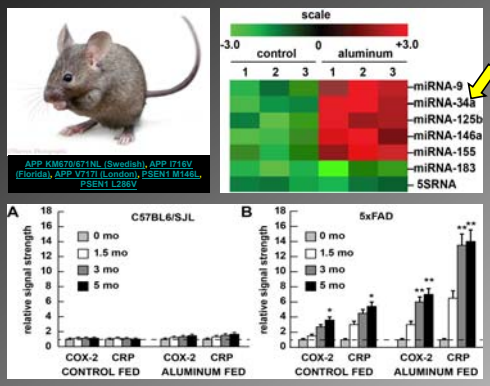
ADDITIONAL EVIDENCE THAT ALUMINUM IS INVOLVED IN NEUROLOGICAL DISEASES OF THE BRAIN (AD) AND RETINA (AMD)

1: Pogue AI, Dua P, Hill JM, Lukiw WJ. Progressive inflammatory pathology in the retina of aluminum-fed 5xFAD transgenic mice. J Inorg Biochem. 2015 Nov;152:206-9. doi: 10.1016/j.jinorgbio.2015.07.009. Epub 2015 Jul 20. PubMed PMID: 26213226; PubMed Central PMCID: PMC4681626.

2: Pogue AI, Lukiw WJ. Aluminum, the genetic apparatus of the human CNS and Alzheimer's disease (AD). Morphologie. 2016 Mar 8. pii: S1286-0115(16)00024-2.doi: 10.1016/j.morpho.2016.01.001. [Epub ahead of print] PubMed PMID: 26969391.

3: Bhattacharjee S, Zhao Y, Dua P, Rogaev EI, Lukiw WJ. microRNA-34a-Mediated Down-Regulation of the Microglial-Enriched Triggering Receptor and Phagocytosis-Sensor TREM2 in Age-Related Macular Degeneration. PLoS One. 2016 Mar7;11(3):e0150211. doi: 0.1371/journal.pone.0150211. eCollection 2016. PubMed PMID: 26949937.





ACKNOWLEDGEMENTS

Human Brain and Retinal Tissues Obtained from N=15:

Coriell Institute for Medical Research, Camden NJ, USA
Harvard University/McLean Hospital Tissue Center, Boston MA, USA
Louisiana State University, New Orleans LA, USA
Lomonosov Institute, Moscow State University, Moscow, RUSSIA
National Disease Research Interchange, Philadelphia PA, USA
Netherlands Brain Research Institute, Amsterdam, NETHERLANDS
New York State Institute for Basic Research, Staten Island NY, USA
Oregon Health Sciences University, Portland OR, USA
Southern Eye Bank, Metairie LA, USA
University of California, Irvine CA, USA
University of Kentucky Alzheimer's disease Brain Bank, Lexington KY, USA
University of Maryland Brain and Tissue Bank, Baltimore MD, USA
University of Massachusetts, Worcester MA, USA
University of Pennsylvania School of Medicine, Philadelphia PA, USA
University of Toronto Brain Bank, Toronto ON, CANADA

Acknowledgements / Collaborators

Peter Alexandrov PhD, Academy of Medical Sciences, Moscow, Russia
Nicolas G. Bazan, MD, PhD, LSU Neuroscience Center, New Orleans LA, USA
Christoph Eicken PhD, LC Sciences, Houston TX, USA
Zhide Fang PhD, LSU Biostatistics, New Orleans LA, USA
Christopher Hebel PhD, LC Sciences, Houston TX, USA
Sebastien Hebert PhD, Laval University Quebec, CANADA
James M. Hill, PhD, LSU Ophthalmology, New Orleans LA, USA
Hilary Thompson, PhD, LSU Ophthalmology, New Orleans LA, USA
Peng Jin, PhD, Emory University, Atlanta GA, USA
Debomoy K. Lahiri, PhD, University of Indianapolis IN, USA
Constantin Tagonov, PhD, David Baltimore, PhD / Caltech, CA, USA

Supported in part by:

COBRE III Pilot Award;
Louisiana Biotechnology Research Network (LBRN);
Research to Prevent Blindness (RPB);
Russian Academy of Medical Sciences (Skoltech; RAMS);
National Institutes of Health (NIH) National Institute of Aging NIA AG018031
National Institutes of Health (NIH) National Eye Institute NEI EY006311