Molecules that make our minds: fighting autism, schizophrenia and Alzheimer’s disease

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Laboratory Members:

TAU collaborators: Dr. David Gurwitz; Dr. Segev Barak; Dr. Rina Rosin-Arbesfeld, Dr. Joel Hirsch; Dr. Leonid Mittelman; Dr. Metsada Pasmanik-Chor and Adva Yeheskel.
Imagine your brain as a large rail transportation system and your 100 billion nerve cells = trains, while your brain “support cells”, are the train tracks.

Now imagine disruption in connectivity ... (~100 trillion points of connection)......

Can we fix it?
Cognitive Development and Aging Dementia

Autism: Intellectual disability and facial dysmorphisms caused by mutations in ADNP

Nature Genetics 46, 380–384 (2014)

Cognitive Development and Aging Dementia

Autism: Intellectual disability and facial dysmorphisms caused by mutations in ADNP

Nature Genetics
46, 380–384 (2014)

Autism spectrum disorder (ASD) refers to a range of disorders: ‘triad of impairments’

1. social and emotional understanding
2. all aspects of communication
3. flexibility in thinking and behavior

(Wing, 1996)
ADNP
Connecting Autism, Cancer and Alzheimer’s disease:
Tau/Autophagy/Apoptosis
Regulating >400 Genes
Our Discovery: Protective Platforms


Neuropeptides

Brain support cell-derived protectants

Activity-Dependent Neuroprotective Protein

- NAPVSIPQ

Activity-Dependent Neurotrophic Factor

- SALLRSIPA

ADNP platform

- Davunetide; NAP; D-NAP
- Dementia/Cognitive impairment
  - Alzheimer’s and Parkinson’s disease, schizophrenia

ADNF platform

- D-SAL; AL-309 (9 a.a.)
- SAL; AL-209 (9 a.a.)
- Neuropathies
1. No ADNP – No brain

2. ADNP is part of a large protein complex - **SWI/SNF** regulating >400 genes and determining nerve cell shape and brain structure

3. As part of the SWI/SNF, ADNP associated with proteins regulating tau splicing

*Our papers:*
- J Pharmacol Exp Ther. 2007;323:438-434
- Pharmacol Ther. 2007 ;114:146-54.
- Brain Res Dev Brain Res. 2003;144:83-90


Gene Array: ADNP Regulates >400 Genes


ADNP regulates ApoE – the major risk gene for Alzheimer’s disease
Bioinformatics: The ADNP Network

A SWI/SNF-related autism syndrome caused by de novo mutations in ADNP

Céline Helsoomert1, Anneke T Velt-o van Silfhout1,2, Bradley P Cox3,4,6, Geert Vandeweyer1,5, Liesbeth Roos2, Janneke van den Ende6, Janneke H M Schuur-van Hooijmakers7, Carlo I. Marcelli2, Marjoen H Willemsen2, Liesenka E L M Vissers8, Helger G Yntema2, Madhura Bakhshi7, Meredith Wilson8, Kali T Witherspoon9,3, Helena Malmgren9, Ann Nordgren8, Göran Annerén10, Marco Fichera11,12, Paolo Bosco11, Corrado Romano14, Bert A de Vries2,25, Tijltske Kleefstra2,25, R Frank Kooy9, Evan E Eichler3,4 & Nathalie Van der Aa1,6

http://www.adnpgene.com/

Activity-dependent Neuroprotective Protein Constitutes a Novel Element in the SWI/SNF Chromatin Remodeling Complex

Shmuel Mandel1 and Ilana Gozes2

From the Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel
Sexual Dichotomy: Gene Expression

**elf4E**

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<td>2^ΔdCt</td>
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**ADNP2**

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**ADNP**

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**APOE**

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Behavior

**Long retention choice phase (24 hours after habituation)**

- **Male**
- **Female**

**D2 (b-a)/(b+a)**

- **ADNP+/+**
- **ADNP+/−**

**Social memory**

- **Male**
- **Female**

**Malishkevich** A., **Amram** N., **Hacohen-Kleiman** G., **Magen** I., **Giladi** I., **Gozes** I. Activity-Dependent Neuroprotective Protein (ADNP) Exhibits Striking Sexual Dichotomy Impacting on Autistic and Alzheimer's Pathologies. Transl Psychiatry. 2015 Feb 3;5:e501. doi: 10.1038/tp.2014.138.


Yang MH et al.: Alzheimer’s disease (AD) serum proteins were analyzed using two-dimensional gel electrophoresis combined with nano-high performance liquid chromatography electrospray ionization tandem mass spectrometry (nano-HPLC-ESI-MS/MS) followed by peptide fragmentation patterning. Results suggested that ADNP may play an important role in slowing the progression of clinical symptoms of AD.
ADNP Haploinsufficiency: Modelling Tauopathy

ADNP partial deficiency: a window to the mouse brain

Tangle-like pathology (not seen in healthy brain)

ADNP decorates microtubules (healthy)

Healthy

Sick: ADNP-deficiency results in brain – cell death (green – fluoro-Jade)

*J Pharmacol Exp Ther.* 2007;323:438-434
Davunetide (NAP) Discovery

- Calculated molecular mass for ADNP-123,562.8 Da
- A large protein (limited bioavailability?)
- Davunetide, NAP VSIPQ is the smallest active fragment of ADNP which provides brain bioavailability and nerve cell protection

NAP = NAPVSIPQ

Contains a small neuroprotective epitope: Protecting against apoptosis

Our papers:
NAP Complements ADNP (+/-) Cognitive Deficiency

A small protecting snippet against protein deficits

ADNP-deficient mice (+/-) display impaired learning and memory in the Morris water maze (latency to reach the hidden platform is not reduced after training)

- Impairment is significantly reversed by treatment with NAP

- Parallel protection against tau pathology is seen also in models of Alzheimer’s disease and related neurodegenerations (tau mutant mice)

J Pharmacol Exp Ther. 2007;323:438-449

Neurobiol Dis. 2009 May;34(2):381-8
Microtubules are Essential Within the Nerve Cell

**Neurodegeneration**
- Destabilization and breakdown of microtubules
- Tau hyperphosphorylation
- Progressive loss of function
- Leads to cell death

**Neuroprotection**
- Davunetide (NAP) crosses the human blood brain barrier
- Reduces Tau hyperphosphorylation
- Stabilizes and repairs microtubules
- Enhances dendritic spine formation
- Restores neuronal structure and function

Gozes I, Barstable CJ, Monoclonal antibodies that recognize discrete forms of tubulin. *Proc Natl Acad Sci USA* 79:2579-83
Microtubules End Binding Proteins: Providing Microtubule Dynamics

NAP increases microtubule end binding protein association with tubulin

NAP increases ADNP Association with microtubule end binding proteins
The NAP-Motif of Activity-Dependent Neuroprotective Protein (ADNP) Regulates Dendritic Spines through Microtubule End Binding (EB) Proteins

NAP/ADNP: increases synaptic connections through EB (green)

“The Molecules that make our minds”

The NAP-Motif of Activity-Dependent Neuroprotective Protein (ADNP) Regulates Dendritic Spines through Microtubule End Binding Proteins
Immunoprecipitation (IP) of ADNP reveals direct interaction with microtubule associated protein 1 light chain 3 (LC3B), a major regulator of autophagy by Western analysis. The ADNP LC3B interaction correlates with the level of ADNP expression, a strong interaction in normal mice (ADNP+/+, right band) and a weaker interaction in ADNP+/− mice (left band). The interaction of ADNP with LC3B was augmented in the presence of NAP.


NAP Enhances Microtubule Invasion to the Tip of the Nerve: Fortifying Cell Communication

Increases microtubule dynamics at the tip of the growth cone

Calculation = Area occupied by dynamic (+Tyr)-tubulin (red, left; grey, right) in the distal-peripheral growth cone (ROI)

Invasion of dynamic microtubules

Enlisting Tau (Ties) Back to the Microtubules (Train Tracks)

NAP increases the tubulin and tau content of microtubules under conditions of zinc intoxication (note: zinc accumulation is a prominent feature of Alzheimer’s disease pathology)


By enlisting tau/building microtubules NAP provides protection against impairments in material (axonal) transport in nerve cells – key to brain communication

S Quraishe, C M Cowan and A Mudher
University of Southampton, Southampton
Molecular Psychiatry, 2013, EPUB
State of the art measurements of axonal transport: inhaled MnCl₂ provides an MRI contrasting agent (white, below). Measurements of progression in the olfactory bulb show disruption by microtubule breakdown (colchicine) and protection by NAP.

Protection against axonal impairment is coupled to protection against tau pathology in an ALS model.

NAP Prevents Axonal Transport Deficits: Mouse

Cognitive Improvement in a Schizophrenia Model

> The stable tubule-only polypeptide (STOP) knockout mice have been shown to provide a valid model for schizophrenia.

> The capacity of the mice to discriminate between novel object and familiar object was calculated as discrimination capacity: D2.

> The “schizophrenic” mice (microtubule-deficient) show markedly decreased memory.

> NAP ameliorates this deficiency.

Merenlender-Wagner et al., Peptides. 2010;31:1368
Gozes, Peptides. 2011;32:428; Olson Prize

Microtubule dysfunction in schizophrenia

DD=placebo
Heterozygous=Schizophrenia model

Working Hypothesis

DD=placebo
Heterozygous=Schizophrenia model
The STOP+/- Mouse Model Mimics Schizophrenia: NAP Compensation

NAP treatment reverses BECN1 reduction in STOP+-/ mouse hippocampus

## Significant Improvement in Schizophrenia Patients

### Daily Activities Change-from-Baseline


Original support: NIA, NIMH, ISOA, Allon Therapeutics

### Translation into clinical trials

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## Conclusion

- Statistically significant treatment effect of davunetide (NAP) 5 mg once daily - QD, (p=0.023) and combined treatment groups (high dose twice daily – BID + low dose, once daily) versus placebo (p=0.015) (mixed model ANCOVA)
- No statistical significance between davunetide groups
- Associated with *in vivo* neuroprotection
Davunetide (15 mg, x2/day) Improves Memory in aMCI Patients

Statistically significant, dose dependent and durable impact seen on memory
We Fight Against Their Disease

Lou Gehrig  Ronald Reagan  Maurice Ravel  Margaret Thatcher

Michael J Fox  Muhammad Ali  John Nash
from the film “A Beautiful Mind”
"It seems that for success in science or art a dash of autism is essential."

~ Hans Asperger
Jarnougkin, Regina Ostritsky & Illana Gozes

Davunetide (NAP) protects the brain in a diabetes rat model

NAP (davunetide) modifies disease progression in a mouse model of severe neurodegeneration: Protection against impairments in axonal transport

A.R. Esteves, I. Gozes, S.M. Cardoso

The rescue of microtubule-dependent traffic recovers mitochondrial function in Parkinson's disease


Clinical Translation: Medical Validation by Others


Effects of Davunetide on N-acetylaspartate and Choline in Dorsolateral Prefrontal Cortex in Patients with Schizophrenia


Effect of the neuroprotective peptide davunetide (AL-108) on cognition and functional capacity in schizophrenia.
Thanks!