UPDATE ON NAF RESEARCH

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University of Florida
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GOALS AND IMPACT OF NAF FUNDING

• Birds-Eye View of Funding Portfolio
  • Making scientific connections between diseases
  • Encouraging young investigators to pursue ataxia research

• Some Examples
  • Genetics 101-2015
  • Four Pioneer Awards
  • Therapeutic strategies to fight ataxia
  • Connections between scientists, diseases, NIH and Industry
NAF’S RESEARCH PROGRAMS

- **Research Seed-Money Grants** – Seed monies in early or pilot phases of studies and ongoing investigations that demonstrate need to attract future funding from other sources.

- **Post-Doc Fellowship Awards** – Intended for researcher to spend a 3rd year in a post-doc position to increase their chance of establishing an independent ataxia research program.
NAF’S RESEARCH PROGRAMS

▪ Young Investigator and Young Investigator SCA Awards - Awarded to encourage young investigators to pursue a career in the field of ataxia

▪ Pioneer SCA Translational Awards - This grant is intended for research investigations that will facilitate the development of treatments for the spinocerebellar ataxias (SCAs).
RESEARCH APPLICATIONS RECEIVED

- 33 Seed-money grant applications were received
- 22 Post-Doc Fellowship Applications received
- 15 Young Investigator (non-SCA) applications received
- 14 Pioneer SCA Translational Research Applications received
- 18 Young Investigator SCA Applications received

TOTAL of 102 Applications were submitted
13 COUNTRIES REPRESENTED

- United States
- United Kingdom
- Brazil
- The Netherlands
- Germany
- Portugal
- Australia
- Canada
- Spain
- France
- Italy
- Cyprus
- Mexico
<table>
<thead>
<tr>
<th>Types of Ataxia</th>
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<tbody>
<tr>
<td>SCAs</td>
</tr>
<tr>
<td>Friedreich Ataxia</td>
</tr>
<tr>
<td>Gordon Holmes Syndrome</td>
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<tr>
<td>Joubert Syndrome</td>
</tr>
<tr>
<td>Mitochondrial</td>
</tr>
<tr>
<td>FXTAS</td>
</tr>
<tr>
<td>A-T</td>
</tr>
<tr>
<td>Episodic Ataxia</td>
</tr>
<tr>
<td>ARSACS</td>
</tr>
<tr>
<td>Spastic ataxia</td>
</tr>
<tr>
<td>AOA</td>
</tr>
<tr>
<td>SCAN</td>
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<tr>
<td>Sporadic</td>
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</tbody>
</table>
REVIEW PROCESS

Reviewers are assigned by Dr. Harry Orr and Dr. Laura Ranum

All applications are peer-reviewed by 2 reviewers

Pioneer applications peer-reviewed by 3 reviewers

More than 60 reviewers worldwide

Conference calls are attended by a sub-group of NAF Medical Research Advisory Board members to make funding recommendations

NAF Board of Directors make the final funding decisions
NAF FUNDED RESEARCH

27 Research Projects were Funded totaling more than $1.1 million of research

9 Seed Money Research Grants
4 Young Investigator Awards
7 Post-doc Fellowship Awards
4 Young Investigator-SCA Awards
3 Pioneer Translational SCA Awards

26% of submitted grants were funded
Success rate is substantially higher than the NIH
**RESEARCH SEED GRANTS 2016**

**Speech Rehabilitation**  
**SCA3**  
**Gene discovery**  
**SCA36**  
**Brain Imaging**  
**Inflammation in Setx**  
**Ataxia Database**

**Vogel, Adam, PhD**  
Centre for Neuroscience of Speech Parkville, Victoria, Australia  
*Intensive home based speech rehabilitation for adults with degenerative ataxia*

**Simões, Ana, PharmD, PhD**  
University of Coimbra, Portugal  
*Calpain-mediated proteolysis in Machado-Joseph disease*

**Karnebeek, Clara, MD, PhD**  
University of British Columbia, Vancouver, BC, Canada  
*Whole Exome Sequencing in the Diagnosis and Management of Atypical Childhood Hereditary Ataxia Conditions*

**He, Fang, PhD**  
Texas A&M University, Kingsville, TX  
*Development of a Drosophila model for Spinocerebellar Ataxia type 36 (SCA36)*

**Oz, Gulin, PhD**  
University of Minnesota, Minneapolis, MN  
*Launching the US-Europe Neuroimaging Partnership in SCA*

**Burmeister, Margit, PhD**  
University of Michigan, Ann Arbor, MI  
*Role of VPS13D in Ataxia*

**Lavin, Martin, PhD**  
University of Queensland Centre for Clinical Research (UQCCR) Australia  
*Assessing the role of senataxin in cellular inflammation, gene regulation, and innate immunity in Setx-/- mice and a human neuronal model*

**Ribeiro, Sandra, PhD**  
Instituto de Biologia Molecular e Celular Porto, Portugal  
*New therapeutic approaches for Machado-Joseph Disease: Chaperoning protein self-assembly*

**Perlman, Susan, M.D.**  
University of California Los Angeles, Los Angeles, CA  
*Web-based National Ataxia Database*
NAF GRANT RECIPIENTS
YOUNG INVESTIGATORS IN 2016

Yao, Bing, PhD
Emory University, Atlanta, GA
Epigenetic Modulation Mediated by RNA-Binding Proteins in Neurodegeneration

Schmidt, Jana, PhD
University of Tuebingen, Germany
Alleviation of proteasomal inhibition as a therapeutic approach for SCA3

Butler, Jill Sergesketter, PhD
University of Alabama at Birmingham, AL
Reduced expression of mitochondrial aldehyde dehydrogenases contributes to metabolic stress in Friedreich’s ataxia

Ben-Johny, Manu, PhD
Johns Hopkins University, Baltimore, MD
Aberrant Regulation of Voltage-gated Na channels in the Pathophysiology of Spinocerebellar Ataxia 27

Cvetanovic, Marija, PhD
University of Minnesota, Minneapolis, MN
Role of astrocyte calcium signaling in the pathogenesis of SCA1

Nageshwaran, Sathiji, MD
Harvard University, Boston, MA
Transcriptional activation using CRISPR/Cas mutant proteins as a novel therapy for Frataxin gene silencing

Khurana, Vikram, MD, PhD
Brigham and Women’s Hospital and Harvard Stem Cell Institute, Boston, MA
Systematic edgotyping of ataxin proteins in cellular systems from yeast to patient neurons

Gennarino, Vincenzo, PhD
Baylor College of Medicine, Houston, TX
PUMILIO1 deficiency: understanding a new ataxia gene and its role in cerebellar dysfunction in mice and humans

INVESTING IN THE NEXT GENERATION OF ATAXIA RESEARCHERS

Gene Regulation
- Removal of toxic proteins in SCA3
- Friedreich’s – mitochondrial stress
- Cell-Cell communication SCA27
- Astrocyte signaling in SCA1
- Turning up Frataxin with Crispr/Cas

ASO SCA3
- Chemical pathways in Friedreich’s
- Pathways for polyQ regulation SCA3
- Protein networks in ataxia
- PUMILIO1 – a new ataxia gene
NAF GRANT RECIPIENTS
POSTDOCTORAL FELLOWSHIPS 2016

INVESTING IN THE NEXT GENERATION
OF ATAXIA RESEARCHERS

Brain Structure
Small Molecule Drugs
Motor Neuron Degeneration
RNA Degradation of CAG repeats
Cell-cell communication and protection
Protein dysregulation

• Anderson, Collin J., PhD
University of Utah, Salt Lake City, UT
Development and mechanistic study of deep brain stimulation of dentate nucleus for the treatment of degenerative ataxia.

• Orengo, James, MD, PhD
Baylor College of Medicine, Houston, TX
Unraveling the mechanisms of motor neuron degeneration in Spinocerebellar Ataxia, type 1

• Chen, Jonathan, PhD
Scripps Florida, Jupiter, FL
Rapid structure-based lead optimization of a small molecule drug that targets r(CAG)exp

• Bott, Laura C., PhD
Northwestern University, Evanston, IL
Transcellular regulation of the proteostasis network in Spinocerebellar ataxia type 3

• Chopra, Ravi, PhD
University of Michigan, Ann Arbor, MI
Identifying Dendro-Protective Ion Channels in Cerebellar Ataxia

• Seminara, Stephanie, MD
Massachusetts General Hospital, Boston, MA
Ataxia with hypogonadotropic hypogonadism due to ubiquitin ligase dysregulation
NAF GRANT RECIPIENTS IN 2016

PIioneer SCA
TRANSLATIONAL
RESEARCH AWARDS
Moving towards therapies

DNA

RNA

Protein

• Orr, Harry, PhD
University of Minnesota, Minneapolis, MN
Towards an ASO Therapy for Spinocerebellar Ataxia Type 1

• Roon-Mom, Willeke M.C. van, PhD
Leiden University Medical Center, The Netherlands
Advancing the therapeutic potential of exon skipping
• for Spinocerebellar ataxia type 3

BASIC RESEARCH STEM CELLS

• Maciel, Patrícia, PhD
University of Minho, Braga, Portugal
Testing the therapeutic potential of Mesenchymal Stem Cells and their secretome in an animal model of spinocerebellar ataxia type 3.
GENE DISCOVERY TO GENE BASED THERAPIES
The DNA Double Helix

- Sugar phosphate backbone
- Bases
- Base pair

Adenine (A)  Cytosine (C)
Thymine (T)  Guanine (G)
Overview
Chromosomes, DNA, Genes, Proteins

Adapted from *Understanding Gene Testing*, NIH, 1995
Central Dogma

DNA

RNA

Protein
GENOMIC SOFTWARE

“The Code”
DNA → RNA  CODONS→Protein

Genomic DNA  THYBIGRYDDOGRANOUT
Messenger RNA  THEBIGREDDOGRANOUT
Translated Message  THE BIG RED DOG RAN OUT
Genetics of Disease
Disease-Associated Mutations

A mutation is a change in the normal base pair sequence

Commonly used to define DNA sequence changes that alter protein function
Disease-Associated Mutations Can Alter Protein Function

Functional protein

Nonfunctional or missing protein
Point Mutations

<table>
<thead>
<tr>
<th>Type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>THE BIG RED DOG RAN OUT.</td>
</tr>
<tr>
<td>Missense</td>
<td>THE BIG RAD DOG RAN OUT.</td>
</tr>
<tr>
<td>Nonsense</td>
<td>THE BIG RED.</td>
</tr>
<tr>
<td>Frameshift (deletion)</td>
<td>THE BRE DDO GRA.</td>
</tr>
<tr>
<td>Frameshift (insertion)</td>
<td>THE BIG RED ZDO GRA.</td>
</tr>
</tbody>
</table>

Point mutation: a change in a single base pair
Autosomal Dominant Inheritance

- Each child has 50% chance of inheriting the mutation
- Equally transmitted by men and women
Autosomal Recessive Inheritance
Genetics 101 → 2017
Repeat Expansion Diseases

Fragile X Syndrome

Huntington Disease

Myotonic Dystrophy

Protein Loss

Protein Gain

RNA Gain
DNA → RNA  CODONS→Protein

Genomic DNA           THYBIGRYDDDOGRANOUT
Messenger RNA         THEBIGREDDDOGRANOUT
Translated Message    THE BIG RED DOG RAN OUT

The code is read three letters at a time and the ATG sets the reading frame.
<table>
<thead>
<tr>
<th>DNA → RNA → Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic DNA</td>
</tr>
<tr>
<td>Messenger RNA</td>
</tr>
<tr>
<td>Translated Message</td>
</tr>
</tbody>
</table>
PolyQ Protein Problems

Mutant RNA problems – myotonic dystrophy

Ranum and Cooper Ann Rev Neurosci, 29:259; 2006
Repeat Expansion Mutations in Neurologic Disease

Protein Loss / Protein Gain / RNA Gain of Function
Bidirectional Expression
Repeat Associated Non-ATG (RAN) translation
Expansion mutations produce unexpected proteins in multiple frames
Spinocerebellar Ataxia Type 8
SCA8
**SCA8: one mutation two genes**

Exon B → Exon A → Exon 1 → (CTG)n → CUG_EXP

Toxic RNA Mechanism
Daughters et al. 2009 *PLoS Genetics*

Toxic PolyQ Mechanism?
Moseley et al., 2006 *Nature Genetics*

*AAAATG*(CAG)_EXP***
Discovery of Repeat-Associated Non-ATG Translation (RAN translation)

<table>
<thead>
<tr>
<th>Frame</th>
<th>Codon</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CAG</td>
<td>Glutamine(Q)</td>
</tr>
<tr>
<td>2</td>
<td>AGC</td>
<td>Serine(S)</td>
</tr>
<tr>
<td>3</td>
<td>GCA</td>
<td>Alanine(A)</td>
</tr>
</tbody>
</table>

**A8(\text{*KKQ}_{\text{Exp}})-endo** 5'...*AAAAAG(CAG)_{103}CGG(CAG),CGG(CAG)_{5}...3'

**A8(\text{*KMQ}_{\text{Exp}})-end** 5'...*AAAAATG(CAG)_{103}CGG(CAG),CGG(CAG)_{5}...3'

Zu et al., PNAS 2011
DNA → RNA → Protein

Genomic DNA

Messenger RNA

Translated Message

Repeat Associated Non-ATG (RAN) translation

THYBIGRYDRYDRYDROYDOGRANOUT

THEBIGREDREDREDREDDOGRANOUT

THE BIG RED RED RED RED DOG RAN OUT

RED RED RED RED DOG RAN OUT

EDR EDR EDR EDD OGR ANO UT

DRE DRE DRE DDO GRA NOU T
RAN Translation in vivo: SCA8 and myotonic dystrophy

α-SCA8 poly-GCA-Ala

Mouse Cerebellum

α-DM1 poly-CAG-Gln

Human Myoblasts

Zu et al., PNAS 2011
Growing trends in many expansion diseases

Framework for understanding disease mutations needs revision.
Bidirectional expression is common
Repeats express unexpected proteins
RAN proteins now found in RNA and “polyQ” diseases
RAN Proteins in Huntington’s Disease

CAGCAGCAG...

HD mutation

...GTGCTGTC

CUG

polyGlutamine mHTT

polyAlanine

polySerine

polyCysteine

polyLeucine

HD pathogenesis

polyCAG

WHAT ABOUT THE POLY-Q ATAXIAS?
Therapy development

New Genetic Tools
  Gene Editing
  RNA Targeting
Targeting the RNA

Targeted degradation of toxic RNA by gapmer ASOs
FDA approval for Biogen and Ionis Pharmaceuticals – Spinaraza
Spinal Muscular Atrophy

ANTISENSE OLIGONUCLEOTIDE THERAPY

DNA → mRNA → Protein

Antisense Oligo

RNase H

SIMILAR DRUGS CAN REMOVE RNA

DRUG WAKES UP A SLEEPING GENE
SCA8 targeting the problems: one or both RNAs?

Toxic RNA & RAN Proteins

Toxic RNA & RAN proteins

Similar strategies being applied for many of the SCAs including SCA1 and SCA3
PARTNERING WITH YOU

GENE DISCOVERY
HOW GENETIC MUTATIONS CAUSE DISEASE
THERAPY DEVELOPMENT
Conclusions

THANK YOU!!!!!!

• NAF funding critically important for understanding the causes of ataxia and developing treatments

• Therapeutic efforts to target RNA and protein pathways and to repair genes are underway.

• Lessons from one disease connect to other diseases
  • How mutations cause disease
  • Therapy development