The CZI Neurodegeneration Challenge Network

A MODEL FOR COLLABORATIVE SCIENTIFIC IMPACT

Chan Zuckerberg Initiative

SEPTEMBER 2025

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Cover: Attendees at the CZI Neuroscience 2025 Meeting in San Jose, California.

Welcome

Above: Attendees at the CZI Neuroscience 2024 Meeting in Monterey, California.

We are proud to present the Neurodegeneration Challenge Network (NDCN) impact report, a comprehensive overview of the significant strides made by the Chan Zuckerberg Initiative's (CZI) NDCN over the last seven years.

Since its inception in 2018, the NDCN has been a pioneering force reshaping the landscape of neurodegenerative disease research. Our commitment has been to foster a new era of understanding, collaboration, and innovation, with the ultimate goal of contributing to effective treatments and cures for these devastating conditions.

There are currently no effective therapies to cure or prevent most neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis (ALS), and the hundreds of other neurodegenerative diseases and conditions. These illnesses affect millions of

Collaborative Pairs Pilot Project Award Cycle 2 grantee Naomi Habib fields a question from the audience at the CZI Neuroscience 2024 Meeting in Monterey, California. Naomi collaborated with Francisco Quintana to integrate computational predictions of molecular drivers from large human datasets with functional screening platforms to identify pathways that operate in astrocytes to drive neurodegeneration.

Despite decades of research and billions of dollars of investment, we still lack effective therapies for most neurodegenerative disorders.

people worldwide, yet their causes are only partly understood. This gap in understanding represents a critical deficiency in current methodologies and resource allocation, and highlights an urgent and compelling need for new approaches.

The core mission of the NDCN has been to pave the way for groundbreaking scientific advances with significant translational applications. The network was created to embrace fresh perspectives that challenge conventional wisdom and foster collaborations across diverse scientific expertise. Through our unique interdisciplinary model, we have accelerated discovery, cultivated new talent, initiated new frameworks and models for collaboration, and developed novel technologies that are reshaping the field.

This seven-year report showcases the tangible impacts of our collective efforts across four key pillars underpinning our strategic approach: 1) investment in high-risk/high-reward science at the frontiers of the field; 2) recruitment of new interdisciplinary talent to the field; 3) collaboration as a vehicle to accelerate discovery; and 4) community resource development.

The scientific breakthroughs emerging from this collaborative network are already directly contributing to novel opportunities in biomarker development and to the identification of innovative therapeutic pathways for rare and common neurodegenerative diseases. This trajectory aligns seamlessly with the ambitious philanthropic interests of CZI, whose audacious goal is to cure, prevent, or manage all disease by the end of this century. The NDCN's dedication to foundational research serves as a cornerstone in the quest to achieve a future where neurological disorders are deeply understood and fully treatable.

We are thankful for our CZI partners, organizations, mentors, external reviewers, and advisors who have helped build the Challenge Network. And we are especially grateful for the extraordinary community of investigators, students, postdocs, and staff scientists whose collective efforts are the bedrock of its achievements. The NDCN investigators and their labs have changed the status quo in neurodegeneration research through their groundbreaking basic science research, broad range of perspectives, and open collaboration. Their passion, innovative spirit, and tireless commitment have transformed our initial aspirational vision into a tangible reality — a network that is far greater than the sum of its parts. Indeed, the remarkable success of the Challenge Network is unequivocally a testament to their invaluable contributions and shared triumphs.

Impact At-A-Glance

Our vision for the Challenge Network has been to build a collaborative research consortium that is more than the sum of its parts. We explicitly cast a wide net aimed at breaking down disease and methodological silos that existed in the field. We brought together world-class scientists, technology developers, and clinicians, with varied and wide-ranging expertise, and empowered them to think creatively and tackle hard problems. This network approach enabled scientists to collaborate in new and exciting ways to propel research around neurodegeneration and fundamental neuroscience. Importantly, many of our grantees have told us that being a part of the Challenge Network has been as impactful for their work and careers as the funding itself.

The success of this network approach is evident across multiple key performance indicators. First, the sheer productivity emerging from this collaborative ecosystem has been exceptional, manifesting in a significant increase in research output, novel methodologies, and groundbreaking discoveries. Second, the tangible achievements realized within this network are a testament to its efficacy, ranging from advancements in understanding complex neurodegenerative processes to the development of innovative therapeutic strategies.

Third, our significant contributions to both supporting the network and developing high-quality shared resources for the broader scientific community reflect our commitment to open science and accelerating progress across the field. This includes the creation of shared datasets, standardized protocols, and accessible research tools, all of which serve to democratize access to vital resources and foster a more efficient research environment.

Finally, and most importantly, the true hallmark of this network's triumph lies in the depth and breadth of collaborations that have organically blossomed within its framework. These partnerships extend far beyond mere professional acquaintances, evolving from initial thought-partner discussions into robust, long-term scientific alliances. This collaborative spirit has culminated in a significant number of co-publications in leading scientific journals. The synergistic efforts born from these collaborations have not only amplified individual strengths but have fostered a dynamic environment where different perspectives converge to tackle complex challenges with considerable creativity and effectiveness.

BUILDING THE NDCN
JANUARY 2018 – AUGUST 2025

4
Number of open-call, investigator-initiated RFA mechanisms introduced to grow the network

287
Grants issued under the four funding mechanisms

62
Grant supplements to support collaborative projects within and outside of the NDCN, as well as across CZI imaging and neuroscience programs

79
Targeted grants to support computational tools, trainings, workshops, mentorships, and resource development

30
Grants to support community building by NDCN students and postdocs

COMPOSITION OF THE NDCN

312
Labs supported

700
Students, postdocs, and staff scientists participated in the Challenge Network

25
Clinician scientists contributed disease expertise

11
Rare and common neurodegenerative diseases are represented within the Challenge Network

25
Mentors provided 1:1 support for Ben Barres Early Career Acceleration Award investigators

NETWORK-SUPPORTED ACTIVITIES

6
Annual meetings

3
Working Groups:
1) iPSC/CRISPR,
2) Computational Biology,
3) Human Microglia Taxonomy

3
Focus Groups (FGs):
1) Organelle and Metabolism FG,
2) RNA and RNA Protein Interaction FG,
3) Sleep Collaborative

RESOURCES DEVELOPED AND SHARED BY NDCN INVESTIGATORS

232
Lab tools including viral vectors, cell lines, mouse models, plasmids, and biomaterials

75
Computer algorithms and code

60
Datasets

83
Laboratory protocols

OUTPUTS FROM THE NDCN

959
Collaborations ranging from thought partners, and resource sharing, to co-submission of grants and publications

76
Co-publications (see right)

61
Co-grant applications

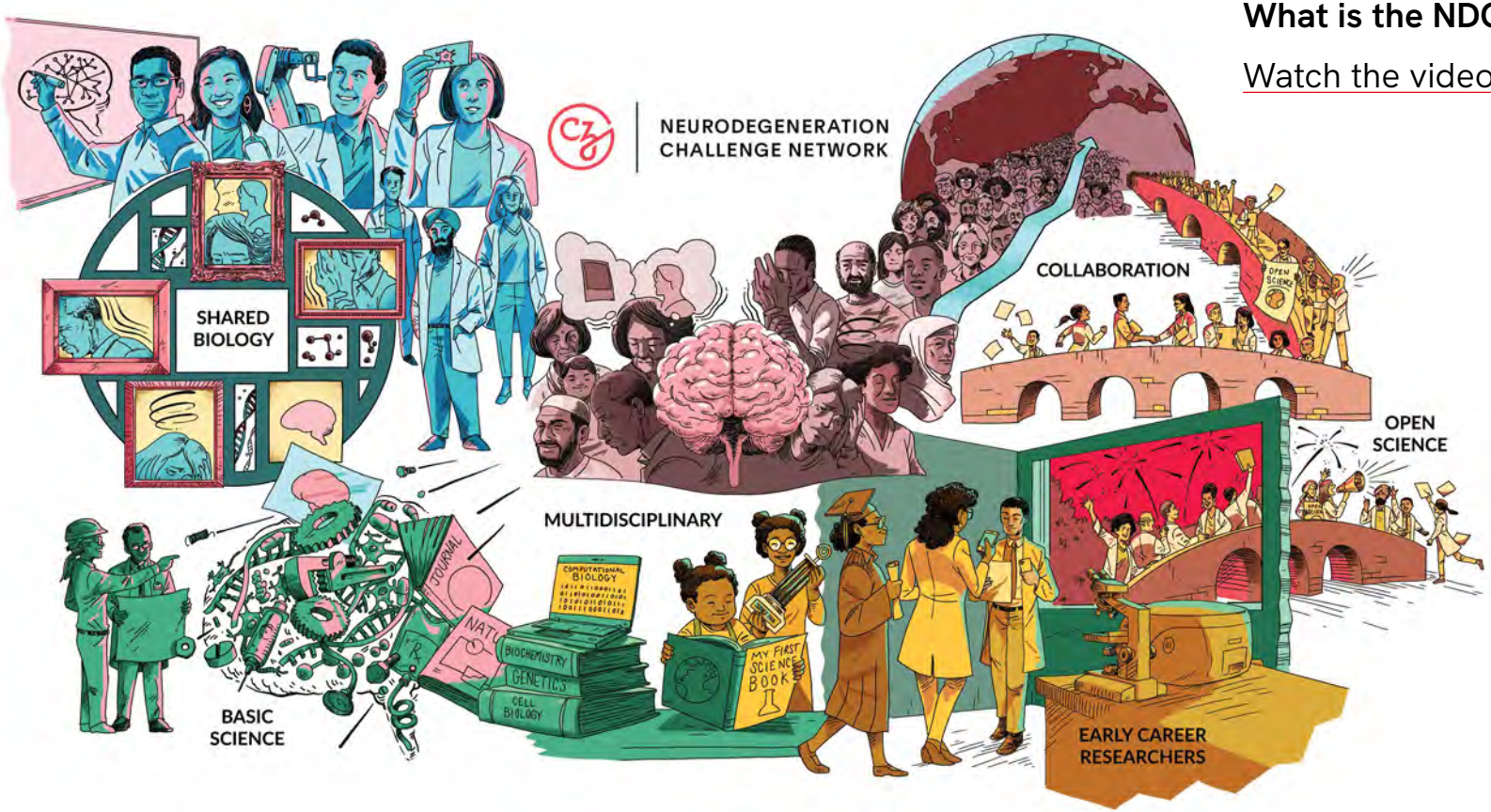
937
Publications including:
22 Cell papers, 35 Nature,
15 Nature Neuroscience,
18 Neuron, and 10 Science

390
Preprints

CO-PUBLICATIONS

As of July 2025, NDCN grantees have published over 900 publications and nearly 400 preprints, with 76 publications from collaborations that emerged from the Challenge Network directly, demonstrating the power of this interconnected community. These collaborations extend beyond individual grants, advancing joint research. For details on these scientific discoveries — including some of the journal covers shown here — please see the report appendix.

THE CHALLENGE NETWORK EFFECT



What is the NDCN?

Watch the video here →

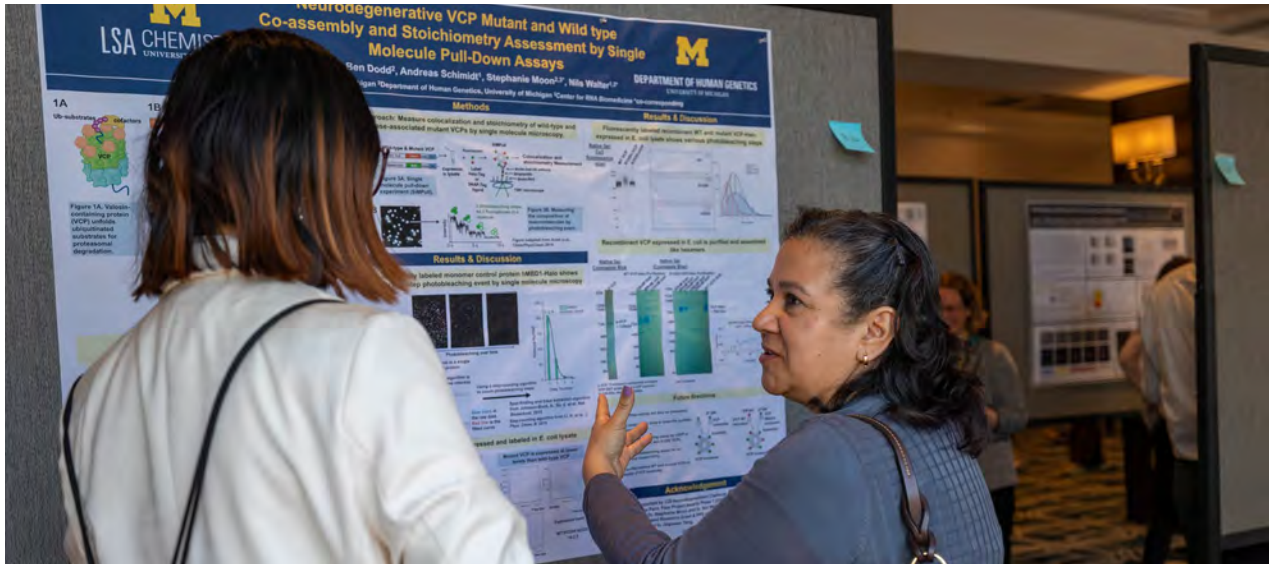
The Neurodegeneration Challenge Network: A Collaborative Frontier in Neurodegenerative Disease Research

The Neurodegeneration Challenge Network (NDCN) has been a groundbreaking, collaborative approach aimed at unraveling the complexities of neurodegenerative diseases. Launched by CZI, the NDCN was conceived with the ambitious goal of pushing beyond traditional research paradigms and fostering interdisciplinary collaboration to tackle one of humanity's most pressing health challenges.

The Challenge Network has united an exceptional cohort of scientists from a diverse spectrum of disciplines. This includes, but is not limited to, neuroscientists dedicated to understanding the brain's intricate workings, engineers developing novel tools and methodologies, cell biologists delving into the fundamental units of life, geneticists exploring the hereditary underpinnings of disease, immunologists investigating the immune system's role, and computational scientists leveraging advanced analytical techniques.

The overarching objective of the NDCN has been to cultivate a dynamic and synergistic network where outstanding, innovative, and forward-thinking scientists could converge and collectively address critical questions related to the basic cell biological mechanisms of neurodegeneration. By fostering this integrated approach, the NDCN sought to accelerate the pace of discovery, translate foundational insights into tangible advancements, and, ultimately, pave the way for more effective treatments and cures for devastating neurodegenerative conditions.

Our network has operated on the principle that breakthrough discoveries require both specialized expertise and cross-disciplinary collaboration. By connecting early-career investigators with established leaders and creating platforms for knowledge exchange, we fostered an ecosystem where transformative ideas can flourish.



Jane Tang (left), a graduate student in the lab of Stephanie Moon, at the University of Michigan, presents a poster at the CZI Neuroscience 2023 Meeting.

The NDCN has had a unique emphasis on:

- Pursuing high-risk, high-reward scientific questions
- Recruiting and supporting talent from both within and outside traditional neuroscience
- Building collaborative teams to tackle complex problems
- Developing community resources that accelerate progress across the field
- Looking across diseases to reconceptualize neurodegenerative disorders as a class of disorders with shared biology, mechanisms, and potentially treatment strategies rather than a collection of individual diseases

To achieve these goals, we utilized a multifaceted approach to grantmaking and program development. Our approach to grantmaking combined both open solicitations for applications and more targeted, invitation-only grantmaking. This dual strategy allowed us to cast a wide net to discover novel ideas while also enabling us to direct funding towards specific, high-priority areas aligned with the Challenge Network's evolving objectives.

In the design of our open calls, specifically our Requests for Applications (RFAs), we sought to develop innovative mechanisms that would inspire new kinds of scientific inquiry and interdisciplinary collaboration. We moved beyond traditional grant structures, encouraging proposals that presented unconventional methodologies, novel hypotheses, and partnerships across a range of scientific fields. This innovative approach was central to our mission of disrupting conventional research silos in neurodegeneration.

As the program developed and matured, our approach to grant-making iterated and evolved. We continuously refined our strategies based on the scientific landscape, emerging discoveries, and the Challenge Network's progressing goals. This adaptive methodology ensured that our funding mechanisms remained relevant, impactful, and responsive to the dynamic challenges within neurodegeneration research.

The RFA grant mechanisms were open to the global research community and served as the main entry point for most grantees in the Challenge Network. Each distinct grant mechanism was carefully designed and used to advance the overall vision of the NDCN, tailored to address specific aspects of our comprehensive research agenda.

Gina Poe, a Collaborative Pairs Investigator from the University of California, Los Angeles, asks a question during the CZI Neuroscience 2024 Meeting.

Stanley Qi (right), a Collaborative Pairs Investigator from Stanford University, connects with a fellow participant between sessions at the Neuroscience 2025 Meeting in San Jose.

1. **Ben Barres Early Career Acceleration Award:** This investigator award was designed for early-career academic researchers, particularly those new to the field of neurodegeneration. Recipients benefited from mentored support and access to NDCN resources. Cycle 1 supported 17 investigators, representing a total investment of $42.5 million over five years; Cycle 2 supported 13 investigators, representing a total investment of $15.5 million over four years.

2. **Collaborative Science Award:** This award provided funding for small interdisciplinary teams of investigators, typically comprising one to four members. Each group included a physician-scientist with active clinical engagement. This program funded nine teams, with a total investment of $12.6 million over four years.

3. **Collaborative Pairs Award:** This grant mechanism featured a two-phased funding structure: an 18-month pilot phase followed by a 4-year acceleration phase in Cycle 1 and a 2-year acceleration phase in Cycle 2. The pilot phase aimed to provide collaborating teams with the autonomy to explore novel, unconventional, and potentially transformative ideas. Each collaborative pair team was required to include an early- or mid-career investigator, and the team members could not have previously received joint grant funding prior to submitting a Collaborative Pairs grant application. Cycle 1 supported 30 pilot phase pairs (60 grants) and 16 acceleration phase pairs (32 grants), with a total investment of $30.1 million. Cycle 2 supported 64 pilot phase pairs (128 grants) and nine acceleration phase pairs (18 grants), with a total investment of $20 million.

4. **Patient-Partnered Collaborations in Rare Neurodegenerative Diseases:** This grant mechanism was established to foster collaborative teams that integrated patient-led rare disease organizations with research teams, thereby accelerating scientific discovery. Patients and patient-led organizations have been central to the research conducted under this mechanism. This program funded five teams, with a total investment of $10 million.

To translate funding opportunities into funded research, we collaborated with the CZI grants team to design the RFAs and establish a comprehensive, rigorous and efficient review process for all applications, regardless of the funding mechanism. This process began with an internal review by CZI program staff to identify applications within the scope of the funding announcement that proposed innovative, high-risk, high-reward research rather than incremental advances. Applications that met these criteria underwent an external review process, each assessed by two to three reviewers with relevant domain expertise. More than 200 external reviewers from the United States, Europe, Asia, and Australia, recognized

Vidhya Rangaraju, a Ben Barres Early Career Acceleration Award recipient from the Max Planck Florida Institute for Neuroscience, presents at the CZI Neuroscience 2024 Meeting in Monterey, California. Vidhya's research investigates metabolic disruptions that impair learning and memory in ALS using super-resolution imaging of mitochondrial stabilization and state-of-the-art mitochondrial ATP imaging.

Jeff Carroll (left), of the University of Washington, Albert "Al" La Spada (center) and Leslie Thompson (right), both, of the University of California Irvine, connect at the CZI Neuroscience 2024 Meeting in Monterey, California.

for their significant contributions to neuroscience research and offering broad domain expertise, participated in evaluating NDCN funding opportunities.

Those applications that advanced underwent a final panel review, with a panel composed of external reviewers and CZI program staff. In many instances, top-tier applicants completed a final interview with an external review panel. These interviews allowed applicants to provide research plan updates and address specific reviewer questions.

In addition to open solicitations through our RFA grant mechanisms, we provided targeted grants to support individuals and teams demonstrating leadership in resource development, technology dissemination, and the promotion of open science. This approach facilitated the creation of new tools, ensured widespread technology adoption, and championed open science through new data-sharing platforms.

Targeted grants have been instrumental in accelerating the integration of AI/ML technologies, particularly in neuropathology. Brain banks, vital resources for the research community, often face challenges with data analysis and sharing, which can impede the swift identification and distribution of human brain samples. To address this, targeted grants were awarded in 2024 to six teams representing brain banks in the U.S. and Europe. Their objective is to leverage AI/ML approaches to enhance data analysis and improve the sharing of both data and samples with the broader research community.

Using these funding and programmatic approaches, we built a network of over 300 researchers from 70 institutions worldwide, an unprecedented collaborative force in the fight against neurodegenerative diseases. Unlike other consortia, which often focus on a single disease, large-scale data generation, or a shared hypothesis — and are supported by a single funding mechanism — our approach was different. We aimed to build and evolve a network that encouraged investigators to openly share expertise, technologies, and scientific interests. We prioritized collaboration

over competition, anchored by strong open science practices. In that respect, the network functioned more as an “institute without walls” than a typical consortium or program-project mechanism.

To accomplish this “institute without walls” function, program staff adopted a high-touch grant and program management approach to foster investigator integration within the Challenge Network. This included annual lab check-in calls, a monthly Challenge Network webinar series for knowledge sharing, and monthly NDCN Newsletters which highlighted upcoming network events and scientific achievements of NDCN labs.

Upon the NDCN's launch, new machine learning and computationally sophisticated methods, such as single-cell biology, were emerging with the potential to revolutionize scientific practices and significantly accelerate discovery. While many labs recognized the promise of these methods for their research, they often lacked the in-house expertise and capacity to leverage these new tools. To address these needs, we developed various grant and programmatic strategies to support computational biology training and explore emerging fields at the intersection of computational biology and bioinformatics. Through these strategic investments, we aimed to modernize research methodologies and foster a more collaborative and efficient scientific landscape.

Our Four Pillars of Impact

This Impact Report is structured around four foundational pillars that defined our strategy for building the Challenge Network. It highlights some of the significant achievements of NDCN investigators and their labs, demonstrating how this network approach has furthered our understanding of the basic science underlying neurodegeneration.

INVESTMENT IN HIGH-RISK/ HIGH-REWARD SCIENCE AT THE FRONTIERS OF THE FIELD

We were committed to supporting audacious ideas by strategically allocating resources to ambitious research initiatives that dared to venture into uncharted scientific territory. These were projects that unlocked new avenues of inquiry and had the potential for transformative breakthroughs, even if they carried a higher degree of risk.

COLLABORATION AS A VEHICLE TO ACCELERATE DISCOVERY

Our model actively promoted and facilitated deep, meaningful collaborations among researchers from various backgrounds and institutions. By breaking down barriers between disciplines and disease and research fields and encouraging open communication and shared problem-solving, we created a dynamic ecosystem where discoveries were accelerated, and complex challenges could be tackled with a multifaceted approach.

RECRUITMENT OF NEW INTERDISCIPLINARY TALENT TO THE FIELD

Recognizing that the most profound insights often emerge at the intersection of disciplines, we actively recruited exceptional individuals with a wide variety of expertise. Our focus was on bringing together scientists, engineers, data specialists, and other innovators who could bridge traditional academic silos to create a rich environment where cross-pollination of ideas could thrive.

COMMUNITY RESOURCE DEVELOPMENT

Beyond individual research projects, we were committed to building and nurturing a robust scientific community. This involved the development and dissemination of cutting-edge tools, platforms, and methodologies that served as shared resources for the broader scientific endeavor. By providing accessible and advanced infrastructure, we empowered not only our immediate grantees and collaborators but also a wider network of researchers.

HIGH-RISK / HIGH-REWARD SCIENCE AT THE FRONTIERS OF THE FIELD

In developing the strategy for what would become the Challenge Network, we consistently heard from experts within and outside the neurodegeneration field that fresh thinking and new ideas were essential. This feedback shaped our approach: the NDCN prioritized unconventional, potentially transformative scientific inquiries that might otherwise go unexplored through traditional funding mechanisms. Our core mission was to disrupt stagnation and cultivate a fertile ground for genuine breakthroughs.

To achieve this, our open solicitation RFA grant mechanisms were designed to cast a wide net, actively seeking researchers from a wide range of academic disciplines and professional backgrounds. We specifically encouraged individuals who possessed unique expertise, specialized skills, or cutting-edge technologies to consider how their knowledge could be applied to the multifaceted challenges posed by neurodegenerative diseases. A hallmark of our RFA grant mechanisms was the unwavering emphasis on truly novel ideas and groundbreaking strategies. Significantly, and a key differentiator from many conventional funding mechanisms, we deliberately eliminated the requirement for extensive preliminary data.

By eliminating the requirement for extensive preliminary data, we shifted the evaluation focus from exclusively current work and past accomplishments in the field, to a researcher's ideas, their expertise and their broader potential, as well as the originality of their proposed work, empowering reviewers to identify and champion groundbreaking inquiries. To maintain focus on core innovation, grant proposals were concise, emphasizing research itself over extensive background. We believed genuine scientific breakthroughs stem from unconventional thinking, and our RFA funding mechanisms enabled forward-thinking individuals to explore uncharted scientific territories.

Statistics for Collaborative Pairs Pilot Program

- **94** paradigm-challenging research projects funded across the network
- **78%** of projects exploring previously untested hypotheses about neurodegeneration mechanisms
- **$17.3** million invested in pilot phase high-risk investigations with potential for outsized impact

Scientific Focus Areas

Neurodegenerative disease research has, to some extent, been characterized by disciplinary research silos, with a strong concentration on a limited range of mechanisms such as protein aggregation in Huntington's disease, amyloid and tau biologies

Our core mission was to disrupt stagnation and cultivate a fertile ground for genuine breakthroughs.

in the case of Alzheimer's disease, and an overall neuron-centric focus on disease mechanisms. While these areas of research remain of interest, we believed it is imperative to expand the scientific aperture and explore critically underfunded yet highly promising areas to advance the cellular biology underlying neurodegeneration.

Seven years into the process, our choice to concentrate on these neglected frontiers has yielded significant advancements:

- **Neuroimmunology:** We placed a significant emphasis on neuroimmunology, recognizing the increasingly vital role of the immune system in the initiation and progression of neurodegenerative processes. This once-overlooked area is now recognized to be central to understanding disease pathogenesis.
- **Brain-Body Physiology:** Our initiative delved deep into the intricate connections of brain-body physiology. This includes the exploration of metabolic dysregulation in disease states and the fascinating complexities

of the gut-brain axis. While the microbiome garnered substantial attention about six years ago, our current primary area of interest has pivoted to a comprehensive understanding of metabolism's profound impact on neurological health and disease.

- **Computational Biology:** Recognizing the exponential growth of data in biological research, we strategically invested in innovative approaches for analyzing complex datasets, modeling disease progression, and identifying novel therapeutic targets.

Finally, the NDCN team recognized the crucial need to advance technology development and strategically apply emerging technologies to neurodegenerative disease research. This commitment went beyond funding; it involved actively supporting the creation and deployment of cutting-edge tools, methodologies, and platforms to accelerate discovery.

We understand that technological innovation — including advanced imaging, sequencing methodologies, cellular biology techniques, novel disease modeling platforms, and innovative computational modeling tools — can serve as the foundation for future breakthroughs. Our investment ensured that supported researchers had access to and contributed to the latest technological advancements, pushing the boundaries in the fight against neurodegenerative diseases.

Impact in Action

The Role of the Immune System in Brain Aging

NDCN COLLABORATIVE PAIRS CYCLE 1, PHASE 1 AND 2

- **Ozgun Gokce, Ph.D., University of Bonn**
- **Mikael Simons, M.D., Institute of Neuronal Cell Biology, Technical University Munich**

White matter degeneration is a hallmark of nervous system aging, yet its underlying mechanisms remain largely unknown. In humans, white matter volume begins to diminish as early as mid-life, and focal lesions begin to appear, which present as bright, high-intensity areas on magnetic resonance images. This focal white matter degeneration is linked to an increased risk of stroke and dementia and contributes to cognitive decline, likely by disrupting crucial connective pathways within the brain.

To uncover mechanisms involved in white matter degeneration, the Collaborative Pair team of Gokce and Simons combined their expertise in single-cell RNA sequencing, computational biology, imaging, and immunology to investigate the role of CD8+ T cells in white matter aging. Using single-cell RNA sequencing, they identified interferon (IFN)-responsive oligodendrocytes and microglia cells in the vicinity of CD8+ T cells. Genetic ablation of functional lymphocytes — achieved using $Rag1^{-/-}$ mice or $CD8^{-/-}$ mice — effectively prevented both aging-induced oligodendrocyte loss and the formation of IFN-responsive oligodendrocytes and microglia. Conversely, T-cell checkpoint inhibition worsened the aging effect.

Mikael Simons (left) and Ozgun Gokce (right), investigators funded under Collaborative Pairs, chat between sessions at the CZI Neuroscience 2022 Meeting.

These perturbation experiments provide compelling support that CD8+ T cells play a direct role in driving white matter aging.

To further these observations, Gokce and Simons' Collaborative Pairs funded research used a mouse model of Alzheimer's disease to demonstrate that amyloidosis could trigger age-related oligodendrocyte and myelin damage. Again, CD8+ T cells appeared to play a role in exacerbating interferon-responsive oligodendrocyte damage to myelin.

Selected Publications

- CD8+ T cells induce interferon-responsive oligodendrocytes and microglia in white matter aging. Kaya, T., et al., Nature Neuroscience (2022) 25, 1446-1457
- T cell-mediated microglia activation triggers myelin pathology in a mouse model of amyloidosis. Kedia, S., et al., Nature Neuroscience (2024) 27, 1468-74

New Tools for Disease Modeling

NDCN COLLABORATIVE PAIRS CYCLE 1, PHASE 1 AND 2

- **Yang Hu, M.D., Ph.D., Department of Ophthalmology, Stanford University**
- **Stanley Qi, Ph.D., Department of Bioengineering, Stanford University**

Developing therapeutic strategies for complex diseases presents numerous challenges for the field of neurodegeneration research. Two of these challenges stand out as high-risk, high-reward research opportunities: creating robust models for identifying genes and pathways involved in the disease; and developing genetic approaches for simultaneous manipulation of multiple genes and their associated pathways.

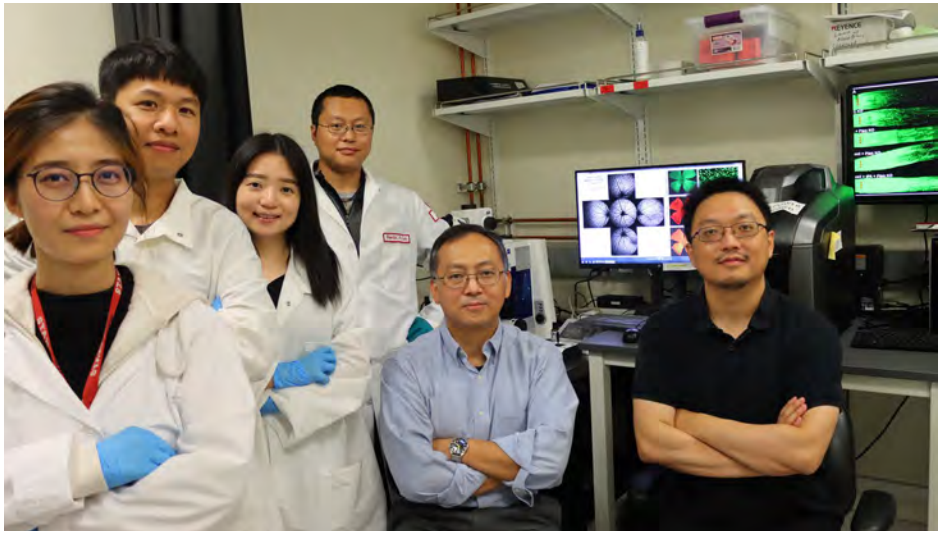
The Collaborative Pair team of Hu and Qi tackled these two challenges by integrating their interdisciplinary expertise in novel glaucoma model development with CRISPR-based engineering technologies. Hu, a physician-scientist, has been instrumental in pioneering new glaucoma models to pinpoint genes involved in axon regeneration. His lab recently developed an axonopathy animal model to investigate late-onset neurodegeneration in retinal ganglion cells (RGCs), the optic nerve (ON), and spinal cord motor neurons, a neurodegenerative process often preceded by a reduction in axonal mitochondria.

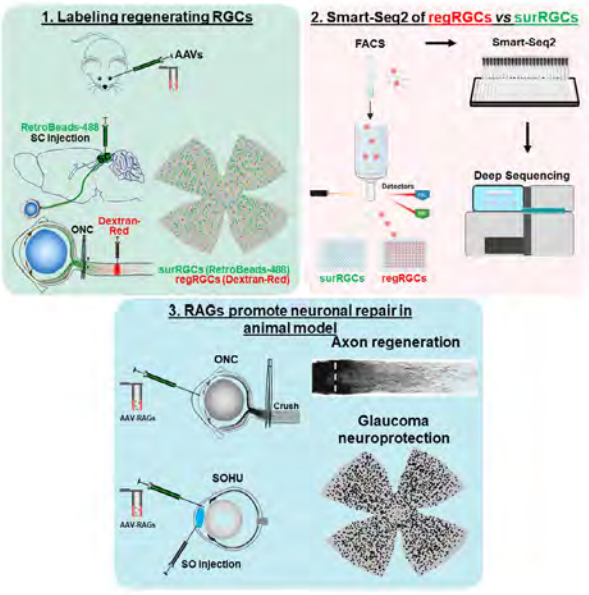
Qi, a bioengineer, has pioneered novel CRISPR-based technologies for multiplexed modulation of endogenous genes. Among other tools, his lab created MEGA, a CRISPR-Cas13d-based platform for multiplexed transcriptome regulation in CAR T-cell engineering which can target multiple RNA transcripts simultaneously.

Together, this pair has made incredible progress modeling complex neurodegenerative diseases and developing tools to genetically manipulate those models.

Selected Publications

- Single-cell transcriptome analysis of regenerating RGCs reveals potent glaucoma neural repair genes. Li , L., et al., Neuron (2022) 110 (16) 2646-2663
- Multiplexed genome regulation in vivo with hyper efficient Cas12a. Guo, L.Y., et al., Nature Cell Biology (2022) 24 590-600.
- A versatile CRISPR-Cas13d platform for multiplexed transcriptomic regulation and metabolic engineering in primary T cells. Tieu, V., et al., Cell (2024) 187 (5) 1278-1295.
- Optineurin-faciliated axonal mitochondria delivery promotes neuroprotection and axon regeneration. Liu, D., et al., Nature Communications (2025) 16 1789





Above: Yang Hu (center), Stanley Qi (right) and colleagues in the lab at Stanford University.

Left: Experimental design for identification of new targets for glaucoma therapy, from Yang Hu and Stanley Qi. Figure 1 shows the differential labeling strategy used to identify surviving retinal ganglion cells (SurRGCs) from retinal ganglion cells that contribute to axon regeneration (regRGCs) in a mouse optic nerve crush (ONC) injury model. Figure 2 demonstrates the use of plate-based single cell sequencing (Smart-Seq2) to identify transcripts associated with axon regenerating RGCs. Figure 3 Regeneration associated genes (RAGs) identified through Smart-Seq2 analysis were validated by expression of these genes, through AAV delivery, in a mouse ONC injury model.

Analyzing Cellular Images Through the Lens of Machine Learning

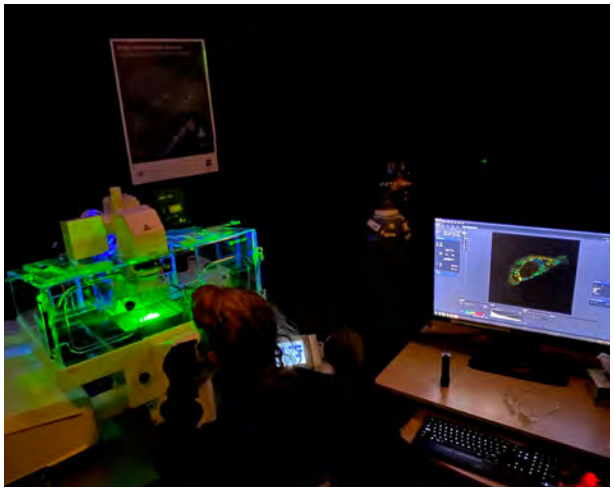
NDCN COLLABORATIVE SUPPLEMENT AND NDCN COLLABORATIVE PAIRS CYCLE 1, PHASE 1 AND 2

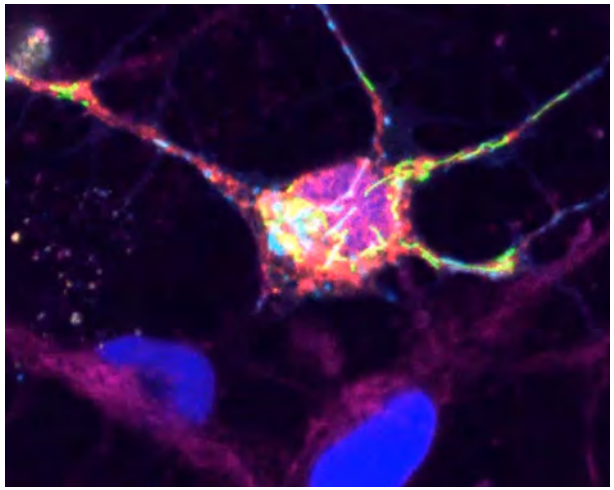
- **Sarah Cohen, Ph.D., Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill**
- **Serena Yeung-Levy, Ph.D., Department of Biomedical Data Science, Stanford University**

Microscopy underpins modern cell biology, as high-throughput imaging is crucial for genetic screens, drug profiling, and creating cellular atlases. Within those applications, quantifying cell and organelle morphology is key to characterizing structural and functional changes to cells from genetic or environmental perturbations.

Autoencoders, popular for unsupervised representation learning, map images to low-dimensional embedding spaces useful for clustering, dimensionality reduction, and outlier detection. However, they don't guarantee orientation-invariant features, meaning similar but rotated shapes (as found with many organelles, like mitochondria) may not be close in the embedding space. In this way, orientation becomes a confounding variable in analysis.

To address this computational hurdle, cell biologist Cohen and computational biologist Yeung-Levy came together to develop a novel machine learning method for image analysis specifically optimized for cell biological challenges. Interestingly, Cohen and Yeung-Levy each joined NDCN through other Collaborative Pairs teams and after meeting through NDCN, joined forces through a Collaboration Supplement (a supplement mechanism we developed to encourage collaboration across the Network). They demonstrated that the O2-variational autoencoder (O2-VAE), an unsupervised method that learns robust, orientation-invariant representations, successfully discovered morphology subgroups in segmented cells and mitochondria, detected outlier cells, and rapidly characterized cellular shape and texture in large datasets. Since mitochondrial morphology varies tremendously depending on cell type, experimental conditions, and location within a cell, this computational image-analysis approach offers a rapid and accurate methodology for quantifying and characterizing organelles.





Left: Postdoctoral Researcher Dr. Maria Clara Zanellati performs multispectral imaging of iPSCs.

Right: An iNeuron: Z-stack image from a live neuron derived from an induced pluripotent stem cell (iPSC) in which seven organelles have been visualized using multispectral imaging, generated by Sarah Cohen's lab and leveraged by Serena Yeung-Levy. Photo credit: Maria Clara Zanellati.

Selected Publication

- Orientation-invariant autoencoders learn robust representations for shape profiling of cells and organelles. Burgess, J., et al., Nature Communications (2024) 15 1022

Innovative Computational Modeling of Disease

NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2018-2023)

Debora Marks, Ph.D., Department of Systems Biology, Harvard Medical School

Understanding genetic contributions to disease is a major challenge, no less so for neurodegenerative diseases where there are complex contributions of genetics to disease risk. Computational biology has the power to decipher the contribution of genetics and the vast sea of genomic data and accelerate discovery across the spectrum of neurodegenerative diseases.

A Ben Barres Early Career Acceleration Award empowered Marks' lab to deploy its formidable computational biology expertise to that goal. The lab aimed to develop novel statistical methodologies to model disease through the application of probabilistic modeling and machine learning techniques.

EVE (Evolutionary model of Variant Effect), an early success for the lab, was a genetic variant pathogenicity prediction model. EVE distinguished itself from many machine learning models of its time by employing deep generative models to predict variant pathogenicity without relying on scarce and often inconsistent disease labels for training. This machine learning approach not only surpassed computational methods that depended on labeled data, but also equaled or exceeded the prediction accuracy from high-throughput experiments, commonly used for variant classification.

Recently, Marks' lab developed popEVE, a new model that leverages evolutionary and population data to predict and rank the effects of variants in human disease. This model, which combines EVE and ESM1v (a large language model), is particularly useful for identifying likely causal variants in individual patients with severe developmental disorders, and it has already uncovered evidence for over a hundred novel developmental disorder genes.

Marks is currently partnering with three NDCN investigators to broaden the application of these computational models to neurodegenerative diseases. These collaborators include Elizabeth Bhoj and Rebecca Ahrens-Nicklas, pediatric physician-scientists at Children's Hospital of Philadelphia (CHOP), and Simone Mayer, an investigator at the Karlsruhe Institute of Technology (KIT). These collaborations will leverage the predictive capabilities of popEVE to pinpoint disease-causing mutations in rare pediatric neurodegenerative disorders.

Selected Publications and Preprints

- Disease variant prediction with deep generative models of evolutionary data. Frazer, J et al., Nature (2021) 599, 91-95
- Large-scale discovery of protein interactions at residue resolution using co-evolution calculated from genomic sequences. Green, A.G., et al., Nature Communications (2021) 12 1396
- Machine learning for functional protein design. Notin P., Nature Biotechnology (2024) 42, 216-228.
- MaveDB 2024: a curated community database with over seven million variant effects from multiplexed functional assays. Rubin A, F., et al., Genome Biology (2025) 26 (13)
- Proteome-wide model for human disease genetics. Orenbuch, R., et al., medRxiv (2025)

Debora Marks (left) with lab members Han Spinner (center) and Rose Orenbuch (right) at the CZI Neuroscience 2023 Meeting in San Diego, California.

A CRISPR View on Cellular Function

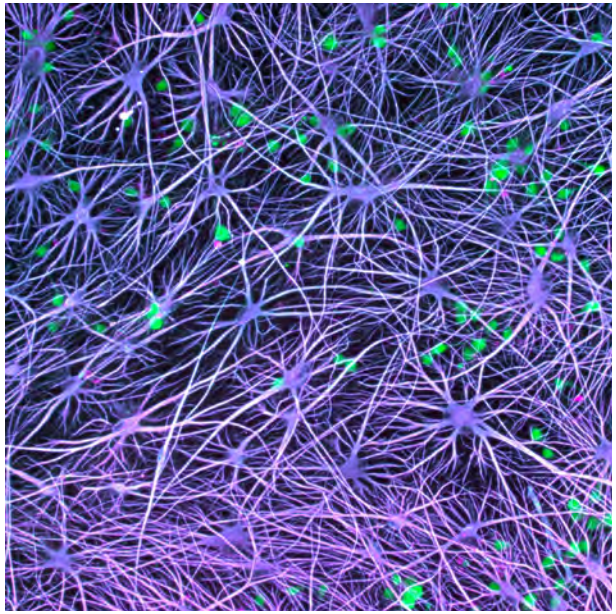
NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2018-2023)

Martin Kampmann, Ph.D., Department of Biochemistry and Biophysics, University of California, San Francisco

CRISPR/Cas9-based functional genomics has revolutionized our ability to understand mammalian cell biology. However, early utilization of CRISPR-based screens primarily relied on cancer cell lines. While useful for basic biology studies, these immortalized cells often failed to fully recapitulate the complexity and physiological relevance of primary human cells or tissues, especially when studying diseases affecting terminally differentiated cells, such as neurons in neurodegenerative disorders.

To address this technical and biological challenge, Kampmann's lab, supported by a Ben Barres Early Career Acceleration Award, developed a transformative CRISPR-based functional genomics platform specifically designed for induced pluripotent stem cell (iPSC)-derived neurons and glia, and 3D assembloids. This platform led to the systematic identification of genes and pathways involved in neuronal survival, axonal integrity, synaptic function, and glial support.

To facilitate the scientific community's access to functional genomics data across diverse human cell types, Kampmann's lab established a robust data commons. This resource, CRISPRbrain, is a centralized repository for the vast amount of data generated from CRISPR screens and other experiments. CRISPRbrain is designed to enable researchers worldwide to explore and compare gene function, accelerate discoveries in neuroscience, and ultimately contribute to the development of new treatments for neurodegenerative diseases.



Left: Martin Kampmann outlines strategies for disease modeling using induced pluripotent stem cells at the 2019 Neuroscience Meeting in Aptos, California.

Right: Human iPSC-derived astrocytes and neurons. Credit: Parker Grosjean, Kampmann Lab, UCSF.

Selected Publications

- CRISPR interference-based platform for multimodal genetic screens in human iPSC-derived neurons. Tian, R., et al., Neuron (2019) 104 (2) 239-255.
- Genome-wide CRISPRi/a screens in human neurons link lysosomal failure to ferroptosis. Tian, R., et al., Nature Neuroscience (2021) 24 1020-1034.
- CRISPRi-based screens in Assembloids to elucidate neuron-glia interactions. Li E., et al., Neuron (2025) 113 (5) 701-718

NEW INTERDISCIPLINARY TALENT

To broaden the scope of neurodegenerative disease research, the NDCN worked to attract outstanding researchers from a range of disciplines and at early stages of career development. Many of these investigators were new to the complex and rapidly evolving field of neurodegeneration.

To ensure their success, the network provided both financial resources and access to cutting-edge technologies, mentorship from seasoned experts, and opportunities for interdisciplinary training. This comprehensive support, combined with a fostered collaborative environment, was designed to be an unparalleled launching pad for the next generation of leaders in neurodegenerative disease research. By strategically investing in nascent careers and fostering a culture of shared discovery, the NDCN has actively ensured a continuous influx of innovative ideas and a steady supply of passionate talent.

Above: NDCN Early Career Investigators gather at CZI headquarters in Redwood City, California in 2024 for the NDCN Early Career Investigator Retreat.

Cori Bargmann (left), Ivan Marazzi (middle) and Adrienne Sussman (right) at the Neuroscience 2019 meeting in Aptos, California.

Ivan Marazzi asks a question during a scientific session at the CZI Neuroscience 2023 Meeting in San Diego.

"As an immunologist who had never worked in neurodegeneration, the NDCN created a pathway for me to apply my expertise to juvenile ALS. Our discovery of the immune system's key role in this disease has completely changed how we think about potential treatments."

— Ivan Marazzi, Ph.D., Ben Barres Early Career Acceleration Award Investigator

Flagship Initiative

Ben Barres Early Career Acceleration Awards provided:

- 4- to 5-year awards to enable transition into a new research field
- Mentorship from established leaders in the field
- Access to collaborative networks and resources
- Dedicated career development support
- Awards to individuals rather than specific projects, encouraging innovation and flexibility

The program comprised two award cycles. The first, from 2018 to 2023, supported 17 investigators. The second, currently underway from 2023 to 2027, is supporting 13 investigators.

Career Development Impact for Cycle 1 NDCN Ben Barres Early Career Acceleration Award Investigators

- **92%** retention rate of early career investigators in neurodegeneration research; notably, most of these investigators transitioned from other fields
- **94%** of funded assistant professors achieved tenure (between 2019-2024)
- **$65M** in independent funding was subsequently secured by Cycle 1 investigators
- **33** awards and recognitions were received by NDCN Early Career Investigators for their contributions to neurodegeneration research, including the National Institutes of Health (NIH) Director's Transformative Research Award, the Paul G. Allen Distinguished Investigator Award, and the Rainwater Prize for Innovative Early Career Scientist

Scientific Output for Cycle 1 Ben Barres Early Career Acceleration Award Investigators

- **318** publications
- **147** preprints
- **77** biological resources developed
- **47** computer code repositories created
- **30** datasets shared

NDCN investigators made truly monumental contributions, so substantive and far-reaching that to individually enumerate each would be an impossible task within the scope of this impact report. Therefore, to offer a comprehensive yet digestible overview of their collective impact, we have selected several compelling vignettes. These narratives serve to illuminate the breadth and depth of expertise within the NDCN early career investigator cohort, and exemplify the transformative power of our investment in fostering a growing and innovative neurodegeneration research community.

Impact in Action

Sergiu Pasca, M.D., Ph.D.

Psychiatry and Behavioral Sciences, Sleep Medicine, Stanford University

NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2018-2023)

Pasca's groundbreaking work centers on a novel approach to studying complex neural systems: the creation of self-organizing 3D human cellular systems called "brain assembloids." These sophisticated models represent a significant leap forward from traditional 2D cell cultures, offering an unprecedented opportunity to delve into neural-glial interactions and the inter-regional neural cross-talk that underpins brain function.

The versatility of brain assembloids has already yielded remarkable insights. By carefully manipulating and observing these assembloids, Pasca's lab began to unravel the molecular and cellular programs that govern the prolonged maturation of neurons and astrocytes in humans. This understanding is crucial for comprehending normal brain development and identifying deviations that contribute to neurological disorders.

Furthermore, these 3D models have so-far proven invaluable in accurately recapitulating specific genetic forms

Sergiu Pasca (center) discusses his research at the 2019 NDCN Investigator Meeting in Aptos, California.

of neurodevelopmental disorders, such as Timothy syndrome. Timothy syndrome type1 (TS1) is caused by a gain-of-function variant in the *CACNA1C* exon 8A. Leveraging a transplantation platform, where human cortical organoids carrying the Timothy syndrome mutation were transplanted into the developing cerebral cortex of early postnatal rats, the lab tested antisense oligonucleotides (ASOs) to reduce exon 8A inclusion.

A single intrathecal injection of ASOs robustly rescued calcium changes and *in vivo* dendrite retraction in patient-derived neurons, suggesting ASOs as a potential TS1 treatment. This research advances our understanding of these conditions and the platform offers a powerful tool for modeling brain maturation and neurodegeneration using patient-derived cells.

Selected Publications

- Generation of human striatal organoids and cortico-striatal assembloids from human pluripotent stem cells. Miura, Y., et al., Nature Biotechnology (2020) 38, 1421-1430
- Generation of functional human 3D cortico-motor assembloids. Andersen J., et al., Cell 2020 183 (7) 1913-1929. 659
- Dissecting the molecular basis of human interneuron migration in forebrain assembloids from Timothy syndrome. Birey, F., et al., Cell Stem Cell (2022) 29 (2), 248-264.
- Antisense oligonucleotide therapeutic approach for Timothy syndrome. Chen, X., et al., Nature (2024) 628 818-825.

Ethan Lippmann, Ph.D.

Department of Chemical and Biomolecular Engineering, Vanderbilt University

NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2018-2023)

At Vanderbilt, Lippmann's lab focuses its attention on the blood-brain barrier (BBB) and its critical role in neurological health and disease. The BBB, a highly selective semipermeable border of endothelial cells, acts as a protective shield for the brain, regulating the passage of substances from the bloodstream into

Ethan Lippman addresses attendees at the CZI Neuroscience 2024 Meeting in Monterey, California.

the central nervous system. Dysfunction of the BBB is a recognized hallmark of numerous neurodegenerative diseases and is a significant obstacle to delivering therapeutic agents to the brain.

Lippmann's lab has taken a multi-disciplinary approach to overcome these challenges. By integrating biomolecular and biomedical engineering strategies, the team has developed sophisticated *in vitro* models, advanced biomaterials, and novel drug delivery systems.

Several key discoveries and innovations to date include:

- In collaboration with Martin Kampmann, Lippmann's team used an iPSC-derived BBB model, to demonstrate that Tumor Necrosis Factor induces inflammatory

astrocytes via STAT3 activation and *SERPINA3* upregulation. Manipulation of *Serpina3n* impacted vascular inflammation in *ex vivo* and *in vivo* models, establishing the clinical relevance of this pathway and opening new therapeutic avenues targeting inflammation-driven BBB breakdown.

- Development of a lipid–siRNA conjugate (L2-siRNA) that, when injected into the cerebrospinal fluid (CSF), effectively transports siRNAs throughout the brain, offering a promising avenue for treating a range of neurological disorders.

Selected Publications

- A simplified, fully defined differentiation scheme for producing blood-brain barrier endothelial cells from human iPSCs. Neal, E.H., et al., Stem Cell Reports (2019) 12 (6) 1380-1388.

- Reactive astrocytes transduce inflammation in a blood-brain barrier model through a TNF-STAT3 signaling axis and secretion of alpha 1-antichymotrypsin. Kim, H., et al, Nature Communications (2022) 13 6581 Collaboration between Martin Kampmann and Ethan Lippmann's labs.

- CRISPRi screen in human iPSC-derived astrocytes elucidate regulators of distinct inflammatory reactive states. Leng, K., et al., Nature Neuroscience (2022) 25, 1528-1542.

- Elucidating brain transport pathways and cell type-dependent gene silencing of a durable lipid-siRNA conjugate administered into the cerebrospinal fluid. Sorets, A.G. et al., Nucleic Acids Research (2025) 53 (12).

Viviana Gradinaru, Ph.D.

Neuroscience and Biological Engineering, California Institute of Technology

NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2018-2023)

Gradinaru, a neuroscientist with a foundational background in physics, stands at the forefront of innovation in neuroscience and has been celebrated for her pioneering work in developing Adeno-Associated Virus (AAV) tools. Her expertise lies in integrating neuroscience, protein engineering, and data science to craft sophisticated methods for the cell-type specific delivery of gene products, targeting both the peripheral and central nervous systems with precision.

When Gradinaru received a NDCN Ben Barres Early Career Acceleration Award, neurodegeneration research predominantly centered on neurons and their circuits within the brain. Gradinaru strategically diverged from this paradigm, investigating the crucial roles of body-to-brain connections, specifically those mediated by non-neuronal brain cells. Utilizing advanced engineering technologies, she explored the periphery's influence on neurodegeneration and engineered non-invasive gene-delivery tools for targeting non-neuronal brain cells, such as immune and brain endothelial cells, thereby opening new therapeutic possibilities.

Recently, Gradinaru's team pioneered a novel strategy to overcome AAV packaging limitations and enhance gene therapy specificity. Their research revealed transcriptional crosstalk between AAV genomes, enabling cell-type specific expression of large DNA cargos. The team successfully demonstrated cell-type specific expression of a 3.2 kb Cas9 cargo through systemic AAV administration, a significant leap forward for precise and effective gene therapy.

Gradinaru and her lab are committed to open science and accelerating research progress by actively disseminating their innovations, and have shared new opsins, viral vectors, and detailed protocols for gene delivery and tissue clearing with the broader research community.

Selected Publications

- Negative feedback control of neuronal activity by microglia. Badimon, A., et al., Nature (2020) 586, 417-423

- Brain-wide Cas9-mediated cleavage of a gene causing familial Alzheimer's disease alleviated amyloid-related pathologies in mice. Duan, Y., et al., Nature Biomedical Engineering (2022) 6, 168-180.

- Functional gene delivery to and across brain vasculature of systemic AAVs with endothelial-specific tropism in rodents and broad tropism in primates. Chen, X., et al., Nature Communications (2023) 14 3345

- Spatial genomics of AAV vectors reveals mechanism of transcriptional crosstalk that enables targeted delivery of large genetic cargo. Coughlin, G.W., et al., Nature Biotechnology (2025)

Viviana Gardinaru presents at the 2019 NDCN Investigator Meeting in Aptos, California.

Vidhya Rangaraju (right) discusses data with a lab member.

Tal Laviv, presents at the 2024 Neuroscience meeting on novel FRET-based biosensor development for monitoring activity in the CNS.

Vidhya Rangaraju, Ph.D.

Neuroenergetics Lab, Max Planck Florida Institute for Neuroscience

NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2023-2027)

Rangaraju's lab has made significant strides in the development of innovative spine- and mitochondrial-ATP reporters. These reporters, combined with super-resolution imaging methods like spt-PALM, allow for direct visualization and precise measurement of brain cell metabolic activity at synaptic sites. This unprecedented spatial resolution enables real-time imaging of ATP within single spines and individual mitochondria during synaptic plasticity, offering a unique opportunity to understand cellular processes critical for healthy brain function.

Supported by a Ben Barres Early Career Acceleration Award, Rangaraju's lab is applying these advanced reporters to investigate mutations in the vesicle-associated membrane protein-associated (*VAP*) gene, which are linked to Amyotrophic Lateral Sclerosis. Their research has revealed that the Vapb paralog is central to stabilizing dendritic mitochondria, which is necessary for sustained synaptic plasticity formation and maintenance. Using these state-of-art ATP reporters, Rangaraju's lab is now using mouse and human models of disease to uncover specific metabolic dysfunctions in ALS and other neurological conditions.

Selected Publications and Preprints

- VAP spatially stabilizes dendritic mitochondria to locally support synaptic plasticity. Bapat, O., et al., Nature Communications (2024) 15, 205.
- Synapses drive local mitochondrial ATP synthesis to fuel plasticity. Ghosh, I., et al., bioRxiv (April 2025)

Tal Laviv, Ph.D.

Physiology and Pharmacology, Faculty of Medicine, Tel Aviv University

NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2023-2027)

Laviv's laboratory uses innovative FRET (Förster Resonance Energy Transfer)-based methodologies for the creation of sophisticated biosensors. These biosensors are designed to enable the real-time monitoring of specific protein activity within intact biological systems, offering an unprecedented window into cellular processes crucial for understanding neurological health and disease.

With the support of a Ben Barres Early Career Acceleration Award, Laviv's lab has successfully developed a highly specialized FRET-based biosensor to monitor phosphatase and tensin homolog (PTEN) activity using two-photon fluorescence lifetime (2pFLIM) imaging. Loss-of-function mutations in the *PTEN* gene are strongly correlated with a spectrum of human pathologies, ranging from cancers to severe developmental disorders. Therefore, the ability to accurately track PTEN activity in living systems is a critical step towards understanding and potentially mitigating these conditions.

A pivotal achievement in the development of this PTEN biosensor was the identification of a specific point mutation within the PTEN protein. This strategic modification enables the real-time monitoring of its activity with minimal interference to the intricate endogenous PTEN signaling pathways. Further enhancing the capabilities of this research, Laviv's team successfully developed a red-shifted variant of the PTEN biosensor, enabling cell-type specific PTEN activity profiling in excitatory and inhibitory cortical cells within the mouse brain at unparalleled resolution.

Building upon his biosensor methodology, Laviv is developing an autophagy biosensor for *in vivo* imaging. This new biosensor will be instrumental in unraveling the multifaceted role of autophagy, a fundamental cellular catabolic process, in both the early and late stages of neurodegeneration. By combining measurements from a novel autophagy biosensor with readouts for neuronal activity, Laviv aims to determine if subtle, early changes in autophagy can serve as predictive markers for the onset and severity of later-stage neuronal and synaptic dysfunction in animal models of neurodegenerative disorders.

Selected Publication

- Genetically encoded biosensor for fluorescence lifetime imaging of PTEN dynamics in the intact brain. Kagan, T., et al., Nature Methods (2025) 22, 764-777

Erin Gibson, Ph.D.

Psychiatry and Behavioral Sciences, Stanford University School of Medicine

NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2023-2027)

Sleep, a cornerstone of biological function, is profoundly impacted by neurological health, particularly in the context of degenerative dysmyelinating disorders such as Alzheimer's disease (AD). Accumulating evidence points to a strong bidirectional relationship between sleep disturbances — including disruptions in circadian rhythms and altered sleep architecture — and the progression of these debilitating conditions.

While the connection between sleep and neuro-degeneration is increasingly recognized, the precise cellular and molecular mechanisms underlying this relationship remain a critical area of investigation. Gibson's lab has made significant contributions to our understanding of sleep biology by establishing a link between myelin-forming glia — specifically oligodendrocytes, the cells responsible for producing the myelin sheath that insulates nerve fibers — and the complex mechanisms that regulate sleep. This pioneering work suggests that the health and function of myelin may play a more direct role in sleep regulation than previously understood.

To delve deeper, Gibson's team, with support from a Ben Barres Early Career Acceleration Award,

Erin Gibson (right) with a colleague, in the lab at Stanford University School of Medicine.

has developed an innovative *ex vivo* model to study human iPSC-derived oligodendrocyte myelination. The model utilizes organotypic brain slices derived from *Shiverer* (MBP$^{shi/shi}$) mice, which lack compact myelin due to a spontaneous mutation in the myelin basic protein (*MBP*) gene. The absence of functional myelin in Shiverer brain slices provides an ideal "blank slate" or de-myelinated environment to precisely evaluate the myelinating capacity of transplanted human oligodendrocytes in a controlled setting, free from confounding effects of endogenous rodent myelin.

By comparing the myelinating potential and functional integration of healthy human oligodendrocytes with those carrying AD-associated mutations, Gibson's lab can directly investigate how AD pathology might impair myelin formation and function, and subsequently, how these changes might contribute to the sleep disturbances characteristic of the disease. This powerful experimental approach paves the way for uncovering the intricate mechanisms by which myelin pathology intersects with AD progression and its associated sleep deficits.

Selected Publication

- Protocol for assessing myelination by human iPSC-derived oligodendrocytes in Shiverer mouse ex vivo brain slice cultures. Tsarouchas, T.M., et al., STAR Protocols (2025)

Sabine Krabbe, Ph.D.

Functional Diversity of Neural Circuits Group, German Center for Neurodegenerative Diseases

NDCN BEN BARRES EARLY CAREER ACCELERATION AWARD (2023-2027)

Krabbe's laboratory is at the forefront of investigating the precise roles of amygdala neuronal subtypes in anxiety-related behaviors in mice. Their innovative approach involves *in vivo* recording of cell-type specific neurons in freely moving mice at single-cell resolution. This feat is achieved by combining a gradient refractive-index (GRIN) lens-based imaging technique and miniaturized microscopes. To ensure the specificity of the observations, distinct cell types within various amygdala subregions are targeted for the expression of genetically-encoded calcium sensors, leveraging the power of cell-type specific promoters. This methodology allows for unprecedented insight into the real-time activity of individual neurons within complex brain circuits during natural behaviors.

With the crucial support of a Ben Barres Early Career Acceleration Award, Krabbe's team has made significant discoveries regarding the dynamic interplay of amygdala interneurons during fear learning and extinction. Their research revealed

Sabine Krabbe in her lab at the German Center for Neurodegenerative Diseases.

that disinhibitory vasoactive intestinal peptide (VIP) expressing cells are predominantly activated during states of high fear. This suggests a critical role for VIP interneurons in amplifying fear responses. In stark contrast, somatostatin (SST) interneurons exhibit a preference for safety cues, effectively suppressing the responsiveness of excitatory neurons when no threat is present. This differential activation highlights a sophisticated mechanism for regulating fear and safety signals within the amygdala.

Building upon this *in vivo* imaging technology, Krabbe's future research endeavors aim to meticulously map the anatomical association of these precisely recorded neurons with pathological proteinaceous aggregates, which are hallmarks of neurodegenerative diseases, with the ultimate goal of providing a comprehensive and multi-level analysis of amygdala circuit dysfunction across a spectrum of distinct neurodegenerative disorders. This integrated approach promises to yield insights into the fundamental mechanisms underlying neuropsychiatric symptoms in these debilitating diseases and could pave the way for novel therapeutic interventions.

Selected Preprint

- Heterogeneous plasticity of amygdala interneurons in associative learning and extinction. Favila, N., et al., bioRxiv (September 2024).

COMMUNITY AND COLLABORATION

CZI believes that the most profound discoveries frequently arise from the convergence of varied expertise and perspectives, nurtured within a supportive and dynamic collaborative ecosystem. With this in mind, the NDCN was designed to foster a dynamic environment for early research sharing and collaborative innovation.

To accomplish this, we developed a diverse array of intentionally curated Challenge Network events and activities designed to provide opportunities for investigators to connect, exchange insights, and forge powerful partnerships. Our annual meetings have been the cornerstone, bringing the entire network together to present nascent findings, engage in spirited discussions, and collectively chart new directions. Regular webinars extended these opportunities, allowing for focused explorations of emerging data and methodologies, and providing venues for the cross-pollination of ideas.

As the Challenge Network expanded, program staff assumed a pivotal role in initiating and nurturing new collaborations. This involved not only connecting NDCN researchers with each other but also strategically linking them with external experts and advisors who can enrich network activities. A key aspect of this strategy was the development of community-focused approaches, such as the establishment of Working Groups and Focus Groups.

The most profound discoveries frequently arise from the convergence of varied expertise and perspectives, nurtured within a supportive and dynamic collaborative ecosystem.

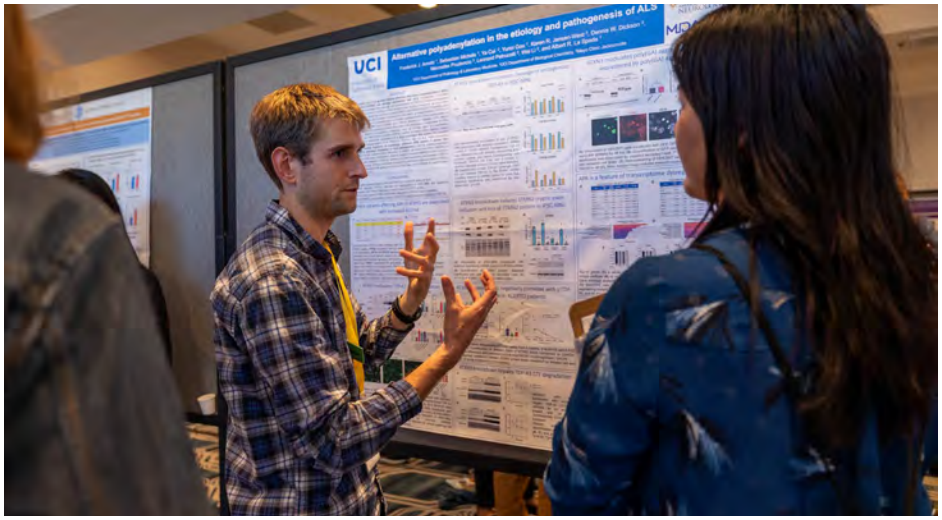
These structures provide dedicated forums for focused discussion, knowledge exchange, and the organic formation of new collaborative projects.

Within this diverse collection of Challenge Network events — from the large-scale gatherings to intimate group discussions — countless collaborations have blossomed. These partnerships, often sparked by a shared curiosity or complementary expertise, have been a defining characteristic of the NDCN's success. New collaborations accelerated the pace of discovery and cultivated a strong sense of community and shared purpose among investigators, ultimately amplifying our collective impact on the fight against neurodegenerative diseases.

This deliberate focus on collaboration-building activities and efforts has also yielded a remarkable programmatic outcome: some of the most exciting and innovative work emerging from the Challenge Network has directly resulted from participation in network-

Ki Coale (left), a member of the cureCADASIL Community Advisory Group, and Fanny Elahi (right), a member of cureCADASIL's Scientific Advisory Board and a physician-scientist at Icahn School of Medicine at Mount Sinai, connect before taking the stage for discussion at the CZI Neuroscience 2024 Meeting. Fanny serves as the Coordinating PI for a Patient-Partnered Collaboration for Rare Neurodegenerative Disease grant. In close partnership with cureCADASIL, Fanny and colleagues are working to reverse engineer CADASIL to discover therapeutic molecular targets using stem cell technologies in a deeply phenotyped cohort of patients.

Shiyi Wang (left), a postdoc in the lab of Cagla Eroglu, a Collaborative Science Investigator from Duke University, connects with Jeff Rothstein (right), a Collaborative Science Investigator, at the CZI Neuroscience 2023 Meeting.



Frederick Arnold, a postdoc in the lab of Albert "Al" La Spada, a Collaborative Science Investigator from the University of California at Irvine, presents a poster on "Alternative polyadenylation in the etiology and pathogenesis of ALS" at the CZI Neuroscience 2023 Meeting.

driven collaborations. This impactful work, often not even directly supported by CZI grant funding, would not have materialized without the facilitating environment and connections nurtured by the Challenge Network.

Beyond the creation of new collaborative opportunities, the community strategy has generated a multitude of other tangible and intangible benefits. These include, but are not limited to, expanded professional networks for investigators, offering them access to a broader range of expertise and opportunities. The Challenge Network has also fostered an environment conducive to informal mentorship and peer support, allowing researchers to learn from each other's experiences and navigate challenges collectively. Furthermore, a crucial impact has been the enhanced engagement between basic scientists and clinicians, bridging the gap between foundational research and its clinical applications, thereby accelerating translational efforts and ultimately benefiting patient care.

An important aspect of the NDCN was the participation of over 700 students, postdocs, and staff scientists. To foster leadership skills and encourage continued engagement in neurodegenerative research, we established a community project funding opportunity that empowered students and postdocs to cultivate local communities focused on neurodegeneration-related topics. We funded 30 diverse projects, ranging from seminar series featuring patients and clinicians, to practical training events on machine learning applications, to omics data analysis.

To illustrate the power of the NDCN collaborative research, we offer the following vignettes to demonstrate how bringing together investigators with diverse interdisciplinary skills, at various career stages, has sparked new scientific directions and driven new types of program engagement.

> "I have been blown away by the network opportunities and engagement levels at CZI, and in particular, by the goals it is trying to establish at an international level across institutions."
>
> — Soyon Hung, Collaborative Pairs investigator

Impact in Action

A Role for the Immune System in Early Onset Neurodegeneration

Collaborators: Jenny Jiang, Ph.D. (Ben Barres Early Career Acceleration Award investigator), Al La Spada, M.D., Ph.D. (Collaborative Science investigator) and Ivan Marazzi, Ph.D. (Ben Barres Early Career Acceleration Award investigator)

While collaborating with an international consortium, virologist Ivan Marazzi identified a senataxin gene (*SETX*) mutation linked to ALS4, a rare juvenile form of ALS. Senataxin, a nuclear ATP-dependent DNA/RNA helicase, is ubiquitously expressed and crucial for cellular processes. Prior studies showed its involvement in RNA metabolism and type I interferon responses to viral infections.

Driven by the compelling hypothesis that alterations in a patient's immune system might contribute to the pathology observed in ALS4 patients with *SETX* mutations, Marazzi forged a strategic collaboration with Jenny Jiang, a bioengineer with a keen interest in immune cell biology, and Al La Spada, a physician scientist at UC Irvine specializing in ALS and other neurodegenerative disorders.

Jiang's lab has pioneered technology for T-cell receptor sequencing. Using this technology on CNS and blood samples from *SETX* knock-in mice (engineered with the human ALS4-causative L389S mutation), the team uncovered a distinct and clonally expanded population of terminally differentiated effector memory (TEMRA) CD8 T cells. These cells were present in significant numbers in both the central nervous system and peripheral blood of the knock-in mice, indicating a systemic immune response. The research further revealed a striking correlation: increased frequencies of these antigen-specific CD8 T cells directly mirrored the progression of motor neuron disease, suggesting a potential link between escalating immune response and worsening neurological deficits characteristic of ALS4.

Building upon these compelling mouse model findings, Jiang's team successfully validated the presence of clonally expanded TEMRA CD8 T cells in the peripheral blood of individuals diagnosed with ALS4. This direct human evidence unequivocally underscored the relevance of their mouse model findings to human disease, bridging the gap between preclinical research and clinical reality.

To further dissect and elucidate the precise role of the immune system in ALS4 neurodegeneration, Marazzi's lab conducted a series of bone marrow transplantation experiments. Findings from these experiments provided compelling evidence for the immune system's role in the progression of ALS4. Further research into this immune signature could lead to novel diagnostic biomarker approaches and targeted immunomodulatory therapies to slow or reverse ALS4 progression.

Selected Publications

- High-throughput and high-dimensional single-cell analysis of antigen-specific CD8+ T cells. Ma, K-Y., et al., Nature Immunology (2021) 22, 1590-1598

- Clonally expanded CD8 T cells characterize amyotrophic lateral sclerosis-4. Campisi, L., et al., Nature (2022) 606,945-952

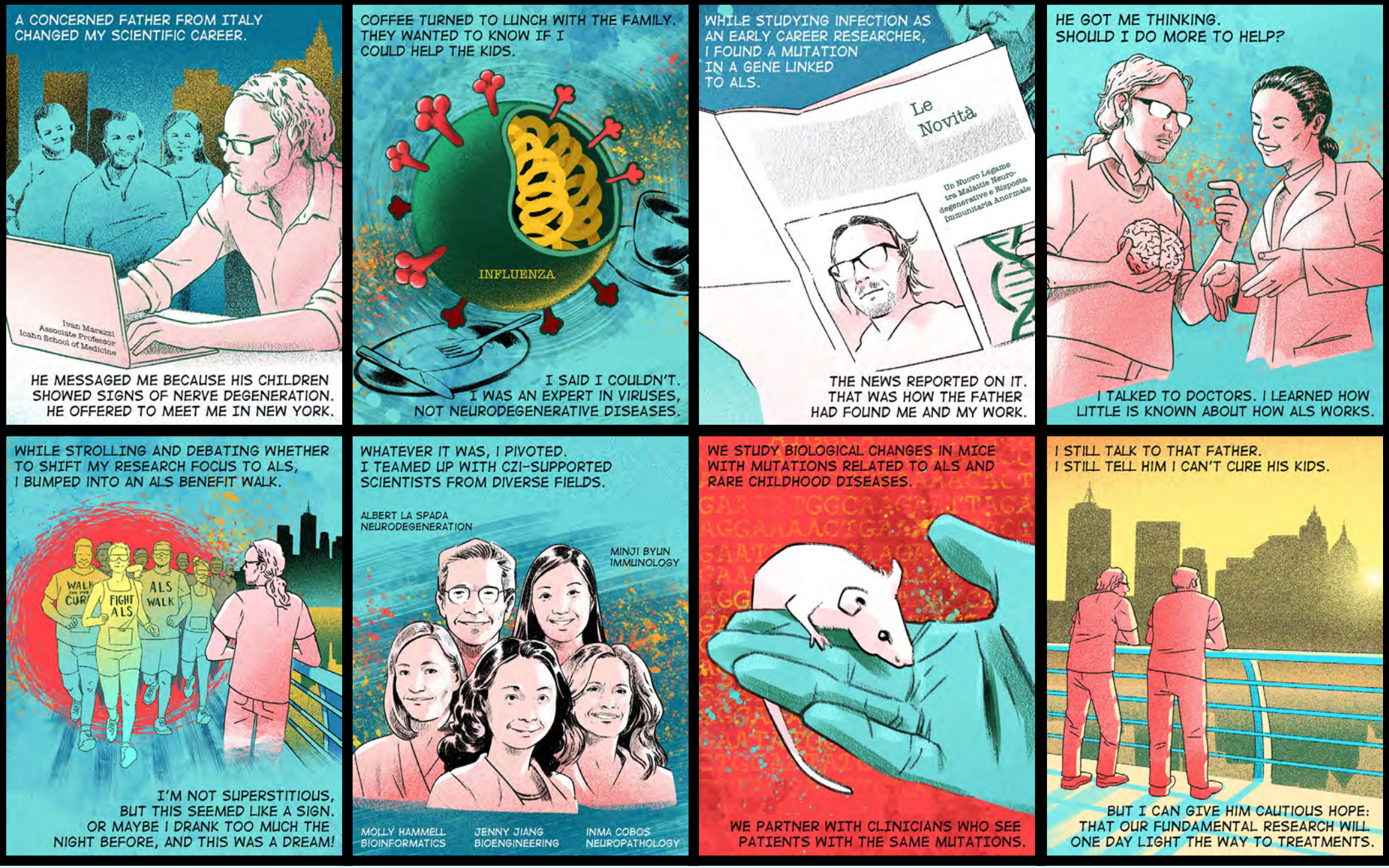
See what inspired Ivan Marazzi's work on the next page.

A Protein Linked to Inflammatory Cell Death

Collaborators: Alice Chen-Plotkin M.D., Ph.D. (Collaborative Science investigator), Isaac Chiu, Ph.D. (Ben Barres Early Career Acceleration Award investigator) , and Clotilde Lagier-Tourene, M.D., Ph.D. (Collaborative Pairs investigator)

The intricate relationship between the nervous system and the immune system is at the forefront of neurodegeneration research. Immunologist Isaac Chiu has dedicated his research to elucidating that interplay, focusing on its implications for pain, host defense, and immunity.

Isaac Chiu (left) with Lani Wu (right) at the NDCN 2019 Meeting in Aptos, California.



How an Urgent Call From a Family in Italy Changed This Scientist's Career

Virologist Ivan Marazzi, a NDCN Ben Barres Early Career Acceleration Award investigator was inspired to change the course of his career after a pivotal conversation with a concerned father seeking answers.

See project

Adapted from a CZI Story originally by Devin Powell and Maki Naro, published in July 2022.

In a groundbreaking collaborative effort, Chiu's laboratory, working alongside clinician-scientists Clotilde Lagier-Tourenne and Alice Chen-Plotkin from the NDCN, has unearthed a critical link between a recently-identified family of proteins called gasdermins (GSDMs) and the progression of Frontotemporal Degeneration (FTD) and Amyotrophic Lateral Sclerosis (ALS).

Gasdermins, which normally form pores within cellular membranes, are fundamental to the processes of inflammation and programmed cell death, particularly a highly inflammatory form of cell death known as pyroptosis. While the vast majority of research on GSDM biology has focused on their roles in pyroptotic cell death within immune and cancer cells, this collaborative team was drawn to a specific member of the protein family, Gasdermin-E (GSDME), which intriguingly and unexpectedly is found within both the brain and spinal cord. This novel distribution hinted at a function beyond the conventionally studied immune and cancer contexts.

Their research utilizing an ALS mouse model and patient-derived ALS/FTD iPSC-derived motor neurons uncovered that proteins linked to ALS/FTD pathology trigger GSDME activation. Genetically reducing GSDME expression showed neuroprotective effects in both *in vitro* (cell culture) and *in vivo* (animal model) studies, indicating that inhibiting GSDME activity can effectively lessen neuronal damage. In this way, GSDME3 may be a new therapeutic target for early stages of ALS/FTD.

Selected Publication

- Gasdermin-E mediates mitochondrial damage in axons and neurodegeneration. Neel, D. V. et al., Neuron (2023) 111(8), 1222-1240.

The Role of Cryptic Exons in Neurodegenerative Diseases

Collaborators: Pietro Fratta, M.D., Ph.D. (Collaborative Pairs), Jenny Jiang, Ph.D. (Ben Barres Early Career Acceleration Award investigator), Alessandro Ori, Ph.D. (Collaborative Pairs), Hemali Phatnani, Ph.D. (Collaborative Pairs), Towfique Raj, Ph.D. (Patient Partnered Collaborations), and Michael Ward, M.D., Ph.D. (Collaborative Pairs)

Since its discovery in 2006, TDP-43 (TAR DNA-binding protein 43) has been a significant focus in neurodegenerative disease research because it accumulates in the cytoplasm in over 97% of ALS patients and up to 50% of FTD patients.

TDP-43 is crucial for RNA processing, specifically in preventing the inclusion of cryptic exons — DNA segments typically excluded from messenger RNAs (mRNAs). Reduced TDP-43 levels lead to abnormal splicing of these cryptic exons into mRNAs, often resulting in frameshifts, premature termination, or decreased RNA stability.

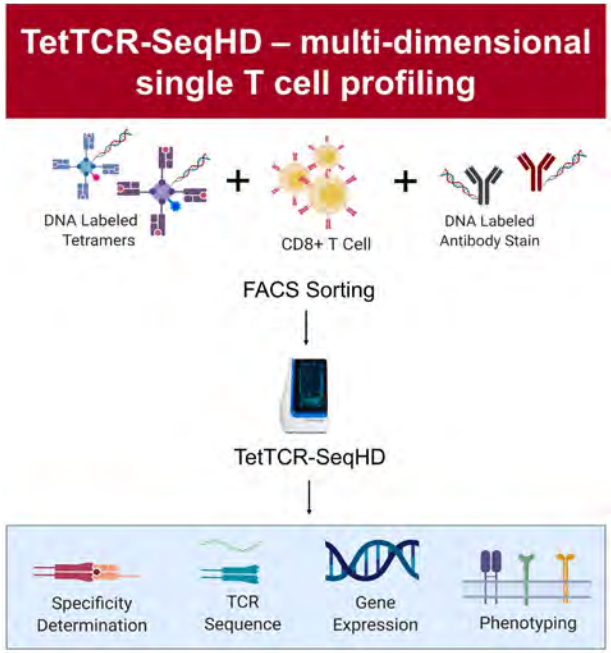
A number of NDCN labs are investigating the connection between cryptic exon inclusion and ALS/FTD risk factors, as well as its potential for diagnostic biomarkers and therapeutic development. For instance, Pietro Fratta's lab, in collaboration with Alessandro Ori, Michael Ward, Hemali Phatnani, and Towfique Raj, demonstrated that TDP-43 depletion causes significant cryptic exon inclusion in the synapse associated gene *UNC13A*, leading to nonsense-mediated decay and a loss of UNC13A protein. Genetic variants in *UNC13A* elevate the risk of ALS and FTD. Two intronic variants

Jenny Jiang in the lab at the University of Pennsylvania.

overlap TDP-43 binding sites and enhance cryptic exon inclusion in the brains and spinal cords of ALS/FTD patients.

Michael Ward's lab, working with Alessandro Ori and Pietro Fratta, showed that in a human iPSC-derived neuronal model lacking TDP-43, transcripts containing cryptic exons could produce de novo proteins. Eighteen of these de novo peptides (derived from 13 genes) were found in the cerebrospinal fluid (CSF) of ALS and FTD patients. This cryptic exon translation suggests new mechanisms of ALS/FTD pathophysiology stemming from TDP-43 dysfunction and offers a potential biomarker strategy for assessing TDP-43 function in patient CSF.

To understand the role of these de novo peptides in disease, Jenny Jiang, with Pietro Fratta, explored whether the peptides act as neo-antigens to trigger an immune response. Previous evidence links the immune system to ALS and inclusion body myositis



Schematic of TetTCR-SeqHD technology — a high-throughput and multi-dimensional method to profile antigen-specific T cells. The Jiang Lab developed and used this method to analyze PBMCs from ALS and IBM patients.

(IBM), showing highly differentiated, clonally expanded CD8+ T cells in patient peripheral blood mononuclear cells (PBMCs), cerebrospinal fluid (CSF), and muscle. Using TetTCR-SeqHD, Jiang's lab revealed that cryptic epitope-specific CD8+ T cells from ALS and IBM PBMCs are polyclonal, highly clonally expanded, and bind to multiple cryptic epitopes. By engineering cells to express those epitope-specific T cell receptors, Jiang's team demonstrated that these cells not only bound the epitopes, but also mediated T cell-specific activation and effector responses. Overall, this research identifies T-cell antigenic targets in ALS and IBM for the first time, directly linking the adaptive immune response to TDP-43 pathology.

Selected Publications and Preprints:

- TDP-43 loss and ALS-risk SNPs drive mis-splicing and depletion of *UNC13A*. Brown, A.L., et al., Nature (2022) 603. 131-137
- Mis-spliced transcripts generated de novo proteins in TDP-43-related ALS/FTD. Seddighi, S., et al., Science Translational Medicine (2024) 16 734
- TDP-43 pathology induces CD8+ T cell activation through cryptic epitope recognition. Chizari, S., et al., bioRxiv (2025)

The Power of Patient-Partnered Collaborations

Collaborations: David Liu, Ph.D. (Patient Partnered Collaboration), Cathleen Lutz, Ph.D. (Patient Partnered Collaboration), Simon Frost (Patient Partnered Collaboration), and Nina Frost (Patient Partnered Collaboration)

In July 2025, a NDCN Patient Partnered Collaboration team led by David Liu (Harvard), Cathleen Lutz (JAX labs), and Simon, and Nina Frost (RARE Hope, f.k.a. Hope for Annabel) published a landmark study reporting the development and deployment of *in vivo* CRISPR-based precision genome engineering therapeutics to rescue disease pathogenesis in mouse and human models of Alternating Hemiplegia of Childhood (AHC).

AHC is an ultra-rare (~1 in 1,000,000), early-onset neurological disease present in childhood with multiple symptoms typically observed in other more common neurological disorders. Approximately 70% of AHC cases are caused by genetic mutations acquired in a gene called *ATP1A3*, encoding an Na+/K+ ATPase subunit.

Currently, no disease-modifying therapies exist to slow or reverse AHC progression. Recently, Liu's team and collaborators harnessed their understanding of AHC genetics and recent advancements in CRISPR-based genome engineering to propose a one-time, precision editing therapeutic for this devastating disorder. They developed genome engineering therapeutics using two refined CRISPR-Cas technologies, prime editing (PE) and base editing (BE).

While the first PE clinical trial is underway as an *ex vivo* treatment for chronic granulomatous disease, a safe and efficacious *in vivo* therapeutic application of PE, particularly in the central nervous system (CNS), remains underexplored. Liu's lab demonstrated that PE can be efficiently and specifically used to correct AHC-causing *ATP1A3* mutations in cultured cells and patient-derived iPSCs *in vitro*. To transition this approach to an *in vivo* setting, the team leveraged recent progress in viral packaging and delivery to develop a dual-AAV platform specifically targeting CNS neurons.

Intracerebral injection of these viral vectors into neonatal *ATP1A3* mutant mice successfully corrected disease-causing mutations across various brain regions, restoring Atp1a3 protein function. While further validation is required for clinical application of prime editing (PE) in AHC, this research underscores its therapeutic promise.

Selected Publication

- *In vivo* prime editing rescues alternating hemiplegia of childhood in mice. Sousa, A.A., et al., Cell (2025) 188 (16), 4275-4294

RESOURCE DEVELOPMENT (TOOLS & TECH) AND TRAINING OPPORTUNITIES

NDCN's strategic approach to resource development and training has been multifaceted, aiming to benefit not only our immediate network but also the broader scientific and stakeholder community, embodying the principle of "lifting all boats." This pillar outlines the key concepts that have guided our efforts in creating and disseminating valuable tools and resources. This pillar also spotlights programmatic efforts to support training across the network, including support for early-stage trainees (students and postdocs).

Identification of Resource Needs: A Top Down and Bottom Up Approach

Our identification of key resource needs was a dynamic and collaborative process. It combined top-down program-staff-led landscaping, which involved an objective evaluation of critical resource gaps and strategic priorities, with a bottom-up approach that was directly informed by the interests and expressed needs of our grantees. This dual approach ensured that our resource development was both strategically aligned and responsive to the evolving requirements of the field.

Theory of Impact: Broad Community Benefit:

A fundamental goal and core component of our theory of impact was the development of resources and tools that transcended the immediate needs of our Challenge Network. We have been committed to creating outputs that are broadly applicable and beneficial to the wider scientific community and ecosystem. This commitment was central to our mission of accelerating progress across the field, fostering collaboration, and maximizing the impact of our initiatives.

Developing Resources through Collaborative Partnerships

Our resource development strategy has been intricately linked with our targeted grant and partnership strategies. We actively sought and cultivated collaborations with other organizations and funders within the field. This partnership-centric approach was crucial for engaging not only the broader research community but also the wide variety of stakeholders working in neurodegenerative diseases. We firmly believe that partnership is paramount; we recognize that achieving our ambitious goals is not possible in isolation. By working alongside others, we can leverage collective expertise, avoid duplication of efforts, and amplify our overall impact.

> "The iNDI cell lines developed by the NDCN have transformed how we model neurodegeneration. These resources address the challenges with other models that fail to replicate human disease, moving the entire field closer to clinically relevant discoveries."
>
> — Dr. Michael Ward, NIH

In addition to the development and dissemination of tools and resources, we recognized that targeted training opportunities could benefit NDCN labs and the broader community, accelerating the adoption of technologies developed by NDCN labs and fostering computational expertise within the neurodegeneration field. Consequently, significant effort was dedicated to providing training in newly developed technologies, such as CRISPR-based genome engineering, 3D tissue modeling, and advanced computational biology applications.

One example of a computational training opportunity supported by CZI is the Neurodegeneration Computational Fellows program. This program focused on expanding the pool of researchers

investigating health disparities in neurodegenerative diseases. It achieved this by supporting a select group of post-Baccalaureate/Master's students interested in developing computational biology skills. Their training began with a four-week orientation bootcamp, followed by placement in an NDCN lab for research experience and career-path mentorship.

Impact of Key Resources Developed and Supported Through the NDCN

Jackson Laboratory iPSC Cell Resource →
This resource was developed and supported through a collaboration with the NIH Center for Alzheimer's and Related Dementias (CARD), the Jackson Laboratory, CZI, and the Aligning Science Across Parkinson's (ASAP) program. IPSC lines are available to both academic and industry investigators.

As of July 2025:
- **507** lines available in the catalog
- **4,075** vials of iPSC lines distributed
- **895** labs utilizing these resources
- **247** repeat-customer labs, showing sustained value
- **32** countries represented, demonstrating global impact
- **37%** of orders request trio sets for comprehensive studies
- **124** publications and preprints using Jackson Laboratory iPSC lines

Protocols.io Neurodegeneration Method Development Community →
This open protocol platform enables researchers to efficiently reuse and reproduce methods developed by the NDCN community and the NIH CARD program.

- **141** members
- **21** protocols
- **23,140** views of protocols in the Neurodegeneration Method Development Community

CRISPRbrain →
Developed by Martin Kampmann's lab in collaboration with DataTecnica International, this CZI-funded open-source data commons houses data from harmonized functional genomic screens conducted in differentiated human cell

Michael Ward, a Collaborative Pairs Investigator from the NIH Center for Alzheimer's and Related Dementias, presents during a CZI Neuroscience 2024 Meeting on the iPSC Neurodegenerative Disease Initiative iPSC lines distributed by Jackson Laboratory with support from CZI, NIH and Aligning Science Across Parkinson's (ASAP).

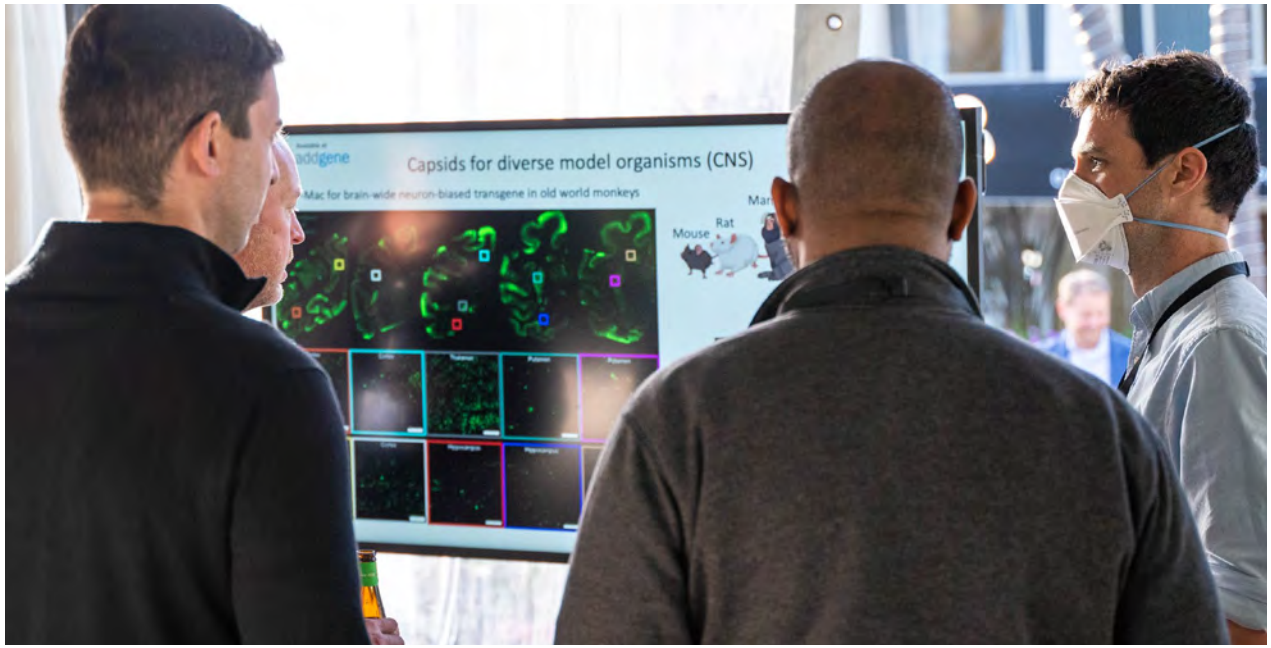
We firmly believe that partnership is paramount; we recognize that achieving our ambitious goals is not possible in isolation. By working alongside others, we can leverage collective expertise, avoid duplication of efforts, and amplify our overall impact.

types. CRISPRbrain has since been integrated into the Open Targets Platform, an open-source knowledge base providing data and tools to streamline drug target identification, annotation, and prioritization.

34 screens representing:
- **6** cell types (astrocyte, glutamatergic neuron, T cells, iPSC, microglia, hematopoietic stem, and progenitor cell)
- **19,901** genes
- **33.5%** of 45,507 indirect gene-disease associations represent novel associations
- **305,038** phenotypes

Neurolipid Atlas →
The Neurolipid Atlas was developed to facilitate insights into the role lipids play in neurodegenerative diseases. Through sharing of data and providing an easily accessible data-exploration tool, the Neurolipid Atlas provides an open-source lipidomics data commons for the neurodegeneration community.

66 datasets representing:
- **4** diseases
- **3** cell types (microglia, astrocytes, neurons)
- **2** species (human and mouse)
- **1307** lipid species



Tim Shay (right) presents on Caltech's Clover Center for AAV Engineering during the Tech Showcase at the CZI Neuroscience 2022 Meeting.

AAV Data Hub →
The AAV Data Hub* is an open-source data commons for experimental details and outcomes generated by the research community using AAV vectors distributed by Addgene.

149 reports representing:
- **62** vectors
- **8** serotypes
- **6** model organisms

**Addgene AAV data hub: a platform for sharing AAV experimental data, Nasse, J.S. et al., Nature Methods (2023) 20, 1271-1272*

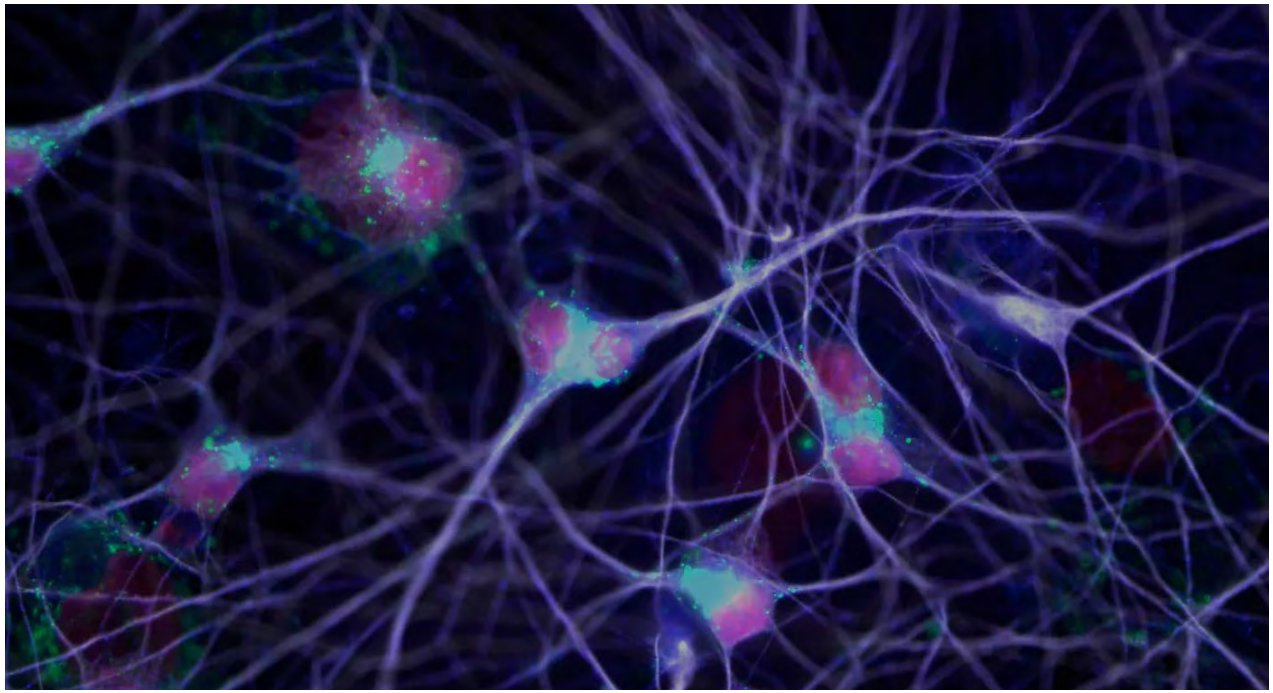
CLOVER (CLarity, Optogenetics and Vector Engineering) Center AAV Repository →
The Caltech CLOVER Center AAV Repository is a repository of published systemic AAV experiments that summarizes the capsid used, tissue target, promoter, cargo, dose used, injection method, and length of incubation, with a link to the publication.

- **25** engineered capsids
- **1000** total entries

Impact in Action

Genetically Engineered Human iPSC Lines for the Global Research Community

NDCN iPSC/CRISPR Working Group Members: Celeste Karch, Ph.D. (Collaborative Science investigator co-chair), Florian Merkle, Ph.D. (Ben Barres Early Career Acceleration Award investigator and co-chair), Michael Ward, M.D., Ph.D. (Collaborative Pairs investigator and co-chair), Ernest Arenas, M.D., Ph.D. (Collaborative Science investigator), Andrew Bassett, Ph.D. (Sanger Institute), Kristen Brennand, Ph.D. (Collaborative Pairs investigator), Alejandro Chavez, Ph.D. (Collaborative Pairs investigator), Sarah Cohen, Ph.D. (Collaborative Pairs investigator), Martin Kampmann (Ben Barres Early Career Acceleration Award investigator), Deborah Kronenberg-Versteeg, Ph.D. (Collaborative Pairs investigator), Manuel Leonetti, Ph.D. (CZ San Francisco Biohub), Esteban Mazzoni, Ph.D. (Collaborative Pairs investigator), Justin McDonough, Ph.D. (Jackson Laboratory), Priyanka Narayan, Ph.D. (Collaborative Pairs investigator), Stanley Qi, Ph.D. (Collaborative Pairs investigator), Birgitt Schuele, M.D. (Collaborative Science investigator), Bill Skarnes, Ph.D. (Jackson Laboratory), Leslie Thompson, Ph.D. (Collaborative Pairs investigator), and Marius Wernig, Ph.D. (Stanford University)



Human neurons, shown here, can now be reliably generated from stem cells and are used by the Ward lab to model neurodegenerative diseases in a dish. Photo provided by Michael Ward, National Institutes of Health.

An inherent challenge of neurodegeneration research is that existing animal and non-CNS cellular models do not often accurately and comprehensively replicate the intricate complexities of human diseases. One of NDCN's scientific objectives, therefore, was to reorient basic science neurodegeneration research toward a deeper understanding of human biology.

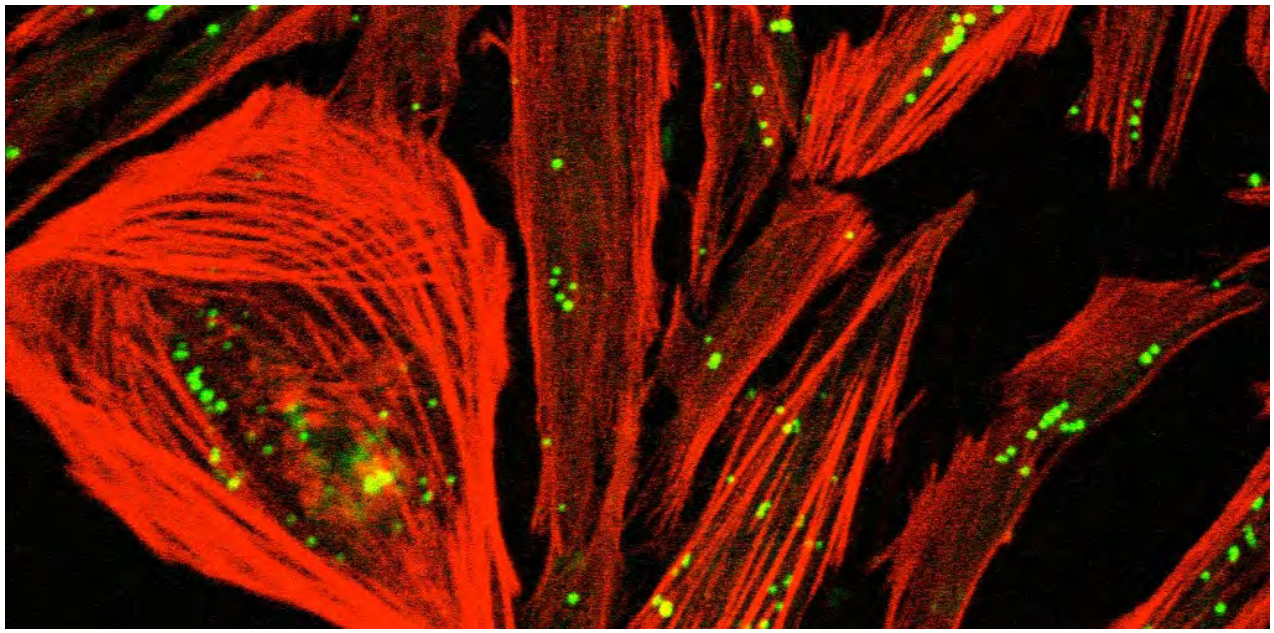
A significant partnership, exemplary of this commitment, was established with Jackson Laboratory, the ASAP initiative, and NIH CARD iPSC Neurodegeneration Disease Initiative (iNDI). This strategic collaboration focused on the systematic generation and broad distribution of high-quality, genetically engineered human iPSC lines for academic and industry use. CZI's funding supported:

- The engineering of iPSC lines beyond those targeting Alzheimer's disease and related dementias. These iPSC lines represent mutations associated with leukodystrophies, vanishing white matter disease, spastic paraplegia, spinocerebellar ataxias, and motor neuron diseases. iPSC lines representing rare diseases studied by NDCN Patient Partnered Collaborations are under development.
- Scaling, quality assessment, catalog development and dissemination by the Jackson Laboratory of over 500 engineered iPSC lines.

- Program-led development and support of a Working Group focused on the critical validation of newly engineered iPSC lines and evaluation of the neuronal and glial differentiation potential of the parental KOLF2.1J iPSC line used for genetic engineering. By acting as an early group of "testers" for these lines, the Working Group's validation efforts dramatically improved the quality of the lines and accelerated their dissemination and early adoption by the research community.

- This working group also developed and validated a number of tools to address key bottlenecks being experienced across labs. These tools included a new expression vector that overcomes transgene silencing during iPSC differentiation, and the identification of novel sites within the genome that enable alterations without phenotypic changes, underscoring the vital role of collaborative science in developing tools for iPSC disease modeling.

The Jackson Laboratory iPSC repository serves as a foundational resource for the global research community, representing a diverse array of neurodegenerative diseases. A key feature of this collection is the inclusion of a comprehensive set of isogenic lines for each single nucleotide variant (SNV) represented in the catalog. This allows researchers to study the precise impact of specific genetic mutations on cellular function and disease progression, minimizing confounding variables. Furthermore, knock-out lines are readily available for most of the genes represented, providing invaluable tools for dissecting gene function and identifying therapeutic targets. To facilitate advanced cellular imaging and functional studies, halo-tagged lines are also provided for both wildtype and mutant alleles, enabling researchers to track specific proteins in live cells.



Staining of lipid storage organelles in human astrocyte cells. Photo provided by Rik van der kant, Amsterdam UMC.

In 2026, the repository will undergo a significant enhancement by incorporating mutations engineered into iPSC lines from individuals of different genetic backgrounds.

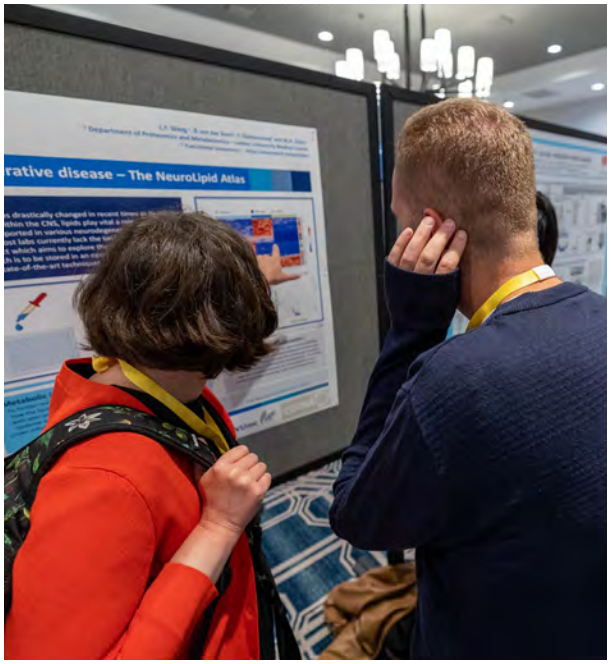
Selected Publications and Preprints

- A reference human induced pluripotent stem cell lines for large-scale collaborative studies. Pantazis, C.B., et al., Cell Stem Cell (2022) 29(16), 1685-1702

- Prevention of transgene silencing during human pluripotent stem cell differentiation. Uenaka, T., et al., bioRxiv (April 2025)

The Neurolipid Atlas: A Comprehensive Data Commons for Neurodegeneration Disease Research

Collaborators: Rik van der Kant, Ph.D. (Collaborative Pairs investigator), Martin Giera, Ph.D. (Collaborative Pairs investigator), Martin Kampmann, Ph.D. (Ben Barres Early Career Acceleration Award investigator), Adrian Isaacs, Ph.D. (Collaborative Pairs investigator), Michael Ward, M.D., Ph.D. (Collaborative Pairs



Sarah Cohen and Rik van der Kant at the NDCN 2023 meeting discuss a poster on the Neurolipid Atlas project.

Martin Giera presents on the Neurolipid atlas at the CZI 2023 Neuroscience Meeting.

investigator), Deborah Kronenberg-Versteeg, Ph.D. (Collaborative Pairs investigator), Leslie Thompson, Ph.D. (Collaborative Pairs investigator), Alessandro Ori, Ph.D. (Collaborative Pairs investigator)

Disruptions in brain lipid metabolism are increasingly recognized as critical factors in the pathology of numerous neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS). The Neurolipid Atlas, an innovative data commons, has been developed and launched by a collaborative team of eight NDCN investigators, led by Martin Giera and Rik van der Kant, to address the urgent need for standardized and accessible comparative lipidomic research across complex brain diseases.

The Atlas houses a rich collection of novel lipidomics data from diverse sources, such as human post-mortem brain tissues, mouse models of neurodegenerative disease, and isogenic iPSC-derived brain cells. This comprehensive dataset facilitates cross-species and cross-model comparisons, offering a more holistic understanding of lipid alterations linked to disease.

While developing the iPSC dataset for the Neurolipid atlas, this team of investigators found that iPSC-derived neurons, microglia, and astrocytes have distinct lipid signatures mirroring their *in vivo* counterparts, validating iPSC models for studying cell-specific lipid dynamics in neurological diseases. They also discovered that cholesterol significantly regulates astrocyte immune processes, with high free cholesterol enhancing immune activation and ApoE4-associated cholesterol esterification buffering it. These findings could lead to new neurodegenerative disease therapies.

Recent research from Adrian Isaacs' lab in collaboration with NDCN investigators Alyssa Coyne, Rik van der Kant and Martin Giera links reduced fatty acid and lipid metabolism to C9orf72-associated ALS/FTD. Studies in C9 *Drosophila*, iPSC neurons, and postmortem FTD brain tissue showed a specific reduction in phospholipid species containing polyunsaturated fatty acids (PUFAs). Increasing PUFA levels, especially in neurons, significantly extended the lifespan of C9 ALS/FTD flies and suppressed stressor-induced neuronal death in patient-derived neurons. These findings suggest that interventions aimed at increasing neuronal PUFA levels could be beneficial in slowing the pathogenesis of ALS/FTD, given the implication of neuronal fatty acid saturation. The supporting data can be found on the Neurolipid Atlas platform.

The Neurolipid Atlas (neurolipidatlas.com) empowers researchers to explore lipidomic data, identify biomarkers, and understand lipid dyshomeostasis. By facilitating collaboration and data sharing, the Atlas will accelerate diagnostics and therapies for neurodegenerative disorders.

Selected Publications

- The Neurolipid Atlas: a lipidomics resource for neurodegenerative diseases uncovers cholesterol as a regulator of astrocyte reactivity impaired by ApoE4. Feringa, F.M., et al., bioRxiv (2024) Accepted in Nature Metabolism.

- Neuronal polyunsaturated fatty acids are protective in ALS/FTD. Giblin A., et al., Nature Neuroscience (2025) 28, 737-747

Translating Basic Science Discoveries

When the NDCN was launched, the focus on basic science — rather than translational or clinical approaches — was an unconventional choice. The decision was met with skepticism by those in the field who said we should focus on new treatment pathways, and by others who questioned the focus on an area already receiving significant investment, and perceived by some as having become ossified.

Yet our conviction was, and remains, that investing in basic science will lay the groundwork for subsequent translational efforts. Neurodegeneration is a disease of cell biology, and therefore NDCN prioritized fundamental discovery over clinical translation, guided by the belief that understanding core biological mechanisms is essential to treating disease and that studying disease pathology reveals new insights into core biology, creating a virtuous cycle of discovery.

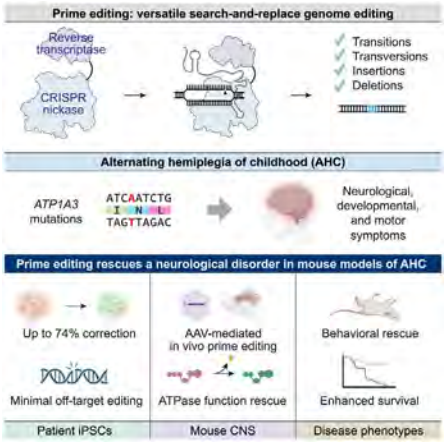
With this impact report, we can proudly showcase how investments in basic science have significantly advanced our understanding of neurodegeneration mechanisms. Here, we describe how our focus on fundamental scientific discoveries has shaped translational and clinical opportunities for neurodegenerative diseases.

Bridging Connections Between Rare and Common Disease Biology Leads to New Treatment Avenues for Neurodegenerative Disease

While common neurodegenerative diseases such as Alzheimer's and Parkinson's are typically associated with aging, rare forms of neurodegenerative diseases can also occur across the lifespan. There are many commonalities between pediatric and adult neurodegenerative conditions, but pediatric neurodegenerative disorders, and especially rare forms, are understudied.

To better investigate those rare forms, physician-scientists Rebecca Ahrens-Nicklas (a specialist in metabolic disorders) and Elizabeth Bhoj (a geneticist) joined forces in a Collaborative Pairs grant to expand the functional genomics pipeline at the Children's Hospital of Philadelphia. They leveraged deep phenotyping, multi-omics and animal models to better understand the mechanisms underlying a collection of rare monogenic pediatric neurodegenerative diseases. In their project, they identified several genes with strong correlations to adult neurodegenerative phenotypes.

Their pipeline was recently put to use when Rebecca participated in a clinical trial using a new CRISPR-based therapy to treat a child with a rare genetic metabolic disorder. The rapid six-month development of a therapeutic strategy from genetic variant identification and diagnosis to treatment yielded very promising initial results for baby KJ. While baby KJ will require lifelong monitoring, this success paves the way for CRISPR-based therapies for other rare genetic metabolic disorders.



Primer editing approach designed by David Liu's lab for a mouse model of alternating hemiplegia of childhood.

Technologies Driving New Directions for Therapeutic Development

New tools facilitate new discoveries, and the NDCN adopted various strategies to foster the creation and application of advanced research tools for challenges related to neurodegeneration. Importantly, we

prioritized rapid and open sharing of tools, methods, and resources with the broader community. By doing so, we hoped to create a ripple effect, extending our impact beyond immediate grantees and improving the caliber of tools and resources available for all neurodegeneration research.

For example, working to raise the standards for human-based cell platforms, we partnered with Jackson Laboratory, the NIH CARD, and the ASAP initiative on the iNDI project, with CZI funding the community development and dissemination efforts in partnership with Jackson Laboratory. We also collaborated with and funded Addgene and the Caltech CLOVER Center to develop open source AAV data hubs for the research community.

Opportunities to work together on technology development was an attractive part of the Challenge Network for many NDCN investigators, who came together to create new tools as well as work through bottlenecks and application challenges with existing tools. Several NDCN research teams focused on iPSC model development and applications of new genome engineering tools (CRISPR, ASOs). Others came together to drive the development of CRISPR screens in human cell types, complex 3D models, and *in vivo*, with the aim of uncovering new pathways and mechanisms — as exemplified by the labs of Martin Kampmann (Tauopathies), Ethan Lippmann (Alzheimer's disease), Clotilde Lagier-Toureene and Paul Blainey (ALS/FTD), Alex Chavez and Serge Przedborski (Spinocerebellar Ataxias), Yang Hu (Glaucoma), and Michael Ward (ALS/FTD). These pathways and mechanisms are leading to the development of new biomarkers and potential therapies for neurodegenerative diseases.

A number of teams are also deploying CRISPR technologies towards more directed translational goals, such as Stanley Qi (CRISPR-Cas systems that allow targeting of multiple genes with a single vector). To deliver these potential therapies to the central and peripheral nervous systems in a cell-type-specific manner, Viviana Gradinaru has been at the forefront of developing adeno-associated viral (AAV) tools. The application of AAV technology for translational purposes is currently under development by David Liu's team in collaboration with the RARE Hope Foundation, who have developed an AAV9 platform for delivering prime editing machinery to correct mutations in the *ATP1A3* gene responsible for Alternating Hemiplegia of Childhood.

Physician Scientists and Patients Driving Clinical Applications

When we launched the NDCN with the vision of bringing new research talent into the neurodegeneration field, we wanted to ensure that researchers who were new to the field were partnered with clinicians and physician-scientists, to ground their work in the critical clinical contexts. To fulfill this goal, NDCN has funded 25 physician scientists, who played an important role in mentorship and in advancing basic science methodologies into clinical practice.

Patients were also central to our effort. The "Patient-Clinician Conversations" brought together patients, patients' families, and clinicians for discussions. Patients and families challenged by a range of diseases — including Parkinson's disease, Frontotemporal Degeneration, Amyotrophic Lateral Sclerosis,

> Our conviction was, and remains, that investing in basic science will lay the groundwork for subsequent translational efforts.

Huntington's disease, Lysosomal Storage Diseases, Spinocerebellar Ataxia, Multiple Dystrophy, and metabolic disorders such as Multiple Sulfatase Deficiency — participated in these discussions. Engagement between patients and clinicians led to the development of the Patient-Partnered Collaborations for Rare Neurodegenerative Disease, a funding mechanism that kicked off in 2022 in collaboration with CZI's Rare as One Project.

Inspired by the power of collaborative networks that bridge research and therapeutic applications, NDCN was a founding funder of the N=1 Collaborative, a new consortium focused on developing ASO therapeutic approaches for rare neurological diseases. Tim Yu, a physician-scientist, and Julia Vitarello, a mother and founder of Mila's Miracle Foundation — who together drove the first *in vivo* application of an ASO therapy for Batten' Disease for Julia's daughter Mila — were part of N1C's founding team. Yu and Vitarello are also part of a team funded by a Patient-Partnered Collaboration to generate a single-cell reference atlas of Batten Disease pathobiology and ASO therapeutic responses based on Mila's case, demonstrating once again the power and impact of basic research and clinical translation working together in a virtuous cycle of learning and innovation.

In Closing: Learnings From an Experiment in Collaborative Science

Above: Attendees at the CZI Neuroscience 2024 Meeting in Monterey, California.

The NDCN was an experimental venture in collaborative science, conceived as a **"network that is more than the sum of its parts."** A key objective has been for the Challenge Network to serve as a model for future initiatives in any scientific field, as its principles and approaches are broadly applicable.

We selected neurodegeneration as the field to test our network model due to clear unmet needs, compelling scientific challenges, and the potential benefit from a fresh approach. Importantly, the NDCN was envisioned as a "learning model." This allowed for experimentation with our programmatic strategy for building, operating, and sustaining a robust scientific network.

A key objective was for the Challenge Network to serve as a model for future initiatives in any scientific field, as its principles and approaches are broadly applicable.

This impact report concludes by highlighting key learnings and lessons from the NDCN, with the hope of inspiring other funders and organizations in science — across all disciplines — to invest in and build impactful initiatives for collaborative research.

Towards a More Holistic Understanding of Neurodegenerative Disease

We've championed a cross-disease, systems-level approach to neurodegenerative disorders that moves beyond siloed, disease-specific models to reframe neurodegeneration as a spectrum of related conditions with shared mechanisms, causes, and therapeutic opportunities. This approach involved reframing neurodegeneration as a class of disorders — both rare and common — with shared cellular mechanisms and not solely associated with aging, but with impact across the lifespan.

Neurodegenerative disorders are systemic conditions with causes and effects extending beyond the nervous system. Just as cancer research evolved from organ-specific silos to a mechanism-driven framework, we've pushed for a similarly integrated view in neuroscience: "putting the brain back in the body" by investigating the roles of the immune system, vascular network, gut, and metabolism.

Focus on Frontiers

The NDCN focused on frontier research areas in the field and actively recruited new kinds of researchers and disciplinary expertise into the field. By focusing on emerging areas and untapped expertise, we aimed to catalyze novel research directions and distinguish our approach from more traditional funders. This commitment to frontier science was embedded in our grant design, selection processes, and community-building strategy.

People First, Talent Forward

Rather than enforce rigid milestones or collaborations, we focused on empowering talented individuals and teams, bringing them together in creative ways and trusting that great science would follow. Our early-career efforts aimed to shape the next generation of field leaders. More than funding grants, we funded people and talent and a way of doing science that is collaborative, bold, and inspired.

Those investments in basic science, collaboration, and a people-first strategy have paid off, not only significantly advancing neurodegeneration mechanisms, but also shaping translational and clinical opportunities for neurodegenerative diseases. We aimed to change how the field views neurodegenerative diseases, and we achieved that goal.

A Network That Is More Than the Sum of Its Parts

Envisioned and executed as an experiment in collaborative science, the Challenge Network approach has proven its impact, adaptability, and durability. There are many more stories and examples of team successes and impacts funded and supported through NDCN than could fit into this report.

Beyond the successful outcomes from grants we funded, some of the most inspiring work and discoveries emerging from the Challenge Network have been projects that were not part of the original

More than funding grants, we funded people and talent and a way of doing science that is collaborative, bold, and inspired.

CZI-funded grants but rather work that was seeded by collaborations within the network that emerged organically. Indeed, we feel that the scientific impact of NDCN was outsized relative to the financial and time investment, especially when one considers that the early years of NDCN straddled a global pandemic that put significant stress on the scientific community, especially around collaborations.

The Challenge Network model is not specific to neurodegeneration or neuroscience, or any specific discipline. It is a readily adaptable model that can be exported to a broad range of scientific challenges where new ideas and new modes of collaborative, interdisciplinary approaches are needed.

We hope this report provides inspiration and a blueprint for other organizations seeking to push scientific boundaries by building collaborative networks in new and exciting ways, beyond the average consortia.

To the grantees, collaborators, and partners that have been a part of the NDCN, we are deeply grateful for your creativity, dedication, and bold thinking that brought this vision to life. Your work is not only advancing the science of neurodegeneration, it is reshaping what's possible. Thank you for being at the heart of this effort and for driving the field forward with passion and purpose.

Appendixes

The NDCN leveraged four distinct funding mechanisms to catalyze paradigm shifts in understanding and addressing neurodegenerative diseases, ultimately leading to new pathways for diagnosis, treatment, and prevention.

The NDCN included over 300 researchers from 70 institutions worldwide. Together, these dedicated scientists changed the face and trajectory of neurodegenerative disease research, and we are profoundly grateful for their depth of engagement and profound spirit of collaboration.

BEN BARRES EARLY CAREER ACCELERATION AWARD

This investigator award was designed for early-career academic researchers, particularly those new to the field of neurodegeneration. Recipients benefited from mentored support and access to resources within the NDCN. Cycle 1 supported 17 investigators, representing a total investment of $42.5 million over five years. Cycle 2 supported 13 investigators, representing a total investment of $15.5 million over four years.

Assembling Human Cellular Models to Study Neurodegeneration

Sergiu Pasca, M.D., Ph.D.,
Stanford University

Description: to use brain assembloids — self-organizing 3D human cellular systems that capture neural-glial interactions and inter-regional neural cross-talk — to uncover the programs underlying prolonged maturation of neurons and astrocytes in humans and model specific genetic forms of neurodegenerative disorders.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Bacterial and Toxin Engineering to Treat Motor Neuron Degeneration

Isaac Chiu, Ph.D.,
Harvard Medical School

Description: to determine whether the gut microbiome alters inflammation, neuronal loss, and disease progression in ALS mice; determine whether bacterial infections trigger neurodegeneration; and engineer a specific bacterial toxin to deliver pro-survival factors into motor neurons to treat ALS.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Brain Resilience and Astrocyte States Across Species

Maria Antonietta Tosches, Ph.D.,
Columbia University

Description: to leverage an evolutionary perspective to discover how the diversity of molecular states in astrocytes may underlie the resilience of the brain to neurodegeneration.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Brain-Body Synchronization as a Novel Mechanism of Neurodegeneration

Li Ye, Ph.D.,
The Scripps Research Institute

Description: to decipher the role of long-term, systemic brain-body crosstalk on neurodegeneration at the organismal level, using whole-body imaging and click chemistry labeling.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Circuit Signatures of Psychiatric Symptoms in Neurodegeneration

Sabine Krabbe, Ph.D.,
German Center for
Neurodegenerative Diseases

Description: to unravel the circuit mechanisms underlying early psychiatric symptoms in proteinopathies by combining longitudinal functional recordings with novel post-hoc anatomical and molecular reconstructions.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Computational Discovery to Accelerate Neurodegenerative Disease Research

Debora Marks, Ph.D.,
Harvard University

Description: to develop novel statistical methods, employing probabilistic modeling and machine learning to find patterns in genomic data that allow us to discover the 3D protein interactions, RNA complexes, and — most critically for neurodegenerative disease pathophysiology — their dynamics, alternative conformations, and propensity to form structures that may progress to fibrils.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Deciphering the Mechanisms of RNA-Mediated Toxicity in Neurodegeneration

Mitchell Guttman, Ph.D.,
California Institute of Technology

Description: to explore known repeat-containing RNAs that are genetically linked to neurodegenerative disorders to understand common features of RNA-induced toxicity in neurons.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Decoding the Systems Pharmacology of Proteinopathy in Human Cells

Michael Keiser, Ph.D.,
University of California, San Francisco

Description: to determine the biological mechanisms of promising new drug-like compounds using interpretable deep learning, to improve understanding of proteinopathies and accelerate drug discovery for neurodegenerative diseases.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Dynamic Visualization of Autophagy During Neurodegeneration

Tal Laviv, Ph.D.,
Tel Aviv University

Description: to utilize an imaging-based approach to monitor *in vivo* subcellular autophagy dynamics in the mouse brain, allowing early detection of cell-specific autophagy failure during neurodegeneration.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Dysfunctions of Brain Energy Supply in ALS

Vidhya Rangaraju, Ph.D.,
Max Planck Florida Institute for
Neuroscience

Description: to investigate metabolic disruptions that impair learning and memory in ALS using super-resolution imaging of mitochondrial stabilization and state-of-the-art mitochondrial ATP imaging.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Elucidating Cellular Mechanisms of Neurodegeneration by Functional Genomics

Martin Kampmann, Ph.D.,
University of California, San Francisco

Description: to reveal cellular mechanisms underlying disease-associated genes, using genetic modifier screens in patient-iPSC derived neurons and glia.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Epigenetic Control of Genomic Repeats in the Human Brain

Christopher Douse, Ph.D.,
Lund University

Description: to identify how the epigenetic regulation of repetitive genetic elements influences human neural transcriptional networks in repeat-associated neurological disorders.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Gridlock in the Nervous System: Altered RNA Localization and Local Translation in Neurodegeneration

Eric Wang, Ph.D.,
University of Florida

Description: to elucidate rules for how cargoes are chosen, for example, which RNAs and proteins are carried by which motor proteins; and to accelerate repeat expansion to better model neurodegenerative processes.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Harnessing Mechanics to Immunomodulate Microglia and Fight Neurodegeneration

Alba Diz Muñoz, Ph.D.,
European Molecular Biology Laboratory

Description: to use cutting-edge mechanobiology approaches to decipher how cell intrinsic and extrinsic mechanical cues regulate microglia activation during neurophysiology and neurodegeneration.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

High-Throughput "3D" Profiling of Single T-cells in Neurodegenerative Diseases

Ning (Jenny) Jiang, Ph.D.,
University of Pennsylvania

Description: to leverage systems immunology tools developed by the Jiang Lab to profile single T-cells that infiltrate into the brain during neurodegeneration.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Identifying the Role of Astrocytes in Neuronal Synapse Loss and Repair in Neurodegenerative Disease

Nicola Allen, Ph.D.,
Salk Institute for Biological Studies

Description: to investigate how astrocytes regulate neuronal synapse formation and function from development to aging, how dysfunctional astrocytes contribute to neurodegenerative disease, and how astrocytes may be used to stimulate synaptic repair in disease.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Intersectionality of Myelin and Sleep in Alzheimer's Disease

Erin Gibson, Ph.D.,
Stanford University

Description: to elucidate the role of myelin-forming glia and myelination in neurodegenerative sleep dysregulation using genetic mouse modeling, viral tracing, and Alzheimer's patient-derived iPSCs.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Investigating Mechanisms of Tau-Mediated Neurodegeneration in the Human Brain

Inma Cobos, M.D., Ph.D.,
Stanford University

Description: to investigate the contributions of distinct cell types to disease pathogenesis and identify the transcriptome changes associated with tau pathology, applying single-cell technology to human brain samples with Alzheimer's disease.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Mapping Cellular Networks for Microbiome Contributions to Neurodegeneration

Elaine Hsiao, Ph.D.,
University of California, Los Angeles

Description: to investigate how genetic and environmental risk factors for neurodegeneration modify the gut microbiome, and how interactions between the gut microbiome and immune and nervous systems contribute to disease initiation and progression.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Mechanisms of Blood-Brain Barrier Transporter Regulation: New Avenues for Understanding and Treating Neurodegenerative Disorders

Ethan Lippmann, Ph.D.,
Vanderbilt University

Description: to use cell engineering strategies to systematically deconstruct mechanisms that govern blood-brain-barrier transporter expression and regulation.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Metabolic Crosstalk between Neurons and Astrocytes in Neurodegeneration

Ghazaleh Ashrafi, Ph.D.,
Washington University in St. Louis

Description: to take a multi-pronged approach using optical physiology to elucidate how dysregulation of metabolic crosstalk between neurons and astrocytes contributes to neurodegeneration.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Non-Canonical Pathways Towards Rescue from Neurodegenerative States

Viviana Gradinaru, Ph.D.,
California Institute of Technology

Description: to understand the role of the periphery in propagating neurodegeneration; and engineer non-invasive gene-delivery tools that specifically target non-neuronal brain cells relevant to neurodegeneration, such as immune cells and brain endothelial cells comprising the vasculature, since an impaired BBB can initiate and/or precipitate neurodegeneration.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Retrotransposon Reactivation in Neurodegenerative Disease

Molly Gale Hammell, Ph.D.,
Cold Spring Harbor Laboratory

Description: to develop novel machine learning software to systematically identify the genetic factors and molecular mechanisms that lead to motor neuron cell death, with a particular focus on retrotransposon reactivation.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Targeting Shared Mechanisms in Neurodegenerative and Metabolic Disease

Florian T. Merkle, Ph.D.,
University of Cambridge

Description: to understand links between obesity in mid-life and increased risk of dementia later in life in order to find interventions that can more potently slow or delay neurodegeneration in mice, and gain molecular understanding between these links using human stem cell-derived cellular models.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Targeting the Gliovascular Interface to Improve Brain Waste Clearance

Rune Enger, M.D., Ph.D.,
University of Oslo

Description: to use *in vivo* brain imaging of natural sleep to elucidate how the gliovascular unit regulates brain waste clearance to identify targets for the prevention of neurodegeneration.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

The Innate Immune System in Neurodegeneration

Ivan Marazzi, Ph.D.,
Icahn School of Medicine at Mt. Sinai,
New York

Description: to understand the pathogenic mechanisms of mutant proteins in neurodegeneration, and test the hypothesis that neurodegenerative diseases can be instigated or accelerated by dysfunction of the innate immune system.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

Viral-Like Mechanisms of Intercellular Communication in the Pathology of Neurodegeneration

Jason Shepherd, Ph.D.,
The University of Utah

Description: to determine the role played by neuronal gene Arc, a master regulator of synaptic plasticity and memory, in a novel form of intercellular communication that resembles retrovirus biology.

Award: Ben Barres Early Career Acceleration Awards (Cycle 1)

What Do Myelinating Glia Do in Neurodegeneration?

Lu Sun, Ph.D.,
University of Texas Southwestern Medical Center

Description: to generate novel genetic toolkits to determine the roles of oligodendrocytes and to spatiotemporally map myelination in a neural circuit-specific manner during neurodegeneration.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Whole-Brain Micron-Scale Functional Imaging with Superresolution Ultrasound

Pengfei Song, Ph.D.,
University of Illinois

Description: to develop an ultrasound microvascular imaging technique that provides structural (vessel morphology) and functional (neural activities) evaluation of brains affected by neurodegenerative diseases.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

Why Can't Microglia Keep Pace with Neurodegenerative Diseases?

Frederick Bennett, M.D.,
University of Pennsylvania

Description: to identify cell engineering strategies that sustain the protective function of microglia as a new therapeutic direction for neurodegenerative diseases.

Award: Ben Barres Early Career Acceleration Awards (Cycle 2)

COLLABORATIVE PAIRS AWARD

Cycle 1 of this grant mechanism featured a two-phased funding structure: an 18-month pilot phase followed by a 4-year acceleration phase. In Cycle 2, the acceleration phase of the award was limited to two years. The pilot phase aimed to provide collaborating teams with the autonomy to explore novel, unconventional, and potentially transformative ideas. Each collaborative pair team was required to include an early- or mid-career investigator, and the team members could not have previously received joint grant funding prior to submitting a Collaborative Pairs grant application. Cycle 1 supported 30 pilot phase teams (60 grants) and 16 acceleration phase teams (32 grants) totaling an investment of $30.1 million. Cycle 2 supported 64 pilot phase teams (128 grants) and 9 acceleration phase teams (18 grants) totaling an investment of $20 million.

A Neurodegenerative Duet: Protein Turnover and miRNAs

Giordano Lippi, Ph.D.,
The Scripps Research Institute

Eugenio Fornasiero, Ph.D.,
University Medical Center Göttingen

Description: to manipulate miRNA networks acting in protein turnover dynamics during aging and neurodegeneration with the aim of favoring neuroprotection.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

A New Sensory Component of Memory and Neurodegeneration

Felipe Almeida de Pinho Ribeiro, Ph.D.,
Washington University in St. Louis

Jonathan Kipnis, Ph.D.,
Washington University in St. Louis

Description: to characterize the role of meningeal peptidergic innervation in memory, cognitive function, and neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

A Plant Small-Molecule Discovery Platform to Study Neurodegeneration

Ankur Jain, Ph.D.,
Whitehead Institute for Biomedical Research

Jing-Ke Weng, Ph.D.,
Whitehead Institute for Biomedical Research

Description: to develop a new peptide screening platform using plant cells that are engineered to express human neurodegenerative disease-causing proteins and RNAs, in the hopes of uncovering a new way for screening small-molecule-protein interactions *in vivo*.

See a graphic representation of their work on the next page.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

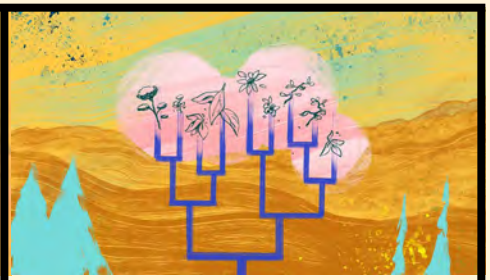
What Can Plants Teach Us About Treating Brain Disease?

Jing-Ke Weng, an expert in plant evolution in MIT's Department of Biology, might seem like a strange person to consult about human brain disease. But he teamed up with MIT biology colleague Ankur Jain to find new ways to study and treat neurodegeneration by harnessing plant cells and the rich diversity of molecules those cells contain.

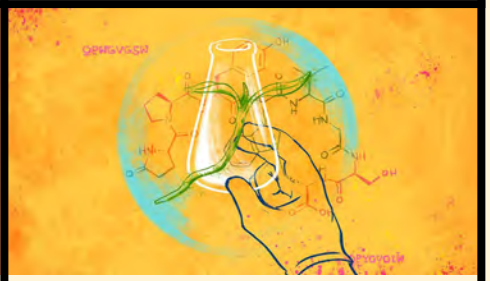
See project

What if we could borrow a superpower from plants to make progress on neurodegenerative disease

In some neurodegenerative diseases, molecules such as proteins and RNA can misfold. They stick to each other and form clumps that can potentially injure or even kill brain cells.



This clumping has rarely been observed in plants, which evolved differently to survive in so many environments on land.



With CZI support, our labs worked together for the first time. We used specially engineered plant cells containing the human molecules that clump together.

In these cells, we can test millions of compounds to see how they interact with the sticky molecules and find ones that stop the sticking.

Some of the compounds we tested come from plants themselves, including traditional medicines. We believe they offer new hope for understanding and treating ALS, Alzheimer's, and other neurodegenerative diseases.

Adapted from a CZI Story originally by Devin Powell and Maki Naro, published in March 2022.

Aging and Neurodegeneration in a Human Brain Tissue Model


Henner Koch, M.D., Ph.D.,
RWTH Aachen University


Deborah Kronenberg-Versteeg, Ph.D.,
Hertie Institute for Clinical Brain Research,
University of Tuebingen


Thomas Wuttke, M.D.,
Hertie Institute for Clinical Brain Research,
University of Tuebingen

Description: to explore cellular interactions, with an initial focus on the role of immune cells (microglia) in Alzheimer's disease.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Altered Corticohippocampal Dialogue in Memory Disorders


Ksenia Kastanenka, Ph.D.,
Massachusetts General Hospital


Simon Schultz, Ph.D.,
Imperial College London

Description: to test the hypothesis that the consolidation of memory traces from the hippocampus into the neocortex depends on a sharp-wave driven dialogue disrupted in memory disorders.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Analysis of Neuro-Specific Splice and 3'UTR Isoforms


Eric Lai, Ph.D.,
Memorial Sloan Kettering Cancer Center


Neville Sanjana, Ph.D.,
New York Genome Center and
New York University

Description: to apply RNA-based CRISPR genomics in hESC-derived neurons for systematic functional analysis of neural-specific splicing and 3'UTR isoforms that are relevant to development and neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Astrocyte Electrical Activity in Plasticity and Learning


Yoav Adam, Ph.D.,
The Hebrew University of Jerusalem


Chris Dulla, Ph.D.,
Tufts University School of Medicine

Description: to define the role of a newly discovered form of astrocyte electrical activity on neuronal function, synaptic plasticity, and learning and memory using novel *in vitro* and *in vivo* imaging approaches.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Astrocyte-Neuron Subproteomes for Stroke Therapies


Elena Blanco-Suarez,
Ph.D., Thomas Jefferson University


Baljit Khakh, Ph.D.,
University of California, Los Angeles

Description: to identify astrocyte-neuron subproteomes in a pre-clinical model of permanent stroke, and find biomarkers and therapeutic strategies to stall neurodegeneration and cognitive decline in the long term.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Autoantibody Biomarkers of Messenger RNA Defects in Neurodegeneration


William Seeley, M.D.,
University of California, San Francisco


Michael Wilson, M.D., Ph.D.,
University of California, San Francisco

Description: to evaluate whether TDP-43 loss-of-function-related cryptic mis-splicing results in autoantibodies to aberrant neoepitopes in patients with frontotemporal lobar degeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Brain-Wide Maps of Myelin Patterns in Plasticity and Repair


Dwight Bergles, Ph.D.,
Johns Hopkins University


Jeremias Sulam, Ph.D.,
Johns Hopkins University

Description: to define changes in the pattern of myelination in the adult mouse brain in the context of remyelination and adaptive plasticity using light sheet imaging and automated cellular reconstruction.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Cell Type-Resolved Circuit Analysis in Huntington's Disease


Irina Dudanova, Ph.D.,
University Hospital Cologne


Takaki Komiyama, Ph.D.,
University of California, San Diego

Description: to perform molecular and functional dissection of cortical and thalamic inputs to the striatum and elucidate their involvement in Huntington's disease pathophysiology.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Cholesterol Dynamics Across Brain-Wide Neural Circuits


Celia Shiau, Ph.D.,
University of North Carolina at Chapel Hill


En Yang, Ph.D.,
University of North Carolina at Chapel Hill

Description: to use *in vivo* whole-brain imaging, whole-body physiology, and computational modeling in zebrafish to map cholesterol dynamics and metabolism across brain regions and cell types, and their impact on cognitive functions.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Chromatin Encoding of Repeat Expansion in Neurodegeneration


Schahram Akbarian, M.D., Ph.D.,
Icahn School of Medicine at Mount Sinai


Kristen Brennand, Ph.D.,
Icahn School of Medicine at Mount Sinai


Esteban Mazzoni, Ph.D.,
New York University


Jennifer Phillips-Cremins, Ph.D.,
University of Pennsylvania

Description: to investigate the emerging link between the genetic sequence's higher-order folding patterns and pathologic repeat instability in trinucleotide repeat expansion disorders.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Correlation to Causation: Astrocytes in Neurodegeneration


Naomi Habib, Ph.D.,
Hebrew University of Jerusalem


Francisco Quintana, Ph.D.,
Brigham and Women's Hospital

Description: to integrate computational predictions of molecular drivers from large human datasets with functional screening platforms to identify pathways that operate in astrocytes to drive neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Cross-Species Approach to Decode Cognitive Rejuvenation


Param Priya Singh, Ph.D.,
University of California, San Francisco


Saul Villeda, Ph.D.,
University of California, San Francisco

Description: to utilize lifespan tracking in a short-lived African killifish model, behavioral paradigms in aging mouse models, and comparative genomics to identify peripheral mechanisms of cognitive rejuvenation.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Deep-Tissue Genetic Reporters of Protein Aggregates

Arnab Mukherjee, Ph.D.,
University of California, Santa Barbara

Laura Segatori, Ph.D.,
Rice University

Description: to engineer a synthetic gene circuit for the noninvasive detection of protein aggregates in neurodegenerative conditions, using magnetic resonance imaging.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Defining the Matrix in Memory Circuits

Anna Victoria Molofsky, M.D., Ph.D.,
University of California, San Francisco

Ye Zhang, Ph.D.,
University of California, Los Angeles

Description: to define what extracellular matrix (ECM) proteins are deposited when memories are formed, which cell types are producing them, and whether ECM determines the strength of the memory trace.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Design and Functions of Neural Circuits in Chimeric Brains

Kristin Baldwin, Ph.D.,
Columbia University

Jun Wu, Ph.D.,
University of Texas Southwestern Medical Center

Description: to engineer chimeric brain models in which specific neural circuits or subtypes are replaced with cells of another species, to identify mechanisms for cognition, memory, and neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Discover Causal Variants and Mechanisms in Neurodegeneration

Douglas M. Fowler, Ph.D.,
University of Washington

Debora Marks, Ph.D.,
Harvard University

Description: to combine deep learning technologies and multiplexed mutational technologies in iPSCs to uncover novel causal genetic variants that drive protective and risk mechanisms across neurodegenerative diseases.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

DNA Repair as a Function of Sleep

Luis de Lecea, Ph.D.,
Stanford University

Nara I. Muraro, Ph.D.,
Biomedicine Research Institute of Buenos Aires, IBioBA-CONICET-MPSP

Description: to test the hypothesis that DNA damage drives sleep pressure and DNA repair mechanisms are more active during sleep in different cell types in both mice and flies.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Elucidating Shared Mechanisms of Pediatric and Adult Neurodegeneration

Rebecca Ahrens-Nicklas,M.D., Ph.D.,
Children's Hospital of Philadelphia

Elizabeth Bhoj, M.D., Ph.D.,
Children's Hospital of Philadelphia

Marylyn Ritchie, Ph.D.,
University of Pennsylvania

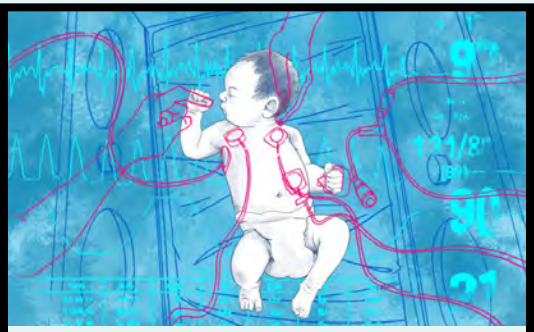
Description: to expand the functional genomics pipeline at the Children's Hospital of Philadelphia to leverage deep phenotyping, multi-omics and animal models to understand the mechanisms underlying a collection of rare monogenic pediatric neurodegenerative diseases.

See a graphic representation of how Rebecca Ahrens-Nicklas and Elizabeth Bhoj came to work together on the next page.

Why These Two Scientists Are Teaming Up To Study, Treat and Prevent Rare Pediatric Diseases

Adapted from a CZI Story originally by Devin Powell and Maki Naro, published in April 2021.

See project



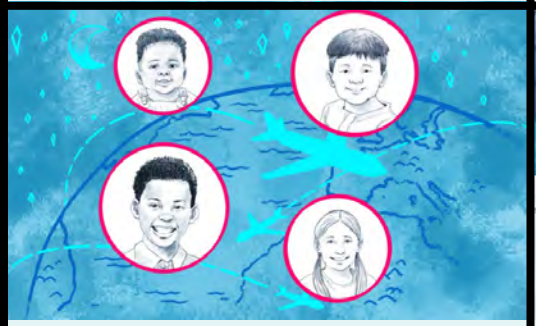
The baby was born healthy. But two hours later, his heart rate and oxygen levels dropped. No one knew what was wrong with him.

We (Rebecca Ahrens-Nicklas and Elizabeth Bhoj) met him as residents. We were two physician-scientists in training: friends, with children who attended each other's birthday and Halloween parties.

His was the first case we solved together. We discovered that he had a rare disease. A small change to his DNA had acidified his blood.

Afterwards, we both went on to study rare pediatric diseases. Rebecca became an expert in metabolics: the food-fueled chemical reactions that keep our bodies running. Elizabeth specialized in genetics, identifying mutations that cause anatomical problems in children.

We each opened up our own labs, right next to each other. We bounced ideas off each other. In texts. With coffee and tea. Over lots of nachos.



And as participants in CZI's NDCN, we combined our skill sets and tracking down 100 kids with rare neurological diseases across the world.

Our goal, like that of our colleagues across the NDCN, was to find out what is going on in each case. To help, if possible. And to break new scientific ground that could help us understand a broad range of neurological diseases, in the young and the old.

This project was built on collaboration: With physicians who bring us their toughest cases, with scientists who share their expertise, and between two friends excited to finally be working together again.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Exploring Convergent Pathways for Sleep Efficiency

Ying-Hui Fu, Ph.D.,
University of California, San Francisco

Arun Padmanabhan, M.D., Ph.D.,
University of California, San Francisco

Description: to initiate the process of identifying convergent molecular pathways for regulating sleep efficiency using multi-omics and computational platforms.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Exploring Mechanisms and Significance of Cerebellar Sleep

Farzaneh Najafi, Ph.D.,
Georgia Institute of Technology

Giulio Tononi, M.D. Ph.D.,
University of Wisconsin-Madison

Description: to study the role of the cerebellum and cerebellar-dependent learning in regulating sleep, and mutually to investigate the role of sleep in modulating cerebellar function during learning.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Functional Genomics of Neural Excitability in Prion Disease

Adriano Aguzzi, M.D.,
University of Zurich

Madhuvanthi Kannan, Ph.D.,
University of Minnesota

Description: to uncover the role of G-protein-coupled receptors in prion disease pathophysiology using high-throughput functional CRISPR assays integrated with spike-resolution optical readout of neuronal activity.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Genetic Modifiers of Microglia-Dependent Disease Etiology

Cody Smith, Ph.D.,
University of Notre Dame

Beth Stevens, Ph.D.,
Broad Institute of MIT and Harvard

Description: to integrate multiple CRISPR-screens on imaging platforms to identify oligogenic combinations that modify microglial behavior and exacerbate pathological functions in neurodegenerative pathologies.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Homeostatic Neuroprotection

Graeme Davis, Ph.D.,
University of California, San Francisco

Jeanne Paz, Ph.D.,
Gladstone Institute of Neurological Disease and University of California, San Francisco

Kira Poskanzer, Ph.D.,
University of California, San Francisco

Description: to leverage systems immunology tools developed by the Jiang Lab to profile single T-cells that infiltrate into the brain during neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Human Proteome Atlas of Maturing Neuroimmune Cells

Alban Ordureau, Ph.D.,
Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center

Lorenz Studer, M.D., Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center

Description: to uncover the unique proteome landscape of subtypes of human iPSC-derived neuron and glial cells that contribute to the neuroimmune axis and are linked to the etiology of neurodegenerative diseases.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Illuminating Organelle Dynamics in Development and Neurodegeneration


Sarah Cohen, Ph.D.,
University of North Carolina at Chapel Hill


Mohanish Deshmukh, Ph.D.,
University of North Carolina at Chapel Hill


Serena Yeung, Ph.D.,
Stanford University

Description: to develop a platform for simultaneous, dynamic imaging of eight organelles in multiple cell types derived from iPSC lines, inviting questions about the contribution of specific genes, genotypes, and organelle dynamics.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Imaging and Modeling Neuromodulation in Zebrafish Sleep


David Prober, Ph.D.,
California Institute of Technology


Matthew Thomson, Ph.D.,
California Institute of Technology

Description: to use zebrafish whole-brain neuromodulator and calcium imaging, analysis, and modeling to identify neuronal mechanisms that underlie circadian and homeostatic regulation of sleep.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Impact of Altered Light Perception on Sleep in Alzheimer's Disease


Antoine Adamantidis, Ph.D.,
University of Bern


Ludovic S. Mure, Ph.D.,
Universitätsklinik für Augenheilkunde,
Inselspital and University of Bern

Description: to characterize the functional alterations of ipRGCs and their brain targets implicated in alertness and sleep in Alzheimer's disease and to determine their time course relative to cognitive decline.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Impact of Sleep on Immune and Cognitive Functions


Shinjae Chung, Ph.D.,
University of Pennsylvania


Yeong Shin Yim, Ph.D.,
University of Pennsylvania

Description: to elucidate how sleep deprivation undermines the immune system and compromises distinct neural circuits regulating cognitive functions in health and neurodegenerative conditions.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

In Vivo CRISPR to Uncover Determinants of Neurodegeneration


Alejandro Chavez, M.D., Ph.D.,
Columbia University


Vincenzo A. Gennarino, Ph.D.,
Columbia University Irving Medical Center


Xin Jin, Ph.D.,
Scripps Research Institute


Vilas Menon, Ph.D..
Columbia University


Serge Przedborski, M.D., Ph.D.,
Columbia University

Description: to develop a new high-throughput *in vivo* (mouse) CRISPR/Cas9 method to screen for genetic modifiers of Spinocerebellar ataxia in the adult mouse brain.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

In Vivo Forward Genetic CRISPR Screens for Glaucoma Modifiers

Xin Duan, Ph.D.,
University of California, San Francisco

Yang Hu, M.D., Ph.D.,
Stanford University

Stanley Qi, Ph.D.,
Stanford University

Description: to deploy CRISPR in an *in vivo* genome-wide screen for modifiers of optic nerve degeneration in mouse glaucoma models, taking advantage of the accessibility of the visual system in the mouse models for screening and intervention.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Increasing mRNA Translation to Treat Neurodegeneration

Sergej Djuranovic, Ph.D.,
Washington University School of Medicine in St. Louis

Timothy Miller, Ph.D.,
Washington University School of Medicine in St. Louis

Slavica Pavlovic Djuranovic, Ph.D.,
Washington University School of Medicine in St. Louis

Description: to develop a new ASO approach to upregulate protein expression for disease mutations affecting FTD and ALS.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Interoceptive Vascular Plasticity in Neurodegeneration

Osama Harraz, Ph.D.,
University of Vermont

Thomas Longden, Ph.D.,
University of Maryland Baltimore

Description: to elucidate how the loss of interoceptive mechanisms driving plasticity in vascular signaling pathways to adapt blood delivery to areas of neuronal energy need contributes to neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Investigating Sleep Through an Evolutionary Approach

Giorgio F. Gilestro, Ph.D.,
Imperial College London

Lucia Prieto-Godino, Ph.D.,
The Francis Crick Institute

Description: to combine CRISPR-powered genetics and machine-learning-powered behavioral analysis to study how sleep evolved in its regulation and function in a selected group of the Drosophila genus.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Lipid Dynamics and Mitochondrial Metabolism in Neurodegenerative Disease

Pietro De Camilli, M.D.,
Yale University and HHMI

Hongying Shen, Ph.D.,
Yale University

Description: to use imaging and cell biology tools, reverse genetics, and metabolomics to learn more about how mitochondrial defects and metabolic perturbation caused by mutations in lipid transport proteins lead to neurodegenerative conditions, in the hopes of identifying novel biomarkers of disease progression.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Long-Term Single-Cell Physiology Recording in Live Brains

Denise Cai, Ph.D.,
Icahn School of Medicine at Mount Sinai

Changyang Linghu, Ph.D.,
University of Michigan

Description: to bring technological breakthroughs to scalably record and analyze spatial and temporal structures of single-cell gene expression in the living brain underlying learning and neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Longitudinal Full-length, Single Molecule RNA Analysis in Neurodegenerative

Robert Spitale, Ph.D.,
University of California, Irvine

Leslie Thompson, Ph.D.,
University of California, Irvine

Description: to deploy cutting-edge transcriptional analyses, including single-molecule long-read sequencing technologies, on human patient-derived samples for Huntington's disease for a longitudinal assessment of the changing RNA landscape in disease progression.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Machine Learning the Biomolecular Basis of Memory Persistence

André Fenton, Ph.D.,
New York University

Stefano Martiniani, Ph.D.,
New York University

Description: to develop machine learning approaches spanning multiple levels of biology (multi-omic/ neurophysiology/behavior) to reveal the biomolecular substrates responsible for memory storage and persistence.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Mapping Expression Dynamics of Neurodegenerative Disease Genes

Atsushi Fukuda, Ph.D.,
Tokai University, School of Medicine

Natsuhiko Kumasaka, Ph.D.,
Institute of Medical Science,
The University of Tokyo

Description: to leverage a CRISPR activation/ interference screening in non-coding GWAS regions to elucidate gene regulations underlying the resilience failure in mid-brain DP neurons derived from hPSCs.

Award: Collaborative Pairs Project Awards (Cycle 2) (Phase 1)

Mapping Non-AUG Translation Initiation in Neurodegeneration

Jay Brito Querido, Ph.D.,
University of Michigan

Peter K. Todd, M.D., Ph.D.,
University of Michigan

Description: to use cryo-electron microscopy and biochemical approaches to elucidate the mechanism underlying repeat-associated non-AUG translation initiation in neurodegeneration associated with short tandem repeat expansion disorders.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Mechanisms of White Matter Aging

Ozgun Gokce, Ph.D.,
Institute for Stroke and Dementia Research

Mikael Simons, M.D.,
Technical University Munich and German Center for Neurodegenerative Diseases

Description: to establish causality of the pathological processes underpinning the finding that white-matter aging is associated with myelin degeneration which results in reactive microglia that accumulate in nodules where they clear myelin debris.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Membrane Damage and Repair in Neurodegeneration


Jeremy Carlton, Ph.D.,
King's College London


Soyon Hong, Ph.D.,
University College London


Adrian Isaacs, Ph.D.,
University College London


Michael Ward, M.D., Ph.D.,
National Institute of Health


Caleb Webber, Ph.D.,
Cardiff University

Description: to test the novel hypothesis that membrane repair pathways are critical in a variety of neurodegenerative conditions, providing insight into if these pathways could be novel neuroprotective factors and lead to new therapeutic interventions.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Metabolic Control of Circuits Supporting Memory


Priyamvada Rajasethupathy, M.D., Ph.D.,
The Rockefeller University


Timothy Ryan, Ph.D.,
Weill Cornell Medicine

Description: to leverage novel metabolic sensors and actuators in defined neural circuits to assess their impact on synaptic function, cellular physiology, and memory performance.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

MicroRNA Regulation of Neuronal Mitochondrial Homeostasis


Tatjana Kleele, Ph.D.,
ETH Zurich


Gerhard Schratt, Ph.D.,
ETH Zurich

Description: to leverage an antisense-based screening platform in human iPSC-derived neurons to elucidate human-specific microRNAs controlling mitochondrial homeostasis in neuronal health and disease.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Molecular Dissection of Metabolic Homeostasis Pathways in Neurons


Marc Hammarlund, Ph.D.,
Yale University


Gulcin Pekkurnaz, Ph.D.,
University of California San Diego

Description: to understand the molecular pathways at the intersection of mitochondrial function, neuronal metabolism, and neurodegeneration *in vivo*, using both mouse and invertebrate systems.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Molecular Mechanisms of Intergenerational Memory


Bianca Jones Marlin, Ph.D.,
Columbia University


Jason Shepherd, Ph.D.,
University of Utah

Description: to investigate the role of Arc capsids in mediating mechanisms of learning and intergenerational inheritance of experiences.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Molecular Systems Neuroscience of Memory Persistence

Kevin Bolding, Ph.D.,
Monell Chemical Senses Center

Olivier Pertz, Ph.D.,
University of Bern

Description: to identify molecular determinants of memory persistence using concurrent *in vivo* imaging of neural activity and intracellular signaling dynamics as novel neural memory traces develop and decay.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Molecularly-Defined Neural Cells Altered by Alzheimer's Disease Pathology

Joseph Castellano, Ph.D.,
Icahn School of Medicine at Mount Sinai

Herbert Wu, Ph.D.,
Icahn School of Medicine at Mount Sinai

Description: to establish the impact of Alzheimer's disease pathology on molecularly-defined neural cells during cognitive tasks using sequentially-coupled calcium imaging with spatial transcriptomics.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Molecules of Mind — Epigenetics of Memory Network Dynamics

David Dupret, Ph.D.,
University of Oxford

Johannes Gräff, Ph.D.,
Ecole Polytechnique Fédérale de Lausanne

Description: to unravel how learning-induced transient neuronal activity is translated into a cell assembly's lasting epigenetic code to store acquired memories.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Neuroimmune Crosstalk Along the Gut-Brain Axis in Parkinson's Disease

Dario Alessi, Ph.D.,
University of Dundee

Tim Bartels, Ph.D.,
University College London and
UK Dementia Research Institute

Kenneth Harris, Ph.D.,
University College London

Soyon Hong, Ph.D.,
University College London and
UK Dementia Research Institute

Neil Ranson, Ph.D.,
Astbury Centre for Structural Molecular Biology, University of Leeds

Description: to study a potential role for the Parkinson's associated gene, LRRK2, in gut macrophages and signaling in the enteric nervous system.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Neuroimmune Mechanisms Linking Sleep and Alzheimer's Disease

Xiaoning Han, M.D., Ph.D.,
Johns Hopkins University

Mark Wu, M.D., Ph.D.,
Johns Hopkins University

Description: to assess the impact of sleep fragmentation on neuroinflammation and BBB function in an Alzheimer's disease mouse model and leverage the discovery of a novel sleep drive circuit to ameliorate these phenotypes.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Neurolipid Atlas

Martin Giera, Ph.D.,
Leiden University Medical Center

Priyanka Narayan, Ph.D.,
National Institutes of Health

Rik van der Kant, Ph.D.,
Vrije Universiteit Amsterdam
and Amsterdam University

Description: to generate the first map of disease-specific, genotype-specific and cell type-specific changes in the human lipidome associated with neurodegenerative diseases.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Neuronal Phenotypes of Impaired Nascent Protein Quality Control

Wade Harper, Ph.D.,
Harvard Medical School

Sichen (Susan) Shao, Ph.D.,
Harvard Medical School

Description: to apply cutting-edge molecular and biochemical methods to investigate how RQC contributes to protein homeostasis in neurodegeneration, exploring a potential new pathway for novel therapeutic targets.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

New Roles of the Proteasome in Preventing Neurodegeneration

Esteban O. Mazzoni, Ph.D.,
New York University

Christine Vogel, Ph.D.,
New York University

Description: to explore their novel hypothesis that membrane-localized proteasome may act as a neuroprotectant in disease-resistant motor neurons.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

New Subcellular Microenvironment-Mapping Tools for CNS

David Shechner, Ph.D.,
University of Washington

Gene Yeo, Ph.D.,
University of California San Diego

Description: to leverage and further develop new RNA-targeted microenvironment-mapping tools in patient-derived neurons, elucidating molecular mechanisms by which repeat-expansion RNAs drive neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Next Generation Neuropathology: Proximity-Proteomics of Proteinopathies

Melissa E. Murray, Ph.D.,
Mayo Clinic Florida

Wilfried Rossoll, Ph.D.,
Mayo Clinic Florida

Description: to establish a next generation approach to neuropathology, developing a method to determine the protein composition of disease-causing aggregates in human brain tissue.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Organelle Metabolism and Neurodegeneration: Lysosomes

Monther Abu-Remaileh, Ph.D., Stanford University

Natalia Gomez-Ospina, M.D., Ph.D., Stanford University

Description: to identify all lysosomal metabolism genes contributing to neurodegeneration by combining high-throughput reverse genetics with functional multi-omics to investigate the lysosomal proteome.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Pooled Mapping of Neuronal Proteome Localization

Pablo Gonzalez Camara, Ph.D., University of Pennsylvania

Ophir Shalem, Ph.D., Children's Hospital of Philadelphia, University of Pennsylvania

Description: to use a novel pooled gene tagging approach with high throughput microscopy and optical sequencing to map subcellular localization of hundreds of endogenous proteins in disease iPSC-derived neurons.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Probing Circuits for Sleep-Dependent Memory Consolidation

Daniel Aharoni, Ph.D., University of California, Los Angeles

Gina Poe, Ph.D., University of California, Los Angeles

Description: to test the mechanisms of sleep-dependent memory consolidation using long-term wearable miniscopes that track and manipulate neural activity with single-cell precision in freely behaving animals.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Probing Sleep Biology Using Temporal Genetic Switches

Karyn Esser, Ph.D., University of Florida

Eric Wang, Ph.D., University of Florida

Description: to leverage tissue-specific and temporal genetic switches to study how the central nervous system and peripheral tissues regulate circadian rhythms and sleep.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Promoting Neuronal Cell Death to Mitigate Widespread Neurodegeneration

David Simon, Ph.D., Weill Cornell Medicine

Trent Watkins, Ph.D., Baylor College of Medicine

Description: to investigate the idea that early cell death of diseased neurons may initially serve as a protective mechanism that slows disease spread.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Quantifying Single-Cell Spatial Brain Aging and Rejuvenation

Anne Brunet, Ph.D., Stanford University

James Zou, Ph.D., Stanford University

Description: to develop innovative ways to quantify brain aging taking into account spatial features, with the goal of developing targeted strategies to counter cognitive decline and neurodegenerative diseases.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1 and 2)

Regulation of Central Nervous System Transcellular Communication

Philip De Jager, M.D., Ph.D.,
Columbia University

Martin Kampmann, Ph.D.,
University of California, San Francisco

Description: to elucidate regulatory mechanisms that enable one brain cell to influence the determination of the state of neighboring cells, and to characterize their perturbation in disease.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

RNA as the Nexus Between Sleep and Neurodegeneration

Ravi Allada, M.D.,
University of Michigan

Swathi Yadlapalli, Ph.D.,
University of Michigan

Description: to apply high throughput genetics in neurodegeneration models, sleep-dependent transcriptomics, and single molecule RNA subcellular localization to reveal the links between sleep and neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

RNA-Protein Interaction Sequencing for Tandem Repeat Disorders

Mandana Arbab, Ph.D.,
Boston Children's Hospital

Richard Sherwood, Ph.D.,
Brigham and Women's Hospital

Description: to study the sequestration of RNA binding proteins as a common pathogenic mechanism of neurodegeneration in tandem repeat disorders using a transformative new technology that leverages genome editing tools.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Search for Sleep-Enabled Memory Engram in the Amygdala

Shawn Liu, Ph.D.,
Columbia University

Yueqing Peng, Ph.D.,
Columbia University

Description: to bridge a knowledge gap between the memory function of sleep and the epigenetic mechanism of memory in the basolateral amygdala, a critical brain structure for emotion and memory.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Sleep and Aging: Can Sleep Ameliorate Age-Driven Neurodegeneration?

Noelle L'Etoile, Ph.D.,
University of California, San Francisco

Brendon O. Watson, M.D. Ph.D.,
University of Michigan

Description: to identify circuit-activity-based tools to leverage sleep as a therapeutic agent for age-dependent neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Sleep and Circadian-Driven Nuclear Multi-Omics Dynamics

Angel Barco, Ph.D.,
Consejo Superior de Investigaciones Cientificas

Maria Robles, Ph.D.,
LMU, Munich

Description: to unravel the role of homeostatic sleep and circadian-driven processes in nuclear function of diverse mouse brain cell populations combining cell-type specific nuclear isolation with deep sequencing and quantitative proteomics.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Sleep-Mediated Brain Functions and Neurocognitive Aging

Sara Aton, Ph.D.,
University of Michigan

Catherine Kaczorowski, Ph.D.,
University of Michigan

Description: to identify sleep-dependent and sleep-independent neurophysiological and biosynthetic processes linked to cognitive resilience during aging, and in the context of Alzheimer's disease risk alleles.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Small RNA-Bound Amyloid Beta Aggregates in Human Alzheimer's Disease Brain

Ryan Flynn, M.D., Ph.D.,
Boston Children's Hospital

Andrew Stern, M.D., Ph.D.,
Brigham and Women's Hospital

Description: to combine biochemical study of postmortem Alzheimer's disease brain tissue with new extracellular RNA analysis to define the RNA-bound diffusible amyloid beta complex and its toxicity.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Solving the Mystery of Immune Regulation by Sleep

Michael Lazarus, Ph.D.,
International Institute for Integrative Sleep Medicine

Haruka Ozaki, Ph.D.,
Institute of Medicine at the University of Tsukuba

Description: to establish a biologic and computational platform to study sleep effects on immune cells, identify molecular factors involved in sleep-immune homeostasis, and elucidate their underlying action mechanisms.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Spatial Analysis of Aberrant RNA Isoforms in ALS Neuromuscular Organoids

Mina Gouti, Ph.D.,
Max Delbrück Center for Molecular Medicine in the Helmholtz Association

Nikolaus Rajewsky, Ph.D.,
Max Delbrück Center for Molecular Medicine in the Helmholtz Association

Description: to combine a novel culture system — 3D human neuromuscular organoids that model the intact neuromuscular circuitry — with spatial transcriptomics and new long-read sequencing methods to understand the gene expression changes and RNA modifications associated with ALS genes implicated in RNA regulation.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Spatial and Functional Identities of Brain Lipid Droplets

Jeeyun Chung, Ph.D.,
Harvard University

Joongkyu Park, Ph.D.,
Wayne State University

Description: to establish cell-type-specific and functional catalogs of lipid droplets in brains and CRISPR-based human iPSC models of lipid dysregulation to elucidate brain lipid metabolism in health and disease.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Synapse-Microglia Signaling Mechanisms for Proteostasis

Takanari Inoue, Ph.D.,
Johns Hopkins University

Shigeki Watanabe, Ph.D.,
Johns Hopkins University

Description: to study interactions between synapses and microglia by developing approaches to induce synthetic synapse-microglia interactions and to visualize the functions of synapses using imaging and neurophysiological methods.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Synaptic Protein Interactions in Hibernation

Fan Liu, Ph.D.,
Leibniz-Forschungsinstitut für Molekulare Pharmakologie

Patrik Verstreken, Ph.D.,
VIB-KU Leuven Center for Brain and Disease Research

Description: to identify pathways and protein interactions hamsters use to degrade and regenerate synapses during cycles of hibernation and test if these can be used to counteract synapse loss in dementia.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Systematic High Content Optical Pooled Genetic Screens in Neurodegeneration

Paul C. Blainey, Ph.D.,
The Broad Institute of MIT and Harvard

Ankur Jain, Ph.D.,
Whitehead Institute for Biomedical Research

Clotilde Lagier-Tourenne, M.D., Ph.D.,
Massachusetts General Hospital

Description: to develop an innovative platform for the discovery of new therapeutic targets in neurodegenerative diseases by applying optical genetic screens in patient fibroblasts and human neurons.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Using Optical Pooled Screening Technology as a Model for Neurodegenerative Research

How do we identify new strategies for treatments of neurodegenerative diseases? Dr. Clotilde Lagier-Tourenne and Dr. Paul Blainey developed a platform to discover new therapeutic targets for ALS and frontotemporal dementia. Their research used optical genetic screens, which combine high-quality imaging with in-situ sequencing analysis.

In their first phase of study, Clotilde and Paul worked to discover how neurodegenerative diseases impact the appearance of a cell and its organelles. In the second phase, they expanded this research to new cellular systems most relevant to disease. They used optical pooled screening technology in neurons made from iPSC, which can then be used as a model for further research on neurodegenerative diseases. Through this technology, it's possible to screen for genetic perturbations that change a cell's structure or behavior, helping identify new disease mechanisms more efficiently. The pair added collaborator Dr. Ankur Jain in this second phase of study.

Systems Genetic Analysis of Alzheimer's Disease Histopathology

Maya Kasowski, M.D. Ph.D.,
Stanford University

Christopher Keene, M.D. Ph.D.,
University of Washington

Description: to leverage a state-of-the-art Alzheimer's disease cell atlas to map cell-type-specific genetic effects in their neuropathologic context.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Targeted Brain Immunotherapy with Engineered Cytokines

Ethan Lippmann, Ph.D.,
Vanderbilt University

Jamie Spangler, Ph.D.,
Johns Hopkins University

Description: to engineer cytokines that elicit immune responses in the brain specifically at sites of neurodegeneration, such as around amyloid beta aggregates.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

TDP-43 and T Cells: Exploring a Bidirectional Link in Neurodegeneration

Pietro Fratta, M.D. Ph.D.,
University College London and
The Francis Crick Institute

Ning (Jenny) Jiang, Ph.D.,
University of Pennsylvania

Description: to uncover the possible bidirectional link between TDP-43 proteinopathy and immune activation/dysregulation in neurodegeneration.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

TDP-43 Mislocalization in Aging and Neurodegeneration

Alessandro Ori, Ph.D.,
Leibniz Institute on Aging,
Fritz Lipmann Institute

Hemali Phatnani, Ph.D.,
New York Genome Center

Lars Steinmetz, Ph.D.,
European Molecular Biology Laboratory
Stanford University

Michael Ward, M.D., Ph.D.,
National Institutes of Health

Description: to address the intersection of aging and genetics using complementary genomic, biochemical, and proteomic approaches in human iPSC-derived neurons, using an *in vivo* killifish model of aging with an initial focus on TDP-43's role in proteostasis, a gene associated with ALS/FTD and other neurodegenerative diseases.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

The Mitochondrial RNA Structurome as Mediator of Neurological Disease

Antoni Barrientos, Ph.D.,
University of Miami Health System

Silvia Rouskin, Ph.D.,
Whitehead Institute for Biomedical Research

Description: to leverage new methods for RNA structure profiling and high throughput sequencing applied to gene-edited human cultured cells to characterize the structural landscape of the mitochondrial transcriptome and how it contributes to mitochondrial neurological disorders.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

The Physical Biology of Neurodegeneration

Liam Holt, Ph.D.,
New York University School of Medicine

Hemali Phatnani, Ph.D.,
New York Genome Center

Description: to take a biophysical approach, using genetically encoded nanoparticles to track what's happening inside neurons during phases of protein aggregation.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

The Role of Pink1/Parkin in the Intestinal Epithelium

Ming Guo, M.D., Ph.D.,
University of California, Los Angeles

Elizabeth J. Videlock, M.D., Ph.D.,
University of California, Los Angeles

Description: to colon biopsy-derived human tissue cultures and organoids to investigate the role of Pink1/Parkin signaling in mitochondrial function in the intestinal epithelium.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Tools to Measure Neural Input-Output Operations

Adam Charles, Ph.D.,
Johns Hopkins University

Kaspar Podgorski, Ph.D.,
Allen Institute for Neural Dynamics

Description: to combine newly developed neurotransmitter indicators, imaging hardware, processing algorithms, and inference methods to build a pipeline for measuring neural computations *in vivo*.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Transcriptional and Epigenetic Encoding of Sleep Loss

Elizabeth Pollina, Ph.D.,
Washington University in St. Louis

Dragana Rogulja, Ph.D.,
Harvard University

Description: to uncover conserved, tissue-specific responses to sleep loss using single-cell genomic profiling in flies and mice and functional screens for factors promoting tissue health during sleep restriction.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Tuning Memory by Altering Amyloid Properties

Lukasz Joachimiak, Ph.D.,
University of Texas Southwestern Medical Center

Kausik Si, Ph.D.,
Stowers Institute for Medical Research

Description: to employ a computational design approach to test the role of amyloid assembly kinetics and stability on the formation and duration of long-term memory in animal models.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Understanding Neuronal Vulnerability to Degeneration in Parkinson's Disease

Maria Soledad Esposito, Ph.D.,
Centro Atomico Bariloche, Consejo Nacional de Investigaciones Cientificas y Tecnicas

Ignacio E. Schor, Ph.D.,
Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas

Description: to address this question of selective vulnerability for Parkinson's disease by using single-cell transcriptomic methods to compare gene expression across multiple vulnerable and resistant neuronal subtypes in a Parkinson's disease mouse model.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Unraveling Anti-IgLON5 Disease Tauopathy with Proteomics

Luís Ribeiro, Ph.D.,
University of Coimbra

Jeffrey Savas, Ph.D.,
Northwestern University

Description: to determine the IgLON5 interactome and identify proteins with impaired turnover in a model of inflammatory-dependent tauopathy (anti-IgLON5 disease) to identify therapeutic targets for tauopathies.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Using Computer Vision to Annotate Cryo-Electron Tomograms of Neurons

Wah Chiu, Ph.D.,
Stanford University

Serena Yeung, Ph.D.,
Stanford University

Description: to develop computer vision and deep learning algorithms to annotate and model subcellular and macromolecular structures in 3D cryo-EM tomograms.

See a graphic representation of their work on the next page.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1)

Using Human and Fly Genetics to Identify New Circadian Genes

Qili Liu, Ph.D.,
University of California, San Francisco

Louis Ptacek, M.D.,
University of California, San Francisco

Description: to use a hybrid approach with human and fly genetics to prioritize and characterize novel candidates regulating circadian entrainment.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

Using Sandpipers to Test the Essential Functions of Sleep

Chiara Cirelli, M.D., Ph.D.,
University of Wisconsin-Madison

John Lesku, Ph.D.,
La Trobe University

Description: to perform molecular and ultrastructural analyses to quantify the synaptic and cellular effects of extreme chronic sleep loss in the sandpiper, a model of sustained high waking performance.

Award: Collaborative Pairs Pilot Project Awards (Cycle 2) (Phase 1)

VCP-Driven RNA: Protein Remodeling in Neurodegeneration

Stephanie Moon, Ph.D.,
University of Michigan

Nils Walter, Ph.D.,
University of Michigan

Description: to deploy multiplexed single-molecule imaging analysis to track the development of RNA-protein stress granules at high spatio-temporal resolution in new cell culture models of ALS and FTD, including human motor neurons and glia derived from iPS cells.

Award: Collaborative Pairs Pilot Project Awards (Cycle 1) (Phase 1 and 2)

Two Stanford Researchers Are Using AI To See More in the Brain

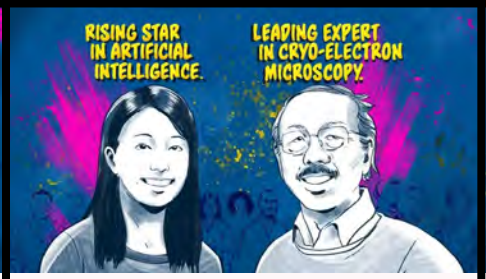
Stanford researchers Wah Chiu and Serena Yeung bring unconventional perspectives to bear on brain research. He has been pioneering the use of cryo-electron microscopy to see molecules at the atomic scale. She explores new approaches to artificial intelligence and machine learning for biomedicine and healthcare. They leveraged their joint technical expertise toward revealing the inner workings of neurons in unprecedented detail, as part of a project supported by the NDCN.

Imagine an alien visiting a city on Earth for the first time. How would it make sense of everything going on?

Neuroscience has a similar problem. Despite a lot of technological progress, people don't really know all the details of what happens inside a neuron.

If we want to understand how the brain works and how to fix it, we need better ways to look inside it and its cells.

Microscopes can show us some pieces, including individual molecules. But putting the pieces together is complicated.



We think artificial intelligence (AI) can help. We are not neuroscientists. But we have a lot of friends who are!

We applied computer vision, a field of AI that enables computers and systems to derive meaningful information from digital images, captured from microscopes, to identify and classify molecules and their interactions.

This allowed us to compare healthy cells to unhealthy cells with mutations linked to Huntington's disease. Our findings can also help us develop data and workflows that could open the opportunity for broader application of computer vision and deep learning algorithms to other neurodegenerative diseases.

Adapted from a CZI Story originally by Devin Powell and Maki Naro, published in January 2022. See project here.

PATIENT-PARTNERED COLLABORATIONS (PPC) IN RARE NEURODEGENERATIVE DISEASES

This grant mechanism was established to foster collaborative teams that integrate patient-led rare disease organizations with research teams, thereby accelerating scientific discovery. Patients are central to the research conducted under this mechanism. Cycle 1 funded five PPC teams, with a total investment of $10 million.

PCH2cure: Revealing Disease Mechanisms to Cure PCH2

Samuel Gröschel, M.D., Ph.D.,
University of Tuebingen

Wibke Janzarik, M.D., Ph.D.,
University of Freiburg

Julia Matilainen, Ph.D.,
PCH-Familie e.V (Patient Organization PI)

Simone Mayer, Ph.D.,
University of Tuebingen (Coordinating PI)

Description: to reveal mechanisms of neurodegeneration in PCH2 through analysis of patient pathophysiology, biosamples and patient-derived cell models.

Closing the Knowledge Gaps in Lafora, a Fatal Neurodegenerative Disease

Maria Chahrour, Ph.D.,
University of Texas-Southwestern

Lena Ismail,
Chelsea's Hope Lafora Children Research Fund (Patient Organization PI)

Berge Minassian, M.D.,
University of Texas-Southwestern (Coordinating PI)

Sharmistha Mitra, Ph.D.,
University of Texas-Southwestern

Felix Nitschke, Ph.D.,
University of Texas-Southwestern

Description: to further research the molecular basis of Lafora disease and thus open the path to therapy by studying and uncovering the basic mechanisms of the disease.

CADASIL-centered Modeling of Immunovascular Neurodegenerative Disease

Joel Blanchard, Ph.D.,
Icahn School of Medicine at Mount Sinai

Fanny Elahi, M.D., Ph.D.,
Icahn School of Medicine at Mount Sinai (Coordinating PI)

Jane Gunther,
CureCADASIL (Patient Organization PI)

Towfique Raj, Ph.D.,
Icahn School of Medicine at Mount Sinai

Shrike Zhang, Ph.D., Brigham Women's Hospital / Harvard Medical School

Description: to reverse engineer CADASIL to discover therapeutic molecular targets using stem cell technologies in a deeply phenotyped cohort of patients.

A Cell Atlas of Batten Pathobiology and Therapeutic Response

Joseph Mazzulli, Ph.D.,
Northwestern University

Larry Sherman, Ph.D.,
Oregon Health and Science University

Julia Vitarello,
Mila's Miracle Foundation (Patient Organization PI)

Timothy Yu, M.D., Ph.D.,
Boston Children's Hospital (Coordinating PI)

Description: to generate a single-cell reference atlas of Batten disease pathobiology and therapeutic response from nonhuman models, patient iPSC neurons, and human autopsy specimens.

Investigating ATP1A3 Diseases in Cell and Animal Models

Simon Frost,
Hope for Annabel (Patient Organization PI)

David Liu, Ph.D.,
The Broad Institute of MIT and Harvard (Coordinating PI)

Cathleen Lutz, Ph.D.,
Jackson Laboratory, Rare Disease Translational Center

Kathleen Sweadner, Ph.D.,
Massachusetts General Hospital / Harvard Medical School

Kathryn Swoboda, M.D.,
Massachusetts General Hospital / Harvard Medical School

Description: to determine how *ATP1A3* mutation dominance causes Alternating Hemiplegia of Childhood by correcting the mutated gene and studying the effects on cell and mouse models.

COLLABORATIVE SCIENCE AWARD

This award provided funding for small interdisciplinary teams of investigators, typically comprising one to four members. Each group included a physician-scientist with active clinical engagement. Cycle 1 funded nine teams, with a total investment of $9.5 million.

Creating and Deploying a Toolkit for Human Microglia in Neurodegeneration


Elizabeth Bradshaw, Ph.D.,
Columbia University


Philip L. De Jager, M.D., Ph.D.,
Columbia University (Lead and Clinical PI)


Vilas Menon, Ph.D.,
Columbia University


Vladislav Petyuk, Ph.D.,
Pacific Northwest National Laboratory

Description: to generate key reference data and a microglia experimental toolkit to identify and target different human microglial subtypes, with plans to make these tools available to the broader research community.

Human Cell-Based Models for Bridging the Gap Between Genetics and Pathology


Steven Altschuler, Ph.D.,
University of California, San Francisco


Aimee Kao, M.D., Ph.D.,
University of California, San Francisco


William Seeley, M.D., Ph.D.,
University of California, San Francisco
(Lead and Clinical PI)


Lani Wu, Ph.D.,
University of California, San Francisco

Description: to use high-dimensional histologic and transcriptomic profiling, analyzed through innovative machine learning-based methods, to identify shared phenotypes within each paired system and across methods and patients.

Investigation of Nuclear Pore Complex Alterations in ALS/FTD


Jeffrey D. Rothstein, M.D., Ph.D.,
Johns Hopkins University (Clinical PI)


Gene Yeo, Ph.D.,
University of California, San Diego (Lead PI)

Description: to use iPSC technology and a combination of molecular, biochemical, and imaging approaches to determine cell type specific alterations in and the effect of ALS/FTD associated mutations on nuclear pores.

Molecular Mechanisms of the Central Regulator of TREM2 Dysfunction


Carlos Cruchaga, Ph.D.,
Washington University in St. Louis


Gregory Day, M.D.,
Mayo Clinic, Florida (Clinical PI)


Oscar Harari, Ph.D.,
Washington University in St. Louis


Celeste Karch, Ph.D.,
Washington University in St. Louis (Lead PI)

Description: to apply a novel systems biology approach that leverages multi-omics in stem cells and human tissues to elucidate the mechanisms by which common genetic drivers of neuroinflammation confer resilience to neurodegenerative disease.

Neuron-Derived Extracellular Vesicles for Biomarker Discovery in Neurodegeneration


Alice-Chen Plotkin, M.D.,
University of Pennsylvania (Clinical PI)


George Church, M.D.,
Harvard University


David Walt, Ph.D.,
Harvard University (Lead PI)

Description: to explore markers for neuronal EV isolation.

Probing Parkinson's Disease with Induced Human Microcircuits on CMOS Chips


Dries Braeken, Ph.D.,
Imec Leuven


Birgitt Schuele, M.D.,
Stanford University


Wim Vandenberghe, M.D., Ph.D.,
KU Leuven (Clinical PI)


Patrik Verstreken, Ph.D.,
VIB-KU Leuven (Lead PI)

Description: to develop the technology to produce mature human neuronal microcircuits relevant to Parkinson's disease on a multi-electrode array chip.

Profiling and Defining Parkinson's Disease


Ernest Arenas, M.D., Ph.D.,
Karolinska Institutet (Lead PI)


Sten Linnarsson, Ph.D.,
Karolinska Institutet


Mats Nilsson, Ph.D.,
Stockholm University


Per Svenningsson, M.D., Ph.D.,
Karolinska Institutet (Clinical PI)

Description: to systematically analyze and provide an unbiased understanding of the cell types and molecular mechanisms involved in Parkinson's Disease, enabling definition of molecular pathways, disease subtypes, markers and possible targets for future therapeutic intervention.

Role of Dysfunctional Astrocyte-Neuron Signaling in Parkinson's Disease


Nicole Calakos, M.D., Ph.D.,
Duke University (Clinical PI)


Cagla Eroglu, Ph.D.,
Duke University (Lead PI)


Albert La Spada, M.D., Ph.D.,
Duke University

Description: to test the functions of Parkinson's Disease genes in astrocytes as controllers of neuronal health and connectivity by applying novel molecular tools to manipulate these genes in both primary rodent and patient cell-derived astrocyte-neuron cultures and in transgenic mice, and utilize cell biological and physiological readouts of astrocyte-synapse signaling, synaptic connectivity, and neuronal health.

The Structural Basis of Protein Pathology in Tauopathy


Marc Diamond, M.D.,
UT Southwestern Medical Center (Lead PI)


Lukasz Joachimiak, Ph.D.,
UT Southwestern Medical Center


Charles White, M.D.,
UT Southwestern Medical Center (Clinical PI)

Description: to develop new tools to analyze tau from human brain tissues, with the goal of achieving more precise diagnosis based on quantitative measures.

OTHER GRANTEES

Separate from our RFA-based grant programs, we partnered with leading institutions and individuals across the neuroscience ecosystem, to advance the work of the NDCN and the broader field. To learn more about these grants, including full descriptions and dates, visit the CZI grants database.

Black in Neuro (2022): for general operating support.

Boston Children's Hospital (2021): to support the N=1 Collaborative, a new consortium of clinical and research experts to advance the development of oligonucleotide drugs as individualized medicine, including for treating rare neurological diseases.

Chan Zuckerberg Biohub Network (2022): to characterize CNS subcellular profiles using genetically engineered iPSC lines carrying fluorescent tags for organelle visualization and isolation and genetic variants causative for neurodegenerative diseases.

Children's Hospital of Philadelphia (2021): to connect postdoctoral fellows and graduate students at the University of Pennsylvania and the Children's Hospital of Philadelphia who research neurodegeneration.

Cold Spring Harbor Laboratory (2020): to provide subsidies for registration fees to the CSHL Neurodegeneration Meeting for attendees from institutions serving underrepresented populations and developing countries; and to support a CSHL Banbury Workshop for 30 practitioners and training course for 20 trainees focused on modernizing the field of neuropathology.

Columbia University (2021): to establish an open platform encouraging collaboration between computational and experimental researchers within the neurodegeneration research community of New York City and surroundings.

Duke University (2021): to expand biomarker research efforts in the neurodegeneration community in the Research Triangle by connecting them with the Cincinnati Cohort Biomarker Program and establishing a partnership.

Harvard Medical School (2022): to systematically optimize CRISPR tools to control inducible gene expression, for application in iPSC models of neurodegeneration.

Howard University (2024): to support a second cohort of the Neurodegeneration Computational Fellows (NDCF) Program.

Icahn School of Medicine at Mount Sinai (2024): Neuropathology: to leverage large digital whole slide image datasets for the development of AI/ML algorithms to measure human brain age-acceleration by linking genetics with histology, thereby identifying novel risk factors for neurodegeneration. (PI: John Crary, Co-PI: Gabriele Campanella)

Institute for Protein Innovation (2024): to develop protein affinity reagents to facilitate the molecular cartography of the neuronal synapse and create a toolbox for detecting glia cell types.

Lieber Institute for Brain Development (2022): for core support of the African Ancestries Neuroscience Research Initiative (AANRI) to ensure collection of datasets representative of differing human ethnicities.

Marine Biological Laboratory (2022–2024): for core support of the Summer Program in Neuroscience, Excellence and Success (SPINES); and to support the "Neurobiology: Mechanisms & Advanced Approaches" research training course for graduate students, post-doctoral fellows, and physician-scientists committed to fundamental, translational, or clinical studies in the field of neuroscience.

National Institutes of Health (2021): to support an entry-level workshop designed to empower NIH neuroscientists to engage with policymakers, advance neuroscience research priorities, and collaborate for larger impact.

Royal Netherlands Academy for Arts and Sciences (2024): to implement advanced AI models (large language models and self-supervised learning models) to process the extensive clinical and neuropathological data from the Netherlands Brain Bank for improved understanding of neurodegenerative disorders.

Society for Black Neuropsychology (2022): for general operating support.

Society for Neuroscience (2022): for core support of the Neuroscience Scholars Program.

Stanford University (2023): to support hands-on training to program 3D brain models for researchers in the NDCN.

The Jackson Laboratory (2021, 2022; 2023; 2023): to support the quality assessment and dissemination strategy for a large collection of genome-edited iPSC lines for the neurodegeneration research community; and to support a newly taught course for the use of iPSC lines to model human neurological disease-associated mutations, democratizing the use of the cell lines; and Neuropathology: to support the development of engineered iPSC lines as *in vitro* disease models for rare and ultra-rare neurodegenerative diseases.

University of California, Davis (2024): Neuropathology: to assess dementia pathology to leverage existing expertise and resources across institutions to develop and validate scalable, generalizable and computationally-efficient ML approaches for quantitative neuropathology assessments of digital whole slide images in Alzheimer's. (PI: Brittany Dugger, University of California, Davis, Co-PI: Chen-Nee Chuah, University of California, Davis, Co-PI: David Gutman, Emory University School of Medicine).

University of California, Irvine (2021): to support a two-day event consisting of lectures, discussion panels, and training sessions that aims to bridge the gap between patients, clinicians, and researchers within the neurodegeneration community.

University of California, San Diego (2022): to systematically optimize CRISPR tools to control inducible gene expression, for application in iPSC models of neurodegeneration.

University of California, San Francisco (2021; 2022; 2024): to forge collaborations and connections between neurodegeneration researchers in the San Francisco Bay Area; and to systematically optimize CRISPR tools to control inducible gene expression, for application in iPSC models of neurodegeneration; and Neuropathology: to accelerate neurodegeneration research by developing a suite of AI/ML tools capable of performing 3D feature segmentation for use on experimental neuropathology datasets, including confocal immunofluorescence images. (PI: William Seeley, UCSF, Co-PI: Serena Yeung-Levy, Stanford).

University of Cambridge (2021): to strengthen connections between groups of common research themes and increase patient and public knowledge of neurodegeneration research in Cambridge.

University of Edinburgh (2024): Neuropathology: to leverage the established SYNMAP method pipeline to generate a synaptome atlas of human cortex characterizing the molecular architecture of synapses across the lifespan. (PI: Colin Smith, Co-PI: Seth Grant).

University of Groningen (2024): Neuropathology: to implement advanced AI models to process the extensive clinical and neuropathological data from the Netherlands Brain Bank for improved understanding of neurodegenerative disorders. (PI: Inge Holtman, University of Groningen, Co-PI: Inge Huitinga, Royal Netherlands Academy for Arts and Sciences)

University of Massachusetts Medical School (2020): to support mentorship for early career grantees as part of the Neurodegeneration Challenge Network Mentors program.

University of Pennsylvania (2021; 2024; 2024): to support a seminar series exploring the intersection of the immune system and neurodegenerative disease; and to support a 2-day workshop focused on the immune system in neurodegeneration; and Neuropathology: to support advanced ultrastructural imaging of human brain tissue to develop advanced electron microscopy (EM) methods for the 3D ultrastructural analysis of pathologic human brain tissue, via the use of fluorescence small molecular chemical mapping methods to guide cryo-FIB milling for cryo-ET imaging. (PI: Edward Lee, Co-PI: Yi-Wei Chang).

University of Texas Southwestern Medical Center (2021): to produce a documentary film series to share the experience of dealing with neurodegenerative diseases through personal stories of patients, as well as scientists and doctors working to find solutions.

Utah Film Center (2024): to sponsor the film "Ask the Question", under development by filmmaker Pamela Green, about the life of neuroscientist Ben Barres, the namesake for the Ben Barres Early Career Acceleration Award.

Vanderbilt University (2021): to provide opportunities for neurodegeneration research trainees from under-represented groups in the Southeast to present research and discuss professional development outside their home institutions.

Vlaams Instituut voor Biotechnologie (2022): to support hands-on training to program 3D brain models for researchers in the NDCN.

Yale University (2021): to support a symposium to bring together trainees from around Connecticut to build connections that will facilitate multi-institutional collaborations focused on the cell biology of neurodegeneration.

ZappyLab (2018, 2019): to build an online community of neurodegeneration researchers and expand an online Human Cell Atlas community to increase knowledge sharing and establish best practices in methods and approaches.

THE SCIENTIFIC MENTORS OF THE NDCN

CZI values the critical role that mentors and mentorship play in career development. We are grateful for the contributions of the NDCN mentors who provided mentorship for the Ben Barres Early Career Acceleration Award grantees, empowering them to reach their full scientific and professional potential.

- Katerina Akassoglou, Ph.D., Gladstone Institutes
- Frank Bradke, Ph.D., Deutsches Zentrum für Neurodegenerative Erkrankungen
- Robert Brown, M.D., University of Massachusetts Medical School
- Yang Dan, Ph.D., University of California, Berkeley
- Beverly Davidson, Ph.D., Perelman School of Medicine, University of Pennsylvania
- Philip De Jager, M.D., Ph.D., Columbia University Medical Center
- Marc Diamond, M.D., The University of Texas Southwestern Medical Center
- Marc Freeman, Ph.D., Oregon Health and Science University
- Li Gan, Ph.D., Weill Cornell Medical College
- Anirvan Ghosh, Ph.D., Unity Biotechnology
- Alison Goate, Ph.D., Icahn School of Medicine at Mount Sinai
- Michael Greenberg, Ph.D., Harvard University Medical School
- Bradley Hyman, M.D., Ph.D., Massachusetts General Hospital
- Wendell Lim, Ph.D., University of California, San Francisco
- Liqun Luo, Ph.D., Stanford University
- Christian Lüscher, M.D., Ph.D., University of Geneva
- Jeffrey Rothstein, M.D., Ph.D., Johns Hopkins University School of Medicine
- Mina Ryten, M.D., Ph.D., University of Cambridge
- Scott Soderling, Ph.D., Duke University
- Beth Stevens, Ph.D., Harvard University Medical School
- Leslie Thompson, Ph.D., University of California, Irvine
- David Walt, Ph.D., Wyss Institute, Harvard University
- Fan Wang, Ph.D., Massachusetts Institute of Technology
- Gene Yeo, Ph.D., University of California, San Diego
- Huda Zoghbi, M.D., Baylor College of Medicine

SELECTED PEER-REVIEWED SCIENCE FROM THE NDCN

The NDCN has demonstrated robust research output, with 937 publications and 390 preprints. Additionally, Challenge Network researchers have engaged in 959 collaborations, encompassing thought partnerships, resource sharing, co-submission of grants, and publications.

A clear indicator of the powerful network effect fostered by this program is the productive connections formed among NDCN grantees, that have resulted in co-publications and preprints. As of July 2025, these grantees have jointly advanced research and published 76 articles with collaborators across the network.

These 76 publications, listed below, serve as positive proof of the effectiveness of the Challenge Network approach in advancing the field.

2020

Scientific Reports: "Heavy Metals Contaminating the Environment of a Progressive Supranuclear Palsy Cluster Induce Tau Accumulation and Cell Death in Cultured Neurons"

Authors include Celeste M. Karch (Collaborative Science) and Aimee W. Kao (Collaborative Science)

Progressive supranuclear palsy (PSP) is a neurodegenerative disorder characterized by the presence of intracellular aggregates of tau protein and neuronal loss leading to cognitive and motor impairment. Occurrence is mostly sporadic, but rare family clusters

have been described. Although the etiopathology of PSP is unknown, mutations in the MAPT/tau gene and exposure to environmental toxins can increase the risk of PSP. Here, we used cell models to investigate the potential neurotoxic effects of heavy metals enriched in a highly industrialized region in France with a cluster of sporadic PSP cases. We found that iPSC-derived iNeurons from a MAPT mutation carrier tend to be more sensitive to cell death induced by chromium (Cr) and nickel (Ni) exposure than an isogenic control line. We hypothesize that genetic variations may predispose to neurodegeneration induced by those heavy metals. Furthermore, using an SH-SY5Y neuroblastoma cell line, we showed that both heavy metals induce cell death by an apoptotic mechanism. Interestingly, Cr and Ni treatments increased total and phosphorylated tau levels in both cell types, implicating Cr and Ni exposure in tau pathology. Overall, this study suggests that chromium and nickel could contribute to the pathophysiology of tauopathies such as PSP by promoting tau accumulation and neuronal cell death.

Nature: "LRP1 Is a Master Regulator of Tau Uptake and Spread"

Authors include Viviana Gradinaru (Early Career Acceleration) and Martin Kampmann (Early Career Acceleration)

The spread of protein aggregates during disease progression is a common theme underlying many neurodegenerative diseases. The microtubule-associated protein tau has a central role in the pathogenesis of several forms of dementia known as tauopathies—including Alzheimer's disease, frontotemporal dementia and chronic traumatic encephalopathy. Progression of these diseases is characterized by the sequential spread and deposition of protein aggregates in a predictable pattern that correlates with clinical severity. This observation and complementary experimental studies3,4 have suggested that tau can spread in a prion-like manner, by passing to naive cells in which it templates misfolding and aggregation. However, although the propagation of tau has been extensively studied, the underlying cellular mechanisms remain poorly understood. Here we show that the low-density lipoprotein receptor-related protein 1 (LRP1) controls the endocytosis of tau and its subsequent spread. Knockdown of LRP1 significantly reduced tau uptake in H4 neuroglioma cells and in induced pluripotent stem cell-derived neurons. The interaction between tau and LRP1 is mediated by lysine residues in the microtubule-binding repeat region of tau. Furthermore, downregulation of LRP1 in an *in vivo* mouse model of tau spread was found to effectively reduce the propagation of tau between neurons. Our results identify LRP1 as a key regulator of tau spread in the brain, and therefore a potential target for the treatment of diseases that involve tau spread and aggregation.

Cell Reports: "*Srebf1* Controls Midbrain Dopaminergic Neurogenesis"

Authors include Sten Linnarsson (Collaborative Science) and Ernest Arenas (Collaborative Science)

Liver X receptors (LXRs) and their ligands are potent regulators of midbrain dopaminergic (M.D.A) neurogenesis and differentiation. However, the molecular mechanisms by which LXRs control these functions remain to be elucidated. Here, we perform a combined transcriptome and chromatin immunoprecipitation sequencing (ChIP-seq) analysis of midbrain cells after LXR activation, followed by bioinformatic analysis to elucidate the transcriptional networks controlling M.D.A neurogenesis. Our results identify the basic helix-loop-helix transcription factor sterol regulatory element binding protein 1 (SREBP1) as part of a cluster of proneural transcription factors in radial glia and as a regulator of transcription factors controlling M.D.A neurogenesis, such as Foxa2. Moreover, loss- and gain-of-function experiments *in vitro* and *in vivo* demonstrate that Srebf1 is both required and sufficient for M.D.A neurogenesis. Our data, thus, identify Srebf1 as a central player in M.D.A neurogenesis.

Nature Neuroscience: "Three-Dimensional Genome Restructuring Across Timescales of Activity-Induced Neuronal Gene Expression"

Authors include Jason D Shepherd (Early Career Acceleration), Jennifer E Phillips-Cremins (Collaborative Pairs)

Neuronal activation induces rapid transcription of immediate early genes (IEGs) and longer-term chromatin remodeling around secondary response genes (SRGs). Here, we use high-resolution chromosome-conformation-capture carbon-copy sequencing (5C-seq) to elucidate the extent to which long-range chromatin loops are altered during short- and long-term changes in neural activity. We find that more than 10% of loops surrounding select IEGs, SRGs, and synaptic genes are induced de novo during cortical neuron activation. IEGs Fos and Arc connect to activity-dependent enhancers via singular short-range loops that form within 20 min after stimulation, prior to peak messenger RNA levels. By contrast, the SRG Bdnf engages in both pre-existing and activity-inducible loops that form within 1–6 h. We also show that common single-nucleotide variants that are associated with autism and schizophrenia are colocalized with distinct classes of activity-dependent, looped enhancers. Our data link architectural complexity to transcriptional kinetics and reveal the rapid timescale by which higher-order chromatin architecture reconfigures during neuronal stimulation.

Neuron: "G4C2 Repeat RNA Initiates a POM121-Mediated Reduction in Specific Nucleoporins in C9orf72 ALS/FTD"

Authors include Alyssa N. Coyne (Collaborative Science), Gene W. Yeo (Collaborative Science), and Jeffrey D. Rothstein (Collaborative Science)

Through mechanisms that remain poorly defined, defects in nucleocytoplasmic transport and accumulations of specific nuclear-pore-complex-associated proteins have been reported in multiple neurodegenerative diseases, including C9orf72 Amyotrophic Lateral Sclerosis and Frontotemporal Dementia (ALS/FTD). Using super-resolution structured illumination microscopy, we have explored the mechanism by which nucleoporins are altered in nuclei isolated from C9orf72 induced pluripotent stem-cell-derived neurons (iPSNs). Of the 23 nucleoporins evaluated, we observed a reduction in a subset of 8, including key components of the nuclear pore complex scaffold and the transmembrane nucleoporin POM121. Reduction in POM121 appears to initiate a decrease in the expression of seven additional nucleoporins, ultimately affecting the localization of Ran GTPase and subsequent cellular toxicity in C9orf72 iPSNs. Collectively, our data suggest that the expression of expanded C9orf72 ALS/FTD repeat RNA alone affects nuclear POM121 expression in the initiation of a pathological cascade affecting nucleoporin levels within neuronal nuclei and ultimately downstream neuronal survival.

Nature Communications: "Single Cell RNA Sequencing of Human Microglia Uncovers a Subset Associated With Alzheimer's Disease"

Authors include Marta Olah (Collaborative Science), Vilas Menon (Collaborative Science), Naomi Habib (Collaborative Pairs), Wassim Elyaman (Collaborative Pairs), Elizabeth M Bradshaw (Collaborative Science), and Philip L De Jager (Collaborative Science)

The extent of microglial heterogeneity in humans remains a central yet poorly explored question in light of the development of therapies targeting this cell type. Here, we investigate the population structure of live microglia purified from human cerebral cortex samples obtained at autopsy and during neurosurgical procedures. Using single cell RNA sequencing, we find that some subsets are enriched for disease-related genes and RNA signatures. We confirm the presence of four of these microglial subpopulations histologically and illustrate the utility of our data by characterizing further microglial cluster 7, enriched for genes depleted in the cortex of individuals with Alzheimer's disease (AD). Histologically, these cluster 7 microglia are reduced in frequency in AD tissue, and we validate this observation in an independent set of single nucleus data. Thus, our live human microglia identify a range of subtypes, and we prioritize one of these as being altered in AD.

2021

Nature Neuroscience: "Molecular Characterization of Selectively Vulnerable Neurons in Alzheimer's Disease"

Authors include William W. Seeley (Collaborative Science) and Martin Kampmann (Early Career Acceleration)

Alzheimer's disease (AD) is characterized by the selective vulnerability of specific neuronal populations, the molecular signatures of which are largely unknown. To identify and characterize selectively vulnerable neuronal populations, we used single-nucleus RNA sequencing to profile the caudal entorhinal cortex and the superior frontal gyrus — brain regions where neurofibrillary inclusions and neuronal loss occur early and late in AD, respectively — from postmortem brains spanning the progression of AD-type tau neurofibrillary pathology. We identified RORB as a marker of selectively vulnerable excitatory neurons in the entorhinal cortex and subsequently validated their depletion and selective susceptibility to neurofibrillary inclusions during disease progression using quantitative neuropathological methods. We also discovered an astrocyte subpopulation, likely representing reactive astrocytes, characterized by decreased expression of genes involved in homeostatic functions. Our characterization of selectively vulnerable neurons in AD paves the way for future mechanistic studies of selective vulnerability and potential therapeutic strategies for enhancing neuronal resilience.

Neuron: "White Matter Aging Drives Microglial Diversity"

Authors include Ozgun Gokce (Collaborative Pairs) and Mikael Simons (Collaborative Pairs)

Aging results in gray and white matter degeneration, but the specific microglial responses are unknown. Using single-cell RNA sequencing from white and gray matter separately, we identified white matter-associated microglia (WAMs), which share parts of the disease-associated microglia (DAM) gene signature and are characterized by activation of genes implicated in phagocytic activity and lipid metabolism. WAMs depend on triggering receptor expressed on myeloid cells 2 (TREM2) signaling and are aging dependent. In the aged brain, WAMs form independent of apolipoprotein E (APOE), in contrast to mouse models of Alzheimer's disease, in which microglia with the

WAM gene signature are generated prematurely and in an APOE-dependent pathway similar to DAMs. Within the white matter, microglia frequently cluster in nodules, where they are engaged in clearing degenerated myelin. Thus, WAMs may represent a potentially protective response required to clear degenerated myelin accumulating during white matter aging and disease.

Nature Methods: "L1CAM Is Not Associated With Extracellular Vesicles in Human Cerebrospinal Fluid or Plasma"

Authors include Alice S Chen-Plotkin (Collaborative Science), George M Church (Collaborative Science), and David R Walt (Collaborative Science)

L1CAM is a transmembrane protein expressed on neurons that was presumed to be found on neuron-derived extracellular vesicles (NDEVs) in human biofluids. We developed a panel of single-molecule array assays to evaluate the use of L1CAM for NDEV isolation. We demonstrate that L1CAM is not associated with extracellular vesicles in human plasma or cerebrospinal fluid and therefore recommend against its use as a marker in NDEV isolation protocols.

2022

Nature: "TDP-43 Loss and ALS-Risk SNPs Drive Mis-Splicing and Depletion of *UNC13A*"

Authors include Hemali Phatnani (Collaborative Pairs), Towfique Raj (Patient Partnered Collaboration), Michael E. Ward (Collaborative Pairs), and Pietro Fratta (Collaborative Pairs)

Variants of *UNC13A*, a critical gene for synapse function, increase the risk of amyotrophic lateral sclerosis and frontotemporal dementia1,2,3, two related neurodegenerative diseases defined by mislocalization of the RNA-binding protein TDP-434,5. Here we show that TDP-43 depletion induces robust inclusion of a cryptic exon in *UNC13A*, resulting in nonsense-mediated decay and loss of *UNC13A* protein. Two common intronic *UNC13A* polymorphisms strongly associated with amyotrophic lateral sclerosis and frontotemporal dementia risk overlap with TDP-43 binding sites. These polymorphisms potentiate cryptic exon inclusion, both in cultured cells and in brains and spinal cords from patients with these conditions. Our findings, which demonstrate a genetic link between loss of nuclear TDP-43 function and disease, reveal the mechanism by which *UNC13A* variants exacerbate the effects of decreased TDP-43 function. They further provide a promising therapeutic target for TDP-43 proteinopathies.

Nature: "Clonally Expanded CD8 T Cells Characterize Amyotrophic Lateral Sclerosis-4"

Authors include Ning Jiang (Early Career Acceleration), Albert R. La Spada (Collaborative Science), and Ivan Marazzi (Early Career Acceleration)

Amyotrophic lateral sclerosis (ALS) is a heterogenous neurodegenerative disorder that affects motor neurons and voluntary muscle control. ALS heterogeneity includes the age of manifestation, the rate of progression and the anatomical sites of symptom onset. Disease-causing mutations in specific genes have been identified and define different subtypes of ALS1. Although several ALS-associated genes have been shown to affect immune functions, whether specific immune features account for ALS heterogeneity is poorly understood. Amyotrophic lateral sclerosis-4 (ALS4) is characterized by juvenile onset and slow progression. Patients with ALS4 show motor difficulties by the time that they are in their thirties, and most of them require devices to assist with walking by their fifties. ALS4 is caused by mutations in the senataxin gene (SETX). Here, using Setx knock-in mice that carry the ALS4-causative L389S mutation, we describe an immunological signature that consists of clonally expanded, terminally differentiated effector memory (TEMRA) CD8 T cells in the central nervous system and the blood of knock-in mice. Increased frequencies of antigen-specific CD8 T cells in knock-in mice mirror the progression of motor neuron disease and correlate with anti-glioma immunity. Furthermore, bone marrow transplantation experiments indicate that the immune system has a key role in ALS4 neurodegeneration. In patients with ALS4, clonally expanded TEMRA CD8 T cells circulate in the peripheral blood. Our results provide evidence of an antigen-specific CD8 T cell response in ALS4, which could be used to unravel disease mechanisms and as a potential biomarker of disease state.

Neuron: "Single-Cell Transcriptome Analysis of Regenerating RGCs Reveals Potent Glaucoma Neural Repair Genes"

Authors include Lei S. Qi (Collaborative Pairs) and Yang Hu (Collaborative Pairs)

Axon regeneration holds great promise for neural repair of CNS axonopathies, including glaucoma. Pten deletion in retinal ganglion cells (RGCs) promotes potent optic nerve regeneration, but only a small population of Pten-null RGCs are actually regenerating RGCs (regRGCs); most surviving RGCs (surRGCs) remain non-regenerative. Here, we developed a strategy to specifically label and purify regRGCs and surRGCs, respectively, from the same Pten-deletion

mice after optic nerve crush, in which they differ only in their regeneration capability. Smart-Seq2 single-cell transcriptome analysis revealed novel regeneration-associated genes that significantly promote axon regeneration. The most potent of these, Anxa2, acts synergistically with its ligand tPA in Pten-deletion-induced axon regeneration. Anxa2, its downstream effector ILK, and Mpp1 dramatically protect RGC somata and axons and preserve visual function in a clinically relevant model of glaucoma, demonstrating the exciting potential of this innovative strategy to identify novel effective neural repair candidates.

Nature Neuroscience: "CD8+ T Cells Induce Interferon-Responsive Oligodendrocytes and Microglia in White Matter Aging"

Authors include Ozgun Gokce (Collaborative Pairs) and Mikael Simons (Collaborative Pairs)

A hallmark of nervous system aging is a decline of white matter volume and function, but the underlying mechanisms leading to white matter pathology are unknown. In the present study, we found age-related alterations of oligodendrocyte cell state with a reduction in total oligodendrocyte density in aging murine white matter. Using single-cell RNA-sequencing, we identified interferon (IFN)-responsive oligodendrocytes, which localize in proximity to CD8+ T cells in aging white matter. Absence of functional lymphocytes decreased the number of IFN-responsive oligodendrocytes and rescued oligodendrocyte loss, whereas T-cell checkpoint inhibition worsened the aging response. In addition, we identified a subpopulation of lymphocyte-dependent, IFN-responsive microglia in the vicinity of the CD8+ T cells in aging white matter. In summary, we provide evidence that CD8+ T-cell-induced, IFN-responsive oligodendrocytes and microglia are important modifiers of white matter aging.

Nature Neuroscience: "CRISPRi Screens in Human Ipsc-Derived Astrocytes Elucidate Regulators of Distinct Inflammatory Reactive States"

Authors include Ethan S. Lippmann (Early Career Investigator) and Martin Kampmann (Early Career Investigator)

Astrocytes become reactive in response to insults to the central nervous system by adopting context-specific cellular signatures and outputs, but a systematic understanding of the underlying molecular mechanisms is lacking. In this study, we developed CRISPR interference screening in human induced pluripotent stem cell-derived astrocytes coupled to single-cell transcriptomics to systematically interrogate cytokine-induced inflammatory astrocyte reactivity. We found that autocrine–paracrine IL-6 and interferon signaling downstream of canonical NF-κB activation drove two distinct inflammatory reactive signatures, one promoted by STAT3 and the other inhibited by STAT3. These signatures overlapped with those observed in other experimental contexts, including mouse models, and their markers were upregulated in human brains in Alzheimer's disease and hypoxic-ischemic encephalopathy. Furthermore, we validated that markers of these signatures were regulated by STAT3 *in vivo* using a mouse model of neuroinflammation. These results and the platform that we established have the potential to guide the development of therapeutics to selectively modulate different aspects of inflammatory astrocyte reactivity.

Nature Communications: "Reactive Astrocytes Transduce Inflammation in a Blood-Brain Barrier Model Through a TNF-STAT3 Signaling Axis and Secretion of Alpha 1-Antichymotrypsin"

Authors include Martin Kampmann (Early Career Investigator) and Ethan S. Lippmann (Early Career Investigator)

Astrocytes are critical components of the neurovascular unit that support blood-brain barrier (BBB) function. Pathological transformation of astrocytes to reactive states can be protective or harmful to BBB function. Here, using a human induced pluripotent stem cell (iPSC)-derived BBB co-culture model, we show that tumor necrosis factor (TNF) transitions astrocytes to an inflammatory reactive state that causes BBB dysfunction through activation of STAT3 and increased expression of SERPINA3, which encodes alpha 1-antichymotrypsin (α1ACT). To contextualize these findings, we correlated astrocytic STAT3 activation to vascular inflammation in postmortem human tissue. Further, in murine brain organotypic cultures, astrocyte-specific silencing of Serpina3n reduced vascular inflammation after TNF challenge. Last, treatment with recombinant Serpina3n in both *ex vivo* explant cultures and *in vivo* was sufficient to induce BBB dysfunction-related molecular changes. Overall, our results define the TNF-STAT3-α1ACT signaling axis as a driver of an inflammatory reactive astrocyte signature that contributes to BBB dysfunction.

Cell Stem Cell: "A Reference Human Induced Pluripotent Stem Cell Line for Large-Scale Collaborative Studies"

Authors include Justin A. McDonough (iPSC/CRISPR WG, Jackson Laboratory), Priyanka Narayan (Collaborative

Pairs, iPSC/CRISPR WG), Rik van der Kant (Collaborative Pairs), Martin Kampmann (Early Career Acceleration, iPSC/CRISPR WG), Patrik Verstreken (Collaborative Science), Birgitt Schüle (Collaborative Science, iPSC/CRISPR WG), Mohanish Deshmukh (Collaborative Pairs), Sarah Cohen (Collaborative Pairs, iPSC/CRISPR WG), Deborah Kronenberg-Versteeg (Collaborative Pairs, iPSC/CRISPR WG), Ernest Arenas (Collaborative Science, iPSC/CRISPR WG), William C. Skarnes (iPSC/CRISPR Working Group, Jackson Laboratory), Michael E Ward (Collaborative Pairs, iPSC/CRISPR WG), and Florian T. Merkle (Early Career Acceleration Award, iPSC/CRISPR WG)

Human induced pluripotent stem cell (iPSC) lines are a powerful tool for studying development and disease, but the considerable phenotypic variation between lines makes it challenging to replicate key findings and integrate data across research groups. To address this issue, we sub-cloned candidate human iPSC lines and deeply characterized their genetic properties using whole genome sequencing, their genomic stability upon CRISPR-Cas9-based gene editing, and their phenotypic properties including differentiation to commonly used cell types. These studies identified KOLF2.1J as an all-around well-performing iPSC line. We then shared KOLF2.1J with groups around the world who tested its performance in head-to-head comparisons with their own preferred iPSC lines across a diverse range of differentiation protocols and functional assays. On the strength of these findings, we have made KOLF2.1J and its gene-edited derivative clones readily accessible to promote the standardization required for large-scale collaborative science in the stem cell field.

Nature Neuroscience: "Integrative Transcriptomic Analysis of the Amyotrophic Lateral Sclerosis Spinal Cord Implicates Glial Activation and Suggests New Risk Genes"

Authors include Hemali Phatnani (Collaborative Pairs), Pietro Fratta (Collaborative Pairs), and Towfique Raj (Patient-Partner Collaboration)

Amyotrophic lateral sclerosis (ALS) is a progressively fatal neurodegenerative disease affecting motor neurons in the brain and spinal cord. In this study, we investigated gene expression changes in ALS via RNA sequencing in 380 postmortem samples from cervical, thoracic and lumbar spinal cord segments from 154 individuals with ALS and 49 control individuals. We observed an increase in microglia and astrocyte gene expression, accompanied by a decrease in oligodendrocyte gene expression. By creating a gene co-expression network in the ALS samples, we identified several activated microglia modules that negatively correlate with retrospective disease duration. We mapped molecular quantitative trait loci and found several potential ALS risk loci that may act through gene expression or splicing in the spinal cord and assign putative cell types for FNBP1, ACSL5, SH3RF1 and NFASC. Finally, we outline how common genetic variants associated with splicing of C9orf72 act as proxies for the well-known repeat expansion, and we use the same mechanism to suggest ATXN3 as a putative risk gene.

2023

eLife: "Maximizing CRISPRi Efficacy and Accessibility With Dual-SgRNA Libraries and Optimal Effectors"

Authors include Michael E Ward (Collaborative Pairs) and Martin Kampmann (Early Career Acceleration)

CRISPR interference (CRISPRi) enables programmable, reversible, and titratable repression of gene expression (knockdown) in mammalian cells. Initial CRISPRi-mediated genetic screens have showcased the potential to address basic questions in cell biology, genetics, and biotechnology, but wider deployment of CRISPRi screening has been constrained by the large size of single guide RNA (sgRNA) libraries and challenges in generating cell models with consistent CRISPRi-mediated knockdown. Here, we present next-generation CRISPRi sgRNA libraries and effector expression constructs that enable strong and consistent knockdown across mammalian cell models. First, we combine empirical sgRNA selection with a dual-sgRNA library design to generate an ultra-compact (1–3 elements per gene), highly active CRISPRi sgRNA library. Next, we compare CRISPRi effectors to show that the recently published Zim3-dCas9 provides an excellent balance between strong on-target knockdown and minimal non-specific effects on cell growth or the transcriptome. Finally, we engineer a suite of cell lines with stable expression of Zim3-dCas9 and robust on-target knockdown. Our results and publicly available reagents establish best practices for CRISPRi genetic screening.

bioRxiv: "Nuclease-Dead *S. aureus* Cas9 Downregulates Alpha-Synuclein and Reduces mtDNA Damage and Oxidative Stress Levels in Patient-Derived Stem Cell Model of Parkinson's Disease"

Authors include Stanley Qi (Collaborative Pairs) and Birgitt Schüle (Collaborative Science)

Parkinson's disease (PD) is one of the most common neurodegenerative diseases, but no disease modifying therapies have been successful in clinical translation presenting a major unmet medical need. A promising target is alpha-synuclein or its aggregated form, which accumulates in the brain of PD patients as Lewy bodies. While it is not entirely clear which alpha-synuclein protein species is disease relevant, mere overexpression of alpha-synuclein in hereditary forms leads to neurodegeneration. To specifically address gene regulation of alpha-synuclein, we developed a CRISPR interference (CRISPRi) system based on the nuclease dead S. aureus Cas9 (SadCas9) fused with the transcriptional repressor domain Krueppel-associated box to controllably repress alpha-synuclein expression at the transcriptional level. We screened single guide (sg)RNAs across the SNCA promoter and identified several sgRNAs that mediate downregulation of alpha-synuclein at varying levels. CRISPRi downregulation of alpha-synuclein in iPSC-derived neuronal cultures from a patient with an SNCA genomic triplication showed functional recovery by reduction of oxidative stress and mitochondrial DNA damage. Our results are proof-of-concept *in vitro* for precision medicine by targeting the SNCA gene promoter. The SNCA CRISPRi approach presents a new model to understand safe levels of alpha-synuclein downregulation and a novel therapeutic strategy for PD and related alpha-synucleinopathies.

Neuron: "A Cellular Taxonomy of the Adult Human Spinal Cord"

Authors include Michael E. Ward (Collaborative Pairs), Hemali Phatnani (Collaborative Pairs), and Vilas Menon (Collaborative Science)

The mammalian spinal cord functions as a community of cell types for sensory processing, autonomic control, and movement. While animal models have advanced our understanding of spinal cellular diversity, characterizing human biology directly is important to uncover specialized features of basic function and human pathology. Here, we present a cellular taxonomy of the adult human spinal cord using single-nucleus RNA sequencing with spatial transcriptomics and antibody validation. We identified 29 glial clusters and 35 neuronal clusters, organized principally by anatomical location. To demonstrate the relevance of this resource to human disease, we analyzed spinal motoneurons, which degenerate in amyotrophic lateral sclerosis (ALS) and other diseases. We found that compared with other spinal neurons, human motoneurons are defined by genes related to cell size, cytoskeletal structure, and ALS, suggesting a specialized molecular repertoire underlying their selective vulnerability. We include a web resource to facilitate further investigations into human spinal cord biology.

Molecular Cell: "What Repeat Expansion Disorders Can Teach Us About the Central Dogma"

Authors include Eric T. Wang (Early Career Acceleration), Ankur Jain (Collaborative Pairs), and Peter K. Todd (Collaborative Pairs)

Pathogenic repeat sequences underlie several human disorders, including amyotrophic lateral sclerosis, Huntington's disease, and myotonic dystrophy. Here, we speak to several researchers about how repeat sequences have been implicated in affecting all aspects of the Central Dogma of molecular biology through their effects on DNA, RNA, and protein.

medRxiv: "MS4A4A Modifies the Risk of Alzheimer Disease by Regulating Lipid Metabolism and Immune Response in a Unique Microglia State"

Authors include Martin Kampmann (Early Career Acceleration), Oscar Harari (Collaborative Science), and Celeste M. Karch (Collaborative Science)

Genome-wide association studies (GWAS) have identified many modifiers of Alzheimer disease (AD) risk enriched in microglia. Two of these modifiers are common variants in the MS4A locus (rs1582763: protective and rs6591561: risk) and serve as major regulators of CSF sTREM2 levels. To understand their functional impact on AD, we used single nucleus transcriptomics to profile brains from carriers of these variants. We discovered a "chemokine" microglial subpopulation that is altered in MS4A variant carriers and for which MS4A4A is the major regulator. The protective variant increases MS4A4A expression and shifts the chemokine microglia subpopulation to an interferon state, while the risk variant suppresses MS4A4A expression and reduces this subpopulation of microglia. Our findings provide a mechanistic explanation for the AD variants in the MS4A locus. Further, they pave the way for future mechanistic studies of AD variants and potential therapeutic strategies for enhancing microglia resilience in AD pathogenesis.

Nature Communications: "CryoET Reveals Organelle Phenotypes in Huntington Disease Patient ipsc-Derived and Mouse Primary Neurons"

Authors include Serena Yeung (Collaborative Pairs), Leslie M. Thompson (Collaborative Pairs), and Wah Chiu (Collaborative Pairs)

Huntington's disease (HD) is caused by an expanded CAG repeat in the huntingtin gene, yielding a Huntingtin protein with an expanded polyglutamine tract. While experiments with patient-derived induced pluripotent stem cells (iPSCs) can help understand disease, defining pathological biomarkers remains challenging. Here, we used cryogenic electron tomography to visualize neurites in HD patient iPSC-derived neurons with varying CAG repeats, and primary cortical neurons from BACHD, deltaN17-BACHD, and wild-type mice. In HD models, we discovered sheet aggregates in double membrane-bound organelles, and mitochondria with distorted cristae and enlarged granules, likely mitochondrial RNA granules. We used artificial intelligence to quantify mitochondrial granules, and proteomics experiments reveal differential protein content in isolated HD mitochondria. Knockdown of Protein Inhibitor of Activated STAT1 ameliorated aberrant phenotypes in iPSC- and BACHD neurons. We show that integrated ultrastructural and proteomic approaches may uncover early HD phenotypes to accelerate diagnostics and the development of targeted therapeutics for HD.

Journal of Biological Chemistry: "Membrane Lipid Remodeling Modulates γ-Secretase Processivity"

Authors include Mikael Simons (Collaborative Pairs) and Martin Giera (Collaborative Pairs)

Imbalances in the amounts of amyloid-β peptides (Aβ) generated by the membrane proteases β- and γ-secretase are considered as a trigger of Alzheimer's disease (AD). Cell-free studies of γ-secretase have shown that increasing membrane thickness modulates Aβ generation but it has remained unclear if these effects are translatable to cells. Here we show that the very long-chain fatty acid erucic acid (EA) triggers acyl chain remodeling in AD cell models, resulting in substantial lipidome alterations which included increased esterification of EA in membrane lipids. Membrane remodeling enhanced γ-secretase processivity, resulting in the increased production of the potentially beneficial Aβ37 and/or Aβ38 species in multiple cell lines. Unexpectedly, we found that the membrane remodeling stimulated total Aβ secretion by cells expressing WT γ-secretase but lowered it for cells expressing an aggressive familial AD mutant γ-secretase. We conclude that EA-mediated modulation of membrane composition is accompanied by complex lipid homeostatic changes that can impact amyloidogenic processing in different ways and elicit distinct γ-secretase responses, providing critical implications for lipid-based AD treatment strategies.

Neuron: "Gasdermin-E Mediates Mitochondrial Damage in Axons and Neurodegeneration"

Authors include: Clotilde Lagier-Tourenne (Collaborative Pairs), Alice S. Chen-Plotkin (Collaborative Science), and Isaac M. Chiu (Early Career Acceleration)

Mitochondrial dysfunction and axon loss are hallmarks of neurologic diseases. Gasdermin (GSDM) proteins are executioner pore-forming molecules that mediate cell death, yet their roles in the central nervous system (CNS) are not well understood. Here, we find that one GSDM family member, GSDME, is expressed by both mouse and human neurons. GSDME plays a role in mitochondrial damage and axon loss. Mitochondrial neurotoxins induced caspase-dependent GSDME cleavage and rapid localization to mitochondria in axons, where GSDME promoted mitochondrial depolarization, trafficking defects, and neurite retraction. Frontotemporal dementia (FTD)/amyotrophic lateral sclerosis (ALS)-associated proteins TDP-43 and PR-50 induced GSDME-mediated damage to mitochondria and neurite loss. GSDME knockdown protected against neurite loss in ALS patient iPSC-derived motor neurons. Knockout of GSDME in SOD1G93A ALS mice prolonged survival, ameliorated motor dysfunction, rescued motor neuron loss, and reduced neuroinflammation. We identify GSDME as an executioner of neuronal mitochondrial dysfunction that may contribute to neurodegeneration.

Acta Neuropathologica: "TREM2 Gene Expression Associations With Alzheimer's Disease Neuropathology Are Region-Specific: Implications for Cortical Versus Subcortical Microglia"

Authors include Vilas Menon (Collaborative Science) and Philip De Jager, (Collaborative Science)

Previous post-mortem assessments of TREM2 expression and its association with brain pathologies have been limited by sample size. This study sought to correlate region-specific TREM2 mRNA expression with diverse neuropathological measures at autopsy using a large sample size (N=945) of bulk RNA sequencing data from the Religious Orders Study and Rush Memory and Aging Project (ROS/MAP). TREM2 gene expression of the dorsolateral prefrontal cortex, posterior cingulate cortex, and caudate nucleus was assessed with respect to core pathology of Alzheimer's disease (amyloid-β, and tau), cerebrovascular pathology (cerebral infarcts, arteriolosclerosis, atherosclerosis,

and cerebral amyloid angiopathy), microglial activation (proportion of activated microglia), and cognitive performance. We found that cortical TREM2 levels were positively related to AD diagnosis, cognitive decline, and amyloid-β neuropathology but were not related to the proportion of activated microglia. In contrast, caudate TREM2 levels were not related to AD pathology, cognition, or diagnosis, but were positively related to the proportion of activated microglia in the same region. Diagnosis-stratified results revealed caudate TREM2 levels were inversely related to AD neuropathology and positively related to microglial activation and longitudinal cognitive performance in AD cases. These results highlight the notable changes in TREM2 transcript abundance in AD and suggest that its pathological associations are brain-region-dependent.

Stem Cell Reports: "Granulin Loss of Function in Human Mature Brain Organoids Implicates Astrocytes in TDP-43 Pathology"

Authors include Martin Kampmann (Early Career Acceleration) and Michael E. Ward (Collaborative Pairs)

Loss of function (LoF) of TAR-DNA binding protein 43 (TDP-43) and mis-localization, together with TDP-43-positive and hyperphosphorylated inclusions, are found in post-mortem tissue of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) patients, including those carrying LoF variants in the progranulin gene (GRN). Modeling TDP-43 pathology has been challenging *in vivo* and *in vitro*. We present a three-dimensional induced pluripotent stem cell (iPSC)-derived paradigm — mature brain organoids (mbOrg) — composed of cortical-like-astrocytes (iA) and neurons. When devoid of GRN, mbOrgs spontaneously recapitulate TDP-43 mis-localization, hyperphosphorylation, and LoF phenotypes. Mixing and matching genotypes in mbOrgs showed that GRN−/− iA are drivers for TDP-43 pathology. Finally, we rescued TDP-43 LoF by adding exogenous progranulin, demonstrating a link between TDP-43 LoF and progranulin expression. In conclusion, we present an iPSC-derived platform that shows striking features of human TDP-43 proteinopathy and provides a tool for the mechanistic modeling of TDP-43 pathology and patient-tailored therapeutic screening for FTD and ALS.

bioRxiv: "RNA- and ATAC-Sequencing Reveals a Unique CD83+ Microglial Population Focally Depleted in Parkinson's Disease"

Authors include P.L. De Jager (Collaborative Science), V. Menon (Collaborative Science), E.M. Bradshaw (Collaborative Science) and S. Przedborski (Collaborative Pairs)

All brain areas affected in Parkinson's disease (PD) show an abundance of microglia with an activated morphology together with increased expression of pro-inflammatory cytokines, suggesting that neuroinflammation may contribute to the neurodegenerative process in this common and incurable disorder. We applied a single nucleus RNA- and ATAC-sequencing approach using the 10x Genomics Chromium platform to postmortem PD samples to investigate microglial heterogeneity in PD. We created a multiomic dataset using substantia nigra (SN) tissues from 19 PD donors and 14 non-PD controls (NPCs), as well as three other brain regions from the PD donors which are differentially affected in this disease: the ventral tegmental area (VTA), substantia inominata (SI), and hypothalamus (HypoTs). We identified thirteen microglial subpopulations within these tissues as well as a perivascular macrophage and a monocyte population, of which we characterized the transcriptional and chromatin repertoires. Using this data, we investigated whether these microglial subpopulations have any association with PD and whether they have regional specificity. We uncovered several changes in microglial subpopulations in PD, which appear to parallel the magnitude of neurodegeneration across these four selected brain regions. Specifically, we identified that inflammatory microglia in PD are more prevalent in the SN and differentially express PD-associated markers. Our analysis revealed the depletion of a CD83 and HIF1A-expressing microglial subpopulation, specifically in the SN in PD, that has a unique chromatin signature compared to other microglial subpopulations. Interestingly, this microglial subpopulation has regional specificity to the brainstem in non-disease tissues. Furthermore, it is highly enriched for transcripts of proteins involved in antigen presentation and heat-shock proteins, and its depletion in the PD SN may have implications for neuronal vulnerability in disease.

Nature Communications: "Functional Gene Delivery to and Across Brain Vasculature of Systemic AAVs With Endothelial-Specific Tropism in Rodents and Broad Tropism in Primates"

Authors include Cagla Eroglu (Collaborative Science) and Viviana Gradinaru (Early Career Acceleration)

Delivering genes to and across the brain vasculature efficiently and specifically across species remains a critical challenge for addressing neurological diseases. We have evolved adeno-associated virus (AAV9) capsids into vectors that transduce brain endothelial cells specifically and efficiently following systemic administration in wild-type mice with diverse genetic backgrounds, and in rats. These AAVs also exhibit superior transduction of the CNS across non-human primates (marmosets and rhesus macaques), and in *ex vivo* human brain slices, although the endothelial tropism is not conserved across species. The capsid

modifications translate from AAV9 to other serotypes such as AAV1 and AAV-DJ, enabling serotype switching for sequential AAV administration in mice. We demonstrate that the endothelial-specific mouse capsids can be used to genetically engineer the blood-brain barrier by transforming the mouse brain vasculature into a functional biofactory. We apply this approach to Hevin knockout mice, where AAV-X1-mediated ectopic expression of the synaptogenic protein Sparcl1/Hevin in brain endothelial cells rescued synaptic deficits.

Clinical Pharmacology and Therapeutics: "A Multistakeholder Perspective on Advancing Individualized Therapeutics"

Authors include Julia Vitarello (Patient-Partnered Collaboration) and Timothy Yu (Patient-Partnered Collaboration)

Precision medicine has evolved from the application of pharmacogenetic biomarkers to the prospective development of targeted therapies in patients with specific molecular/genetic subtypes of disease to truly "N-of-1" medicines targeted to very small numbers of patients — in some cases, a single identified patient. This latter iteration of precision medicine presents unprecedented opportunities for patients with severe, life-threatening, or life-limiting diseases. At the same time, these modalities present complex scientific, clinical, and regulatory challenges. To realize the promise of individualized medicines, a multistakeholder approach to streamlining medical diagnoses, advancing the technologies that enable development of these therapeutic modalities, and re-envisioning collaborative environments for access and evidence generation is of critical importance. Herein, we highlight some of these challenges and opportunities.

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Science Translational Medicine: "Mis-Spliced Transcripts Generate de Novo Proteins in TDP-43–Related ALS/FTD"

Authors include Alessandro Ori (Collaborative Pairs), Pietro Fratta (Collaborative Pairs) and Michael E. Ward (Collaborative Pairs)

Functional loss of TDP-43, an RNA binding protein genetically and pathologically linked to amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), leads to the inclusion of cryptic exons in hundreds of transcripts during disease. Cryptic exons can promote the degradation of affected transcripts, deleteriously altering cellular function through loss-of-function mechanisms. Here, we show that mRNA transcripts harboring cryptic exons generated de novo proteins in TDP-43–depleted human iPSC–derived neurons *in vitro*, and de novo peptides were found in cerebrospinal fluid (CSF) samples from patients with ALS or FTD. Using coordinated transcriptomic and proteomic studies of TDP-43–depleted human iPSC–derived neurons, we identified 65 peptides that mapped to 12 cryptic exons. Cryptic exons identified in TDP-43–depleted human iPSC–derived neurons were predictive of cryptic exons expressed in postmortem brain tissue from patients with TDP-43 proteinopathy. These cryptic exons produced transcript variants that generated de novo proteins. We found that the inclusion of cryptic peptide sequences in proteins altered their interactions with other proteins, thereby likely altering their function. Last, we showed that 18 de novo peptides across 13 genes were present in CSF samples from patients with ALS/FTD spectrum disorders. The demonstration of cryptic exon translation suggests new mechanisms for ALS/FTD pathophysiology downstream of TDP-43 dysfunction and may provide a potential strategy to assay TDP-43 function in patient CSF.

bioRxiv: "TDP-43 Dysregulation of Polyadenylation Site Selection Is a Defining Feature of RNA Misprocessing in ALS/FTD and Related Disorders"

Authors include Molly Gale Hammell (Early Career Acceleration) and Albert R. La Spada (Collaborative Science)

Nuclear clearance and cytoplasmic aggregation of the RNA-binding protein TDP-43 are observed in many neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS) and fronto- temporal dementia (FTD). Although TDP-43 dysregulation of splicing has emerged as a key event in these diseases, TDP-43 can also regulate polyadenylation; yet, this has not been adequately studied. Here, we applied the dynamic analysis of polyadenylation from RNA-seq (DaPars) tool to ALS/FTD transcriptome datasets, and report extensive alternative polyadenylation (APA) upon TDP-43 alteration in ALS/FTD cell models and postmortem ALS/FTD neuronal nuclei. Importantly, many identified APA genes highlight pathways implicated in ALS/FTD pathogenesis. To determine the functional significance of APA elicited by TDP-43 nuclear depletion, we examined microtubule affinity regulating kinase 3 (MARK3). Nuclear loss of TDP-43 yielded increased expression of MARK3 transcripts with longer 3′UTRs, resulting in greater transcript stability and elevated MARK3 protein levels, which promotes increased neuronal tau S262 phosphorylation. Our findings define changes in polyadenylation site selection as a previously unrecognized feature of TDP-43-driven disease pathology in ALS/FTD and highlight a potentially novel mechanistic link between TDP-43 dysfunction and tau regulation.

Nature Communications: "Orientation-Invariant Autoencoders Learn Robust Representations for Shape Profiling of Cells and Organelles"

Authors include Sarah Cohen (Collaborative Pairs) and Serena Yeung-Levy (Collaborative Pairs)

Cell and organelle shape are driven by diverse genetic and environmental factors and thus accurate quantification of cellular morphology is essential to experimental cell biology. Autoencoders are a popular tool for unsupervised biological image analysis because they learn a low-dimensional representation that maps images to feature vectors to generate a semantically meaningful embedding space of morphological variation. The learned feature vectors can also be used for clustering, dimensionality reduction, outlier detection, and supervised learning problems. Shape properties do not change with orientation, and thus we argue that representation learning methods should encode this orientation invariance. We show that conventional autoencoders are sensitive to orientation, which can lead to suboptimal performance on downstream tasks. To address this, we develop O2-variational autoencoder (O2-VAE), an unsupervised method that learns robust, orientation-invariant representations. We use O2-VAE to discover morphology subgroups in segmented cells and mitochondria, detect outlier cells, and rapidly characterize cellular shape and texture in large datasets, including in a newly generated synthetic benchmark.

bioRxiv: "NeuroTri2-VISDOT: An Open-Access Tool To Harness the Power of Second Trimester Human Single Cell Data To Inform Models of Mendelian Neurodevelopmental Disorder"

Authors include Rebecca C. Ahrens-Nicklas (Collaborative Pairs) and Elizabeth J. Bhoj (Collaborative Pairs)

Whole exome and genome sequencing, coupled with refined bioinformatic pipelines, have enabled improved diagnostic yields for individuals with Mendelian conditions and have led to the rapid identification of novel syndromes. For many Mendelian neurodevelopmental disorders (NDDs), there is a lack of pre-existing model systems for mechanistic work. Thus, it is critical for translational researchers to have an accessible phenotype- and genotype-informed approach for model system selection. Single-cell RNA sequencing data can be informative in such an approach, as it can indicate which cell types express a gene of interest at the highest levels across time. For Mendelian NDDs, such data for the developing human brain is especially useful. A valuable single-cell RNA sequencing dataset of the second trimester developing human brain was produced by Bhaduri et al in 2021, but access to these data can be limited by computing power and the learning curve of single-cell data analysis. To reduce these barriers for translational research on Mendelian NDDs, we have built the web-based tool, Neurodevelopment in Trimester 2 — VIsualization of Single cell Data Online Tool (NeuroTri2-VISDOT), for exploring this single-cell dataset, and we have employed it in several different settings to demonstrate its utility for the translational research community.

Journal of Neuroscience Methods: "Human Organotypic Brain Slice Cultures: A Detailed and Improved Protocol for Preparation and Long-Term Maintenance"

Authors include Henner Koch (Collaborative Pairs) and Thomas V. Wuttke (Collaborative Pairs)

The investigation of the human brain at cellular and microcircuit level remains challenging due to the fragile viability of neuronal tissue, inter- and intra-variability of the samples and limited availability of human brain material. Especially brain slices have proven to be an excellent source to investigate brain physiology and disease at cellular and small network level, overcoming the temporal limits of acute slices. Here we provide a revised, detailed protocol of the production and in-depth knowledge on long-term culturing of such human organotypic brain slice cultures for research purposes. We highlight the critical pitfalls of the culturing process of the human brain tissue and present exemplary results on viral expression, single-cell Patch-Clamp recordings, as well as multi-electrode array recordings as readouts for culture viability, enabling the use of organotypic brain slice cultures of these valuable tissue samples for basic neuroscience and disease modeling.

Molecular Cell: "Glycerophosphodiesters Inhibit Lysosomal Phospholipid Catabolism in Batten Disease"

Authors include Alessandro Ori (Collaborative Pairs) and Monther Abu-Remaileh (Collaborative Pairs)

Batten disease, the most prevalent form of neurodegeneration in children, is caused by mutations in the CLN3 gene, which encodes a lysosomal transmembrane protein. CLN3 loss leads to significant accumulation of glycerophosphodiesters (GPDs), the end products of glycerophospholipid catabolism in the lysosome. Despite GPD storage being robustly observed upon CLN3 loss, the role of GPDs in neuropathology remains unclear. Here, we demonstrate that GPDs act as potent inhibitors of glycerophospholipid catabolism in the lysosome using human cell lines and mouse models. Mechanistically, GPDs bind and competitively inhibit the lysosomal phospholipases PLA2G15 and PLBD2, which we establish to possess phospholipase B activity. GPDs effectively inhibit the rate-limiting

lysophospholipase activity of these phospholipases. Consistently, lysosomes of CLN3-deficient cells and tissues accumulate toxic lysophospholipids. Our work establishes that the storage material in Batten disease directly disrupts lysosomal lipid homeostasis, suggesting GPD clearance as a potential therapeutic approach to this fatal disease.

bioRxiv: "MASTR-Seq: Multiplexed Analysis of Short Tandem Repeats With Sequencing"

Authors include Kristen J. Brennand (Collaborative Pairs) and Jennifer E. Phillips-Cremins (Collaborative Pairs)

More than 60 human disorders have been linked to unstable expansion of short tandem repeat (STR) tracts. STR length and the extent of DNA methylation is linked to disease pathology and can be mosaic in a cell type-specific manner in several repeat expansion disorders. Mosaic phenomenon have been difficult to study to date due to technical bias intrinsic to repeat sequences and the need for multi-modal measurements at single-allele resolution. Nanopore long-read sequencing accurately measures STR length and DNA methylation in the same single molecule but is cost prohibitive for studies assessing a target locus across multiple experimental conditions or patient samples. Here, we describe MASTR-seq, Multiplexed Analysis of Short Tandem Repeats, for cost-effective, high-throughput, accurate, multi-modal measurements of DNA methylation and STR genotype at single-allele resolution. MASTR-seq couples long-read sequencing, Cas9-mediated target enrichment, and PCR-free multiplexed barcoding to achieve a >ten-fold increase in on-target read mapping for 8-12 pooled samples in a single MinION flow cell. We provide a detailed experimental protocol and computational tools and present evidence that MASTR-seq quantifies tract length and DNA methylation status for CGG and CAG STR loci in normal-length and mutation-length human cell lines. The MASTR-seq protocol takes approximately eight days for experiments and one additional day for data processing and analyses.

bioRxiv: "Lipid-siRNA Conjugate Accesses a Perivascular Transport Mechanism and Achieves Widespread and Durable Knockdown in the Central Nervous System"

Authors include Timothy M. Miller (Collaborative Pairs) and Ethan S. Lippmann (Early Career Acceleration)

Short-interfering RNA (siRNA) has gained significant interest for treatment of neurological diseases by providing the capacity to achieve sustained inhibition of nearly any gene target. Yet, achieving efficacious drug delivery throughout deep brain structures of the CNS remains a considerable hurdle. We herein describe a lipid-siRNA conjugate that, following delivery into the cerebrospinal fluid (CSF), is transported effectively through perivascular spaces, enabling broad dispersion within CSF compartments and through the CNS parenchyma. We provide a detailed examination of the temporal kinetics of gene silencing, highlighting potent knockdown for up to five months from a single injection without detectable toxicity. Single-cell RNA sequencing further demonstrates gene silencing activity across diverse cell populations in the parenchyma and at brain borders, which may provide new avenues for neurological disease-modifying therapies.

Nature Neuroscience: "T Cell-Mediated Microglial Activation Triggers Myelin Pathology in a Mouse Model of Amyloidosis"

Authors include Gokce O (Collaborative Pairs) and Simons M (Collaborative Pairs)

Age-related myelin damage induces inflammatory responses, yet its involvement in Alzheimer's disease remains uncertain, despite age being a major risk factor. Using a mouse model of Alzheimer's disease, we found that amyloidosis itself triggers age-related oligodendrocyte and myelin damage. Mechanistically, CD8+ T cells promote the progressive accumulation of abnormally interferon-activated microglia that display myelin-damaging activity. Thus, our data suggest that immune responses against myelinating oligodendrocytes may contribute to neurodegenerative diseases with amyloidosis.

Biological Psychiatry: "A Single-Nucleus Transcriptome-Wide Association Study Implicates Novel Genes in Depression Pathogenesis"

Authors include Charles C. White (Collaborative Science), Naomi Habib (Collaborative Pairs), Vilas Menon (Collaborative Science) and Philip L. De Jager (Collaborative Science)

Depression, a common psychiatric illness and global public health problem, remains poorly understood across different life stages, which hampers the development of novel treatments. To identify new candidate genes for therapeutic development, we performed differential gene expression analysis of single-nucleus RNA sequencing data from the dorsolateral prefrontal cortex of older adults (n = 424) in relation to antemortem depressive symptoms. Additionally, we integrated genome-wide association study results for depression (n = 500,199) along with genetic tools for inferring the expression of 14,048 unique genes in seven cell types and 52 cell subtypes to perform a transcriptome-wide association study of depression followed by Mendelian randomization. Our single-nucleus transcriptome-wide association study analysis identified 68 candidate genes for depression

and showed the greatest number being in excitatory and inhibitory neurons. Of the 68 genes, 53 were novel compared to previous studies. Notably, gene expression in different neuronal subtypes had varying effects on depression risk. Traits with high genetic correlations with depression, such as neuroticism, shared more transcriptome-wide association study genes than traits that were not highly correlated with depression. Complementing these analyses, differential gene expression analysis across 52 neocortical cell subtypes showed that genes such as KCNN2, SCAI, WASF3, and SOCS6 were associated with late-life depressive symptoms in specific cell subtypes. These two sets of analyses illustrate the utility of large single-nucleus RNA sequencing data both to uncover genes whose expression is altered in specific cell subtypes in the context of depressive symptoms and to enhance the interpretation of well-powered genome-wide association studies so that we can prioritize specific susceptibility genes for further analysis and therapeutic development.

bioRxiv (accepted in Nature Metabolism): "The Neurolipid Atlas: A Lipidomics Resource for Neurodegenerative Diseases Uncovers Cholesterol as a Regulator of Astrocyte Reactivity Impaired By ApoE4"

Authors include Derks RJE (NDCN iPSC/CRISPR Working Group), Ward M (Collaborative Pairs), Isaacs AM (Collaborative Pairs), Kampmann M (Early Career Acceleration), Kronenberg-Versteeg D (Collaborative Pairs), Thompson LM (Collaborative Pairs), Ori A (Collaborative Pairs), Mohammed Y, Giera M (Collaborative Pairs) and van der Kant R. (Collaborative Pairs)

Lipid changes in the brain have been implicated in many neurodegenerative diseases including Alzheimer's Disease (AD), Parkinson's disease and Amyotrophic Lateral Sclerosis. To facilitate comparative lipidomic research across brain-diseases we established a data commons named the Neurolipid Atlas, that we have pre-populated with novel human, mouse and isogenic induced pluripotent stem cell (iPSC)-derived lipidomics data for different brain diseases. We show that iPSC-derived neurons, microglia and astrocytes display distinct lipid profiles that recapitulate *in vivo* lipotypes. Leveraging multiple datasets, we show that the AD risk gene ApoE4 drives cholesterol ester (CE) accumulation in human astrocytes recapitulating CE accumulation measured in the human AD brain. Multi-omic interrogation of iPSC-derived astrocytes revealed that cholesterol plays a major role in astrocyte interferon-dependent pathways such as the immunoproteasome and major histocompatibility complex (MHC) class I antigen presentation. We show that through enhanced cholesterol esterification ApoE4 suppresses immune activation of astrocytes. Our novel data commons, available at neurolipidatlas.com, provides a user-friendly tool and knowledge base for a better understanding of lipid dyshomeostasis in neurodegenerative diseases.

American Journal of Medical Genetics: "Utility of Genome Sequencing in Exome-Negative Pediatric Patients With Neurodevelopmental Phenotypes"

Authors include Ahrens-Nicklas RC (Collaborative Pairs) and Bhoj EJK (Collaborative Pairs)

Exome sequencing (ES) has emerged as an essential tool in the evaluation of neurodevelop-mental disorders (NDD) of unknown etiology. Genome sequencing (GS) offers advantages over ES due to improved detection of structural, copy number, repeat number and non-coding variants. However, GS is less commonly utilized due to higher cost and more intense analysis. Here, we present nine cases of pediatric NDD that were molecularly diagnosed with GS between 2017 and 2022, following non-diagnostic ES. All individuals presented with global developmental delay or regression. Other features present in our cohort included epilepsy, white matter abnormalities, brain malformation and dysmorphic features. Two cases were diagnosed on GS due to newly described gene-disease relationship or variant reclassification (MAPK8IP3, CHD3). Additional features missed on ES that were later detected on GS were: intermediate-size deletions in three cases who underwent ES that were not validated for CNV detection, pathogenic variants within the non-protein coding genes SNORD118 and RNU7-1, pathogenic variant within the promoter region of GJB1,and a coding pathogenic variant within BCAP31 which was not sufficiently covered onES. GS following non-diagnostic ES led to the identification of pathogenic variants in this cohort of nine cases, four of which would not have been identified by reanalysis alone.

Journal of Neuroinflammation: "Morphotype-Specific Calcium Signaling in Human Microglia"

Authors include Thomas V. Wuttke (Collaborative Pairs) and Henner Koch (Collaborative Pairs)

Key functions of Ca2+ signaling in rodent microglia include monitoring the brain state as well as the surrounding neuronal activity and sensing the danger or damage in their vicinity. Microglial Ca2+ dyshomeostasis is a disease hallmark in many mouse models of neurological disorders but the Ca2+ signal properties of human microglia remain unknown. We developed a novel genetically-encoded ratiometric Ca2+ indicator, targeting microglial cells in the freshly resected human tissue, organotypically cultured tissue slices and analyzed in situ ongoing Ca2+ signaling of decades-old microglia dwelling in their

native microenvironment. The data revealed marked compartmentalization of Ca2+ signals, with signal properties differing across the compartments and resident morphotypes. The basal Ca2+ levels were low in ramified and high in ameboid microglia. The fraction of cells with ongoing Ca2+ signaling, the fraction and the amplitude of process Ca2+ signals and the duration of somatic Ca2+ signals decreased when moving from ramified via hypertrophic to ameboid microglia. In contrast, the size of active compartments, the fraction and amplitude of somatic Ca2+ signals and the duration of process Ca2+ signals increased along this pathway.

Nature Medicine: "How To Pay for Individualized Genetic Medicines"

Authors include Julia Vitarello (Patient-Partnered Collaboration), and Timothy W. Yu (Patient-Partnered Collaboration)

For precision genetic medicines to fulfill their potential as treatments for ultra-rare diseases, fresh approaches to academic–industry partnerships and data sharing are needed, together with regulatory change and adaptation of reimbursement models.

eNeuro: "Phenotype Distinctions in Mice Deficient in the Neuron-Specific α3 Subunit of Na,K-ATPase: Atp1a3tm1Ling/+ and Atp1a3+/D801Y"

Authors include Cathleen M. Lutz (Patient-Partnered Collaboration), Natalia S. Morsci (Patient-Partnered Collaboration) and Kathleen J. Sweadner (Patient-Partnered Collaboration)

ATP1A3 is a Na,K-ATPase gene expressed specifically in neurons in the brain. Human mutations are dominant and produce an unusually wide spectrum of neurological phenotypes, most notably rapid-onset dystonia parkinsonism (RDP) and alternating hemiplegia of childhood (AHC). Here we compared heterozygotes of two mouse lines, a line with little or no expression (Atp1a3tm1Ling/+) and a knock-in expressing p.Asp801Tyr (D801Y, Atp1a3+/D801Y). Both mouse lines had normal lifespans, but Atp1a3+/D801Y had mild perinatal mortality contrasting with D801N mice (Atp1a3+/D801N), which had high mortality. The phenotypes of Atp1a3tm1Ling/+ and Atp1a3+/D801Y were different, and testing of each strain was tailored to its symptom range. Atp1a3tm1Ling/+ mice displayed little at baseline, but repeated ethanol intoxication produced hyperkinetic motor abnormalities not seen in littermate controls. Atp1a3+/D801Y mice displayed robust phenotypes: hyperactivity, diminished posture consistent with hypotonia, and deficiencies in beam walk and wire hang tests. Symptoms also included qualitative motor abnormalities that are not well quantified by conventional tests. Paradoxically, Atp1a3+/D801Y showed sustained better performance than wild type on the accelerating rotarod. Atp1a3+/D801Y mice were overactive in forced swimming and afterward had intense shivering, transient dystonic postures, and delayed recovery. Remarkably, Atp1a3+/D801Y mice were refractory to ketamine anesthesia, which elicited hyperactivity and dyskinesia even at higher dose. Neither mouse line exhibited fixed dystonia (typical of RDP patients), spontaneous paroxysmal weakness (typical of AHC patients), or seizures but had consistent, measurable neurological abnormalities. A gradient of variation supports the importance of studying multiple Atp1a3 mutations in animal models to understand the roles of this gene in human disease.

Nature: "Cellular Communities Reveal Trajectories of Brain Ageing and Alzheimer's Disease"

Authors include Mariko Taga (Collaborative Science), Vilas Menon (Collaborative Science), Naomi Habib (Collaborative Pairs) and Philip L. De Jager (Collaborative Science)

Alzheimer's disease (AD) has recently been associated with diverse cell states, yet when and how these states affect the onset of AD remains unclear. Here we used a data-driven approach to reconstruct the dynamics of the brain's cellular environment and identified a trajectory leading to AD that is distinct from other ageing-related effects. First, we built a comprehensive cell atlas of the aged prefrontal cortex from 1.65 million single-nucleus RNA-sequencing profiles sampled from 437 older individuals, and identified specific glial and neuronal subpopulations associated with AD-related traits. Causal modelling then prioritized two distinct lipid-associated microglial subpopulations — one drives amyloid-β proteinopathy while the other mediates the effect of amyloid-β on tau proteinopathy — as well as an astrocyte subpopulation that mediates the effect of tau on cognitive decline. To model the dynamics of cellular environments, we devised the BEYOND methodology, which identified two distinct trajectories of brain ageing, each defined by coordinated progressive changes in certain cellular communities that lead to AD dementia or alternative brain ageing. Thus, we provide a cellular foundation for a new perspective on AD pathophysiology that informs personalized therapeutic development, targeting different cellular communities for individuals on the path to AD or to alternative brain ageing.

Immunity: "Innate Immune Training Restores Pro-Reparative Myeloid Functions To Promote Remyelination in the Aged Central Nervous System"

Authors include Gokce O (Collaborative Pairs) and Simons M (Collaborative Pairs)

The reduced ability of the central nervous system to regenerate with increasing age limits functional recovery following demyelinating injury. Previous work has shown that myelin debris can overwhelm the metabolic capacity of microglia, thereby impeding tissue regeneration in aging, but the underlying mechanisms are unknown. In a model of demyelination, we found that a substantial number of genes that were not effectively activated in aged myeloid cells displayed epigenetic modifications associated with restricted chromatin accessibility. Ablation of two class I histone deacetylases in microglia was sufficient to restore the capacity of aged mice to remyelinate lesioned tissue. We used Bacillus Calmette-Guerin (BCG), a live-attenuated vaccine, to train the innate immune system and detected epigenetic reprogramming of brain-resident myeloid cells and functional restoration of myelin debris clearance and lesion recovery. Our results provide insight into aging-associated decline in myeloid function and how this decay can be prevented by innate immune reprogramming.

Molecular Therapy: "Hematopoietic Stem Cell Gene Therapy Improves Outcomes in a Clinically Relevant Mouse Model of Multiple Sulfatase Deficiency"

Authors include Cathleen Lutz (Patient-Partnered Collaboration) and Rebecca C. Ahrens-Nicklas (Collaborative Pairs)

Multiple sulfatase deficiency (MSD) is a severe, lysosomal storage disorder caused by pathogenic variants in the gene SUMF1, encoding the sulfatase modifying factor formylglycine-generating enzyme. Patients with MSD exhibit functional deficiencies in all cellular sulfatases. The inability of sulfatases to break down their substrates leads to progressive and multi-systemic complications in patients, similar to those seen in single-sulfatase disorders such as metachromatic leukodystrophy and mucopolysaccharidoses IIIA. Here, we aimed to determine if hematopoietic stem cell transplantation with *ex vivo* SUMF1 lentiviral gene therapy could improve outcomes in a clinically relevant mouse model of MSD. We first tested our approach in MSD patient-derived cells and found that our SUMF1 lentiviral vector improved protein expression, sulfatase activities, and glycosaminoglycan accumulation. *In vivo*, we found that our gene therapy approach rescued biochemical deficits, including sulfatase activity and glycosaminoglycan accumulation, in affected organs of MSD mice treated post-symptom onset. In addition, treated mice demonstrated improved neuroinflammation and neurocognitive function. Together, these findings suggest that SUMF1 HSCT-GT can improve both biochemical and functional disease markers in the MSD mouse.

Immunity: "Apolipoprotein E Aggregation in Microglia Initiates Alzheimer's Disease Pathology by Seeding *β*-amyloidosis"

Authors include Giera M (Collaborative Pairs) and Simons M (Collaborative Pairs)

The seeded growth of pathogenic protein aggregates underlies the pathogenesis of Alzheimer's disease (AD), but how this pathological cascade is initiated is not fully understood. Sporadic AD is linked genetically to apolipoprotein E (APOE) and other genes expressed in microglia related to immune, lipid, and endocytic functions. We generated a transgenic knockin mouse expressing HaloTag-tagged APOE and optimized experimental protocols for the biochemical purification of APOE, which enabled us to identify fibrillary aggregates of APOE in mice with amyloid-*β* (A*β*) amyloidosis and in human AD brain autopsies. These APOE aggregates that stained positive for *β* sheet-binding dyes triggered A*β* amyloidosis within the endo-lysosomal system of microglia, in a process influenced by microglial lipid metabolism and the JAK/STAT signaling pathway. Taking these observations together, we propose a model for the onset of A*β* amyloidosis in AD, suggesting that the endocytic uptake and aggregation of APOE by microglia can initiate A*β* plaque formation.

Genomics, Proteomics & Bioinformatics: "ProtPipe: A Multifunctional Data Analysis Pipeline for Proteomics and Peptidomics"

Authors include Fratta P (Collaborative Pairs), Narayan P (Collaborative Pairs) and Ward ME (Collaborative Pairs)

Mass spectrometry (MS) is a technique widely employed for the identification and characterization of proteins, with personalized medicine, systems biology, and biomedical applications. The application of MS-based proteomics advances our understanding of protein function, cellular signaling, and complex biological systems. MS data analysis is a critical process that includes identifying and quantifying proteins and peptides and then exploring their biological functions in downstream analyses. To address the complexities associated with MS data analysis, we developed ProtPipe to streamline and automate the processing and analysis of high-throughput proteomics and peptidomics datasets

with DIA-NN preinstalled. The pipeline facilitates data quality control, sample filtering, and normalization, ensuring robust and reliable downstream analyses. ProtPipe provides downstream analyses, including protein and peptide differential abundance identification, pathway enrichment analysis, protein–protein interaction analysis, and major histocompatibility complex (MHC)–peptide binding affinity analysis. ProtPipe generates annotated tables and visualizations by performing statistical post-processing and calculating fold changes between predefined pairwise conditions in an experimental design. It is an open-source, well-documented tool available at github.com/NIH-CARD/ProtPipe, with a user-friendly web interface.

Nature Neuroscience: "Implementation and Validation of Single-Cell Genomics Experiments in Neuroscience"

Authors include Kampmann M (Early Career Acceleration), Quintana FJ (Collaborative Pairs), Tosches MA (Early Career Acceleration) and Gokce O (Collaborate Pairs)

Single-cell or single-nucleus transcriptomics is a powerful tool for identifying cell types and cell states. However, hypotheses derived from these assays, including gene expression information, require validation, and their functional relevance needs to be established. The choice of validation depends on numerous factors. Here, we present types of orthogonal and functional validation experiment to strengthen preliminary findings obtained using single-cell and single-nucleus transcriptomics as well as the challenges and limitations of these approaches.

Nature Neuroscience: "Opportunities and Challenges of Single-Cell and Spatially Resolved Genomics Methods for Neuroscience Discovery"

Authors include Gokce O (Collaborative Pairs) and Habib N (Collaborative Pairs)

Over the past decade, single-cell genomics technologies have allowed scalable profiling of cell-type-specific features, which has substantially increased our ability to study cellular diversity and transcriptional programs in heterogeneous tissues. Yet our understanding of mechanisms of gene regulation or the rules that govern interactions between cell types is still limited. The advent of new computational pipelines and technologies, such as single-cell epigenomics and spatially resolved transcriptomics, has created opportunities to explore two new axes of biological variation: cell-intrinsic regulation of cell states and expression programs and interactions between cells. Here, we summarize the most promising and robust technologies in these areas, discuss their strengths and limitations and discuss key computational approaches for analysis of these complex datasets. We highlight how data sharing and integration, documentation, visualization and benchmarking of results contribute to transparency, reproducibility, collaboration and democratization in neuroscience, and discuss needs and opportunities for future technology development and analysis.

Nature Reviews Immunology: "Neuroinflammation in Alzheimer Disease"

Authors include Cruchaga C (Collaborative Science), De Jaeger PL (Collaborative Science), Simons M (Collaborative Pairs) and Kipnis J (Collaborative Pairs)

Increasing evidence points to a pivotal role of immune processes in the pathogenesis of Alzheimer disease, which is the most prevalent neurodegenerative and dementia-causing disease of our time. Multiple lines of information provided by experimental, epidemiological, neuropathological and genetic studies suggest a pathological role for innate and adaptive immune activation in this disease. Here, we review the cell types and pathological mechanisms involved in disease development as well as the influence of genetics and lifestyle factors. Given the decade-long preclinical stage of Alzheimer disease, these mechanisms and their interactions are driving forces behind the spread and progression of the disease. The identification of treatment opportunities will require a precise understanding of the cells and mechanisms involved as well as a clear definition of their temporal and topographical nature. We will also discuss new therapeutic strategies for targeting neuroinflammation, which are now entering the clinic and showing promise for patients.

Neuron: "Inhibition of RNA Splicing Triggers CHMP7 Nuclear Entry, Impacting TDP-43 Function and Leading to the Onset of ALS Cellular Phenotypes"

Authors include Jeffrey D. Rothstein (Collaborative Science), Alyssa N. Coyne (Collaborative Science) and Gene W. Yeo (Collaborative Science)

Amyotrophic lateral sclerosis (ALS) is linked to the reduction of certain nucleoporins in neurons. Increased nuclear localization of charged multivesicular body protein 7 (CHMP7), a protein involved in nuclear pore surveillance, has been identified as a key factor damaging nuclear pores and disrupting transport. Using CRISPR-based microRaft, followed by gRNA identification (CRaft-ID), we discovered 55 RNA-binding proteins (RBPs) that influence CHMP7 localization,

including SM.D.1, a survival of motor neuron (SMN) complex component. Immunoprecipitation-mass spectrometry (IP-MS) and enhanced crosslinking and immunoprecipitation (CLIP) analyses revealed CHMP7's interactions with SM.D.1, small nuclear RNAs, and splicing factor mRNAs in motor neurons (MNs). ALS induced pluripotent stem cell (iPSC)-MNs show reduced SM.D.1 expression, and inhibiting SM.D.1/SMN complex increased CHMP7 nuclear localization. Crucially, overexpressing SM.D.1 in ALS iPSC-MNs restored CHMP7's cytoplasmic localization and corrected STMN2 splicing. Our findings suggest that early ALS pathogenesis is driven by SMN complex dysregulation.

bioRxiv: "Type 1 Lymphocytes and Interferon-γ Accumulate in the Thalamus and Restrict Seizure Susceptibility After Traumatic Brain Injury"

Authors include Anna V. Molofsky (Collaborative Pairs) and Jeanne T. Paz (Collaborative Pairs)

Traumatic brain injury (TBI) is a leading cause of mortality and disability worldwide and can lead to secondary sequelae such as increased seizure susceptibility. Emerging work suggests that the thalamus, the relay center of the brain that undergoes secondary damage after cortical TBI, is involved with heightened seizure risks after TBI. TBI also induces the recruitment of peripheral immune cells, including T cells, to the site(s) of injury, but it is unclear how these cells impact neurological sequelae post-TBI. Here, we characterize the identities and kinetics of lymphocytic infiltrates into the cortex and thalamus using a mouse model of cortical TBI. We identify a population of IFNγ-producing type 1 lymphocytes that infiltrate specific thalamic subregions over weeks following injury, where they elicit a local IFNγ response in microglia and neuronal subset(s). Depletion of CD4+ T cells protects mice from TBI-induced seizure susceptibility by de-repressing other non-CD4+ type 1 lymphocytes and disease-associated microglia (DAMs) in the thalamus. Strikingly, we find that a single dose of IFNγ prior to challenge with a proconvulsant agent was sufficient to reduce TBI-induced seizure incidence, severity, and mortality. This work identifies IFNγ as a direct modulator of TBI-associated seizure susceptibility, which could have therapeutic implications for the treatment of TBI patients.

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Nature Neuroscience: "Aberrant Splicing in Huntington's Disease Accompanies Disrupted TDP-43 Activity and Altered m6A RNA Modification"

Authors include Lagier-Tourenne C (Collaborative Pairs), Spitale RC (Collaborative Pairs) and Thompson LM (Collaborative Pairs)

Huntington's disease (HD) is caused by a CAG repeat expansion in the HTT gene, leading to altered gene expression. However, the mechanisms leading to disrupted RNA processing in HD remain unclear. Here we identify TDP-43 and the N6-methyladenosine ($m6^a$) writer protein METTL3 to be upstream regulators of exon skipping in multiple HD systems. Disrupted nuclear localization of TDP-43 and cytoplasmic accumulation of phosphorylated TDP-43 occurs in HD mouse and human brains, with TDP-43 also co-localizing with HTT nuclear aggregate-like bodies distinct from mutant HTT inclusions. The binding of TDP-43 onto RNAs encoding HD-associated differentially expressed and aberrantly spliced genes is decreased. Finally, $m6^a$ RNA modification is reduced on RNAs abnormally expressed in the striatum of HD R6/2 mouse brain, including at clustered sites adjacent to TDP-43 binding sites. Our evidence supports TDP-43 loss of function coupled with altered $m6^a$ modification as a mechanism underlying alternative splicing in HD.

Analytical Chemistry: "iSODA: A Comprehensive Tool for Integrative Omics Data Analysis in Single- and Multi-Omics Experiments"

Authors include Harari O (Collaborative Science), Cruchaga C (Collaborative Science), Ori A (Collaborative Pairs), Henrie A (Single Cell Working Group) and Giera M (Collaborative Pairs)

Thanks to the plummeting costs of continuously evolving omics analytical platforms, research centers collect multiomics data more routinely. They are, however, confronted with the lack of a versatile software solution to harmoniously analyze single-omics and interpret multiomics data. We have developed iSODA, a web-based application for the analysis of single- and multiomics data. The tool emphasizes intuitive interactive visualizations designed for user-driven data exploration. Researchers can access a variety of functions ranging from simple visualization like volcano plots and PCA to advanced functional analyses like enrichment analysis and lipid saturation analysis. For integrated multiomics, iSODA incorporates multi-omics factor analysis and similarity network fusion. The ability to adapt the data on-the-fly allows for tasks, such as the removal of outlier samples or failed features, imputation, or normalization. All results are presented through interactive plots, the modular design supports extensions, and tooltips and tutorials provide comprehensive guidance for users. iSODA is accessible under isoda.online.

Nature Communications: "Optineurin-Facilitated Axonal Mitochondria Delivery Promotes Neuroprotection and Axon Regeneration"

Authors include Xin Duan (Collaborative Pairs) and Yang Hu (Collaborative Pairs)

Optineurin (OPTN) mutations are linked to amyotrophic lateral sclerosis (ALS) and normal tension glaucoma (NTG), but a relevant animal model is lacking, and the molecular mechanisms underlying neurodegeneration are unknown. We find that OPTN C-terminus truncation (OPTNΔC) causes late-onset neurodegeneration of retinal ganglion cells (RGCs), optic nerve (ON), and spinal cord motor neurons, preceded by a decrease of axonal mitochondria in mice. We discover that OPTN directly interacts with both microtubules and the mitochondrial transport complex TRAK1/KIF5B, stabilizing them for proper anterograde axonal mitochondrial transport, in a C-terminus dependent manner. Furthermore, overexpressing OPTN/TRAK1/KIF5B prevents not only OPTN truncation-induced, but also ocular hypertension-induced neurodegeneration, and promotes robust ON regeneration. Therefore, in addition to generating animal models for NTG and ALS, our results establish OPTN as a facilitator of the microtubule-dependent mitochondrial transport necessary for adequate axonal mitochondria delivery, and its loss as the likely molecular mechanism of neurodegeneration.

Nature Neuroscience: "Neuronal Polyunsaturated Fatty Acids Are Protective in FTD/ALS"

Authors include Alyssa N. Coyne (Collaborative Science), Rik van der Kant (Collaborative Pairs), Martin Giera (Collaborative Pairs) and Adrian M. Isaacs (Collaborative Pairs)

Here we report a conserved transcriptomic signature of reduced fatty acid and lipid metabolism gene expression in a Drosophila model of C9orf72 repeat expansion, the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD), and in human postmortem ALS spinal cord. We performed lipidomics on C9 ALS/FTD Drosophila, induced pluripotent stem (iPS) cell neurons and postmortem FTD brain tissue. This revealed a common and specific reduction in phospholipid species containing polyunsaturated fatty acids (PUFAs). Feeding C9 ALS/FTD flies PUFAs yielded a modest increase in survival. However, increasing PUFA levels specifically in neurons of C9 ALS/FTD flies, by overexpressing fatty acid desaturase enzymes, led to a substantial extension of lifespan. Neuronal overexpression of fatty acid desaturases also suppressed stressor-induced neuronal death in iPS cell neurons of patients with both C9 and TDP-43 ALS/FTD. These data implicate neuronal fatty acid saturation in the pathogenesis of ALS/FTD and suggest that interventions to increase neuronal PUFA levels may be beneficial.

Nature Medicine: "The Rare Therapies Launchpad: A Pilot Program for Individualized Medicines in the UK"

Authors include Tim Yu (Patient-Partnered Collaboration) and Julia Vitarello (Patient-Partnered Collaboration)

Twenty-first century technologies increasingly allow the identification and treatment of the underlying genetic causes of disease. Millions of children around the world are affected by rare diseases, some of which are caused by 'private mutations' that are so rare that they can often only be treated with individualized therapies. Disappointingly, these scientific breakthroughs have not yet led to an increase in the use of individualized medicines owing to regulatory, commercial and economic barriers to adoption. The Rare Therapies Launchpad (RTLP) is a UK pilot program set up to help identify sustainable and scalable approaches to address these barriers, to allow the establishment of an equitable and sustainable national infrastructure to ensure that UK children will benefit from the life-saving potential of cutting-edge science.

Journal of Neurophysiology: "Comprehensive Analysis of Human Dendritic Spine Morphology and Density"

Authors include Kronenberg-Versteeg D (Collaborative Pairs), Wuttke TV (Collaborative Pairs) and Koch H (Collaborative Pairs)

Dendritic spines, small protrusions on neuronal dendrites, play a crucial role in brain function by changing shape and size in response to neural activity. So far, in-depth analysis of dendritic spines in human brain tissue is lacking. This study presents a comprehensive analysis of human dendritic spine morphology and density using a unique dataset from human brain tissue from 27 patients (8 females, 19 males, aged 18–71 yr) undergoing tumor or epilepsy surgery at three neurosurgery sites. We used acute slices and organotypic brain slice cultures to examine dendritic spines, classifying them into the three main morphological subtypes: mushroom, thin, and stubby, via three-dimensional (3-D) reconstruction using ZEISS arivis Pro software. A deep learning model, trained on 39 diverse datasets, automated spine segmentation and 3-D reconstruction, achieving a 74% F1-score and reducing processing time by over 50%. We show significant differences in spine density by sex, dendrite type, and tissue condition. Females had higher spine densities than males, and apical dendrites were denser in spines than basal ones. Acute tissue showed higher spine densities compared with cultured human brain tissue. With time in culture, mushroom

spines decreased, whereas stubby and thin spine percentages increased, particularly from 7–9 to 14 days *in vitro*, reflecting potential synaptic plasticity changes. Our study underscores the importance of using human brain tissue to understand unique synaptic properties and shows that integrating deep learning with traditional methods enables efficient large-scale analysis, revealing key insights into sex- and tissue-specific dendritic spine dynamics relevant to neurological diseases.

bioRxiv: "Prevention of Transgene Silencing During Human Pluripotent Stem Cell Differentiation"

Authors include Ernest Arenas (Collaborative Science), Andrew R. Bassett (iPSC/CRISPR WG), Martin Kampmann (Early Career Acceleration), Deborah Kronenberg-Versteeg (Collaborative Pairs), Florian T. Merkle (Early Career Acceleration), Birgitt Schüle (Collaborative Science), Leslie M. Thompson (Collaborative Pairs), William C. Skarnes (iPSC/CRISPR WG), Michael E. Ward (Collaborative Pairs) and Marius Wernig (iPSC/CRISPR WG)

While high and stable transgene expression can be achieved in undifferentiated pluripotent stem cells, conventional transgene expression systems are often silenced upon differentiation. Silencing occurs with both randomly integrated transgenes, introduced via transposase or lentiviral methods, and with transgenes targeted to specific genomic sites, including at commonly used safe harbor loci. The challenge to robustly express experimental transgenes in differentiated pluripotent stem cells is a major bottleneck in the field for applications such as CRISPR screening. Here, we conducted a comparative analysis to systematically evaluate the impact of various promoters, transcriptional regulatory elements, insulators, and genomic integration sites on transgene silencing during neuronal differentiation. Our findings reveal that specific combinations of promoters and transcriptional stability elements are able to prevent transgene silencing during differentiation, whereas chromatin insulators had less impact on silencing and three novel safe harbor integration sites performed similarly to the CLYBL locus. Guided by these insights we developed the PiggyBac vector TK4, which showed complete resistance to transgene silencing across various neuronal and microglial differentiation protocols from six different pluripotent stem cell lines, as independently confirmed by seven different laboratories. This construct will be highly useful for assays requiring stable transgene expression during differentiation, and holds the potential for broad applications in various research fields.

European Journal of Paediatric Neurology: "Brain Morphometry and Psychomotor Development in Children with PCH2A"

Authors include Julia Matilainen (Patient-Partnered Collaboration) and Simone Mayer (Patient-Partnered Collaboration)

Pontocerebellar hypoplasia type 2A (PCH2A) is a rare neurogenetic disease characterized by severe cognitive and motor impairment. This study reports on brain morphometry and psychomotor development of affected children. We analyzed 78 cerebral MRI datasets of 57 patients with genetically confirmed PCH2A. Volumetric and in-plane measurements were conducted in cerebellum, neocortex and pons. Supratentorial width and width of the anterior horns of the lateral ventricles was used to calculate the Evans index. Caregivers of 65 patients (aged 7 months to 33 years) filled in a survey assessing motor and cognitive development. Developmental status was compared to MRI measurements. In children with PCH2A, cerebellar volume was markedly smaller than in healthy children at birth, with slower increase and stagnation at around 12 months. No cerebellar growth was observed in the cranio-caudal axis. Longitudinal data did not reveal a decrease in cerebellar volume or in-plane measurements. Supratentorial measurements showed progressive microcephaly and a continuous increase of the Evans index, reflecting progressive cerebral atrophy. Patients demonstrated severe cognitive and motor impairments, with developmental regression reported in only a minority. No statistical relationship between brain measurements and cognitive or motor development was observed. MRI in PCH2A patients shows limited cerebellar growth during infancy, especially restricted along the cranio-caudal axis. After infancy, cerebellar volume remains relatively stable. Supratentorial measurements indicate slowly progressive atrophy. Psychomotor development is significantly impaired, but regression is rare.

HGG Advances: "Coupling Deep Phenotypic Quantification With Next-Generation Phenotyping for 192 Individuals With Germline Histonopathies"

Authors include Rebecca C. Ahrens-Nicklas (Collaborative Pairs) and Elizabeth J.K. Bhoj (Collaborative Pairs)

Mendelian histonopathies are rare neurodevelopmental disorders (NDDs) caused by germline variants in histone-encoding genes. Here, we perform a more expansive pan-histonopathy interrogation than previously possible. We analyze data from 192 individuals affected by histonopathies. This analysis includes representation of the 185 published individuals with HIST1H1E syndrome, Bryant-Li-Bhoj syndrome, and Tessadori-Bicknell-van Haaften NDD; as well as from seven unpublished individuals, five

of whom harbor variants in genes not previously associated with disease (HIST1H2AL/H2AC16, H2AFZ/H2AZ1, HIST1H3D/H3C4, and HIST3H3/H3-4). By intersecting clinician-reported phenotypic data with next-generation phenotyping of published 2D facial photographs (n = 98), we sought to address the lack of established craniofacial gestalts or characteristic phenotypic patterns for this community. While these analyses may suggest a histone core versus linker protein basis of delineation, they more strikingly highlight data gaps that confound the identification of phenotypic patterns at this time. Based on this, we developed an updated standardized clinical survey, which allowed us to identify the second known individual with a germline histonopathy and a cancer diagnosis. Notably, the community-wide cancer incidence is currently 1%, which falls below the recommended 5% cut off for routine surveillance. Ultimately, this work highlights the ways in which histonopathy-associated phenotypes change throughout the lifespan, necessitating longitudinal re-evaluation; that every identified individual shapes our understanding of these syndromes in a way that improves care for this community; and the value of ongoing translational work to address the outstanding question of cancer predisposition for individuals living with germline histonopathies.

Neurobiology of Disease: "Alternating Hemiplegia of Childhood Associated Mutations in *Atp1a3* Reveal Diverse Neurological Alterations in Mice"

Authors include Natalia S. Morsci (Patient-Partnered Collaboration), Kathleen J. Sweadner (Patient-Partnered Collaboration), and Cathleen M. Lutz (Patient-Partnered Collaboration)

Pathogenic variants in the neuronal Na+/K+ ATPase transmembrane ion transporter (ATP1A3) cause a spectrum of neurological disorders including alternating hemiplegia of childhood (AHC). The most common de novo pathogenic variants in AHC are p.D801N (~40 % of patients) and p.E815K (~25 % of patients), which lead to early mortality by spontaneous death in mice. Nevertheless, knowledge of the development of clinically relevant neurological phenotypes without the obstacle of premature death, is critical for the identification of pathophysiological mechanisms and ultimately, for the testing of therapeutic strategies in disease models. Here, we used hybrid vigor attempting to mitigate the fragility of AHC mice and then performed behavioral, electrophysiological, biochemical, and molecular testing to comparatively analyze mice that carry either of the two most common AHC patient observed variants in the Atp1a3 gene. Collectively, our data reveal the presence but also the differential impact of the p.D801N and p.E815K variants on disease relevant alterations such as spontaneous and stress-induced paroxysmal episodes, motor function, behavioral and neurophysiological activity, and neuroinflammation. Our alternate AHC mouse models with their phenotypic deficits open novel avenues for the investigation of disease biology and therapeutic testing for ATP1A3 research.

Nature Communications: "Genetically Encoded Fluorescent Reporter for Polyamines"

Authors include Jing-Ke Weng (Collaborative Pairs) and Ankur Jain (Collaborative Pairs)

Polyamines are abundant and evolutionarily conserved metabolites that are essential for life. Dietary polyamine supplementation extends life-span and health-span. Dysregulation of polyamine homeostasis is linked to Parkinson's disease and cancer, driving interest in therapeutically targeting this pathway. However, measuring cellular polyamine levels, which vary across cell types and states, remains challenging. We introduce a genetically encoded polyamine reporter for real-time measurement of polyamine concentrations in single living cells. This reporter utilizes the polyamine-responsive ribosomal frameshift motif from the OAZ1 gene. We demonstrate broad applicability of this approach and reveal dynamic changes in polyamine levels in response to genetic and pharmacological perturbations. Using this reporter, we conduct a genome-wide CRISPR screen and uncover an unexpected link between mitochondrial respiration and polyamine import, which are both risk factors for Parkinson's disease. By offering a lens to examine polyamine biology, this reporter may advance our understanding of these ubiquitous metabolites and accelerate therapy development.

bioRxiv: "Cell-Surface Proteomic Profiling Identifies CD72 as a Regulator of Microglial Tiling"

Authors include Stevens B (Collaborative Pairs), Kronenberg-Versteeg D (Collaborative Pairs) and Wernig M (iPSC/CRISPR WG)

Microglial tiling — the phenomenon of consistent cell-to-cell distances and non-overlapping processes — is regarded as a qualitative indicator of homeostasis, but mechanisms of microglial tiling are unknown. We used cell-surface proximity labeling and mass spectrometry to profile the microglial cell-surface proteome in an *in vitro* model of homeostatic glial physiology and used single-cell RNA sequencing and public databases to identify candidate cell-surface proteins that might modulate tiling. We designed an image-based functional assay which measures six morphological/spatial readouts to screen these proteins for modulation of tiling. CD72, a coreceptor to the B cell receptor that is expressed by microglia, disrupted tiling; we validated its effects *in vitro* and in situ in organotypic hippocampal

brain slices. Phosphoproteomic studies revealed that CD72 modulates pathways associated with cell adhesion, repulsive receptors, microglial activation, and cytoskeletal organization. These results lay the groundwork for further investigation of the functional roles of tiling in homeostasis and disease.

bioRxiv: "Maintenance of Neuronal TDP-43 Expression Requires Axonal Lysosome Transport"

Authors include Kampmann M (Early Career Acceleration) and Ward ME (Collaborative Pairs)

TDP-43 mislocalization and pathology occurs across a range of neurodegenerative diseases, but the pathways that modulate TDP-43 in neurons are not well understood. We generated a Halo-TDP-43 knock-in iPSC line and performed a genome-wide CRISPR interference FACS-based screen to identify modifiers of TDP-43 levels in neurons. A meta-analysis of our screen and publicly available screens identified both specific hits and pathways present across multiple screens, the latter likely responsible for generic protein level maintenance. We identified BORC, a complex required for anterograde lysosome transport, as a specific modifier of TDP-43 protein, but not mRNA, levels in neurons. BORC loss led to longer half-life of TDP-43 and other proteins, suggesting lysosome location is required for proper protein turnover. As such, lysosome location and function are crucial for maintaining TDP-43 protein levels in neurons.

Journal of the American Chemical Society: "A Molecular Glue Approach to Control the Half-Life of CRISPR-Based Technologies"

Authors include Martin Kampmann (Early Career Acceleration Award) and David R. Liu (Patient Partnered Collaboration)

Cas9 is a programmable nuclease that has furnished transformative technologies, including base editors and transcription modulators (e.g., CRISPRi/a), but several applications of these technologies, including therapeutics, mandatorily require precision control of their half-life. For example, such control can help avert any potential immunological and adverse events in clinical trials. Current genome editing technologies to control the half-life of Cas9 are slow, have lower activity, involve fusion of large response elements (>230 amino acids), utilize expensive controllers with poor pharmacological attributes, and cannot be implemented *in vivo* on several CRISPR-based technologies. We report a general platform for half-life control using the molecular glue, pomalidomide, that binds to a ubiquitin ligase complex and a response-element bearing CRISPR-based technology, thereby causing the latter's rapid ubiquitination and degradation. Using pomalidomide, we were able to control the half-life of large CRISPR-based technologies (e.g., base editors and CRISPRi) and small anti-CRISPRs that inhibit such technologies, allowing us to build the first examples of on-switch for base editors. The ability to switch on, fine-tune, and switch-off CRISPR-based technologies with pomalidomide allowed complete control over their activity, specificity, and genome editing outcome. Importantly, the miniature size of the response element and favorable pharmacological attributes of the drug pomalidomide allowed control of activity of base editor *in vivo* using AAV as the delivery vehicle. These studies provide methods and reagents to precisely control the dosage and half-life of CRISPR-based technologies, propelling their therapeutic development.

bioRxiv: "TDP-43 Pathology Induces CD8+ T Cell Activation Through Cryptic Epitope Recognition"

Authors include Michael Ward (Collaborative Pairs), Pietro Fratta (Collaborative Pairs) and Ning Jiang (Early Career Acceleration)

Aggregation and nuclear depletion of the RNA binding protein TDP-43 are the crucial pathological features of amyotrophic lateral sclerosis (ALS) and inclusion body myositis (IBM), two degenerative diseases of the CNS and muscle. The loss of TDP-43 nuclear function results in the aberrant inclusion of cryptic exons in mRNA transcripts, leading to the expression of de novo proteins. Clonally expanded and highly differentiated CD8+ T cells have been observed in individuals with TDP-43 proteinopathies and therapeutics modulating the T cell response have recently been found to extend survival. However, the target antigens mediating T cell activation have remained elusive. Here, we investigate whether the de novo proteins induced by aberrant cryptic splicing due to TDP-43 nuclear loss can act as neo-antigens. We detect the HDGFL2 cryptic peptide and multiple other TDP-43 cryptic exons in IBM skeletal muscle, where their presence correlates with enrichment of T cells and class I antigen presentation pathways. Furthermore, we identify epitopes deriving from HDGFL2 and IGLON5 cryptic peptides which are recognized by clonally expanded and functionally differentiated populations of CD8+ T cells in ALS and IBM Patients. Finally, we demonstrate that T cells engineered to express the identified TCRs can bind and activate in response to the cryptic peptide derived epitopes (cryptic epitopes) and are able to kill TDP-43 deficient astrocytes. This work identifies for the first time specific T cell antigens in ALS and IBM, directly linking adaptive immune response to TDP-43 pathology.

Developmental Medicine & Child Neurology: "Constructed Growth Charts and Nutrition for Pontocerebellar Hypoplasia Type 2A"

Authors include Julia Matilainen (Patient-Partnered Collaboration) and Simone Mayer (Patient-Partnered Collaboration)

Aim: To calculate growth charts for pontocerebellar hypoplasia (PCH) type 2A (PCH2A) and compare them to German reference charts, especially with regard to nutritional aspects. Data were gathered from a cohort of patients with genetically confirmed PCH2A, who were predominantly recruited through the German PCH patient network (65 patients [33 female, 32 male] at a mean age of 8 years 7 months). We collected data retrospectively using a parent questionnaire, and from medical records (December 2020–September 2022). Disease-specific growth charts were prepared from predominantly longitudinal data using the gamlss package implemented in R. Sex-disaggregated growth charts for PCH2A were compared to German reference data from the Kinder- und Jugendgesundheitssurvey (German Health Interview and Examination Survey for Children and Adolescents). The height and weight of participants with PCH2A were within the reference range at birth. Mean height was significantly lower at 6 months of age, weight at 3 months, and body mass index at 4 months. These deviations were also mostly significant later on. Head circumference in individuals with PCH2A was significantly below average at birth; all participants showed severe and progressive microcephaly in the further course. Caloric intake was within or above reference values. Participants with PCH2A exhibited progressive microcephaly and frequently failed to thrive. Disease-specific growth charts are a helpful tool to monitor those with PCH2A.

Cell: "*In Vivo* Prime Editing Rescues Alternating Hemiplegia of Childhood in Mice"

Authors include Simon Frost (Patient Partnered Collaboration), Nina Frost (Patient Partnered Collaboration), Kathleen J Sweadner (Patient Partnered Collaboration), Cathleen M Lutz (Patient Partnered Collaboration) and David R Liu (Patient Partnered Collaboration)

Alternating hemiplegia of childhood (AHC) is a neurodevelopmental disorder with no disease-modifying treatment. Mutations in ATP1A3, encoding an Na+/K+ ATPase subunit, cause 70% of AHC cases. Here, we present prime editing (PE) and base editing (BE) strategies to correct ATP1A3 and Atp1a3 mutations in human cells and in two AHC mouse models. We used PE and BE to correct five prevalent ATP1A3 mutations with 43%–90% efficiency. AAV9-mediated *in vivo* PE corrects Atp1a3 D801N and E815K in the CNS of two AHC mouse models, yielding up to 48% DNA correction and 73% mRNA correction in bulk brain cortex. *In vivo* PE rescued clinically relevant phenotypes, including restoration of ATPase activity; amelioration of paroxysmal spells, motor defects, and cognition deficits; and dramatic extension of animal lifespan. This work suggests a potential one-time PE treatment for AHC and establishes the ability of PE to rescue a neurological disease in animals.

Acta Neuropathologica: "Single-Cell Transcriptomic Landscape of the Neuroimmune Compartment in Amyotrophic Lateral Sclerosis Brain and Spinal Cord"

Authors include Wassim Elyaman (Collaborative Pairs), Elizabeth M. Bradshaw (Collaborative Science), Hemali Phatnani (Collaborative Pairs), Philip L. De Jager (Collaborative Science), Serge Przedborski (Collaborative Pairs), Vilas Menon (Collaborative Science) and Marta Olah (Collaborative Science)

Development of therapeutic approaches that target specific microglia responses in amyotrophic lateral sclerosis (ALS) is crucial due to the involvement of microglia in ALS progression. Our study identifies the predominant microglia subset in human ALS primary motor cortex and spinal cord as an undifferentiated phenotype with dysregulated respiratory electron transport. Moreover, we find that the interferon response microglia subset is enriched in donors with aggressive disease progression, while a previously described potentially protective microglia phenotype is depleted in ALS. Additionally, we observe an enrichment of non-microglial immune cell, mainly NK/T cells, in the ALS central nervous system, primarily in the spinal cord. These findings pave the way for the development of microglia subset-specific therapeutic interventions to slow or even stop ALS progression.

Neuron: "Accelerating Biomedical Discoveries in Brain Health Through Transformative Neuropathology of Aging and Neurodegeneration"

Authors include Melissa Murray (Collaborative Pairs), Colin Smith (Neuropathology), Vilas Menon (Collaborative Science), Dirk Keene (Collaborative Pairs), Adriao Aguzzi (Collaborative Pairs), Maria Cobos (Early Career Acceleration), John Crary (Neuropathology), Phillip De Jager (Collaborative Science), Ozgun Gokce (Collaborative Pairs), Seth Grant (Neuropathology), David Gutman (Neuropathology), Edward Lee (Neuropathology), William Seeley (Collaborative Science), Micahel Keiser (Early Career Acceleration), Brittany Dugger (Neuropathology), Hemali Phatnani (Collaborative Pairs)

Transformative neuropathology is redefining human brain research by integrating foundational descriptive pathology with advanced methodologies. These approaches, spanning multi-omics studies and machine learning applications, will drive discovery for the identification of biomarkers, therapeutic targets, and complex disease patterns through comprehensive analyses of postmortem human brain tissue. Yet critical challenges remain, including the sustainability of brain banks, expanding donor participation, strengthening training pipelines, enabling rapid autopsies, supporting collaborative platforms, and integrating data across modalities. Innovations in digital pathology, tissue quality enhancement, harmonization of data standards, and machine learning integration offer opportunities to accelerate tissue-level “pathomics” research in brain health through cross-disciplinary collaborations. Lessons from neuroimaging, particularly in establishing common data frameworks and multi-site collaborations, offer a valuable roadmap for streamlining innovations. In this perspective, we outline actionable solutions for leveraging existing resources and strengthening collaboration -where we envision future opportunities to drive translational discoveries stemming from transformative neuropathology.

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