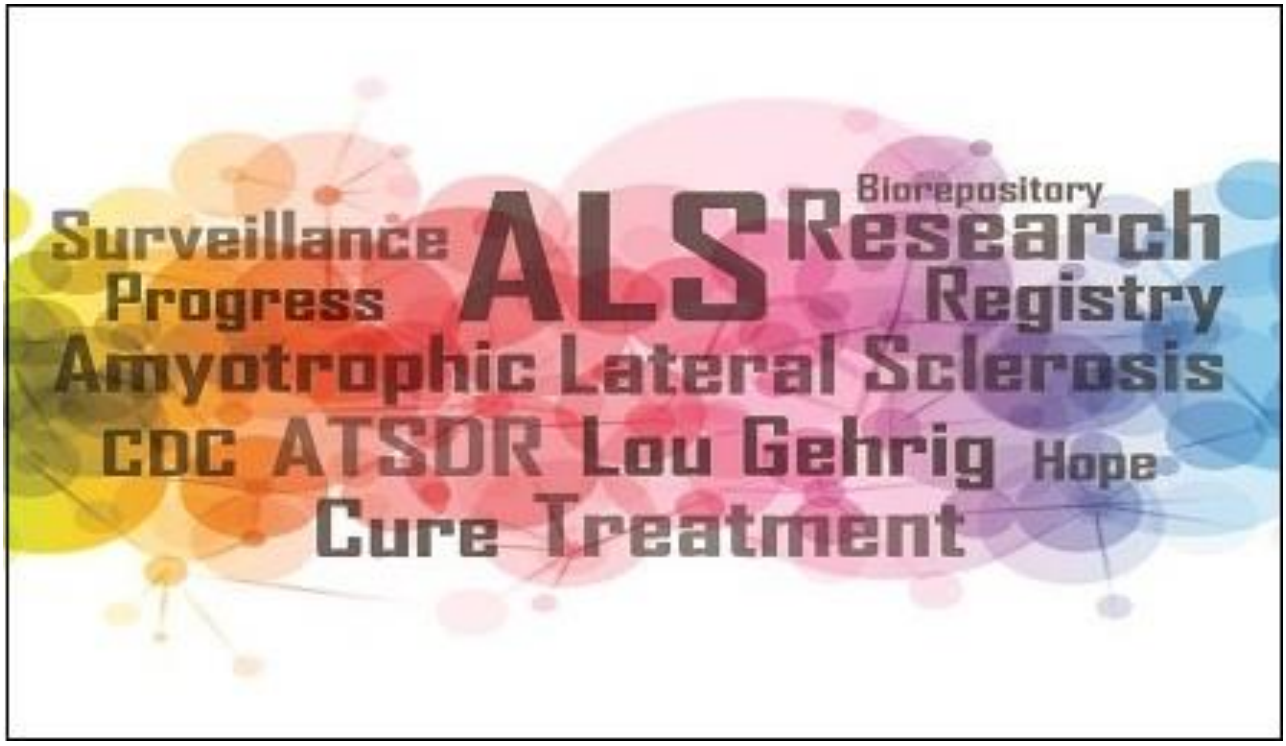
Department of Health and Human Services
Centers for Disease Control and Prevention
Agency for Toxic Substances and Disease Registry

National Amyotrophic Lateral Sclerosis (ALS) Registry Annual Research Symposium and Meeting



August 29-30, 2022
Summary Report

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Acronyms Used in this Document

Acronym	Expansion
ADDF	Alzheimer's Drug Discovery Foundation
AE	Adverse Event
AFM	Acute Flaccid Myelitis
AI	Artificial Intelligence
ALS	Amyotrophic Lateral Sclerosis
ALSRP	Amyotrophic Lateral Sclerosis Research Program
ALSA	Amyotrophic Lateral Sclerosis Association
ALSFRS	ALS Functional Rating Scale
ALSFRS-R	Revised ALS Functional Rating Scale
ATSDR	Agency for Toxic Substances and Disease Registry
BBB	Blood-Brain Barrier
BKMR	Bayesian Kernel Machine Regression
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CDMRP	Congressionally Directed Medical Research Programs
CMS	Centers for Medicare & Medicaid Services
CNS	Central Nervous System
COD	Cause of Death
CSF	Cerebrospinal Fluid
CVD	Cardiovascular Disease
Danish EPIC	Danish European Prospective Investigation into Cancer and Nutrition
dbGaP	Database of Genotypes and Phenotypes
DMSS	Defense Medical Surveillance System
DNA	Deoxyribonucleic Acid
DoD	Department of Defense
DoDSR	DoD Serum Repository
DPH	Department of Public Health
DUA	Data Use Agreement
EBV	Epstein-Barr virus
ECAS	Edinburgh Cognitive and Behavioural ALS Screen
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ERS	Environmental Risk Score
ET	Eastern Time
EU	European Union
EVs	Extracellular Vesicles
fALS	Familial ALS
FDA	Food and Drug Administration
FMC	Finnish Mobile Clinic Health Examination Survey
FMCF	Finnish Mobile Clinic Follow-Up Survey
GIS	Geographic Information System
GLAST	Glial Glutamate Aspartate Transporter
GUID	Globally Unique Identifier
GWAS	Genome-Wide Association Study
HAPs	Hazardous Air Pollutants
HBCUs	Historically Black Colleges and Universities
HHS	(Department of) Health and Human Services
HIPAA	Health Insurance Portability and Accountability Act
HMO	Health Maintenance Organization
HWE	Hardy–Weinberg Equilibrium

IgG	Immunoglobulin G
IP	Immunoprecipitation
iPS	Induced Pluripotent Stem Cells
IRB	Institutional Review Board
ISMMS	Icahn School of Medicine at Mount Sinai
JHU	Johns Hopkins University
JMIR	*Journal of Medical Internet Research*
KMR	Kernel Machine Regression
LAENALS	Latin American Epidemiology Network of ALS
MA ALS Registry	State of Massachusetts Department of Health ALS Registry
MD	Muscular Dystrophy
MDA	Muscular Dystrophy Association
ME	Maine
MFS	Mini-Finland Health Survey
MGH	Massachusetts General Hospital
MII	Maryland Innovation Initiative
miRNA	Micro Ribonucleic Acid
ML	Machine Learning
MLB	Major League Baseball
MMWR	*Morbidity and Mortality Weekly Report*
MND	Motor Neuron Disease
MoCA	Montreal Cognitive Assessment
MS	Multiple Sclerosis
MSG	Marketing Systems Group
MTA	Material Transfer Agreement
NAAMC	National Ambient Air Monitoring Conference
NAICS	North American Industry Classification System
NASA	National Aeronautics and Space Administration
NATA	National Air Toxics Assessment
NCEH	National Center for Environmental Health
NDI	National Death Index
NEALS	Northeast Amyotrophic Lateral Sclerosis Consortium
NFL	National Football League
NfL	Neurofilament Light Chain
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NLP	Natural Language Processing
NMD	Neuromuscular Diseases
NPL	National Priority List
NYGC	New York Genome Center
OC	Organochlorine
OMB	Office of Management and Budget
ORs	Odds Ratios
PALS	Persons with Amyotrophic Lateral Sclerosis
PBDEs	Polybrominated Diphenyl Ethers
PBMCs	Peripheral Blood Mononuclear Cells
PCBs	Polychlorinated Biphenyls
PDA	Physical Disability Agency
PDC	Parkinson's Dementia Complex
PFAS	Perfluoroalkyl and Polyfluoroalkyl Substances
PFOA	Perfluorooctanoic Acid
PGS	Polygenic Scores

PHI	Protected Health Information
PI	Principal Investigator
PII	Personally Identifiable Information
POC	Point of Contact
POP	Persistent Organic Pollutant
PPO	Preferred Provider Organization
PRMRP	Peer Reviewed Medical Research Program
QC	Quality Control
RFA	Request for Application
RNA	Ribonucleic Acid
RNA-Seq	RNA Sequencing
RNM	Research Notification Mechanism
sALS	Sporadic ALS
SBIR	Small Business Innovation Research
SES	Socioeconomic Status
SNPs	Single-Nucleotide Polymorphisms
SOCs	Standard Occupational Classifications
SONEPSYN	Sociedad de Neurología, Psiquiatría and Neurocirugía de Chile SONEPSYN
SOPs	Standard Operating Procedures
SPH	School of Public Health
STD	Sexually Transmitted Disease
TBI	Traumatic Brain Injury
TRI	Toxics Release Inventory
UK	United Kingdom
US	United States
USGS	US Geological Surveys
VA	(United States Department of) Veterans Affairs
VASRD	Veteran Affairs Schedule for Rating Disabilities
VBA	Veterans Benefits Administration
VHA	Veteran Health Administration
VT	Vermont
WGS	Whole Genome Sequence
WONDER	Wide-ranging ONline Data for Epidemiologic Research

Centers for Disease Control and Prevention (CDC)
Agency for Toxic Substances and Disease Registry (ATSDR)
Annual Amyotrophic Lateral Sclerosis (ALS) Surveillance Meeting

Minutes of the Meeting
August 29-30, 2022

Welcome and Introductions

Danielle Boyce, DPA, MPH
Moderator
Johns Hopkins School of Medicine

Dr. Boyce called to order the 2022 National ALS Registry annual research symposium and meeting at 8:15 AM Eastern Time (ET) and welcomed everyone. She emphasized what a great honor it was to moderate this meeting. She thanked Drs. Mehta and Horton for making this a public meeting so that people affected by ALS could join and participate, which she acknowledged as a very important and appreciated gesture. After reviewing the agenda, she called upon Tori Bahe from Ross Strategic who explained the Zoom meeting process. A participant roster is appended to the end of this document.

Opening Remarks

Patrick Breysse, PhD
Director, National Center for Environmental Health (NCEH)/Agency for Toxic Substances and Disease Registry (ATSDR)

Dr. Breysse greeted everyone and expressed appreciation for their virtual attendance at ATSDR's annual National ALS Registry meeting. Because of the pandemic, this is the third year this meeting has been conducted virtually. While the hope was to meet this year in person, due to the recent COVID-19 BA.5 variant circulation, the decision was made to meet virtually for the safety of attendees and persons with amyotrophic lateral sclerosis (PALS). Due to the virtual nature of the meeting, Dr. Breysse pointed out that it would be more interactive so that attendees could ask questions to all presenters. The National ALS Registry is an innovative effort to help scientists better understand the epidemiology of ALS and to help identify possible etiologies and risk factors as researchers work toward a cure for ALS.

He indicated that during this meeting, there would be updates on new products and initiatives that are helping ATSDR to better strengthen the National ALS Registry. Some of these would include presentations on an updated statistical analyses and epidemiological reports for this year and moving forward to help more accurately describe the incidence and prevalence of ALS in the United States (US); understanding new public engagement sessions with stakeholders for the past few months to obtain detailed feedback from patients and caregivers on how to improve the National ALS Registry; working with Kaiser Permanente, one of the largest health maintenance organizations (HMOs) in the country, to solicit private pay information on PALS to help better understand a part of the population to which ATSDR never has had access; improving state collaborations by working with the State of Massachusetts Department of Health

ALS Registry (MA ALS Registry) to help determine where there may be gaps in the data at the state level; awards to new research grants to various institutions to help better understand the links between the environment and ALS; and updates from existing grantees on exciting and groundbreaking work they are doing to better understand causes of ALS and associated risk factors.

In conclusion, Dr. Breysse emphasized that external feedback is invaluable. Having clinicians, researchers, and especially PALS together in a forum such as this helps ATSDR better shape the National ALS Registry. It cannot be emphasized enough that the success of the National ALS Registry depends upon effective collaboration from all ALS stakeholders, including PALS, caregivers, clinicians, researchers, patient organizations, and others.

National ALS Registry Research Update

Paul Mehta, MD
National ALS Registry, Principal Investigator
Registries and Surveillance Section, OIA
Division of Toxicology and Human Health Sciences
Agency for Toxic Substances and Disease Registry

Dr. Mehta began by thanking patients, caregivers, and those who are impacted by ALS for joining the meeting. He recognized that it is not easy and expressed gratitude for their time and willingness to ask questions and make comments freely and openly. As a reminder, the National ALS Registry was enacted by Public Law 110-373 that was signed by Congress in October 2008. The US ALS Registry Act (the Act) directed CDC/ATSDR to create a population-based US registry. The National ALS Registry was launched in October 2010. The purpose of the Registry as specified by the Act is to: 1) describe the incidence and prevalence of ALS; 2) describe the demographics of ALS patients; and 3) examine risk factors for the disease.

ALS is non-notifiable to CDC in the US. In terms of what this means within the public health system, diseases are categorized as "reportable" or "notifiable." Reportable diseases are mandatorily reported to jurisdictions by individuals in the health care community, including providers, facilities, and laboratories.[1] Each state determines which diseases/conditions must be reported. ALS is reportable in the state of Massachusetts (MA), Vermont (VT), and Maine (ME), but not to the Registry. VT and ME came online this year through the efforts of Dr. Elijah Stommel. MA has had a registry for over 12 years, which is now very robust. ATSDR is working with them as well. Notifiable diseases are reported to the CDC on a voluntary basis by each jurisdiction by health departments. Case records are reported in a de-identified format and include limited information about the patient and the case. At this time, MA is not reporting their cases to the National ALS Registry. Given that states and environmental factors differ, it is very important to look at local settings in terms of the epidemiology of ALS. Given that ALS is not reportable or notifiable to CDC, the Registry had to establish novel case-finding methods.

[1] NNDSS, CDC.gov

Inclusion of cases in the National ALS Registry is a 2-pronged effort. Currently, Registry cases come from national administrative databases such as the Centers for Medicare & Medicaid Services (CMS), Veterans Affairs (VA) through the Veterans Health Administration (VHA), and the National ALS Registry Web Portal where patients enter through the Registry and answer a series of questions voluntarily to be added to the Registry. From the portal side, patients can take risk factor surveys, acquire information about clinical trials and epidemiological study, and/or join the National ALS Biorepository. There are numerous ALS research initiatives underway at the federal level, such as the following:

CDC/ATSDR
- ❑ Epidemiology of ALS at the state and national level – public health impact
- ❑ Identification of risk factors and etiologies for ALS (e.g., environmental exposures, heavy metals, et cetera)
- ❑ Identify biomarkers via the Biorepository

National Institutes of Health (NIH):
- ❑ Basic sciences biomedical research
- ❑ Looking at effective approaches to halt cell death
- ❑ Slow disease progression
- ❑ Identify gene mutations and cellular defects
- ❑ Develop biomarkers
- ❑ How ALS changes over time (e.g., symptoms)

Department of Defense (DoD) Congressionally Directed Medical Research Program (CDMRP):
- ❑ Pre-clinical development of therapeutic agents
- ❑ Steps required before Food and Drug Administration (FDA) approval of a new drug
- ❑ Stability, toxicology, pharmacokinetics, efficacy in cell and animal models

In terms of revised prevalence of ALS in the US, CDC/ATSDR has been publishing ALS prevalence in the US using a standard-based methodologies to assess the number of cases. This time, the capture-recapture methodology was used to estimate the number of missing cases in the US.[2] Based on this methodology, the prevalence estimate was revised to 31,843 cases as of 2017 for a rate of 9.9 per 100,000 US population. Approximately 14,043 cases were estimated to be missing primarily among patients who receive care outside of CMS, VHA, and Veterans Benefits Administration (VBA) through private insurance (e.g., PPOs, HMOs). To break down how the total of 31,843 was estimated, in addition to the 14,043 identified through the novel capture-recapture model, a total of 5,323 patients were self-enrolled and another 12,477 patients were identified through administrative databases. Approximately 5% of the total number of definite cases are in both the web portal and administrative databases.

The new estimates show that the undercount is comprised of approximately 10,000 males, over 5,000 females, over 6000 whites, and over 600 African Americans. The pattern of patient characteristics remains unchanged. ALS continues to be more common among whites, males, and person 60–69 years of age. The adjusted prevalence rates per 100,000 are 9.9/100,000 overall, 13.3/100,000 among males, 7.6/100,000 among females, 8.2/100,000 among whites, and 4.4/100,000 among African-Americans. Based on the revised prevalence of ALS in the US, prevalence rates were corrected for the previous years of 2010–2016 as reflected in this table:

[2] Paul Mehta, Jaime Raymond, Reshma Punjani, Moon Han, Theodore Larson, Wendy Kaye, Lorene M. Nelson, Barbara Topol, Oleg Muravov, Corina Genson & D. Kevin Horton (2022) Prevalence of amyotrophic lateral sclerosis in the United States using established and novel methodologies, 2017, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, DOI: 10.1080/21678421.2022.2059380

	Established algorithm		Capture–recapture method			Mean estimation	
Year	No. cases/observed count	Prevalence[a]	No. cases, missing[b]	No. cases, corrected[b]	Prevalence[a], corrected[b]	No. cases[c]	Prevalence[a,c]
2010–2011	12,187	3.9	9614	21,801	7.0	16,994	5.5
2012	14,713	4.7	11,607	26,320	8.4	20,517	6.5
2013	15,908	5.0	12,550	28,458	9.0	22,183	7.0
2014	15,927	5.0	12,565	28,492	8.9	22,209	7.0
2015	16,583	5.2	13,082	29,665	9.2	23,124	7.2
2016	16,424	5.2	12,957	29,381	9.1	22,903	7.1

[a]Cases per 100,000 population.

[b]Estimation based on capture–recapture methodology.

As a reminder, incidence is the number of new cases diagnosed and prevalence refers to the number of people who are currently living with the disease. For the first time, incidence was measured for the entire US for 2014-2016.[3] Capture-recapture was not used for incidence to estimate missing cases, but is of interest for use in the future. Incidence was made possible by re-evaluating through pilot testing those patients who entered through CMS, VHA, and VBA. Verified patients were not new cases in any other databases or across the years. From there, the investigators felt comfortable saying that these were incidence cases coming in through the Registry. For 2014-2016, the age-adjusted incidence rate was 1.7 in 2014, 1.57 in 2015, and 1.56 in 2016. This is probably a slight underestimation of incidence. Once capture-recapture is used and there are better ways of identifying missing cases, that will be revised and will be upward as well most likely.

The research notification mechanism has been used by 68 organizations to date for their clinical trials, including numerous pharmaceutical companies. A paper titled, "Recruitment of Patients With Amyotrophic Lateral Sclerosis for Clinical Trials and Epidemiological Studies: Descriptive Study of the National ALS Registry's Research Notification Mechanism" was published in the *Journal of Medical Internet Research* (*JMIR*) in July 2021[4] that conservatively estimated that thousands of patients have been recruited for clinical trials and epidemiological studies through this system. This was based on feedback from pharmaceutical companies and academic institutions.

Regarding the Registry's current projects, the Registry's algorithm for case-ascertainment is being updated. The existing algorithm has been used for about 12 years and was thought to need an update to adapt to the disease. A group of neurologists was convened to discuss the current algorithm and what updates to make, including Drs. Benjamin Brooks, James Berry, Bjorn Oskarsson, and Stephen Goutman. Changes include how ALS cases are classified from 3 groups currently to 4 groups and moving away from "Definite" and "Possible" ALS to "Confirmed," "Likely," "Undetermined," and "Not" ALS. "Confirmed" and "Likely" will be in the numerator, meaning that they will be counted in terms of cases. "Undetermined" and "Not" ALS will not be counted. A change also will be made regarding how a single prescription of Riluzole will be added to "Likely" case category. A manuscript for this project already has been submitted to and accepted by the journal *ALS/FTD* and should be published soon.

[3] Paul Mehta, Jaime Raymond, Reshma Punjani, Theodore Larson, Moon Han, Frank Bove & D. Kevin Horton (2022) Incidence of amyotrophic lateral sclerosis in the United States, 2014–2016, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 23:5-6, 378-382, DOI: 10.1080/21678421.2021.2023190

[4] Mehta P, Raymond J, Han M, Larson T, Berry J, Paganoni S, Mitsumoto H, Bedlack R, Horton D Recruitment of Patients With Amyotrophic Lateral Sclerosis for Clinical Trials and Epidemiological Studies: Descriptive Study of the National ALS Registry's Research Notification Mechanism J Med Internet Res 2021;23(12):e28021 URL: https://www.jmir.org/2021/12/e28021 DOI: 10.2196/28021

The comparison study with the Massachusetts ALS Registry is currently under analyses by the Registry team. This is a joint collaboration between the National ALS Registry and state health department in Massachusetts for which Alicia Fraser is the primary point of contact (POC). The objective of this study is to compare MA ALS patients identified in the Massachusetts ALS Registry with MA ALS patients identified in the National ALS Registry. The ongoing analyses require matching of ALS case definitions across both sources, which is time-consuming. The hope is to have a publication later in 2022.

Given that ALS patients represent a vulnerable population, a new publication is under development that assesses the impact of the COVID-19 pandemic on ALS deaths. Data have been requested from the National Death Index (NDI). This assessment compares calendar years 2020 prior to vaccines to 2019 in terms of whether the pandemic increase motor neuron disease (MND) deaths in 2020 when compared to 2019 and is anticipated to be published later in the year. Data also will be requested from 2021 when they become available.

Some user-friendly enhancements have been made to the Registry to ensure that people can quickly see the number of ALS cases in the US. A Registry dashboard was developed that can be found on the homepage that provides helpful information such as the number of estimated persons living with ALS in the US, prevalence, incidence, mortality, completed risk factors surveys, published journal articles, number of notifications, funded studies, Biorepository participants, and data requests. This is updated quarterly and is a way to let the public know the current ALS situation. User-friendly enhancements to the Registry include a research publication page and searchable functions including title, author, and year. This is updated each time a new article is published. To date, 92 articles have been included here. Another enhancement is the inclusion of a separate landing page for patients[5] and researchers.[6]

As a reminder, the Office of Management and Budget (OMB) approves all federal data collection activities for the US federal government to ensure that data collection is not a burden to the public. A request was submitted to the OMB over a year ago to add new data sources, update surveys from 18 to 5 named categories, and release state data on incidence and prevalence. If the Fall approval date comes to fruition, it will be possible to report data at the state level. This should offer a lot more information and granularity in terms of the epidemiological burden of ALS at the state level.

Regarding public engagement, listening sessions were launched in Summer 2022 with patients and caregivers. Led by Dr. Danielle Boyce, the purpose of these sessions was to provide input about where the Registry could make improvements. The 4 sessions allowed for open dialogue between the Registry and stakeholders. More sessions are planned for Fall 2022 to continue these dialogues.

To date, the Registry has funded 21 research projects and hopes to fund 1 to 3 new grants in Fall 2022 depending upon the availability of funds. In Fall 2021, 2 grants were funded. The Karolinska Institute was funded to examine military services, traumatic brain injuries (TBI), chronic neuroinflammation, and infections as potential risk factors for ALS. The Karolinska Institute is in Stockholm, Sweden and is one of the preeminent research institutions in Scandinavia. Sweden is an interesting model in that it has a single-payer healthcare system that has robust information on individuals from birth to death. This project is led by Principal Investigator (PI) Dr. Fang Feng. The University of Michigan was funded to investigate long-term

[5] cdc.gov/als
[6] cdc.gov/alsresearch

air pollution as a potential risk factor for ALS. The PIs for this project are Drs. Eva Feldman and Stephen Goutman. In previous years, the research sessions of the National ALS Registry annual meetings have been closed. They heard input that these sessions should be open, which they will be from now going forward. This is a way for ATSDR to report openly on what is being funded by having the investigators present updates on their research.

Regarding overall funding, $10 million was appropriated in Fiscal Year 2021 (FY21) for the National ALS Registry. Of this, $2.5 million was allocated to ATSDR for overhead and $7.5 million was allocated to the Registry. The majority of funding (>60%) is allocated to research funding. About 12% is allocated to personnel, 12% to outreach and education, 9% to information technology (IT) support, 6% to communications, and less than 1% to miscellaneous activities.

Dr. Mehta reviewed the following table that summarizes the recommendations, action items, lead organization, and status from the previous meeting:

Recommendation	Action Items	Lead Organization	Status	Year
Have a webinar that steps partners/clinic staff through registration and a sample of surveys	-Ask partners, what are the most frequent problems encountered by patients with registering and taking risk factor surveys -Develop webinar to show how the Registry works	ATSDR	Ongoing	2020
ALS Registry Webpage updates	-Revamp Landing Page -Revamp FAQ page -Create Dashboard -Add additional Registry fact pages	ATSDR	Ongoing	2021
ALS Registry Road Tour	-Reengage with public by going to local/regional partner meetings and events. Goal is to get face-to-face with patients and -care providers	ATSDR	Ongoing with Public Engagement Session	2021
ALS Registry Rebranding	-Updating branding of the ALS Registry to improve public perception and to educate the public more on what the Registry does	ATSDR	Ongoing	2021
ALS Registry presence at national conferences	-Adding more presentations and increasing our presence at meetings such as AAN, ANA, MND, etc.	ATSDR	Ongoing	2021
ALS Registry Partners - Training program and outreach metrics	-Create training for partners to ensure that the Registry is correctly and fully represented by partners and care providers -Come up with some form of metrics of partner performance to measure the effectiveness of their outreach on behalf of the Registry	ATSDR	Ongoing	2021

In terms of impact, Dr. Mehta reminded everyone that the Registry does more than just count ALS cases. There is the National ALS Biorepository and the Registry includes assessment of the epidemiology of ALS in the US in terms of incidence, prevalence, and mortality. The Registry also works with its partner organizations, neurologists, and academia partners. There are currently over 100,000 completed surveys in the Registry, with data published on these for review. The National ALS Registry is the largest database of ALS patients in the US for research. The Registry collaborates with pharmaceutical companies and academia to provide recruitment assistance for clinical trials and epidemiological studies. The Registry also partners with the largest ALS patient organizations to raise awareness and inform and educate patients and caregivers about the Registry and is always open to partnering with others as well. The Registry advances ALS research through the National ALS Biorepository on biomarkers, genetics, and environmental exposures. The Registry also funds research grants for leading academic institutions to learn more about risk factors and possible etiologies, such as cyanobacteria, heavy metals, persistent organic pollutant (POPs), genetics, and others. In

addition, the Registry provides updated national epidemiological estimates of ALS cases in the US using novel methodologies.

In closing, Dr. Mehta played a video that was developed of Brad Dusek who played for Texas A&M University and the Washington Redskins and was a Super Bowl winner. ATSDR worked with the National Football League (NFL) Alumni Association in order to arrange a visit to Brad's homestead in Texas during the summer. Dr. Mehta noted that he met Brad in 2018 at Methodist Hospital in Houston when he was newly diagnosed with ALS. This video tells the story of the great determination and resiliency of someone with ALS.

Discussion Summary

Dr. Stephen Finger requested clarification on the incidence reports in terms of whether these numbers were meant to be an estimate of new patients with ALS or if they were simply meant to inform on how many new patients are entering the Registry.

Dr. Mehta clarified that these are new patients with ALS from ATSDR's definition of incidence in terms of looking at the data they had. When these patients were entering the Registry, they wanted to make sure that they were not duplicates. There is a delay in diagnosis of ALS for about 12 months that was not factored in. They were looking at the exact number of cases entering the Registry for particular years. There probably is an underestimation regarding these numbers, so they want to adjust them as possible in future years as well. The mortality of ALS is about 7,000 in the US. Mortality can be a surrogate of incidence, so it is important to have better ways to identify new cases and so forth. One of the ways to do this is reflected in the Registry's work with Kaiser Permanente, one of the largest HMOs on the West Coast. Kaiser Permanente's cases and data will be entered into the Registry as well. This will help in terms of having cases from a new source and a geographical area that has a lot more diversity.

For a frame of reference, Dr. Finger asked whether the number of missing cases would be roughly the 44% calculated for the prevalence cases.

Dr. Mehta indicated that these cases would not be considered incidence. This is a different methodology. The question was asked of research partners. The 44% cannot be used to extrapolate incidence in terms of number of new cases in the US. They want to better define incidence in terms of future years. They are working with a new partner, Emory University, that is currently analyzing capture-recapture for successive years. ATSDR has requested that they assess the incidence as well.

Ms. Cathy Collet asked whether ATSDR would be publishing the actual demographics of the people who entered the portal and the actual demographics of the people found in the payer group; whether Dr. Brooks' COVID-19 ALS Registry data were included in the ATSDR COVID-19 analysis; and whether there is any hope for seeing some functional accounting of Registry aspects so that it is not just a research category. That is, more businesslike aspects of the Registry such as how much the portal costs in general, how much is being spent on data mining and clean-up, and so forth.

Dr. Mehta responded that in terms of publishing demographics, because OMB requires them to publish the Registry as not just the portal because it is not representative, the data are an amalgamation from the portal, CMS, VHA, and VBA together. Demographically, the portal is skewed to a higher socioeconomic status (SES) than cases coming from the other sources. ATSDR helped to recruit for Dr. Brooks' COVID-19 Registry out of North Carolina, which is

listed on the ATSDR website. ATSDR's data will be included in that. His data will not be included in the NDI analysis because ATSDR cannot see exactly who has passed away from Dr. Brooks' cohort. Because ATSDR is providing recruitment assistance, their patients can take the survey that Dr. Brooks is administering, but ATSDR cannot obtain their NDI data because that would be a transmission of PII back to ATSDR. ATSDR gets NDI for the entire country as opposed to a specific study. They would have to obtain special approval from Dr. Brooks' Institutional Review Board (IRB) and ATSDR's IRB to include the cases in the National ALS Registry to NDI comparison. Nevertheless, this is a good question to answer to determine whether there is an uptick or impact that Dr. Brooks' registry is seeing. Regarding functional accounting, this certainly would be feasible for ATSDR to do. The way this is done currently is looking at the entire portfolio of the Biorepository in terms of research, funded grants, and other elements of the pie chart. These are taxpayer dollars and the information is available and transparent.

Regarding a question about prevalence, Dr. Han answered that in previous *MMWRs*, prevalence was reported at 5.2/100,000. Using the new method, 9.9/100,000 is the new estimate based on the data available at the time of the analysis.

Dr. Mehta added that the new prevalence rate is 9.9/100,000 or approximately 32,000 cases in the US. This was discussed in the patient engagement sessions. There are different numbers in the research article. Everyone must do a better job to make sure this is uniformly reported so the public is aware that 32,000 is the number they should be using rather than 17,000. They are aware that there is some confusion that can lead to frustration, so they want to make sure that the media and the public are aware of the new number as well.

Pre-Disease Biomarkers of Persistent Organic Pollutants (POPs), Immune System, and Amyotrophic Lateral Sclerosis (ALS)

Marc Weisskopf, PhD, ScD
Professor of Epidemiology
Harvard University

Dr. Weisskopf presented on pre-disease biomarkers of POPs, the immune system, and ALS. He pointed out that POPs can be organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), and other compounds. They are organic in nature and persist in the body and the environment. PBDEs represent some of the newer compounds and are well-known for being flame retardant. These compounds are of interest in ALS for a variety of reasons, such as having known neurotoxic and immunotoxic effects. While OC pesticides and PCBs are largely banned in the US, they are still found in many people in the US because of their persistence. PBDEs are a newer set of compounds, there are many of them, and they are not all banned. Therefore, they are more common in people today.

There has been some history of examining the role of pesticides and ALS. There was a meta-analysis by Kamel et all in 2013[7] summarizing many studies at tried to assess exposure to pesticides and risk of ALS. Overall, they found a positive increased risk of ALS with exposure to pesticides, but many of these are fairly crude measures of pesticides such as "ever exposures." A lot of the studies in this meta-analysis were based on job history or simply were questionnaires of past exposures. A couple of the most recent ones (Weisskopf et all 2009,

[7] Kamel et al.,Neurotoxicology 2013

Present study, and Kamel et al 2013) were questionnaire-based. Weisskopf's was a very broad question in the Cancer Prevention II study about pesticides. The Present study was the Agricultural Health Study that looked at pesticides among pesticide applicators and their spouses. Both of those studies found elevated but not significant risk. When the Agricultural Health Study was broken down, there were signals for certain types of pesticides, including OCs. A more recent paper by Andrew et al in 2021[8] looked at US Geological Surveys (USGS) of pesticide use in different parts of the US in a broad analysis. This showed weighting to the excess risk side, suggesting that something is going on with pesticides. While it can be hard to identify exactly which pesticide because there is correlation between these, this certainly suggests a pesticide signal.

Dr. Stephen Goutman[9] published a paper with their cohort in Michigan looking at blood samples taken from ALS cases and controls to measure a suite of OC pesticides, PCBs, and PBDEs. This study found some signals suggesting increased risk of ALS with some of these compounds. The focus of this work was on the incidence of ALS in the Michigan case-control cohort. Dr. Goutman and this same group[10] followed up on that study to look also at the relationship to survival with ALS just among ALS cases. This study grouped exposure levels summing over all of the compounds they were measuring. Those who were in the higher level of having many of these compounds had a shorter survival period with ALS, suggesting that these compounds affected the rate of progression of ALS.

Michigan was the only group using biomarkers of exposures from cases and controls, so the cases already had ALS. That was the lay of the land when Dr. Weisskopf and colleagues decided to explore similar questions but wanted to find out whether they could identify indicators prior to ALS onset in terms of the pesticide profile and how that related. To do that, they collaborated with groups in Finland and Denmark and took advantage of existing cohorts that were set up many years ago for cancer and cardiovascular disease (CVD). These studies enrolled thousands of people and collected blood samples at the time of enrollment. These samples were stored and were made available for use. Dr. Weisskopf and colleagues proposed to identify ALS cases in 3 cohorts in Finland and 1 cohort in Denmark. The prospective cohort studies in Finland with serum samples included the 1966-1972 Finnish Mobile Clinic Health Examination Survey (FMC), 1973-1977 follow-up FMC (FMCF), and the 1978-1980 Mini-Finland Health Survey (MFH) for a total of 56,862 people. The Denmark cohort came from the Danish European Prospective Investigation into Cancer and Nutrition (Danish EPIC) with a total of 57,053 people.

This resulted in a combined cohort of nearly 120,000 people. An advantage of the national health care system that tracks everyone's medical involvement in Finland and Denmark is that it is possible to identify the people in these cohorts who developed ALS later through linkages with the national registries there. Another advantage is blood samples were collected and stored at the time of enrollment, which made it possible to identify who has ALS in these cohorts, randomly match a couple of controls to each case, and then pull those samples specifically to analyze them for a suite of pesticides before ALS onset. Approximately 265 ALS cases total would be expected from these combined cohorts. Because these studies focus on a variety of data, it is possible to analyze a number of variables including the following:

[8] Andrew et al., Neurotoxicology 2021
[9] Su et al., JAMA Neurology 2016
[10] Goutman et al., J Neurol Neurosurg Psychiatry, 2019

POPS:
- ❑ Organochlorine pesticides (DDT, DDE, HCB, HCH, trans-nonachlor)
- ❑ PCB congeners (118, 138, 153, 180, 105, 118, 156, 157, 167, 189)
- ❑ PBDE congeners (47, 99, 100, 153, 154)

Covariates:
- ❑ Age
- ❑ Sex
- ❑ Smoking
- ❑ Education
- ❑ Body mass index (BMI)
- ❑ Occupation

Drs. Bradley and Stommel have conducted studies recently on airborne PCBs from some of these toxic release inventories that suggested a signal with ALS, so Dr. Weisskopf and colleagues are very interested in the PCB question among the others. With that in mind, the first 2 specific aims are related to pre-disease POP exposure and ALS risk/survival and the third aim focuses on immune-status in relation to POPs and ALS. Aim 1 is focused on ALS risk and is a nested case-control study using conditional logistic regression that includes the cohorts of cases and controls matched 1:2 on age, sex, and municipality using the national ALS registries in Finland and Demark. Because the cohorts in Finland were set up by municipality, the controls were matched this way as well to account for the time of blood collection. Conditional logistic regression with the blood analysis of the POPs is done to determine whether higher levels of certain POPs or certain combinations of POPs predict higher risk of having ALS. Aim 2 is focused on ALS survival using all available cases. Using a Cox proportional hazards analysis, cases are followed to time of death or end of follow-up time to determine whether their exposures identified in the blood samples predict a different survival rate.

Aim 3 branches into the interesting new area of assessing immune function at the time of blood draw in the cases via extracellular vesicles (EVs). These are nano-scale particles secreted by all cells in the body that are present in peripheral blood and carry cargo from the parent cell, including proteins, micro ribonucleic acid (miRNA), and surface markers. EVs are interesting for a variety of reasons, one of which is that they can come from a neuron from an immune cell and can wind up in circulation and can be identified from the blood samples. Dr. Weisskopf likens these to Star Wars space pods that come off of ships. From any cell in the body, the EVs release vesicle balls from the cells from the parent cells. During the release process, they capture little pieces and parts of the cells they are coming from that can be inside the EVs or on the surface membrane of the vesicle.

The EVs are different sizes, are released in different ways, may mean different things, are involved in signaling between cells at a very long distance, and/or may be jettisoning things from cells. Ultimately, they offer a way in peripheral blood to potentially assay things happening with the parent cells. All sorts of things can be found in these cells such as small RNAs and miRNA that relate to signaling function or proteins and enzymes. Because some of the proteins or enzymes are actually in the membrane, they are identifiable from the outside. That raises the interesting possibility that based on the things in someone's membranes, the cell type they come from can be identified because certain proteins only come from certain cells. The idea with Aim 3 is to try to identify whether exposures to POPs are related to the profile of the EVs in general, the profile of the ones thought to be coming from the immune cells, and whether the extracellular vesicle profile is related to subsequent risk of developing ALS or potential survival with ALS.

This is driven in part by investigators who have been trying to identify where these EVs come from. There are certain surface markers on those EVs that probably indicate they are coming from immune cells. There have been studies suggesting that the number and percent of these immune-derived EVs decrease with age and that this may be an indication of immunosenescence or "inflammaging." Dr. Weisskopf and colleagues wanted to find out whether they could do the same thing to determine whether there is a different immunosenescence signal in the EVs in people exposed to POPs or people who are going to go on to develop ALS afterwards. This is done with a colleague of Dr. Weisskopf's, Dr. Quan Lu, who has done a lot of work with EVs. They have begun to do some of this work with Dr. Lu with some of the samples. The process essentially is to dilute the serum sample, spin it in an ultracentrifugation to separate out the EVs, and then count the vesicles that remain. So far in the pilot samples from the Finnish cohort, there is a very strong signal. Tagging now must be done to determine whether these have markers on their surface indicating where they came from.

One of the key elements of the study is ability to assess mixtures in terms of multiple pollutants at a time and their possible synergy/antagonism and multiple EVs and characteristics. The methods utilized for this are dimension reduction (PCS, SEM) and kernel machine regression (KMR)-non-parametric that allows for non-linearity and interactions. Rajarshi Mukherjee, who is an expert in mixtures analyses of dimension reduction and KMR in the Harvard University Biostatistics Department, is working with Dr. Weisskopf and colleagues to assess interactions, synergies, patterns, et cetera. This will allow for assessment of EVs as possible mediators with a "meet in the middle" approach: POPs→ EVs, EVs→ALS, commonalities. Several findings could come out of this, one of which is that higher pesticide exposure may be related to ALS or the pesticide exposures are related to the EV profile. Another is that the EV profile may predict high risk of ALS, which would be very interesting for many reasons, not the least of which is having a biomarker in the near future of who may be more likely to develop ALS. Part of the analysis approach would be to assess whether any signals seen would suggest that the effect on risk of ALS is acting through these effects on the EVs.

Around the time this funding was awarded, the European Union (EU) changed its rules about data privacy and sharing. This resulted in the need to work through this process to be allowed to get these samples and the data. Each European country is dealing with this differently, given that they each have their own lawyers who interpret the rules differently. Finland has now said this can be done much more rapidly, the investigators are still dealing with their Danish colleagues and lawyers. While progress has been made, several hurdles have arisen along the way. The analyses have begun on the Finnish cohort, so there are some preliminary results for that cohort. At the time of this presentation, there were 97 cases and 94 controls, a slight predominance of women, some education data (the first cohort did not collect these data, so other ways are being considered to tackle this), smoking status, and a lot of follow-up data with an average of about 34 years from when the cohort was recruited. Once the cases developed ALS, the average survival was just over 2 years. In terms of preliminary data from the Finland Cohort on the analysis of POPs, in general with most of these compounds, there are much higher levels than would be seen currently in the US based on the 90th percentile from National Health and Nutrition Examination Survey (NHANES) from 2003-2004. That makes sense in terms of when these samples were collected, with some as far back as 1970 or so. The levels of PBDEs in the Finland cohort are extremely low.

Discussion Summary

Dr. Dave asked whether there are plans to measure neurofilament light chain (NfL) protein in these serum samples and related to that whether they have deoxyribonucleic acid (DNA) from the same participants in the Finnish and Danish studies so that they can assess predisposition.

Dr. Weisskopf indicated that measuring NfL protein was not part of the plan. It certainly is a possibility in the sense of the samples in that they did not use it all for the EV analysis. To the extent that this could be done in leftover samples, it could be considered. The efficacy of doing this in serum and the cost are unclear. There is a slight issue in that the protocol was written for certain analyses, so both Finland and Denmark would have to agree to the additional analyses. He did not believe extensive genetics had been done in Finland and Denmark or whether they have DNA in order to do that. It certainly is something they can look into that would be very interesting, but more funding probably would be needed to do this. There is a potential problem of age and when the samples were collected in terms of measuring NfL. This also has been an issue with the POPs. There is an interesting scenario that the sample was collected well before ALS onset, but there are a variety of times. To some degree, they can explore in the data whether a signal shows up if the blood sample which was taken 5 years versus 20 years before ALS developed. The complication pertains the age of the person when the blood was drawn and how long before ALS onset the sample was obtained. There is a plan to try to tease this apart from the POPs and certainly could be analyzed if they performed the NfL analysis. Denmark data were collected in 1993-1995, so it would be the same era and the age distribution was much tighter. There is age distribution in the Finland cohort that overlaps with the Danish cohort.

Dr. Dave observed that this raised a good point about when the potential exposure occurred and when the serum may have been collected. He asked whether they have epidemiological information from this cohort on where the person lived, if they worked at a particular fertilizer plant, et cetera similar to the type of information that CDC collects.

Dr. Weisskopf responded that they do have the type of information that CDC collects, though it may not be in exactly the same format. They have the occupation history of the individuals in these cohorts—maybe not before because of the timing, but from the survey. After a certain date in each country, it is possible to link back to national registry data on occupation. They also have data on where people lived, what part of the country, et cetera. It is interesting that even the earliest blood collected in Finland is around the time that most OC pesticides and PCBs were banned in Finland and Denmark. These are very long half-life compounds, so the likelihood of newer exposures to these after the time the blood was collected is actually relatively low. They still will persist in the body for many years, but probably the majority of exposure to both OC pesticides and PCBs probably came prior to when the blood was collected.

Dr. Stommel asked for Dr. Weisskopf's opinion on whether some type of an epidemic is being created of neurodegenerative disease jets dumping huge amounts of flame retardants on the wildfires that are occurring.

Dr. Weisskopf answered that there is a lot to juggle there in terms of stopping wildfires. While he did not know exactly what formulation has been dropped, certainly PBDEs are limited. Although, PBDEs may not be in the formulation since they are not great at being flame retardants. Nevertheless, what is being dropped on wildfires is certainly worth paying attention to. However, this is even more difficult than that because even some of the organic burning that comes from the wildfires just in and of itself produces dioxins. Regardless of what is been being dropped on

the fires, the fires themselves are producing this from the combustion process. There are many reasons that these wildfires are problematic.

Dr. Michael Benatar asked Dr. Weisskopf to comment on the stability of POPs in biobank samples; the use of biobank as opposed to fresh samples for EV isolation; and the methodological idea of the normal level of the circulation of POPs based on the general population in terms of the stability of POPs in biobank samples, the requirements for sample collection, processing, and storage, and whether contemporaneous normative population level data are needed to know how to interpret this.

Dr. Weisskopf indicated that the biobank samples work to assess EVs. He did not know whether anyone had done this with samples that have been held for 30 or 40 years, so that is an issue. Matching is based on when the blood was collected, so there probably are no differences between the cases and controls in this respect. They are building on the idea that it has been shown that this can be done on frozen samples, although admittedly not shown for 40-year-old samples. They seem to be getting a pretty robust signal of EVs, so he hopes that still bears out once they do the flow cytometry to identify what is on the surface and verify that they really are the EVs. POPs are persistent, so the thought is that they probably survive freezing and long-term storage. There are studies on this suggesting that POPs are quite stable and this is one of the reasons for the focus on POPs instead of organophosphates that have a much shorter half-life and are probably not as stable if kept for a long time. With the caveat that one has studied this over 40 years, there have been studies of several years of storage at -80^0 showing that this seems to be fine. In terms of contemporaneous normative levels, it is good to have these older samples that have much higher levels in that it provides more power to look at whether these are in fact associated. As levels come down, it becomes harder to do this because the range of exposures becomes smaller. That said, looking at whether dose responses, even down to the levels that people are showing in their systems now, still would be having an effect will need to be assessed. They are doing this with the idea that if signals like this can be identified, and in particular identify biological mechanisms such as things in the immune system that can be assayed through EVs or other approaches like NfL, this may offer an indication of mechanisms that might be at play. Identification of biological mechanisms that might be at play could have implications for other factors that are perhaps higher exposures today that might be relevant. It is important also to pay attention to the intervention side in terms of whether there is some sort of fingerprint that identifies who may be at higher risk for whom interventions could be targeted.

Dr. Finger emphasized that regarding the policy implications of the findings, there is the logic of even if certain compounds are known to be very harmful, identifying the mechanism also is very important.

Ms. Collet asked whether these concepts perhaps apply to ALS in the military in any way.

Dr. Weisskopf replied that he does think the concepts apply. One issue of concern is the massive amount of potentially toxic substances to which military personnel might be exposed. Delving into the military experience is of interest because of the signal there seems to be for general military experience. Toxic exposures are certainly high on the list of possibilities.

Dr. Boyce added that her upcoming presentation on the exploration of the qualitative answers received on the open-ended question analysis may shine some light on this as well.

Dr. Mehta noted that perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been in the news in terms of the potential relationship to cancers and so forth. He has not heard anything about PFAS and ALS, but this potential could arise in the future if a link is identified. This substance is ubiquitous in people's blood, cookware, et cetera. He asked whether there was anything in Dr. Weisskopf's experience related to PFAS or perfluorooctanoic acid (PFOA) at this time.

Dr. Weisskopf said he did not know whether there were any specific studies on PFAS and ALS and it was not in their thinking when they developed the protocol for this study. He was not certain whether these samples may be too old to have much PFAS. In theory, this is absolutely an issue that should be considered. The laboratory in which they are doing their analyses does not do PFAS. If they do identify immune-related signals in their work, this may provide an indication that PFASs are even more important to examine. A key aspect of PFAS that has been identified that seems to be a strong signal is immune dysfunction that is caused by PFAS.

Dr. Goutman added that in the metabolomic data he and his colleagues generated through the CDC grant, they were able to measure PFAS and PFOA in untargeted metabolomics and there were high numbers in their cases and controls. These seem to be widely disseminated toxins with a lot of exposures, so they did not detect any differences. This is untargeted metabolomics, so they are not seeing exact concentrations. But, it may be difficult to sort this out since the population is so widely exposed.

Dr. Weisskopf noted that Dr. Alberto Ascherio has done a lot of work as well. He has done a lot of work in different arena with Epstein-Barr virus (EBV) and multiple sclerosis (MS). The levels of EBV are extremely high, yet he still sees very distinct signals suggesting that it is related to MS. Given that PFAS is ubiquitous, consideration has to be given to finding a way to get good discrimination.

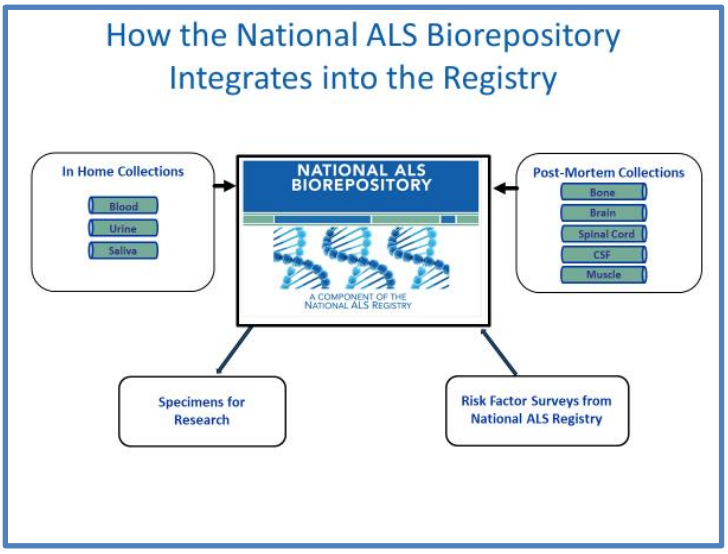
Dr. Eva Feldman emphasized that their data are robust and the levels are extremely high. They did not see a case-control difference and the numbers are shockingly high and robust, so she asked Dr. Goutman to mention this to the group. While they can look further at their data, she and Dr. Goutman did not think there would be a signal.

National ALS Biorepository Update

Laurie Wagner, MPH
Coordinator, National ALS Biorepository
McKing Consulting Corporation

Ms. Wagner presented an update of the previous year's activities in the National ALS Biorepository, which is a component of the National ALS Registry; provided an overview of the biospecimens and post-mortem specimens that have been collected and are being stored currently in the Biorepository; reviewed the results from Expert Working Groups convened earlier in 2022; reviewed researcher requests and supplies that have been distributed; and briefly discussed the partnership with the ALS Postmortem Tissue Core at Johns Hopkins University (JHU).

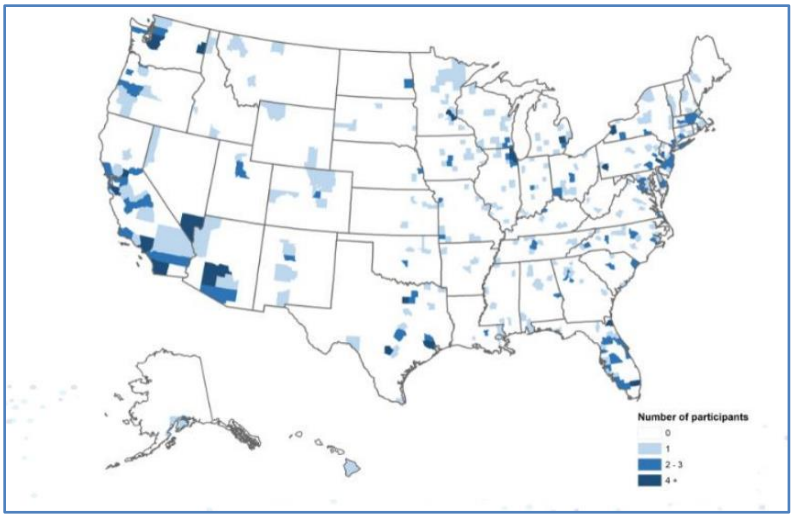
This diagram illustrates the inputs and outputs of how the National ALS Biorepository integrates into the National ALS Registry, with the exception of the new activity with the ALS Postmortem Tissue Core at JHU that makes additional post-mortem samples available to investigators for ALS research:



Risk factor survey data can be distributed with the post-mortem collection samples. In-home collections include blood, urine, and saliva. At this point, only blood is being collected. From the blood samples, DNA, peripheral blood mononuclear cells (PBMCs), plasma, and serum are processed. There are some metals-free samples in the Repository. Post-mortem collections include brain, spinal cord, cerebrospinal fluid (CSF), and muscle samples. Now additional post-mortem samples are available through the new activity with JHU.

COVID-19 has continued to impact in-home collections. While in-home collections were restarted in June 2021, there has been increased rescheduling of appointments due to illness of either participants or phlebotomists. There continues to be low interest from Registry enrollees, and illnesses among participants and phlebotomists continue to impact in-home collections. Pre-COVID-19, there was an average of about 20 collections per month. This has decreased to about 8 to 10 collections per month at this time. To help address this, they obtained permission from the IRB for blood draws to be completed in physicians' offices during participants' already scheduled appointments. Kits were sent to participants to take to their doctors' offices. If needed, doctors could contact the Repository Participant Coordinator for guidance with the process. The samples are then shipped to the Biorepository laboratory. An opportunity also was added for home health care workers or nurses who went to participants homes already to collect the samples. These alternatives have kept things moving, but there remains lower interest from Registry enrollees.

When a registrant clicks the box in the ALS Registry to sign up for the Biorepository, a packet is sent out to them. In terms of Biorepository participation from October 1, 2021 – July 31, 2022, the Biorepository team has consented 81 participants and 75 collections have been completed. The goal for this year is 100, which they hope to achieve by the end of this fiscal year. Of the 75 participants for whom in-home blood collections have been completed, about 59% are male, 41% are female, and 49% are in the age range of 60–69 years. This map shows the historical distribution of all participants:



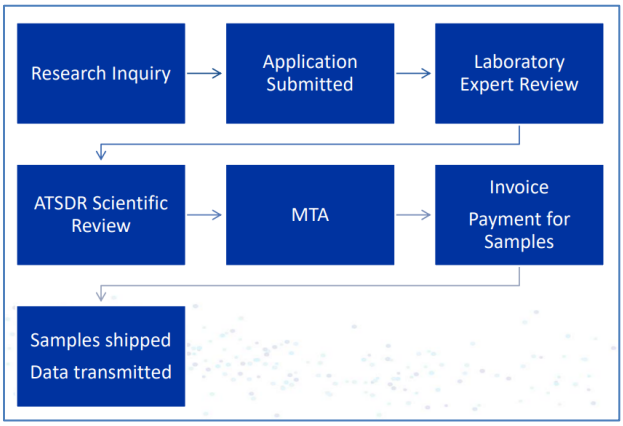
The Biorepository Team has been fairly successful, even during COVID-19, in maintaining geographic representation. Collections have been obtained from participants in all of the contiguous states and Puerto Rico. This table delineates the Biorepository sample inventory from a total of 1,942 participants as of July 31, 2022:

Sample Type	# of aliquots	Aliquot size	# participants
DNA	22,870	2 µg	1,428
Fingernails	268	10 nails/vial	268
Hair	241	40 strands/vial	157
PBMCs	1,106	500,000 cells/vial	148
Plasma	9,130	0.5 ml	1,230
RBC	3,856	1.0 ml	1,229
RNA	10,070	2.0 µg	1,157
Serum	7,172	0.5 ml	1,223
Urine	10,046	1.8 ml	1,062
Urine w/preservative	690	4.5 ml	687
Whole Blood	2,754	1.8 ml	1,199

There are still stored samples of hair, urine, and fingernails for researchers to request even though these are no longer collected. A total of 51 participants have donated post-mortem brain, spinal cord, CSF, bone, muscle, human primary cells (n=28) samples. A total of 7 participants withdrew for various reasons and did not donate, and 6 participants continue to be followed. Among the 51 post-mortem participants, 51% are female and 49% are male. Almost half of the participants are in the 60–69 years of age category. While the Biorepository is no longer recruiting for post-mortem participants, those who have been enrolled continue to be followed.

Given that the Biorepository has been functioning for 10 years, it was felt that there was a need to reevaluate the operations and the samples being collected. Therefore, 2 Expert Working Groups were convened virtually in January 2022, one for biospecimens and another for post-mortem samples. A lot of positive feedback was received from both sessions. In 2021, collection of metals-free biospecimens was stopped. The Expert Working Groups suggested that this be reinstated, so metals-free collections will be added back to the sample collection kit. This should begin again in October 2022. Collection of PAXgene tubes for RNA extraction also had been stopped. The Expert Working Groups also suggested that this be added back. Previously, there were 2 tubes and only 1 will be added back in October 2022 as well. There were no changes to the current post-mortem collections. In terms of data changes, samples will be linked to the Northeast Amyotrophic Lateral Sclerosis (NEALS) Consortium and NIH Globally Unique Identifier (GUIDs). NEALS already has been added in and will be promoted as being available for researchers when they are collecting samples so that there will not be so much overlap. They are in the process of working on the NIH GUIDs, though this will take longer.

Regarding the researcher requests and sample distribution process, after the pilot study was completed in 2007, ATSDR launched the online system through which scientists could request samples to use in ALS-related research. One of the most successful ways information was disseminated about the Biorepository was through attending scientific conferences, although this was limited early in the COVID-19 pandemic. While Ms. Wagner attended a few conferences virtually, this platform is not quite as successful as being able to talk to researchers in person. In addition, several scientific journal advertisements have been posted over the years. Partners also help to promote the Biorepository. CDC and Twitter are used for social media, and there are other means by which the Biorepository is promoted. The Biorepository also is mentioned in Request for Application (RFA) notices. This schematic illustrates the sample request process:



The applications are typically received by Ms. Wagner and Dr. Wendy Kaye. Many researchers reach out to them with inquiries before submitting an application. This helps to guide researchers in terms of letting them know if their samples of interest are available. There is an online system for application submission. Once received, the application is submitted for Laboratory Expert Review and any recommendations and if approved, is submitted for ATSDR Scientific Review. Once that is completed, any edits have been made, and the application is approved, the application is then submitted to the Biorepository. At that point, a (Material Transfer Agreement) MTA is created and executed. An invoice is then created, which involves a minimum fee for pulling and shipping the samples. There are no charges for the actual samples, but they do have to reimburse the laboratory and other groups for pulling and shipping samples.

Once the invoice is paid, the samples are pulled and shipped, and then the data are transmitted. This process typically takes up to 3 months from start to finish. There are always factors that cannot be controlled, but an effort is made to get the samples out as quickly as possible.

From October 1, 2021–August 25, 2022, there were 12 inquiries specific to the Biorepository and samples, 8 applications were received, 1 application was transferred to JHU, 6 applications were approved, and samples have been shipped to 4 researchers. The 2 applications that are pending were approved but are awaiting funds to pay their invoices. These tables show the types of biospecimens and post-mortem samples that have been requested from the Biorepository:

Biospecimens

Sample Type	Approved and Distributed	Applications in Process	Inquiries, but no application to date
DNA	3	n/a	1
RNA	2	n/a	2
PBMCs	2	n/a	3
Plasma	6	2	4
Serum	3	1	2
Whole Blood	5	n/a	3
Urine	3	1	2
Fingernails	1	n/a	n/a
Hair	1	n/a	n/a

Post-Mortem Samples

Sample Type	Approved and Distributed	Applications in Process	Inquiries, but no application to date
Brain	6	n/a	1
Spinal Cord	2	n/a	3
CSF	3	n/a	5
Muscle	n/a	n/a	1
Human Primary Cells	2	n/a	1

Recently, there seem to have been a lot of requests for plasma from the biospecimens, which the Biorepository Team has been trying to track in case any changes need to be made. There is 1 application in process, but samples are pending for the others. About 8 researchers have made inquiries and are working on their applications, but a lot of times they are waiting for funding and this can take longer than expected. While there have not been any requests yet for bone or muscle post-mortem samples, there have been inquiries for muscles. Samples have been requested from a diverse and balanced set of groups (e.g., pharma, private, government, and universities).

As mentioned earlier, the Biorepository now has a partnership with the ALS Postmortem Tissue Core at JHU. The benefits of this partnership with Dr. Ostrow and his team is that it expands the number of post-mortem collection samples that are available. Sometimes researchers contact the Biorepository and need more samples than are available. Partnering with JHU enables researchers to have access to samples from both JHU and the National ALS Biorepository. An effort is being made to streamline the process by which samples can be requested. Meanwhile, it has been possible to supply the samples needed from the Biorepository and JHU. It has been very helpful to be able to collaborate with Dr. Ostrow and his team. Sometimes researcher requests are received that need some fine-tuning or more interaction, and calls with Dr. Ostrow and the researchers have been very helpful in terms of streamlining requests and keeplng everyone moving in the right direction.

Early on in the COVID-19 pandemic, sample distribution was more challenging. However, there were still limited opportunities to promote the Biorepository. Shipment of samples outside of the US was affected by customs limitations. Ms. Wagner currently has a shipment headed to Canada, which is more challenging in general. She is trying to make sure that all documents are in order. The Biorepository laboratory assists with this, but it is still sometimes difficult to get samples outside of the US. Another challenge is that laboratories receiving samples may have limited staff working to receive shipments. She tries to keep shipments to the middle of the week and ship as quickly as possible, but there is still not full coverage in all laboratories at this point.

In terms of future directions for the National ALS Biorepository, there are not a lot of changes anticipated at this time. Recommendations from the Expert Working Groups will be implemented. In-home blood collections will continue, as will follow-up of post-mortem participants. The Biorepository Team will continue to collaborate with the JHU Team. A process will be established to accept samples from outside ALS studies and repositories is under consideration, which has been recommended a number of times throughout the years. While this has not yet been implemented, processes, permissions, and other documents that will be needed to do this are being evaluated.

CDC National ALS Registry – JHU ALS Postmortem Tissue Core Collaboration

Lyle Ostrow, MD, PhD
Johns Hopkins University School of Medicine
Director, Johns Hopkins University Tissue Repository

Dr. Ostrow began with some background about why JHU established the ALS Postmortem Tissue Core Collaboration. ALS is a fatal disorder with variable presentations, rates of progression, and biological "causes." ALS can present and progress in different ways, numerous biological pathways are implicated in causing ALS in various patients, there are different genes, there are different exposures, and so forth. All of the information that is known about ALS comes from laboratory experiments. Despite promising pre-clinical data in laboratory assays and animal models, countless drug candidates have failed to translate into successful clinical trials. This raises a lot of questions about why that is, such as:

- ❑ Are the animal models and cell cultures created by genetic manipulation a good model for what happens in people with ALS?
- ❑ Are we testing the right treatments in the right people with ALS and at the right times?
- ❑ Can we do a better job of validating new laboratory results with relevant patient-derived laboratory tools?

It is possible that patient-derived laboratory tools like biosamples can help with this. There are many cells and pathways that are likely to contribute to neurodegeneration in ALS. The following is a partial list of examples of "pathogenic" mechanisms that have been implicated in ALS:

- ❑ Glutamate induced excitotoxicity
- ❑ Reactive gliosis and other astrocyte dysfunction
- ❑ Oxidative stress
- ❑ Protein aggregation / misfolded proteins
- ❑ ER stress
- ❑ Mitochondrial dysfunction
- ❑ Activation of neuro-inflammation
- ❑ Impaired axonal transport
- ❑ Oligodendrocyte dysfunction
- ❑ Axonal degeneration
- ❑ Dysfunctional RNA processing
- ❑ Endogenous retroviruses
- ❑ Abnormal nucleocytoplasmic transport
- ❑ TDP-43 mis-localization and loss of function
- ❑ Microglial activation
- ❑ Dysfunctional protein quality control
- ❑ Autophagy
- ❑ Cortical hyperexcitability
- ❑ Environmental exposures

Some of these mechanisms are very general and every cell in the body uses them, but an abnormality someplace in the right circumstances and person is somehow contributing to causing ALS. While the SOD1 gene was discovered in 1994 and many genes have been discovered since then, a genetic abnormality is not detected in a large portion of ALS patients. In most cases, the disease may be the product of multiple inter-related factors. Many efforts are focused on identifying distinct patient subsets, such as with various integrated –omics approaches. In terms of why patient-derived biosamples are important, no laboratory model or assay represents the findings of every person with ALS. Comparisons are not unlike an apple and an orange. The problem is that tissues have many types of cells in the human brain. Neurons are only one kind of cell in the brain tissue. Is a ground up piece of apple pie the same as apple sauce?

As the disease progresses, the ratios of the different ingredients in the pie change. Therefore, it is important to be careful about designing experiments and figuring out how to test whether something seen in a cell culture is actually going on in a patient's tissues. Rigorous and standardized clinical, epidemiological, and pathological data linked to the biosamples are needed. There is a substantial unmet need for high quality matched biosamples (blood, CSF, tissues) and data for ALS research. Given that no laboratory model or assay can recapitulate the findings of every patient with ALS, it is important to validate findings with relevant patient-derived laboratory tools, including postmortem tissues, induced pluripotent stem cells (iPS) cells, and biofluids. There also is a need for rigorous and standardized clinical, epidemiological, and pathological data linked to the biosamples. It is not just about having a lot of data. The data have to be the same, with everyone using the same words for the clinical and neuropathology elements. At the end of the day, there are just not enough samples.

The JHU ALS Postmortem Tissue Core now has a goal to provide high-quality, well-characterized post-mortem tissues and data that specifically meet the needs of academic and industry ALS researchers around the world while maximizing the use of every case by as many researchers as possible, ensuring responsible use of the tissues, fostering collaboration, and promoting open science and the rapid sharing of data to accelerate ALS research. Standard operating procedures (SOPs) are in place for tissue dissection, processing, quality control (QC) analysis, clinical data elements, and neuropathological characterization that are specifically optimized for ALS research. Whole genome sequencing (WGS) and multiple Central Nervous System (CNS) region RNA sequencing (RNA-Seq) are made immediately and freely available without embargo for every autopsy and are linked to bar-coded tissue/slide inventories, QC measures, and de-identified clinical, demographic, and neuropathological metadata. All policies are designed to provide samples and data as quickly as possible, while ensuring responsible, open, and unbiased use of all Core resources. Researchers using samples and data retain full ownership of their ideas and results, without authorship/IP requirements.

The JHU ALS Postmortem Tissue Core collects frozen and fixed CNS tissues, liver, and several muscles from ALS/MND and non-neurologic control autopsies. In standard autopsies, things are dissected in a way that is not very useful. ALS researchers are interested in particular parts of the brain, parts of parts, or tiny pieces. The Core finds the parts that researchers need and dissects them initially into tiny pieces that are the exact size needed for research so that this does not have to be done later. The goal is to produce the maximum number of individual, optimally-sized tissue samples from each ALS-relevant region, while preserving the architecture of the tissue. This minimizes subsequent freeze-thaw and labor that otherwise is necessary when re-dissecting frozen slabs or larger tissue regions. They have a bar-coded inventory system that is maintained by NEUROBANK™ that is de-identified. When a researcher has a particular need, the Core's goal is to have the tissues ready to go and not have to do anything else. They know what researchers need because they periodically survey the research community to ask them about what assays they are using, sample sizes, QC variables, clinical data needed, et cetera in order to meet evolving needs.

The JHU ALS Postmortem Tissue Core says "yes" to every request and does its best to meet the needs of every researcher. Requests are evaluated based on feasibility rather than the science in terms of whether the investigators can do the experiments and whether the result will be interpretable. There is a need to ensure that scarce and precious samples are not wasted, experimental plans are optimized, and staining protocols and tissue assays are validated. If a researcher has not validated their assay/staining techniques using human CNS tissues, the Core provides test tissues before providing more high-demand/scarce samples. Experimental designs always should consider the next steps, such as identifying potential biomarkers for eventual clinical trials. The JHU ALS Postmortem Tissue Core will work with researchers to optimize their experimental design. Existing data and analyses are leveraged to provide robust complimentary results, such as using genomics and neuropath data to plan "Black/White" test sample sets. This is the ultimate donation. None of this would be possible without the generosity of incredible patients. If patients and families are donating their tissue, it is imperative to make sure that samples are being used responsibly by as many researchers as possible and not wasted.

Rather than focusing primarily on sample procurement and analysis, most of JHU ALS Postmortem Tissue Core's efforts are devoted to working with researchers to select the optimal samples and data. They started using Zoom before the pandemic began because of the frequent interactions with the research teams using the samples. It has been amusing to watch the world go through this process of learning to use Zoom effectively. Requests for samples and data are reviewed on a rolling basis, using established criteria that emphasize experimental feasibility and appropriateness of sample sizes and quantities. The Core formulated standard MTA for both academic and industry collaborators for all sites with common language. For a post-doc, new investigator, or established scientist with an entirely new idea, they can provide the resources and data rapidly to obtain preliminary
results, and then foster further development and real-time collaboration as the idea evolves. Often, the Core can facilitate collaborations with established academic or industry laboratories already using its resources to provide robust complementary data and results. New ideas can be tested rigorously within weeks, rather than the months to years that normally would be needed to apply for grants, get funded, establish laboratory assays locally, and conduct preliminary experiments.

The Core spends a lot of time considering what it means to "validate" an experimental result using human tissue in terms of what types of measurements to make; how to relate those to a particular type of ALS; whether the questions can be used to design the optimal tests; how the postmortem results will affect what a researcher does next; whether a researcher thinks a mechanism is specific to a genetic subtype, gender, or other subgroups; how far iPSCs were differentiated; and the nature of the samples used in the original experiment. If cell lines are apples, bulk tissue is apple pie, is it reasonable to assume that the ratio of apples to other ingredients changes as the disease progresses? The Core spends a lot of time talking about what the actual go/no go decision will be based on these data. There are numerous anatomical considerations involved, including conserving the scarcest resources, understanding that bulk tissue assays on spinal cord have lots of variability, thinking about when it is appropriate to use thoracic instead of cervical or lumbosacral spinal cord, and considering whether to use primary motor cortex versus spinal cord. Decedent selection considerations involve consideration of what "controls" would provide the best comparison for the question being asked, pathological variability versus assay variability, and the meaning of "end stage" tissue.

There are a number of autopsy selection considerations as well. Consideration must be given to what "controls" provide the best comparison for the question being asked. What is a control? Should a control be someone who died from a non-neurologic cause? Maybe not because maybe what is being assayed is just a sick brain. If it is an autopsy and someone died, that means something went wrong. Perhaps they had pneumonia, cancer, or something else that might affect their brain cells. A lot of times, a better control is to take regions of the brain that are involved in ALS and compare them to regions from those same brains that are not involved. This also is true of the clinical data in terms of how the time of the clinical data relates to the autopsy tissues. What happened around the time of death? Was the person sick, in a nursing home, having breathing trouble? This is all very important information.

Assay validation considerations also are important. Initial slide/sample requests are frequently calculated based on looking for large panels of targets in multiple CNS regions. The Core provides test sample/slide sets to help optimize staining. "Quantitative immunohistochemistry" remains challenging, especially in spinal cord. Frozen tissue-based assays or "bucket biology" on homogenates and batch effects must be considered. They also spend some time trying to determine ways to get results that are invalid, because this helps them to know how to design assays. When in doubt about QC or comparison variables, they do try to screw up the experiments purposely to determine the variables that matter when providing more samples in order to be responsible. Here is an example of an email recap following a Zoom call about a new request, which follows the discussions the Core has with every researcher for every request:

Example Recap Email Following Zoom TC About New Request

From: Lyle Ostrow <lostrow1@jhmi.edu>
To: XXX
Subject: Recap of Zoom TC to discuss XXX Core Request

It was great talking just now! Here is a quick recap of our discussion/considerations, and plan:

You have used iPS MN lines from sALS, controls, and SOD1 to look at mitochondrial protein expression and identified ~10 targets that appear different between ALS and CONTROLS, but not really a clear difference between SOD1 fALS and sALS iPS lines.

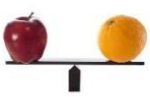
It isn't clear whether these changes are UMN/LMN specific from the iPSC-MN data, but you do see a different pattern when you differentiate iPSCs into dopaminergic neurons. You did not see a clear difference in RNA expression patterns between your iPSC groups, and thus you think that these changes are perhaps regulated at the protein rather than the RNA level.

You have previously isolated mitochondria for analysis from human frozen banked caudate autopsy tissue for an HD-related project, but not from cortical regions.

We discussed a few specific caveats:

- The majority of mitochondria in a bulk tissue sample may not be from motor neurons vs other cell types. This can be somewhat mitigated by normalizing your data to cell-type specific protein levels.
- The MNs won't be a constant proportion of the cells – even in different regions of the motor cortex in the same decedents.
- The MNs remaining at autopsy may in fact be ones that have a "protective" phenotype/expression pattern.

We are going to start with a test sample set (see below) – to optimize methods and see what is feasible given these possibilities.



PLAN GOING FORWARD:

1. We will send a test sample set comprised of:
   a. Frozen primary motor cortex from a single sALS case and a single control case. We will include two different motor cortex regions from each. (4 samples total). We will try to send larger samples (~400mg).
   b. We will send an "uninvolved" cortical region from each of these two cases as well (the same region for both cases).
   c. We will send a large sample of frozen THORACIC spinal cord from each of these two cases to see whether the thoracic tissue has enough quantifiable grey matter signal.

   Do you want us to keep the identity of the test samples blinded? **We can unblind at any time.**
2. Attached to this email is the form to request the genetic raw data. Just fill it out and send it to the address listed on the email.
3. Kathryn will send you the Hopkins MTA right away.

FEW MORE QUICK QUESTIONS:

1. Have you ever looked in non-CNS tissues? Would you like to? We also have muscle samples from these cases if there is a rationale for analyzing it. Since you said that differentiating into other neuron classes looked different, I expect that the answer is "no" – but I figured I would ask. To that end… did you ever do your mitochondrial analysis straight on the fibroblasts/PBMCS or non-differentiated iPSCs?
2. Here is a link to a manuscript doing unbiased RNA analysis of our postmortem cortex samples that I thought might interest you – mainly because it found that there is a specific subset of patient samples that showed an oxidative stress signature: https://doi.org/10.1016/j.celrep.2019.09.066. **We have annotated the samples in our biorepository with their cluster identity from this study.** Thus, I wonder if it might be interesting to specifically choose samples for your mitochondrial analysis from decedents in the different clusters.

Best wishes,
Lyle

Lyle W. Ostrow, M.D., Ph.D.
Director, JHU ALS Postmortem Core

The JHU ALS Postmortem Tissue Core would not be able to do any of this if not for the collaboration and support that they receive from the CDC/ATSDR ALS Registry. Since April 1, 2021, the Core has provided 2,638 slides and 215 frozen tissue samples to researchers. They have provided letters of support (LOS) for 15 grant and fellowship applications to the NIH, including 4 R01s, 2 Small Business Innovation Research (SBIR), and 1 R21; CDC/ATSDR; Amyotrophic Lateral Sclerosis Association (ALSA); Congressionally Directed Medical Research Programs (CDMRP): ALSRP and PRMRP; TEDCO's Maryland Innovation Initiative (MII), Alzheimer's Drug Discovery Foundation (ADDF)-Harrington; and others. There have been at least 12 manuscripts published, 1 PhD thesis defense, and several abstracts/posters presented at meetings. Between October 1, 2021 and August 15, 2022, the Core discussed ongoing and new requests via Zoom for 59 different ALS research projects. In most cases, this includes multiple zoom meetings about each ongoing research program.

The following lists illustrate some of the laboratories and institutions working the Postmortem Core and the research projects using Postmortem Core resources over the past 16 months:

Labs/Institutions working with our Postmortem Core over past 16 months *(list is incomplete)*

- ABBVie
- Barrow Neurological Institute
- Biogen
- Celdara
- Codiak Biosciences
- Cold Spring Harbor Labs
- Columbia University
- Harvard / MGH
- Dartmouth Hitchcock Medical Center
- Janssen R&D
- Johns Hopkins University (8 labs)
- LSU Health Science Center
- Mount Sinai Medical Center
- New York Genome Center
- NIH (NIA and NINDS)
- North Carolina State
- Northwestern University (2 labs)
- Takeda
- Tufts
- Ulm University (Germany)
- UC San Diego
- Unity Biotechnology
- University of Chicago
- University of Colorado
- University of Massachusetts
- University of Southern California
- Uniformed Services University
- University of Texas at Arlington
- University of Texas HSC Houston
- Verge Genomics
- Van Andel Research Institute
- Quralis

Examples of Research Projects using our PM Core Resources over past 16 months *(list is incomplete)*

- Dipeptide Repeat trafficking in ALS and FTD
- SOD1 & VCP interactions and protein persistence
- Molecular mechanisms underlying DPR synthesis in C9orf72
- Immune dysfunction in ALS4 and other MNDs
- ALS pathway enrichment from exposure to exogenous compounds
- PFN1 aggregation in ALS tissues
- Methyltransferases and LINE1 in C9orf72 ALS
- Cryptic Exons splicing in ALS and IBM muscle
- rhMG53 as potential novel ALS therapeutic
- Senescence /Senolytics in ALS
- RAGE dependent microglial signaling
- Nucleolar stress activated by proteotoxicity
- GDE2 Regulation of TDP-43 localization
- P2X7 on spinal motor neurons
- Blood Brain Barrier disruption in ALS
- Deciphering genetic risk in sporadic ALS
- Somatic mutations in sALS ventral horn MNs
- Therapeutic potential of targeting ISG15 in ALS, and biomarkers linking ALS and TBI
- Development of novel SOD1 Antibody development
- SPTLC1 in ALS
- Pathology of SBMA
- Spatial transcriptomics of ALS motor cortex and lumbar spinal cord
- Cryo-EM of pathogenic cellular aggregates in ALS
- Novel ALS Therapeutic target proteins in neurons and microglia

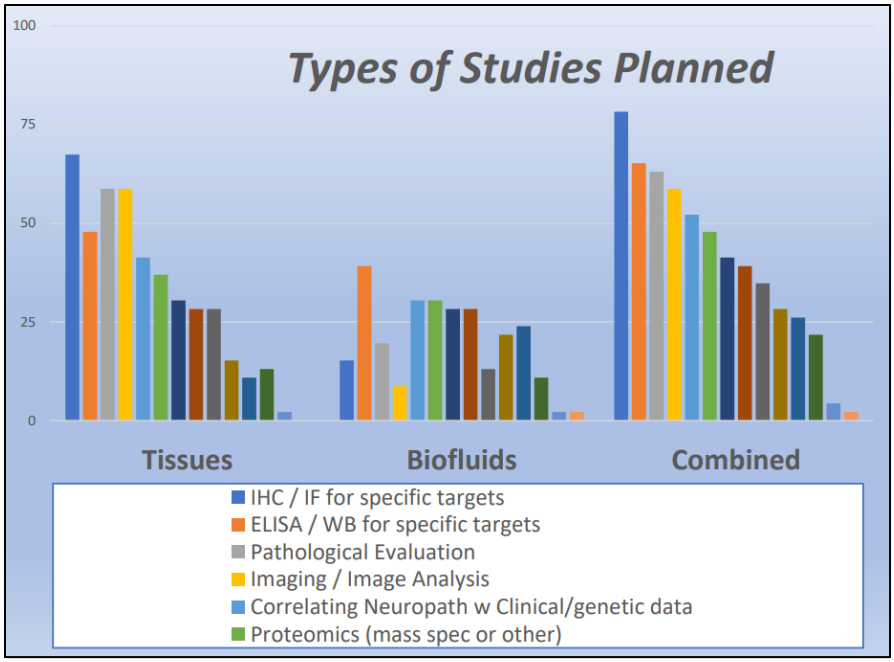
As identified during the 2020 VA Biorepository ALS Brain Bank meeting of all of the brain banks on ALS to discuss how to do this better and how to work better together, it is vital to find ways to work together and to collaborate to make samples and data interchangeable so that researchers in the research community can get what they need. There was a lot of discussion about different ways to compare different decedent groups and different pathologies. Ideally if something is discovered in a subset of tissues, perhaps there is something in the biofluids from those same patients that could be used to identify which patients are more likely to respond to a given therapy. In order to do that, matched biofluids, tissue, and clinical data are needed. The thought has been that the plan should be to collect and analyze everything thought to be important to understand ALS from everyone and to:

- ❑ Recruit as many subjects and relevant controls as possible
- ❑ Find ways to combine efforts with other biobanks and data collection and analysis efforts to increase numbers, breadth of samples and data, and analytical capabilities
- ❑ Make all data, samples, and analyses broadly available
- ❑ Establish central platforms / "data hubs" to combine and analyze multimodal data, and to define and compare subsets of samples and patients
- ❑ Leverage the results, tools, and wisdom of the global ALS research community for everyone
- ❑ Educate and encourage participants in established longitudinal data and biosample collection efforts to consider postmortem tissue donation

JHU and many others spent several months creating a detailed survey to assess ALS researchers' biosample needs. The first 80 responses are included in these analysis results. For the first question, 71% of respondents anticipate needing biosamples in the next 6-12 months. Of those, post-mortem tissues comprised about a third and post-mortem tissues, biofluids, cells, and non-CNS tissue comprised about 44%. Those who selected post-mortem tissues were asked how they needed them (e.g., fresh frozen, unstained, glutaraldehyde-fixed, fixed and frozen from the same regions, et cetera). Importantly, over 50% of respondents indicated matched fixed and frozen tissues from the same region of the brain. Post-mortem banks generally do not do this because generally for an autopsy, half the brain is fixed and half is frozen. That does not work in order to correlate something seen on a slide of fixed tissue with a biochemical assay done on frozen tissue. Therefore, the Core has adapted its SOPs to make sure that there are frozen and fixed tissues from the same hemispheres from the same regions.

The top 5 specific anatomical regions needed included primary motor cortex, spinal cord, frontal pole cortex, muscle, and cerebellum. Muscle is an evolving and growing interest among researchers. A third of respondents said they wanted muscle. Muscle can be sampled during life and also has lower motor neuron branches in it. There is a lot of interest in determining whether patient subsets can be identified and tracked. For the biofluids, the responses were much broader. The top selections were serum, CSF, whole blood, iPSCs from fibroblasts. Muscle was included again because this question asked about tissues collected during life. In terms of post-mortem and biofluid QC measures, "required" and "good to have" seemed to correlate with what people need. Respondents were asked what biosamples they anticipated needing from a long list of samples (e.g., Non-Neuro Controls, C9orf72 ALS, TDP43 ALS, Pre-Symptomatic ALS, ALS + FTD, Sporadic ALS, Other Neurodegen Disorders, SOD1 ALS, FUS ALS, Other MND, Other mutations unspecified, MATR3, Ubqln2, VCP, SQSTM1, ANG, chmp2b, DNAJC7) and the greatest need was identified as being for non-neurological controls. These are the hardest biosamples to get, but they are working to improve this. There also was demand for different types of ALS, which are scarce. The only way to meet researchers' needs is by working together.

When asked about the types of studies planned, the list was very long and illustrates that people are using the samples for the same purposes. These -omics types of studies take a lot of tissue. If ways can be found to do these things centrally and in a highly curated fashion, it will conserve resources and will make the data more valuable and easier for everyone to use. Anything that is going to be done by more than 25% of people who request the samples, it is much more sensible to do that for everyone. The types of studies planned are reflected in this graphic:



Of note, pathological evaluation (gray bar) is high. If standardized neuropath across all of the various repositories, it will help everyone. Responders also were asked what information would be most useful to search for samples across different biorepositories and what clinical metadata linked to biosamples would be most useful. About 50% of people showed interest in new scales and if they can be linked to biosamples, it may be possible to learn how to use them better in clinical trials. In terms of biosamples needed, the top responses were post-mortem tissue, CSF, blood, and cell lines. Of the respondents, 63% indicated that they previously have used biosamples previously for ALS research. Most comments were very positive and researchers were generally satisfied with the quality of samples and data. However, several concerns also were noted as illustrated in the following responses:

- ❑ Samples requested from multiple biorepositories, some not ALS focused. There were challenges and delays in receiving samples from some of these biorepositories, and the type of information and samples collected were not harmonized across the biorepositories. This made standardization of experiments a challenge for the study.
- ❑ It is difficult to obtain samples from patients with certain mutations (TDP-43, FUS…)
- ❑ We have only been able to access our own samples.
- ❑ Mutation information is not always available.
- ❑ Some frozen spinal cord tissue was difficult to immunostain due to poor conditions and presence of excess non-neuronal tissue around it.
- ❑ For some cases received, only scraps of tissue were provided on paraffin slides. Often thoracic cord was received when cervical cord was requested.
- ❑ We have a biobank here… Many of the tissues are from patients many years ago before a lot of the standardized testing… Postmortem interval would be super helpful (we do not have this for 99% of our samples)… Sometimes fixation of tissues (if they've been in formalin for years) can be an issue for staining.
- ❑ Local samples from hospital. Was satisfied in the quality, but numbers were too low.
- ❑ Limited availability of some clinical information (ALSFRS scores, UMN/LMN, etc.). Limited availability of matched biofluid samples… Samples from some repositories are very expensive. Time to retrieve metadata was several months beyond arrival of tissue.

- ❑ Limited/No availability of control samples.
- ❑ Strict rules regarding sharing results and the storage/access of genomic data created from samples (more than is typical for standard genomic data privacy compliance.
- ❑ Only have used samples collected as part of our own research.
- ❑ I've used samples collected by my own organization with variable quality and results.

Regarding a question about how long on average it took to receive samples from biorepositories around the world, generally the time to receive samples requested has generally been okay. The delays are typically regulatory-related, but efforts are underway to try to streamline these issues. Of the respondents, 12% said they had a request for biosamples denied. It was reassuring that only 2 respondents said that the biobank said they were not interested in the proposed line of research or that they wanted credit for authorship. Beyond that, the reasons for refusal were scarcity or unavailability—the types of issues that can be addressed by working together. So far, 17 respondents indicated that they are affiliated with an ALS Brain Bank or Repository and would be interested in contributing to harmonized ALS biosample efforts. This included repositories in Canada, Italy, the US, and the UK. The final question posed on the survey was, "Do you have any additional thoughts related to ALS biosample access that you would like to share?" Some of the responses included the following:

- ❑ Standardized nomenclature and samples collected across the biorepositories, especially those focused on ALS, would be highly beneficial to the field.
- ❑ Linking patient clinical scores to patient tissue samples especially biofluids will be very useful.
- ❑ We are always in need of more healthy and non-neurological controls for biofluids and post-mortem tissue.
- ❑ Post-mortem intervals are important to know and match when looking at protein modifications.
- ❑ Thank you for doing this.
- ❑ Greater availability is always helpful – thanks for your efforts.
- ❑ Thanks for providing this service!
- ❑ Samples with more metadata have been most helpful.
- ❑ I realize there's a limited amount of material and lots of researchers hoping to do similar studies. However, it would be wonderful if there could be some sort of centralized source that directs researchers to studies that have been done and where the open-access data has been made available. For researchers (like me) that need matched samples, this might help us to identify samples that have been profiled in some way, but for which additional material is available to follow up on those samples – through, e.g. Neurobank ID tracking.

The last bullet describes to Dr. Ostrow a modern day registry and biorepository—something that collects and curates all different types of data about people with ALS to look at risks, demographics, and the distribution of epidemiology studies and provides samples and links to clinical trials. If someday there is a global unique identifier that is administered by an entity such as CDC, a 1-stop shop could bring everyone together—Dr. Ostrow's personal dream for the future. In that regard, this is an excerpt from a LOS that he wrote a couple of weeks previously for someone who was applying for a grant:

> As you intend to compare analyses of CNS and muscle tissues, we have identified suitable muscle samples (paraspinal and/or psoas muscles) from 47 decedents whose CNS genetic profiling data is already available from the NYGC ALS Consortium, matched via de-identified GUIDs.
>
> We also can provide both muscle and CNS tissues from an additional 12-15 autopsies whose genomic analysis is not yet completed. Altogether, this will enable you to analyze matched muscle and CNS tissues from at least 50 ALS, 4 neurological controls, and 6 non-neurological control decedents.
>
> You have also requested tissues from decedents who participated in the Answer ALS project during life, for which the de-identified data has already been made publicly available. By matching their de-identified GUIDs, we have identified 8 decedents who also participated in that study, from whom we can supply matched postmortem frozen motor cortex and muscle tissue samples.
>
> All of the samples requested are autopsy material, thus any genomic data (e.g. DNA-seq, RNA-seq, and proteomics) generated from these tissues by your lab may be made publicly available at one of the standard genomic data sharing portals that ensure adherence to NIH genomic data privacy policies (such as through the NYGC's ALS Consortium data portal or through the NIH's dbGAP and Short Read Archive). We encourage you to share this data through one of those portals, as part of our commitment to open data sharing policies within the ALS community.

In terms of future plans, the survey data will be used to harmonize and prioritize the collaborative growth of existing ALS biosample efforts. Efforts also will be made to correlate samples and data collected during life with findings in autopsy tissues across established biorepository efforts; standardize SOPs and methods to share data and resources across efforts; harmonize ALS pathology nomenclature and clinical data elements; centralize analyses and link researcher results back to samples in biorepositories; adopt unified GUIDs; create regulatory "checklists" that enable sharing of components of data between efforts and platform linking via APIs; and establish SOPs and regulatory practices that allow the Core to accept autopsies from other academic centers.

As an example of how other samples can be incorporated, Dr. Ralph Garruto at Binghamton University has been the curator for the entire collection of the Guam ALS and Parkinson's Dementia Complex (PDC) autopsies collected by the NIH center on Guam over many decades. This collection includes frozen and fixed postmortem specimens from 250-300 autopsies of ALS-PD-dementia (Lytico-Bodig), ALS, and control decedents and all of their associated data. Dr. Garruto is retiring and approached the CDC about incorporating these samples and tissues into the JHU resources. This involves a considerable amount of tissue, paper records, filing cabinets, autopsy reports, handwritten information, et cetera and JHU has had to map out a plan to ingest this into its system. Many researchers already have expressed interest in this because it is the prototype of environment and perhaps genomics combining to produce this disease.

In closing, Dr. Ostrow thanked the entire academic, industry, consortia, and nonprofit ALS foundation community, and emphasized that none of this could be done without all of the amazing PALS who donate to these efforts.

Discussion Summary: Wagner & Ostrow

Dr. Siddique asked what the outcome has been in terms of publications, if anything has changed in the way that Dr. Ostrow looks at the disease, and how the field has changed with all of the work put in.

Dr. Ostrow indicated that he started doing this in about 2010 when he was a fellow at JHU when he needed post-mortem samples for his own research for which he was submitting a proposal. He collected and curated samples from JHU laboratories and about a year later, the Target ALS Foundation wanted to create a multi-center resource. He helped design and direct that 6-center project, which is still ongoing and they still work together. As of April 2022, the JHU site

separated from the multi-center effort. Everything he just presented was work that everyone involved in the Target ALS Foundation effort did and would not have been possible without them. He credited the Target ALS Foundation for laying the groundwork for the idea that these samples can be shared without worrying about ownership, authorship, and IP. The New York Genome Center (NYGC) that did all of the genomics for this would not have been able to do all of the work that they did without support from ALSA and others. They all still work together today. There have been numerous publications and at pretty much every ALS science meeting he has attended, at some point over the last decade, they have provided samples or genomic data to 80% of the researchers presenting. In terms of any changes as a result, the way that experiments are designed using these tissues has changed. Originally, everyone was doing everything with an SOD mouse and would request SOD tissues even if their experiments were not specific to an SOD pathway. They would request spinal cord because what they had done in the mouse was homogenized to the mouse's spinal cord. As they have worked with researchers to figure out what techniques work, they also have provided protocols from other researchers for staining, tissue processing, et cetera. No one will be happy until there are treatments for this disease. It is easy to say that validating things in the laboratory with post-mortem tissues or other patient-derived tools is going to result in treatments. Until it does, all they can do is the best they can do. His goal is to be able to provide researchers with the resources they need.

Dr. Siddique emphasized that while treatment is the ultimate goal, there is a step before that to understand the pathology, mechanism, or both because that is what the tissue and biospecimen collection is about.

Dr. Ostrow said he would be happy to provide a bibliography or it is easy to search Scholar for JHU, Target ALS Foundation, and/or the Postmortem Core. He stressed that while he was delighted to provide a bibliography, his metric is not papers, studies, or even grants that have been funded with support from JHU. His metric is once things are added to the clinic that work, though they are not there yet.

Open Ended Question Analysis

Danielle Boyce, DPA, MPH
Executive Director
Robert Packard Center for ALS Research

Dr. Boyce thanked everyone for their attention to what has become one of her favorite projects ever because it has truly helped her to get to know people with ALS in a really meaningful way and possibly in some small way, help the brilliant researchers they were hearing from during this meeting by improving the dataset that they have to work with. Her objective for this presentation was to describe a qualitative analysis project, "Survey 16" that she was in the process of doing using the CDC National ALS Registry data. The question that she analyzed for Survey 16 was, "Please enter your ideas or thoughts regarding the factors that may have causes your ALS." There were a total of 3,154 responses. A veteran who has ALS said that when she signed up for the Registry and answered the questions, she wondered if anyone would ever actually read it. Dr. Boyce stressed that she had read every word and that it was her honor to do so.

Why would anyone read more than 3,000 paragraphs containing self-reported theories when there already are more than a dozen surveys to capture this information? This project has 2 major aims: 1) It could be hypothesis-generating. Some of the comments revealed risks or exposures that had not been considered previously or may provide nuance or specificity to some of the things that already are known; and 2) Even for the comments that do not provide scientific enlightenment, from a patient advocacy and education perspective, it is important to know what is going on in the minds of people with ALS. Many studies show that there is a significant relationship between patients' self-blame and anxiety, depression, and psychological distress. Even if the responses do not shed new light on research hypotheses, risks, or exposures, it is good to know what people are thinking. She asked everyone to consider as she went through her presentation how lucky there were to have the privilege to have such an abundance of patient voices and the ability to do so much good for both research and the advocacy and support communities with the wisdom shared here.

This is a really large project that Dr. Boyce started working on this Spring, which was at a good point to share some findings. The first step was to develop themes and code them, which she was in the process of wrapping up. Then they can explore different analysis approaches and incorporating with other surveys, sharing the data and documentation, and repeating the analysis as more data come in. For the last 6 months or so, she read each and every comment as it was important for her not to assume that she knew what people would think about this. She wanted to go into this with an open mind and be as unbiased as possible. She knew that if she jumped in to set the codes, she would have introduced bias. For example, she assumed that some people would say genetics, exercise, or military. But she wanted to wait to find out what they would tell her. Her methodology could be described as inspired by Grounded Theory. She tried to develop some larger themes and the key words associated with those themes. She coded key words and overarching themes as an interim but not final step. This code was written to flag sub-themes and overarching themes. After the initial key word coding. Dr. Boyce re-reviewed every single comment to confirm that she got it right.

Why could she not code mother, father, brother and save all of those as genetic without having to read all of them? Because that can lead to mistakes. Mother, as she learned from reading through more than 3,000 comments, might actually have nothing to do with familial ALS. It could be someone saying "my mother was older when she gave birth to me" rather than "my mother had ALS" or "my mother had Parkinson's disease." It was good to do the keywords because it facilitated faster coding. For example, she flagged "I don't know," "IDK," "who knows," and "don't know" as a 1. Then she could sort and quickly confirm that was in a big chunk. The little codes were like a crutch and nice to have, meaning they have every reference now coded to all of those in case someone needs them. While much of this is already collected elsewhere in the Registry, she did not feel that her time was wasted in doing this. As a reminder, it was not her job to comment on whether people were right or wrong in their responses or to read more meaning into their comments. Her job was to be an objective observer, which required her to put her own theories aside and let the participants speak. Although she has expertise in artificial intelligence (AI) and natural language processing (NLP) techniques and is a data analyst and engineer by training, her concern was that some of the subtle meaning would be dropped if she did not go through this initial manual exercise first. Knowing what she knowns now based on the complex coding approach, they might be in a better position to take the more automated approach. Dr. Boyce thinks that after almost 30 years of doing this kind of work, sometimes it is necessary to do it manually and read and listen. As someone who works in the data science field, the advantage of using sophisticated technology are not lost on her.

In terms of the initial coding, about a quarter of people said they did not know and that remained accurate. Some people said they did not know, but then gave a very detailed reason that moved them out of the "I don't know" category. "Genetic" meant a lot of things and "occupational" may have meant working in an old building or specific like working in a nuclear power plant or specific chemicals, so she had separate codes for those things even if they were captured under "occupation." Many people said that they thought their ALS was caused by a combination of genetic and environmental factors, so she made that its own code. A lot of people said that it was familial but did not say what their mutation was, though that might be captured elsewhere in the surveys. A lot of people talked about mutation and their perceived triggers. Not surprisingly, there was a lot of mention of other familial neurological conditions. During the survey dates 12/02/2013 through 12/31/2021, participant characteristics included 58% male, 96% White, 79% married, 62% college educated, 7% family history of ALS, and 19% family member diagnosed with a neurological disease. Among the 3,154 participants, 3% were diagnosed between 18–39 years of age, 11% between 40–49 years of age, 29% between 50–59 years of age, 38% between 60–69 years of age, 17% between 70–79 years of age, and 2% at ≥80 years of age.

Again, a lot of these codes will not add more information to what is already in the other survey. "Unknown" was the most common response. About 25% of respondents simply do not know what caused their ALS. Many think that it is a combination, but cited no other specific exposures. Other general theories stated included environmental, occupational, and lifestyle with no specific exposures cited. Some theories related to food people ate, water they drank, immunizations they received (influenza, COVID-19, generic references). Some suggested comorbidities such as autoimmune, cardiovascular, or Lyme disease. Others thought perhaps medications played a role, especially statins and antibiotics. Military service in general or military immunization/medications were cited. A really rich finding is that there were a lot of specific military exposures cited, such as exposures to gas chambers, fumes from firing ranges, and high stress levels. Because Dr. Boyce does not fully understand all of these, she is going back to the community for member checking to help refine her codes. People mentioned exercise, sports, athletics, and heavy physical labor. A lot of people cited accidents with and without head injury. Emotional trauma and stress such as child abuse, divorce, stressful occupations, death, and other emotional and mental challenges came up a lot. In the open-ended analysis, people made observations such as "A lot of questions have to deal with physical trauma, what about emotional trauma? . . . prior to the first symptom, I lost my wife suddenly to a motor vehicle accident, putting me in the single parenthood game, added with stress/anxiety/with a side order of depression. Could any of that have contributed to my 'brain short circuit?"

There are a lot of limitations. For instance, Dr. Boyce may have introduced bias as a researcher. Perhaps participants who were more motivated to fill out the optional survey might have had stronger opinions, although 25% still said they did not know. Previous surveys may have introduced bias. There were a lot of posts in which people said. "Well, after reading all the risk factors mentioned in the surveys here . . . toxins!" or "The surveys I have thus far completed seem to be pursuing causes, e.g., chemical exposures, that make sense to me." This is all self-reported clinical, medical/family history, and exposure information. It also is possible that disease stage and ability to communicate in paragraphs may impact bias toward those with fewer communication challenges.

In terms of future opportunities, after hearing the presentations during this meeting, Dr. Boyce may be able to include some additional codes. It is interesting that there are multiple codes and triggers across one participant and people with familial ALS, so there may be something there. She wants to return the coded data back to the Registry Team, and potentially impute missing data in supplemental questionnaires (e.g., military, sports, hobbies, occupation). In addition, she would like to translate the results into patient education and reassurance to lessen the emotional impact of self-blame. She emphasized that she is happy to share her findings with everyone and expressed her gratitude to the CDC/ATSDR Registry Team and all of the participants who took the survey for taking the time to share their thoughts and feelings and provide such valuable information for the research community.

Discussion Summary

Regarding a question about whether within military exposure there was a subclass for "firefighters," Dr. Boyce indicated that she did not get many, but there were some so she does have "firefighter" flagged.

Lauren Webb asked whether Dr. Boyce thought that this research could help inform programs, services, and possible interventions to better support families and people living with ALS to address the issue of sampling. Having clinicians change that trajectory would be very powerful.

Dr. Boyce said that to share her own lived experience, she is the mom of a child with a catastrophic illness. The best thing a neurologist ever did for her was, unsolicited, to tell her there was nothing she could have done to cause his disease. That really set her free from something she did not realize she was doing. Reading all of these comments was sacred because seeing so much pain and self-blame mixed in with other comments that were more objective was important. It would be great to have a webinar with clinicians and patients to hear their perspectives. Addressing this and allowing people to voice it would be really helpful in the community, which perhaps the Les Turner Foundation could do.

Michelle Lorenz asked whether Dr. Boyce is still looking for more participants and this would be an ongoing IRB that would allow for adding to the current 3,100 patients.

Dr. Boyce indicated that it would be ongoing as long as the Registry would give her the next round of data. Her thought was now that they have good codes pulled together, they might try other techniques. With the next round of 1 more year of data, perhaps they could do NLP using the codes and then compare the results to the Grounded Theory approach.

Dr. Siddique thought that if there was an insight and this was a disease that had a commonality, she would have found it. This suggests that there is great heterogeneity and insight about the disease. The other thing she mentioned that was very interesting, which anecdotally he thought most ALS neurologists know, is the association of anxiety, stress, and depression as a harbinger of this disease. He wondered if that is a prodrome of the disease rather than a cause of the disease. It could be a self-perpetuating mechanism, but he thinks it is more likely to be true. He wondered if she could address her question in a way that would bring that out. It is known from other neurological disorders now and from ALS that it is not really just an MND. The people who talked about stress may not be able to cope with the stress they have had all of their lives or just the complexity of the stress is too much. It also could be that they receive it with the dispersion of frontal lobe abilities. Their relationships fall apart, there is divorce, there is separation, and other things happen. Is it a coping mechanism because of environmental pressure or is it an internal problem that is a prodrome for disease that may exist for years. It is

known that people with Parkinson's disease can have prodromes going back 20 years. It is known from with psychiatric disorders such as schizophrenia, psychosis, and so forth that increasing stress brings it out. It is like the dopamine that people use for Huntington's disease. The question is the same. Is there environmental stress bringing out the disease because of a weakness, the stress is just too much and it could happen to anybody, it is unrelated, or it is because of the disease?

Dr. Boyce said that her hope is that by providing all of these codes in the dataset that she can share back because these are all Registry participants, others can use it for their own research. That includes the occupational and military exposures giving a little more nuance and subtlety to some of their answers that they provide in the quantitative questionnaires. She hopes that some of the work that she is doing because of the amazing people who participated in the Registry will help in some small way to shed some light on this and to help people in a practical clinical way to get more support that they need so that they can face this better.

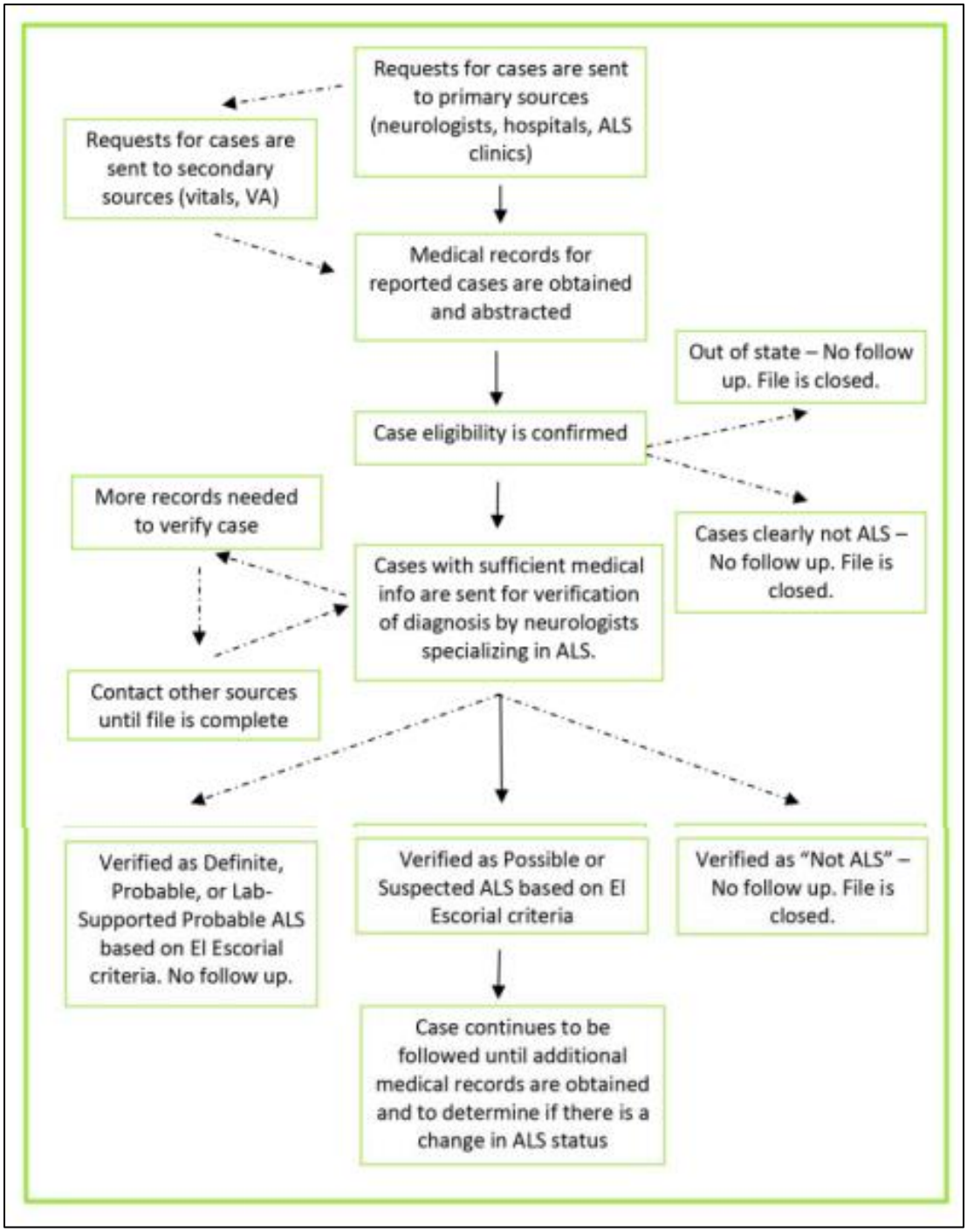
State-Based ALS Registries

Elijah Stommel, MD, PhD
Professor of Neurology
Geisel School of Medicine at Dartmouth

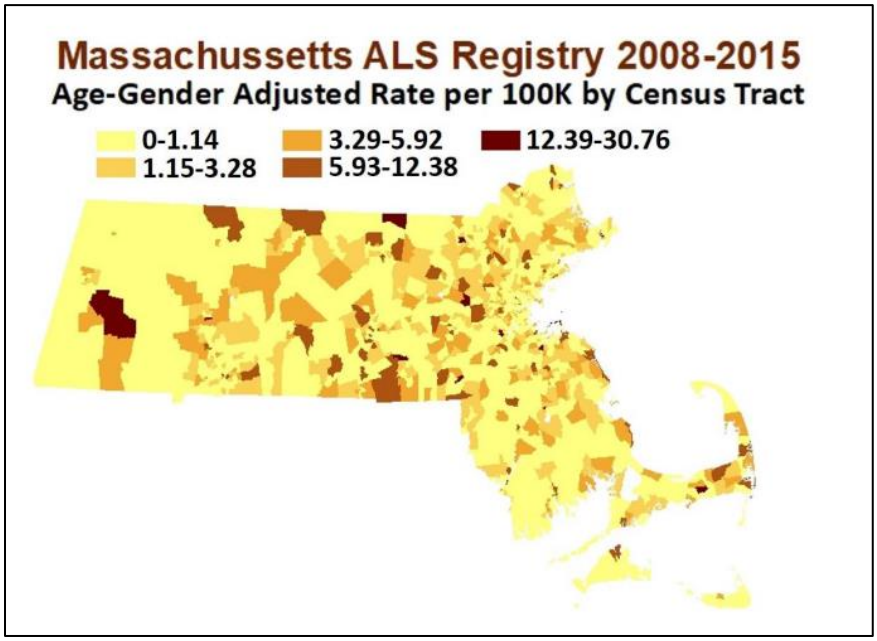
Dr. Stommel described his experience with trying to start up state-based registries. He has been working closely with Dr. Mehta in an effort to leverage his clout as a member of the National ALS Registry to help convince statewide politicians to take up the cause. Reportable diseases are diseases considered to be of great public health importance. In the US, local, state, and national agencies (for example, county and state health departments or the US CDC) require that these diseases be reported when they are diagnosed by doctors or laboratories. Reporting allows for the collection of statistics that show how often the disease occurs. This helps researchers identify disease trends and track disease outbreaks. This information can help understand the underpinnings of disease causation, control future outbreaks and mitigation efforts. There are many types of registries such as those related to cancers, a variety of infectious diseases, animal control, food handling, immunization programs, insect control, sexually transmitted disease (STD) tracking, water purification, et cetera. It is somewhat of a foreign idea to have reportable registries for neurodegenerative diseases, given that they are classically not thought to have clearcut environmental influences linked to the diseases to the same degree as cancers and infectious diseases. Unfortunately, the healthcare system in the US is such that there is no way to get a complete dataset other than to try to create registries that are as robust as possible. Healthcare systems in parts of Europe are able to collect all of the data for complete datasets.

The MA ALS Registry that was started in 2008 was largely political because Paul Cellucci, the Governor of MA, died of ALS and there were many friends, ALS patients, families, and patient advocacy groups that pushed hard for the passing of a registry there. This led to a legislative mandate that established a statewide surveillance system for ALS (M.G.L. c. 111, § 25A). The MA ALS Registry collects statewide information on patients with ALS identified by neurologists, hospitals, ALS clinics, and vital records. This registry had a lot of power behind it in terms of getting it to fruition and the cost of starting this registry was about $250,000. Dr. Stommel found this to be a major concern when approaching politicians in Northern New England states about having a registry for ALS. Based on a literature review of ALS studies using the MA ALS Registry, there have been very few thus far. Dr. Mehta and his colleagues at the National ALS

Registry are in the process of comparing an overlay of cases collected by the MA ALS Registry with the National ALS Registry to ascertain what type of overlay there may be in cases. In fact, the MA ALS population is poorly reported to the National ALS Registry. Dr. Stommel was surprised to learn that while MA has the most complete dataset, they do not share those data very quickly with the National ALS Registry. This schematic represents the way the cases are reviewed in MA:



Dr. Stommel and colleagues have an IRB set up with the MA ALS Registry and have done some preliminary work looking at the population there. This map represents about 7 years of ALS patients. When they started culling through the data, there were cases that were both in nursing homes and their home address. They tried meticulously to remove cases that were duplicates or had multiple addresses. Based on age and gender, they were able to develop this geospatial map, which they ultimately would like to overlap with potential environmental toxins:



The MA ALS Registry has ways to protect patient privacy. All information in the MA ALS Registry is kept secure, yet access to data may be granted for approved research studies that could not otherwise be easily conducted. As the first and only statewide system in the country to have complete capture of all known ALS cases, this registry produces high-quality, unbiased, and reliable surveillance data. Patient information contained in the MA ALS Registry includes patient demographics (age, gender, address), clinical symptoms and laboratory data, diagnosis, and treatment. While this is confidential information, the US Health Insurance Portability and Accountability Act (HIPAA) allows physicians to report to DPH for the purpose of disease surveillance. Researchers must apply for access to Registry data through the Department of Public Health (DPH) IRB. To be approved, studies must be able to guarantee the continuous protection of patient confidentiality. If a researcher requires contact with patients to complete a study, they must go through a rigorous approval process through the DPH IRB in order to contact patients to obtain informed consent. This type of research can help patients enroll in clinical trials and epidemiological research studies related to the causes of or treatments of ALS. Any reportable registry has the potential to help patients and families who are unaware of available help such as ALS Clinics of Excellence and other resources such as VA benefits.

When Dr. Stommel and colleague began to try to start registries in Northern New England, they spent a good deal of time talking to the MA ALS Registry team about how one might do this and what their own experience was. The first time Dr. Stommel's team tried to establish a registry in Vermont, they met with resistance from the DPH in Vermont which said it would cost too much money, that there were only a few researchers who selfishly want the information, the research being done was not good—though it is not clear that they read the papers. To reapply a second time, they found politicians who had friends and relatives with ALS who supported them. They discussed the financial issues associated with registries in detail with many politicians. Vermont has a population of about 700,000 people, so there will not be a huge number of patients. Dr. Stommel and Dr. Rup Tandan, a well-known ALS neurologist from the University of Vermont Medical Center, attended several virtual meetings to discuss the various issues that people had. Dr. Mehta was involved in some of those meetings during which he described the National ALS Registry and how it might be able to help start a state-wide registry, which was very helpful.

Maine was less of a problem, which surprised Dr. Stommel because Maine has fairly strict politics and a strict budget. However, the Governor of Maine was close friends with one of Dr. Stommel's patients who strongly advocated for them before her recent death, so politics can make a huge difference. The ALS Association also supported them in Maine. This registry was approved the first time around. Interestingly, Maine has one of the worst records of reporting cases to the National ALS Registry. Some arguments for statewide registries at that research is beneficial for patients and families, the price a of a registry can be kept small by using academic medical centers that have the infrastructure to help and to collaborate with the National ALS Registry, and more effort has to be made by states to work with the National ALS Registry in order to create state registries. The National ALS Registry could help state registries by storing and securing the state data, which both Vermont and Maine liked.

State registries are pending in Alabama, Michigan, and New Hampshire. Drs. Feldman and Goutman were trying diligently in Michigan to set up a registry. The population of Michigan is many times more than Vermont or Maine, so the expense is going to be at least somewhat more. There was discussion with Vermont and Maine about how academic medical centers like the University of Vermont, Dartmouth-Hitchcock Medical Center, and some of the neurologists in Maine who specialize in ALS could look through the charts of all of the patients within their infrastructures and their neurologists, residents, and fellows who are interested in ALS. There were problems with the legislature the first time in New Hampshire, but the plan is to go back to

ask them again. Alabama is in the process of trying to establish a registry. While Dr. Stommel did not have any details, he had one conversation with them about a year ago. In terms of how new state reportable registries are being set up, the registries in Maine and Vermont will hopefully have an alliance with the National ALS Registry. While the infrastructure has not yet been determined, it is likely to be largely through academic medical centers where there is existing infrastructure. Secure data storage can be established with the National ALS Registry.

In terms of law, there is precedence for setting up mandatory registries. Small states setting up registries can have an effect on larger states. It is likely that more states will follow suit, especially if the price can be kept low. Working directly with academic medical centers that have the infrastructure and expertise is crucial. Having the National ALS Registry advocating for state registries was a win-win situation in Maine and Vermont, so it could be for other states as well. The epidemiology in big states with mandatory registries will be very important. Imagine if California, Florida, Ohio, Georgia, Texas, New York, Pennsylvania and Illinois (CA, FL, OH, GA, TX, NY, PA, IL) had registries. Getting other states on board will require convincing the state politicians that it is affordable and worthy of the effort. There is no doubt that information about ALS will be helpful to understand other neurodegenerative diseases such as Parkinson's Disease or Alzheimer's Disease. Because ALS is relatively rare, the work involved to collect and analyze data should be less labor-intensive than for other diseases as well.

Discussion Summary

Dr. Thakur noted that in addition to the presentation they heard earlier in the data about numerous potential exposures, Dr. Stommel mentioned the intent to overlap the Massachusetts map of prevalence across the state with environmental exposures. It seemed that when this was complete, all they would have would be more associations between environmental exposures and ALS prevalence—something for which they already had a lot of data. If he went to the Environmental Protection Agency (EPA) with all of this to say there is an association between X chemical and ALS so they should do something about this chemical, the EPA would say it is just an association. He wondered how coming up with more associative data would help with efforts to reduce the risks associated with ALS.

Dr. Stommel clarified that ultimately, they would be looking at geospatial associations and tissues. There are several brain banks that have ALS cases from Massachusetts. They can look for persistent longstanding toxins that do not degrade. They also can do combinatorial evaluations of various toxins they might see. A patient who has high lead levels and high mercury levels and lived near a coal burning plant or near a forest that was burned and had flame retardants spread over it, over time these combinatorial effects can be assessed. Machine learning could suggest temporally what was important and what combinations of toxins were important. While research is one reason for having a registry, another reason is to help patients and families.

Dr. Thakur agreed that a detailed understanding of exposures people went through is really important, it was not clear to him how this could be derived from a registry for mandatory reporting. It seemed like the National ALS Registry with its elaborate surveys and specimen collection, as well as other brain banks, would be able to answer some of this by giving people who agree to join a survey to collect some of that information. As far as he could tell, that was not happening from a state registry, so it was not clear how those two things were related.

Dr. Mehta pointed out that Dr. Thakur's question basically regarded the question, "When does enough association lead to causation?" It took 30 years to say that cigarette smoking is bad and it causes cancer. While they do not want to wait 30 years, that is one of the policy questions. The hope with a state-based registry is that there would be more data points. The premise is that having more data points offers more to evaluate. They want to figure out what it means to have an association and potentially a causation in order to determine what can be done, such as not producing whatever compound or substance it may be in order to prevent ALS in the future. The question is whether rich state registries with more data endpoints and background information will provide insight on what is happening in certain states that have more environmental pollutants. For example, farm states have more farm runoff. Perhaps ALS could follow the cancer model in which state registries report to CDC. MGH is using the cancer model in their trials.

Dr. Thakur said he thought there was a lot happening in Dr. Mehta's statement. First, there is not a clear path for how this information is going to dramatically change the current understanding of ALS. If they are going to ask a state to pass a law to make care more affordable for people with ALS, they could ask states to pass a law to collect information that may lead to more associated data that may help in the future, but he would want them to be careful about that. The analogy between what they were talking about here and the cancer model are not necessarily the same. The great thing about state cancer registries is that there is usually some phenotyping of the tumors that is done with a lot more specificity and a deeper understanding of what is going on with cancer that what there is with ALS right now. It just seemed like they were skipping some steps and asking states to do things when they could be asked to do things that are going to help people living with ALS today rather than something that may have some pay off in the future. The Massachusetts example is great. They have a registry that is working and has been in place for a long time. However, the work for that is incomplete and its scientific value is unclear or not realized yet. Those funds could have been spent on other things.

Dr. Goutman pointed out that registries also have additional benefits. In this era with an interest in assessing patterns of care and health disparities, finding areas in certain states where ALS may not be getting enough coverage or enough clinical coverage is especially important. Incredible health disparities may be found in the way people receive treatment for their ALS based on geographic boundaries or hurdles. Answering *where* people are developing ALS may allow for connecting certain environmental risk factors to hotspots or clusters of ALS. Those data then could be used further to determine prevalence and incidence of ALS in a state. Without knowing definitively how many people are developing ALS and where they are developing it, it will not be possible to decrease incidence through prevention efforts. It is possible that there are areas that do not get coverage from the ALS clinics, so there may be incredible gaps or disparities in care that could be addressed through a rigorous registry effort.

Dr. Thakur agreed that there are clearly gaps in coverage for people with ALS and there are entire areas of the country that do not have access to care. There is always value in more scientific data, but there is a question of timing and whether a registry needs to be created in a state to say that there are not enough clinics in that state. Every state in the country needs more ALS care and coverage, but a registry is not needed to demonstrate this. Often the limiting factor in establishing a clinic is that there are no neurologists or specialists in the geographic area of interest and/or there is not enough funding.

Ms. Gwen Peterson recalled that mention was made earlier that it would be easier to collect data from larger diseases such as Alzheimer's and Parkinson's. She emphasized the importance of borrowing models that have been successful from other diseases.

Dr. Mehta clarified that he was trying to point out that because they are relatively less rare diseases, it might be easier to collect the cases on Parkinson's and Alzheimer's. In addition to higher burden, these diseases also have a lot more research dollars. Nevertheless, there is much that could be learned from other diseases in terms of making ALS much more prominent and so forth.

Dr. Horton added that most non-communicable diseases do not have registries or surveillance systems in the US. ALS is fortunate to have a system through which to collect this information on a national scale. There are upwards of 600 neurological diseases, but very few of them have any kind of system that collects data on a national scale. It would be great to have a system that collects information on a number of diseases.

National ALS Registry Funded Research Presentations: Part 1

Serological Profiling of the Human Virome and ALS Risk in a Military Population

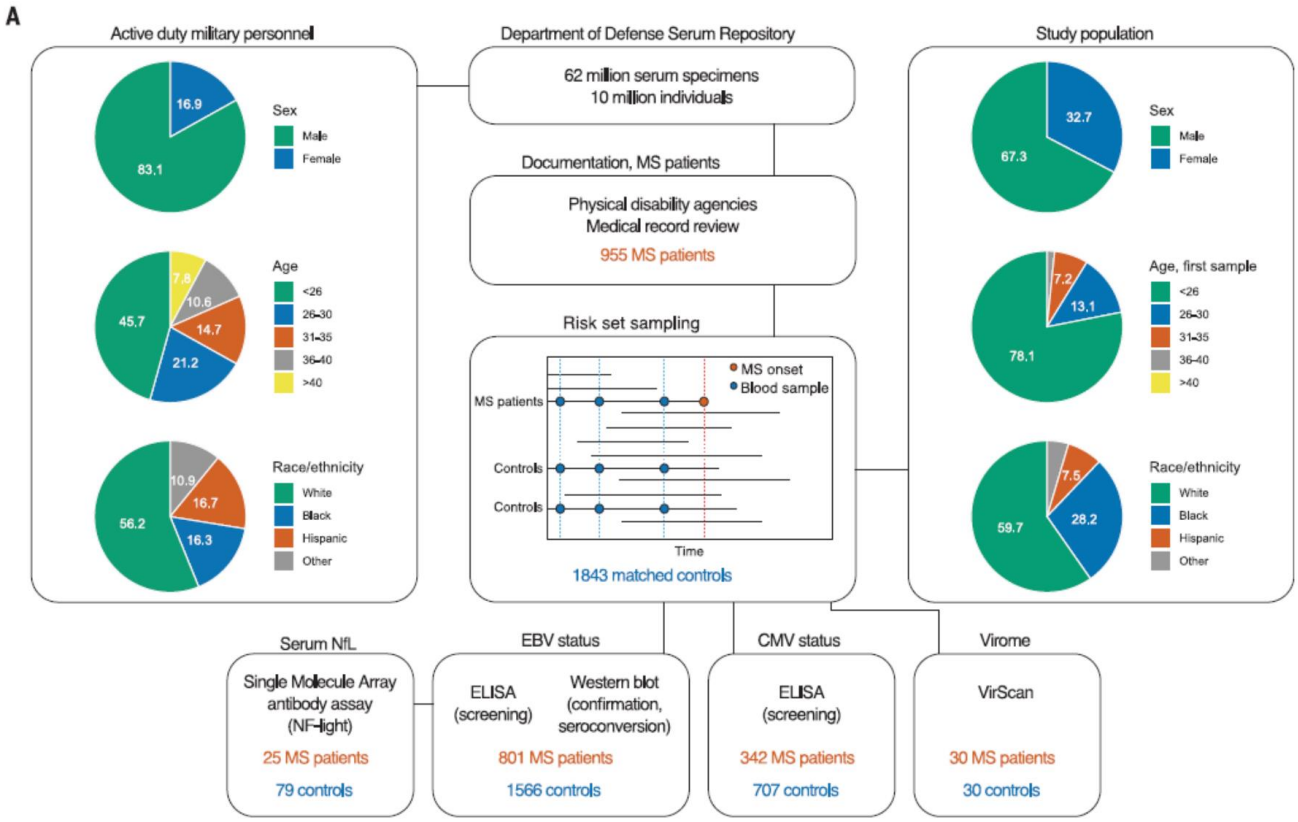
Alberto Ascherio, MD, DrPH
Professor of Epidemiology
Harvard University

Dr. Ascherio indicated that the purpose of this project was to investigate the possibility that viral infection could have a role in the etiology of ALS, in which there is an increasing interest. The thought is that infection in general can be related to risk of neurodegenerative diseases, including Alzheimer's, Parkinson's, and ALS. This is because particular viral infections can cause several hallmarks that have been associated with neurodegeneration going from protein aggregation to oxidative stress (OS). Neurodegeneration can be triggered by a viral infection—not necessarily an acute infection, but there are 2 mechanisms that could be hypothesized. One is that there could be persistent latent infection that has been accumulated from several viruses, and the persistent infection can create a stimulus for the degenerative process. The other is that virus would trigger cells in an attempt to protect itself from viral infection and control the infection in the sense that there is not a cytosis, but at the same time can remain with the center of the protein that can cause the disease. For example, TDP43 is a hallmark of ALS. The movement from the nucleus to the cytoplasma of TDP43 can be caused by coxsackievirus B3 infection and excrete the TDP43 into 2 segments and continue the process of aggregation. The right amounts of these mechanisms is more speculative, so Dr. Ascherio said he was just offering the theoretical argument that prompted him and his colleagues to conduct a broad exploratory study rather than focusing on a specific virus to determine whether they could find a signal.

The specific aims of this study are to: 1) assess whether enteroviruses associated with acute flaccid myelitis (AFM) (e.g., coxsackievirus B3, enterovirus A71 and enterovirus D68) contribute to predicting ALS risk; neurotropic herpesviruses (e.g., herpes simplex virus 1 and varicella zoster virus) contribute to predicting ALS risk; viral infection profile (virome) at baseline or its changes during the follow-up are associated with ALS risk; or 4) incident viral infections are associated with increases in serum levels of NfL; and 2) assess potential confounding by TBI, deployment history, smoking, body mass index (BMI), diabetes, and/or family history of ALS. This study is conducted in a unique population of Active Duty military who are exposed to other

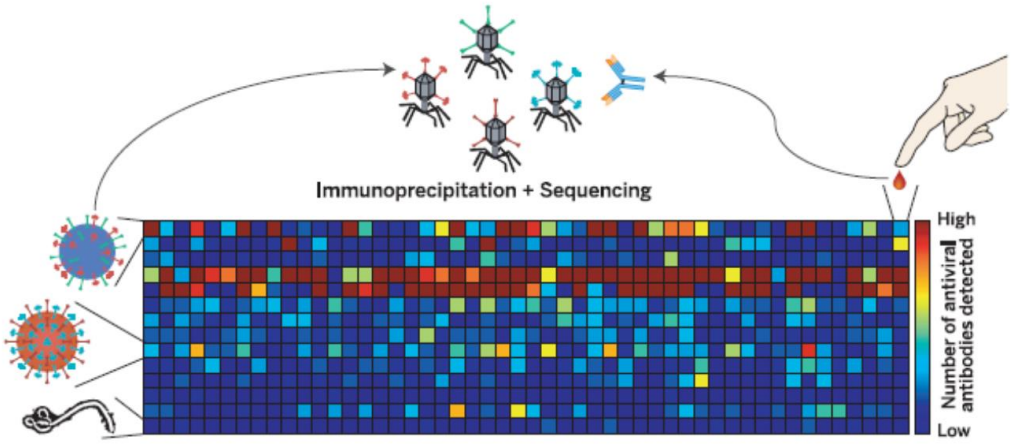
potential risk factors for ALS. ALS has been of interest to the military historically as recognized by the Gulf War paper a few years ago.

Dr. Ascherio highlighted a study by Bjornevik et al titled, *Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis*.[11] This study demonstrated that MS is a complication of infection with EBV. This study was made possible by the fact that the Active Duty military population is comprised of over 10 million young men and women, about 6.8% of whom were EBV-negative at the time of recruitment. This population was followed for incidence of MS for 20 years. EBV infection was monitored by using samples collected by the DoD Serum Repository (DoDSR) that has over 60 million serum samples collected from a population of several million young men and women who served on Active Duty in the US Army, Navy, and Air Force. The unique feature of the DoDSR is that it contains repeated blood samples collected over the years. The Bjornevik study showed that the risk of MS increased 32-fold after infection with EBV, but was not increased after infection with other viruses, including the similarly transmitted cytomegalovirus. This diagram from the paper shows the composition of this population:



[11] Bjornevik et al, SCIENCE, 13 Jan 2022, Vol 375, Issue 6578. pp. 296-301, DOI: 10.1126/science.abj8222

A common aspect of this type of study is the use of viral epitope scanning (VirScan), which is a technology that allows for the use of a small amount of serum to allow for a comprehensive analysis of antiviral antibodies in human sera. This exploratory tool is being successfully employed in several studies and is expected to provide a big picture view of whether any signal is seen for association with ALS:

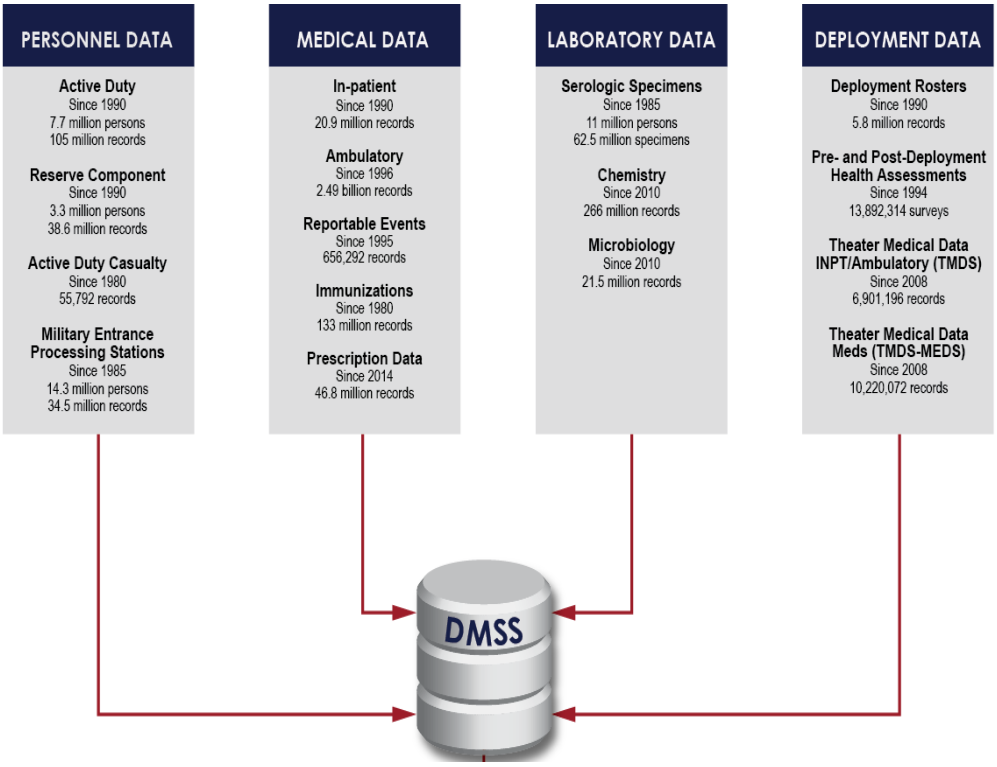


The following table is a breakdown of the DoDSR population in person-years of follow-up by branch and age strata from 1990 through 2019. This is a very large population that is skewed toward a younger age group, with about half being <26 years of age:

Age, Years	Army, n	Navy, n	Marine Corps, n	Air Force, n	Total, n
<26	6,975,170	5,105,019	3,650,605	4,271,397	20,002,192
26-30	3,229,407	2,349,052	860,244	2,396,353	8,835,057
31-35	2,350,524	1,671,655	495,586	1,690,929	6,208,693
36-40	1,790,823	1,335,919	327,744	1,486,190	4,940,675
>40	1,414,566	1,036,089	208,997	1,207,817	3,867,469
Total	15,760,490	11,497,734	5,543,176	11,052,686	43,854,086

Another interesting feature of the military population is that they have a relatively high rate of deployment abroad where they experience exposure to various infectious agents and different strains of infectious agents.

The following diagram provides an overview of the data available in the Defense Medical Surveillance System (DMSS):[12]



The DMSS is a comprehensive surveillance system with a very large amount of data that have been collected in a systematic manner since the 1990s. The main constraint in the serological profiling of the human virome and ALS risk in the military population investigation is not the availability of data, but the need for protecting human subject identification. Therefore, the investigators for this study are being very selective in including only a limited set of exposures allowed by negotiation with the IRB committee within the military.

Looking at the practical aspects of the proposed investigation that is ongoing, Dr. Ascherio provided a progress report. When an Active Duty member develops ALS, they go through a rigorous fitness for duty medical examination through the Physical Disability Agency (PDA). If the diagnosis is confirmed, the individual will be released for medical reasons from Active Duty. The investigators will retrieve medical records for individuals who have a Veteran Affairs Schedule for Rating Disabilities (VASRD) code "8017 (ALS)" from the PDA evaluation. The ALS diagnosis will be confirmed according to revised El Escorial criteria by 2 independent reviews of the medical records. Confirmed ALS cases will be included in the study. The following table depicts the number of cases that are expected in each age and branch from 1990 through 2019:

[12] (Figure retrieved from: https://health.mil/Military-Health-Topics/Combat-Support/Armed-Forces-Health-Surveillance-Branch/Data-Management-and-Technical-Support/Defense-Medical-Surveillance-System on February 20, 2020)

Table 3: Projected number of ALS cases by branch and age from 1990 to 2019.

Age, years	Army, n	Navy, n	Marines, n	Air Force, n	Total, n
<26	14.2	8.9	6.4	7.7	37.3
26-30	10.3	6.4	2.3	6.9	25.9
31-35	6.6	4.0	1.3	4.0	15.9
36-40	10.1	6.4	1.7	7.1	25.3
>40	30.5	18.9	4.0	22.5	75.9
Total	71.7	44.6	15.6	48.2	180.2

There is a disadvantage in studying such a young population in that it will not be representative of the general population. However, there is an advantage in the sense that a shorter period of exposure may make it easier to determine if there is a viral infection that is related to ALS risk. For each case of ALS, the investigators will look at the same point in time, age, sex, race, ethnicity, branch, and time of collection of the blood sample. Also important to assess will be estimated time from the first blood sample collection until ALS onset. The study should have sufficient power to detect associations between infection with certain enterovirus species and ALS risk.

In terms of the study's status, Data Use Agreements (DUAs) have been completed with US Army and Air Force. DUA negotiation is still in process with the US Navy. Review of ALS medical records is expected to start in the Fall of 2022. The investigators are engaged in advanced negotiations with the VA to extend the study to ALS cases diagnosed after retirement from Active Duty.

Discussion Summary

Dr. Siddique noted that in certain arms of the military, there is extraordinarily high representation of African Americans and other people of color. He asked whether the investigators saw this in their 180 cases. Related to the viruses being studied, he noted that EV-A71 cross-reacts in its serology with polio as noted in the paper published by Amy Rosenfeld and by Vincent Racaniello from Columbia who discovered the poliovirus receptor. He asked how the investigators were dealing with that conundrum. Another issues is similar in terms of herpes or varicella related to vaccination. One would expect that the infection would be over and they would be seeing immunoglobulin G (IgG).

Dr. Ascherio said that in relation to the first question, the 180 cases is a projected number. They do not have the cases yet. It is anticipated that minorities will represent about 30% of the study population. They did not project the number of minority cases because they would have to rely on the relative incidence and were not sure whether the data are very good for this. They expect to have cases in African Americans and minorities at a range of around 15% to 20% (~30 cases). Regarding the second question, VirScan is a screening method. In the case of EV-A71, EV-D68, and others, there was a paper a couple of years ago on AFM and VirScan. There has been a fair amount of validation. VirScan itself is insufficient to disentangle viral infection with cross-reacting antibodies. The solution would be to look at more specific serum samples. In

terms of cross-reactivity with poliovirus, the signal could be diluted. They will have to look at this in more detail.

Dr. Lorenz asked whether in their studies of the military, Dr. Ascherio and colleagues knew of the cluster of the 4 C-130 pilots at the Charleston Air Force Base. There also appears to be a higher prevalence amongst C-130 pilots and crew at the Altus Air Force Base in Oklahoma. With that in mind, she asked whether Dr. Ascherio and colleagues would be collecting data and biological samples from healthy controls who were in those same squadrons for comparison.

Dr. Ascherio indicated that as he hinted at in his presentation, they will not be able to get the details of specific assignments because of the protection of the identity of human subjects. An exception potentially could be negotiated in theory, but it would take a lot of time to get a DUA approved by everyone. The short answer is that they cannot match on specific locations.

Novel Extracellular Vesicle and Molecular Biomarkers of Environmental Exposure and Disease Progression in ALS

Diane B. Re, PhD
Assistant Professor
Columbia University

Dr. Re presented some of the work she and her colleagues have done with funding from the ATSDR and the CDC to investigate novel EV and molecular biomarkers of environmental exposure and disease progression in ALS. This work is part of R01 work with Neil Shneider at Columbia. For this presentation, Dr. Re focused on Specific Aim 2 of this project, which is to investigate CNS-derived EVs as metal exposure and ALS progression biomarkers. She explained that while her role in this project was to lead the method development for the EV selection and the processing of the samples, she is not an epidemiologist. The data analyses could not have been performed without Dr. Kioumourtzogiou and a team of great graduate students. In the context of environmental, this project focuses on metals that have been linked to ALS etiology and potentially progression in case reports and in some epidemiological studies. There are no accurate and non-invasive biomarkers of central CNS metal burden. The role of metals remains unclear, though circulating EVs could address this urgent research gap. As noted earlier in the day by one of her collaborators, Dr. Stommel, it is important to have more information about toxicant exposure.

EVs are nano-sized membrane-bound vesicles released by virtually all cell types into the extracellular space. They are often referred to as exosomes, microvesicles, or apoptotic bodies depending on their mechanism of release or size. They have been found in nearly all biofluids tested including plasma, urine, breast milk, and saliva. EVs play roles in regulation of gene expression, activation of signaling, and distribution of catalytic activity. EVs also operate as "trash bags," allowing cells to eliminate unwanted materials. It is their role as a cellular disposal system on which this aim focuses. EVs play local and also long-distance roles. They can cross the blood-brain barrier (BBB), enter the bloodstream, and be detected in the periphery. As reported by several groups, EVs that originated from the brain can be isolated from peripheral blood because of their expression of different membrane surface markers of their cell type of origin, such as L1CAM for neurons and glial glutamate aspartate transporter (GLAST) for astrocytes.

In terms of what the link is between EVs and metals, several years ago Dr. Re was inspired by the article, "The secret life of extracellular vesicles in metal homeostasis and neurodegeneration."[13] What really impressed her was a series of publications, including one showing that Cisplatin-resistant tumors extrude Pt metal via increased EV release. EVs contain many proteins involved in metal metabolism (e.g., EVpedia, Exocarta, and vesiclepedia). Perhaps CNS cells also use EV production as a mechanism of metal homeostasis. Astrocytes are key players in metal homeostasis.[14] For this study, primary astrocytes were cultured and treated with 2.5 µM of either As or Mn (or vehicle – saline) for 3 or 7 days. The investigators first confirmed isolated EVs by transmission electron microscopy showing the presence of rounded vesicles ~50nm in diameter and by Western Blot showing that the EV prep was enriched in the EV markers CD9 and Flotillin-1, but not in calnexin, an endoplasmic reticulum protein not expected to be enriched in EVs. Then they assessed EV release over time. From Day 3 to Day 7, they saw an increase in EV release in astrocytes exposed to vehicle, and As (assessed by Flotillin-1 levels) (less clear here for Mn). Furthermore, they measured metals in these EVs by ICP-MS, and found that the levels of both Mn and As in treated astrocyte EVs increased over time, whereas these metals were very low or not detected at all in control cells. Mn is an essential metal, so they do not release it right away until day 7 when it is too high vs As excreted right away because there is no biological reason. This in vitro study supported the hypothesis that CNS cells exposed to an excess of metals can eliminate them via the release of EVs.

In the context of the research proposal, this led to the formulation of 2 research objectives which were to: 1) pilot the testing of whether CNS-EV metal load could serve as a biomarker of CNS metal burden in paired patient/blood CNS samples; and 2) test if circulating CNS-EV metal load in longitudinal blood samples correlate with ALS progression, duration and other clinical data. Samples were obtained from the pilot study of the National ALS Registry from patients from across the US (N=180) with 2 serial visits and collection of whole blood samples in metal-free condition (N=7 for matched CNS samples). Each visit was separated by 6 months and included metal-free blood collection and clinical stage assessment using the ALS Functional Rating Scale-Revised (ALSFRS-R). Other clinical, epidemiologic, and risk factor survey data were available for these patients through the National ALS Registry. The first step was to develop and validate a reliable and reproducible method to isolate CNS-EVs from whole blood and measure their metal content. There was a lot of work to develop an as metal-free as possible EV isolation method. To decrease metal contamination, only certified metal-free reagents were used whenever possible (15 mL tubes and tips). Metal-free gloves were used as personal protective equipment (PPE). All the other tubes used along the EV isolation process are pre-washed with 2M HNO3. Buffers are prepared in 2M HNO3 pre-washed glassware. EV isolation work is performed under a hood to prevent potential environmental contamination. Background metal levels measured in experimental negative controls were substantially decreased.

They had to characterize and select the best CNS-EV isolation methods for whole blood. They first determined whether a 2-step[15] or 1-step[16] method of CNS-EV isolation was best based on previously published literature. Both started from plasma or serum. The 2-step method started with 0.5 mL of plasma. The first step consists of isolating total EVs by ExoQuick, which is a commercial reagent. Once there is a total EV fraction, the second step involves characterizing L1CAM EV immunoprecipitation (IP) or GLAST EV IP. It was found that direct IP was more

[13] Bellingham SA, Guo B, Hill AF. The secret life of extracellular vesicles in metal homeostasis and neurodegeneration. Biol Cell. 2015 Nov;107(11):389-418. doi: 10.1111/boc.201500030. Epub 2015 Jun 24. PMID: 26032945.
[14] Front. Neurosci., 01 June 2016
[15] Mustapic et al., 2017
[16] Shi et al., 2017

efficient to enrich in Astro markers and less in neuro markers. L1CAM is less efficient to isolate EVs based on CD81 and is enriched in neuro and astro markers to a lesser extent than GLAST EVs. An enzyme-linked immunosorbent assay (ELISA) was used to be more quantitative. The L1CAM was found to be less efficient to isolate EVs based on CD81 and was enriched in neuro and astro markers to a lesser extent than GLAST EVs. In terms of selecting the best normalization factor for GLAST-EV isolation efficiency, the levels of CD81, CD63, GLAST, and the nanoparticles were compared in independent preparations of GLAST-EVs isolated from 40 control or 40 ALS patients. Only CD81 and CD63 were not significantly different between ALS patients and controls. The efficiency of CD81, CD63, GLAST, and the EV protein were compared in normalizing GFAP and GLUL levels in 5 independent IPs of GLAST-EVs isolated from the same human sample. CD81 always was the best normalization factor leading to the smaller STDEV.

With this method in hand, they decided to complete Research Objective 1 using an exploratory design to determine whether metal levels in CNS-EVs could predict patient brain metal burden. The subjects were comprised of 7 ALS patients from the National ALS Registry. Samples from these patients included whole blood, cortex, spinal cord, and CNS EVs of astrocytic origin (GLAST) isolated via the direct IP method modified from Shi et al (2014). Metals included As, Cu, Fe, Mn, Pb, and Se measured by ICP-MS. While this showed that GLAST-EV Mn levels may better predict spinal Mn levels than total blood Mn levels, this needs to be confirmed with a larger number of patients. It is less clear whether GLAST-EV Mn levels may better predict cortex Mn levels than total blood Mn levels and also needs to be confirmed in a larger number of patients. GLAST-EV Cu levels may better predict cortex Cu levels than total blood Cu levels, but again must be confirmed in a larger sample size. Se blood levels predict well CNS Se load.

In terms of Research Objective 2 to test whether CNS-EV metal load correlates with ALS progression, duration, and other clinical data, a total of 97 National ALS Registry patients were assessed. Samples included whole blood at 2 time points 6 months apart. The exposures of interest included levels of Al, Cd, Cu, Fe, Hg, Mn, and Pb in GLAST-EVs isolated via direct IP and measured by ICP-MS at Time 1, or Time 2 or Delta Metal (T2 –T1). The outcomes of interest included ALSFRS-R at Time 1 or Time 2, disease progression (Delta ALSFRS-R T2 – T1), disease duration (Age at death- Age at diagnosis), age at death, and age at diagnosis. The main findings for GLAST-EV metal levels at T1 are that Al, Cu, and Mn moderately positively correlated with change in ALSFRS (disease progression). Hg moderately positively correlated with duration of disease. Mn and Cd moderately negatively correlated with age at death. Gender moderately positively correlated with change in ALSFRS score. In terms of findings for GLAST-EV metal levels at T2, Hg moderately positively correlated with duration of disease. Regarding findings for Delta metal levels (T2-T1), Hg Delta was positively correlated with duration of disease. Mn Delta was positively associated with age at death. No significant linear regressions were found, indicating that if there are relationships, they are not linear.

The next steps are to: 1) perform an exploratory analysis using Bayesian Kernel Machine Regression (BKMR) to assess any potential non-linear associations between metals and ALS-related outcomes and test the potential effects of the metals as a mixture that may be associated with ALS-related outcomes; and 2) validate with 147 paired blood and CNS samples from the VA Brain Bank and Biorepository whether CNS-EV metal levels can serve as a biomarker of CNS metal burden, comparing the biomarker potential of astro vs. neuro and microglia EVs.

Discussion Summary

Regarding a request for clarification of what was meant by "paired blood and brain samples," Dr. Re clarified that this meant that blood was obtained from the same patient when the person was living and after autopsy samples were obtained. This is in order to test the biomarkers that they are trying to develop from blood to be non-invasive and perhaps later to follow ALS patients over time.

Dr. Han asked whether the investigators have any guess about the target of these EVs circulating with this high level of metals in terms of whether they are targeting neurons or muscles and what the mechanism could be.

Dr. Re indicated that while they do not know, they are hoping to determine this through an elimination pathway. Hopefully, after circulation, some of them can be eliminated. Perhaps some are eliminated through urine and others through the gastrointestinal (GI) system. They are struggling with this, given that the EV field is so novel and it is not clear where the EVs coming out of the brain are going.

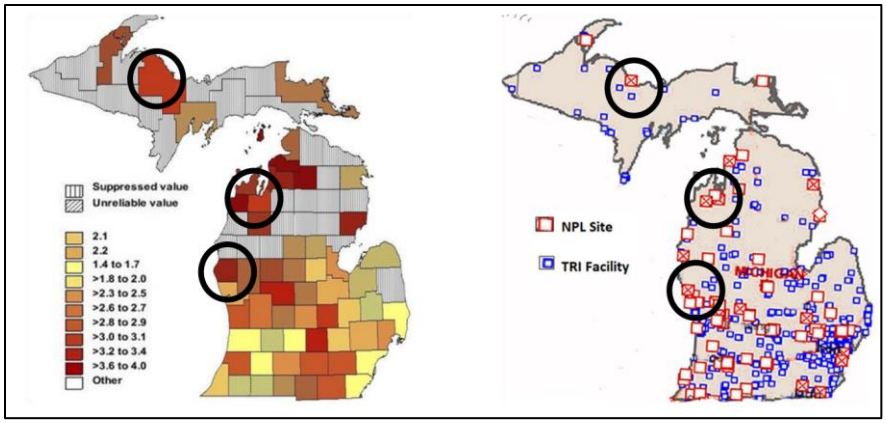
Targeting the ALS Exposome for Disease Prevention: Air Pollution Update

Eva Feldman, MD
Professor of Neurology
University of Michigan

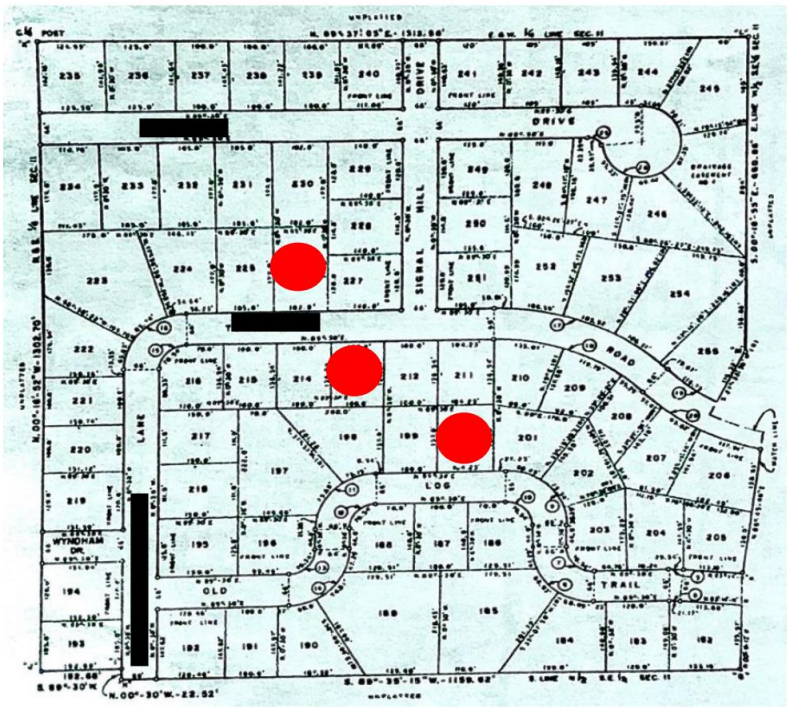
Stephen Goutman, MD
Assistant Professor of Neurology
University of Michigan

Dr. Feldman expressed appreciation for the opportunity to speak to the entire ALS community about their work on targeting the ALS exposome for disease prevention, and to provide an update on a recently funded air pollution grant. She has been in the ALS research community for 2 decades and has spent a great deal of her time trying to understand new treatments for ALS. The field could not be more exciting right now regarding new treatments in terms of the increased understanding of the genetic architecture of the disease, defining and designing new gene therapies, a great increase in the understanding of the pathophysiology of the diseases and the role of inflammation that allows for involvement in new therapeutics, and the development of new clinical progressions scores. They also firmly believe at the University of Michigan that they need to look at new ideas for ALS prevention and for what increases ALS risk, which brought them to their interest in exposome science.

The exposome is the cumulative effect of environmental exposures and corresponding biological responses across the entire lifespan in terms of what people ingest, breath, and/or put on their skin. As Dr. Mehta noted earlier in the day, ALS prevalence could be as high as 9.9 cases per 100,000 population. The University of Michigan has always been driven by the fact that the Midwest has the highest prevalence of ALS at an estimated prevalence of 8.8 (8.6-9.0). The State of Michigan has a very high prevalence and incidence of ALS. For the last 2 decades Dr. Feldman has asked herself why that is. Michigan is an industrial and agricultural state. On the following 2 maps, she circled the areas with the highest age-adjusted death rates for MND in Michigan per 100,000 from 1999–2010 using 2000 US standard population on the left and major toxic emissions in Michigan based on the Toxics Release Inventory (TRI) facilities, National Priority List (NPL), and Superfund sites on the right:



ALS clusters are common in Michigan. The red circles in the map below indicate the childhood homes of 3 friends who later developed ALS around the same time who self-identified and approached Dr. Feldman and colleagues in 2018.[17] Just 2 weeks ago, Dr. Feldman diagnosed another person from this neighborhood with ALS who knows 2 of the other people. These individuals shared their story with the media. There is a PBS broadcast on these women.[18] Clear clusters of disease makes one think that there has to be more than just a genetic cause and illustrates why registries with known geographical locations are so important:



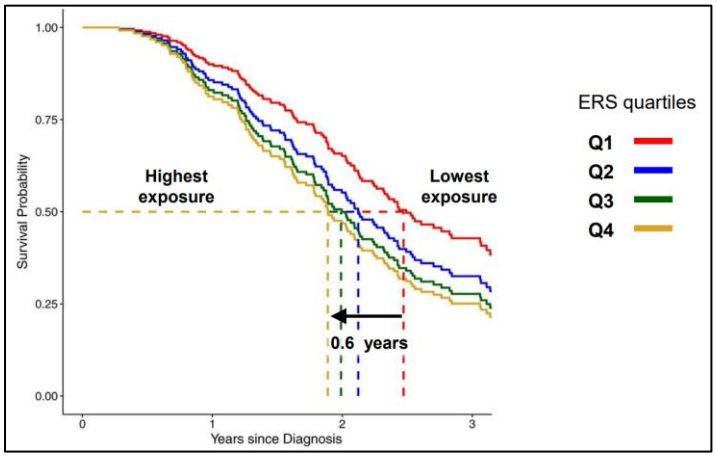
This makes Dr. Feldman and colleagues believe in the ALS gene-environment hypothesis in which the exposome, or cumulative lifetime exposure, makes people more susceptible to neurodegenerative disease as they age.

[17] Yu et al. PLoS One, 2014
[18] https://www.pbs.org/video/als-xmw7rw/

Dr. Goutman provided highlights of the University of Michigan's ATSDR/CDC—funded research in the past and their current application. He explained that one way they have been looking at measuring the exposome is by actually measuring biomarkers of exposure in people's blood. They have been measuring POPs, which are persistent chemicals that get into the blood and last for decades in terms of their half-life. They have looked at 3 categories of POPs. The first is organochlorine pesticides, which have been banned but last in the environment. They collect in the Great Lakes and people living there are exposed through the air and their diets. The second is flame retardants, PBDEs, that were mentioned earlier. These were created to help prevent the environment around us from burning, so they are in couches, clothing, et cetera. The third is PCBs, which were used in coolants and lubricants. While PCBs were banned in 1979, they continue to persist in the environment.

The University of Michigan's initial publication[19] included 156 individuals with ALS and 128 healthy controls for whom blood was collected and targeted analyses were performed on POPs. In a follow-up analysis on an additional dataset, the investigators looked at categories of pollutants. A lot of the chemicals have a somewhat higher increase in the risk or odds of ALS, particularly among pesticides. Of great interest is the mixture effect, which is essentially a combination of all of the pollutants together to understand what they do as a whole to the contribution of ALS. With the help of their colleagues in Environmental Health and Biostatistics at the University of Michigan, the investigators developed a multi-pollutant Environmental Risk Score (ERS). This analysis showed that the multi-pollutant environmental risk score is strongly associated with ALS risk. The ERS summated marker of exposure was greater than an odds ratio of 7, meaning that when someone changes their category of POPs from the 25th to the 75th percentile of exposure, their odds of having ALS increases by 7 times.

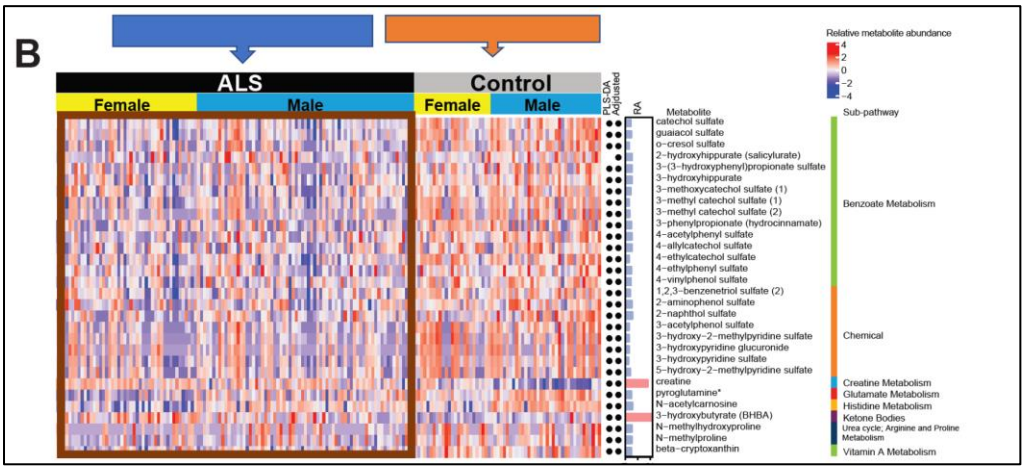
The investigators also looked at how these POPs impact someone's survival with ALS.[20] With the same 167 individuals with ALS, the same analysis was performed of 122 pollutants in which the ERS was calculated for combined scores. The calculated risk scores were placed into ERS quartiles to create a survival plot. In terms of the lines in the following graphic, a 1.00 indicates that the individual continues to live. As it decreases, that means someone passed away:



[19] Su et al. JAMA Neurol 2016
[20] Goutman et al. J Neurol Neurosurg Psychiatry 2019

Quartile 1 (Q1) is the lowest category of exposure. Individuals in Q1 have the longest survival in contrast to individuals in Q4, who have the highest level of exposure and for whom the curve falls at the fastest pace. It turns out that the difference is about 0.6 years, meaning that those individuals who have the highest category of exposure have 0.6 years decreased survival compared to those in the lowest quartile of exposure. This suggests that not only does the effect of these pollutants increase someone's risk of ALS, but also that it has an impact on their overall survival. Moving from the lowest quartile to the highest quartile, one's rate of mortality increases by 2 times, which is a significant increase in the hazard ratio.

The investigators next looked at metabolomics, which represent the exogenous and endogenous compounds in one's body that are involved in metabolism, dietary intake, and all of the function of the body's energy. Untargeted metabolomics can be performed in which a sample of blood is run through a detailed protocol to look for all of the compounds that are parts of different forms of metabolism (glucose, fatty acid, et cetera). In this analysis[21] of unique ALS metabolomic signatures for 125 ALS cases compared to 71 controls, there is a very clear difference in the coloring on the left (ALS cases) of the following heat map compared to the right (controls) side:



This indicates that the metabolomic signatures of individuals who have ALS are very different from individuals who are not living with ALS. This can provide some insight into the mechanistic pathways that are involved in ALS specifically in terms of what types of mechanisms are changing in individuals with ALS. Goutman et al published a validation in *Brain* in 2022 with an additional cohort with these similar metabolic signatures. They are now going further to determine how these metabolic signatures change over time during disease course, as well as correlating these metabolic signatures with individuals' chemical exposures and exposures to other chemicals or exposures in their occupational or residential settings.

The University of Michigan team is currently funded to study individuals' occupations and air pollution exposures. For this analysis,[22] they have looked at individuals who filled out a detailed survey about where they have lived and worked and were able to assess individual self-reported occupational exposures and classify individuals into standard occupational classifications (SOCs). What they found is that certain occupations have a higher risk of ALS. More particularly, persons who work in production occupations have a much higher risk of ALS compared to individuals who are in other occupational categories. Looking at the phenotypes of

[21] Goutman SA et al., J Neurol Neurosurg Psychiatry, 2020; Brain, 2022
[22] Goutman et al. Int J Occup & Enviro Exposures, 2022

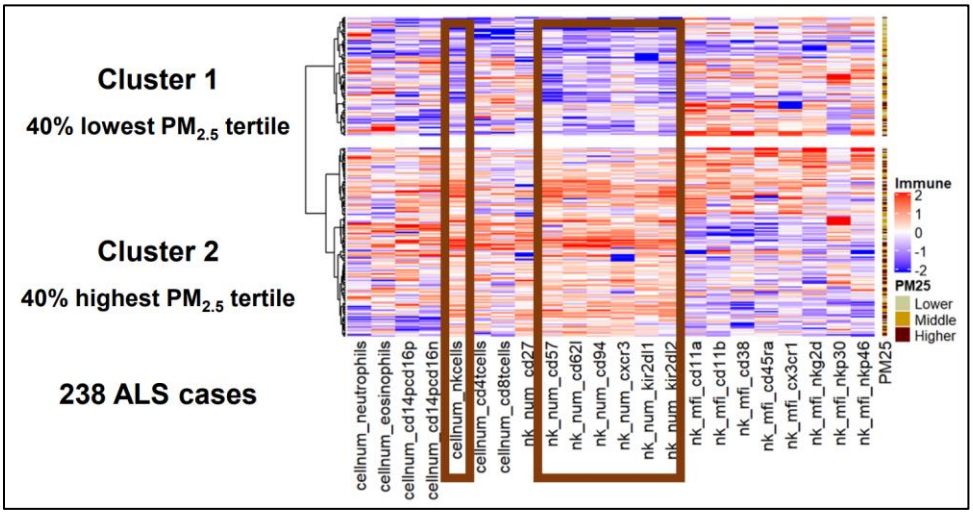
individuals who work in these occupations and their form of ALS, individuals who are employed in building and grounds cleaning and maintenance, construction and extraction, and production occupations tend to have a higher likelihood of having cervical onset disease compared to lumbar or bulbar onset disease. This offers some insight into perhaps why disease starts in one region of the body versus another and in terms of whether there could be an occupational connection or at least some connection to the types of activities one performs. This clearly needs further validation in other cohorts. Looking at occupations and sites of onset, other cohorts have shown some similar associations as well. Notably, the proportion of individuals working in productions occupations in Michigan is some of the highest in the entire nation.[23] In fact, the Midwest tends to have a very high prevalence of people working in production occupations—even out of proportion to larger states such as California. The investigators are particularly interested in why there is an increased risk of ALS in individuals who are production workers and whether that in part explains some of the higher prevalence rates seen in individuals in Michigan and the Midwest.

Turning to air pollution and neurodegenerative disease, particulate matter is a common form of air pollution that is released from vehicles and burning oil, fossil fuels, and wood. These can be measured by satellites, making it possible to see the levels of particulate matter that sit over the US. A study looking at Parkinson's disease[24] shows that the same areas that have very high levels of particulate matter exposures also have very high levels of Parkinson's disease. This peaked the University of Michigan team's interest in trying to understand whether there is an association between ALS and air pollution as well. There is an existing literature that tends to support this theory as well. Looking at areas of Michigan where nitrogen dioxide is of higher concentration, very high levels of nitrogen dioxide have been identified around the Detroit area.[25] The lifetime residential history from participants with ALS can be overlaid on the map to gain a better understanding of where people have lived in relationship to their historical air pollution. These air pollution models date back at least a couple of decades, so it is possible to get great insight into where people have lived and their exposure to air pollution over time.

National Aeronautics and Space Administration (NASA) has satellites that are circling the globe that are measuring the concentration of particulate matter in the atmosphere down to a resolution of a kilometer. As these satellites collect data, there are models that can be used to reconstruct someone's exposure to pollutants over time. Individuals can be placed on timelines of where they lived to understand from a week-to-week timepoint how much air pollution they have been exposed to. At this point, the investigators asked individuals with ALS and control subjects to provide a lifetime residential history. Over 80% of the addresses that are matched to $PM_{2.5}$ estimates have been geocoded and these are being aligned with the models of air pollution that are developed from the satellites.[26] The hypothesis is that air pollution interacts with the immune system, triggering an inflammatory response that influences an individual's risk of developing ALS and their survival. Individuals with ALS and healthy controls who present to the University of Michigan clinic provide information about where they live and work and provide blood samples on which very detailed immunophenotyping that can be correlated with the air pollution models being developed.

[23] https://www.bls.gov/oes/2020/may/oes_mi.htm#51-0000
[24] Shi et al. The Lancet Planet Health 2020
[25] Unpublished data: van Donkelaar et al. Environ Sci Technol 2016; Bechle et al. Environ Sci Technol 2015
[26] Dalhousie University, Aaron van Donkelaar

Some preliminary unpublished data[27] that was provided in the grant application showed that there are very clear clusters of certain types of immune cells that tend to correlate with the $PM_{2.5}$ exposure tertile as shown in the heat map below. For example, someone who has high levels of NK cells tends to be in the highest tertile of $PM_{2.5.}$ These are the types of data the investigators believe they will be able to investigate using their dataset and historical cases:



To summarize what has been learned, the Michigan team believes that if they take the combined exposome approach that includes someone's underlying genetics, their measures of pollutants (pesticides, air pollution, occupational exposure), it will be possible to understand how these all relate to ALS risk. The team believes that these ALS registries can facilitate the correlating of measures of the ALS exposome to documented ALS cases, which can then be linked to banked biosamples. They want to translate their findings from association to causation and believe that there is a need to establish enriched longitudinal cohorts of individuals at higher risk of developing ALS based on occupation (production workers, construction workers) in order to capture occupational, residential, and lifestyle ALS risk factors in real time using validated exposure assessment tools. This will allow for the collection of markers of early ALS phenoconversion and how such exposures influence someone's neurological functioning and match that to their polygenic scores (PGS) and underlying genetics., including body mass index, and mild motor and cognitive impairment, – Collect buccal swabs to complete PGS and biobank hair and nails for exposure measures.

This raises the idea of whether ALS can be made preventable. Using all of the information presented during this session, the Michigan team is firmly of the belief that if people who are at higher risk of developing ALS can be identified, targeted approaches can be taken to modifying behaviors and the environment to reduce people's likelihood of developing ALS. This is going to take detailed assessments of cohorts to monitor for exposures and personal histories and align that with detailed omics data (DNA, transcriptome, metabolome) to develop a personalized prevention plan to one day make ALS a preventable disease.

[27] Murdock et al, unpublished data

Discussion Summary

Dr. Ostrow emphasized how amazing Michigan's work is with the genotyping and the incredible way they are able to make use of these different types of data. Regarding air pollution specifically as it may relate to the progression of ALS, as the disease progresses people have respiratory compromise and they do not ventilate as much. He asked whether it is possible that there is a "chicken and egg issue" in that it may not be that air pollution is increasing the risk, but that that as someone's ventilation is diminished as their disease progresses, these toxins perhaps are less clear. He also wondered with regard to the seeming increased risk of cervical onset identified for the particular occupations whether it was that those occupations increase the risk of having cervical onset of ALS, or perhaps those are the people who are having diminished ventilation.

Dr. Feldman indicated that Dr. Goutman brought this up to her the previous week, which she had not previously considered. They do not have the answer to that, but it is a very important question that they can look at since they have a very robust clinical phenotype of their patients every 3 months with detailed respiratory parameters.

Dr. Goutman added that when they saw these data that are now published, they were trying to speculate or hypothesize why that would be. There was no literature they could turn to try to figure out why those regions were more susceptible to cervical onset disease. He was thinking more in terms of physical activity in that people in these occupations would be utilizing their arms more than other areas of their bodies. He is open to any ideas.

Dr. Ostrow pointed out that another thing that happens as ALS progresses is that people become less mobile. If looking at interactions with the environment, whether it is air pollution or something else, his guess is that there is some sort of measure that says that as ALS progresses, people with ALS spend more time inside than outside. He wondered if this was something that could be controlled for, if patients with ALS do spend more time indoors, and if that matters.

Dr. Feldman agreed that this was a good point and something that Dr. Azar, an Associate Professor in the School of Public Health (SPH), has mentioned. She also has said that in previous work that she has done, particularly with other chronic diseases, there does not appear to be a difference between whether someone is more stationary within the home or sitting outside on their back porch. This is a very good point that has been raised. While there does not appear to be a distinct answer, it likely is not a major factor. In terms of whether people with ALS do spend more time indoors, it probably is regional and seasonal.

Regarding the ERS with survival plotted from diagnosis, Dr. Benatar wondered if the same effect is observed when survival is plotted from symptom onset.

Dr. Goutman indicated that in that model, they adjusted from symptom onset to diagnosis. They used survival time from diagnosis as more of a uniform period of time.

Dr. Benatar clarified that he was wondering about adjusting for time from onset to diagnosis, because that is a measurable rate of disease progression, so it seemed that this is adjusting out a lot of what they are trying to measure.

Dr. Goutman pointed out that what they wanted to do was take into account other validated associations with survival in that particular model, so they adjusted for time from symptom onset to diagnosis, onset segment, use of non-invasive ventilation, and other factors that are associated with ALS survival.

Regarding an inquiry about whether the length of exposure matters, Dr. Feldman said she thought it definitely does. As shown in the gene-time-environment hypothesis, one's exposure to toxicants/pollutants is going to increase with age over time. The basis of the gene-time-environment hypothesis is that the longer one is exposed, the more likely they are to have some sort of neurodegenerative disorder. As Dr. Mehta showed earlier, the highest prevalence of ALS was between males between 60–69 years of age. While this is a well-known fact, prevalence was as high as 14%.

Regarding the plots of POPs exposure mixtures versus survival, Dr. Stommel asked whether there was a difference in the phenotypes in those exposures.

Dr. Goutman indicated that in that paper, they showed that the phenotypes were fairly similar across the 4 quartiles.

Dr. Feldman added that they did not see a difference between limb, cervical, or lumbar onset.

Thinking about the industrial revolution in London and everyone burning coal, Dr. Stommel noted that the air pollution around the time that Charcot described ALS was terrible. He tried to obtain some brain tissue from Salpêtrière from that era, but was unable to get any.

Regarding a question about whether the Michigan investigators have assessed the impact of in utero exposure, Dr. Goutman responded that they have not. That is an excellent question because the exposome accounts for lifetime exposure from in utero onward. While they do not have a way of capturing that in their cohort, it is an outstanding question.

Dr. Feldman added that they used laser technology to examine metal exposure in teeth. They examined adult teeth, which can be for metal exposure throughout the lifetime. They found a correlation between certain metals and ALS risk, one of which is manganese. While that is the only type of work they have done to look at early lifetime exposures, there was a fairly strong signal with that work.

Identification and Characterization of Potential Environmental Risk Factors for ALS Using the ALS Registry Cases and a Control Population

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Dr. Talbott emphasized that she was completely energized by this by this conference. During this session, she provided an update on the University of Pittsburgh's study that has been underway since early 2018. However, COVID-19 impacted them considerably in 2020 when the IRB shut their blood collection and interview processes. They did rebound and are now pleased to be back on track. As a reminder, the goal of this study is to examine environmental and occupational risk factors for ALS by conducting a case-control study of cases from the ATSDR National ALS Registry and population-based matched controls. The specific aims of the study are to: 1) (a) evaluate self-reported environmental/occupational exposure to metals, pesticides,

and solvents as independent risk factors for ALS; and (b,c) examine exposures to ambient air pollution (fine $PM_{2.5}$ and ozone) (2002-2015) and air toxics from the National Air Toxics Assessment (NATA) in cases vs controls; 2) measure exposures to pesticides in samples with a battery of tests using blood concentrations of persistent environmental pollutants (pesticides) in cases and controls; and 3) examine the functional relationship between environmental toxicants in human biological samples and key biological pathways and common genes associated with the development of ALS among ALS cases.

ALS case data were provided by CDC from an ALS Registry survey (demographics, employment, military history, smoking, residential history, occupational exposures, home and work pesticide exposures, and hobbies) for individuals with ALS in the Biorepository Pilot (n=80) and National Biorepository (n=200). Results from analyses of blood specimens for organic pesticides (n=280) and genetic material for further DNA testing of cases of ALS were provided from the Biorespository pilot (n=330) and the National Biorepository samples. Controls matched on birth year, gender, and county were identified by Pittsburgh researchers using databases from a sample vendor, Marketing Systems Group (MSG). Samples were drawn every 2 months from both cell and landline numbers, and controls were interviewed using a similar survey to cases. Blood specimens were obtained and analyzed for pesticides in the same laboratory as cases. In terms of control recruitment, a total of 7965 pre-notification letters were mailed. Up to 10 call attempts were made to each sample (n=6949). There were 284 completed interviews, 1071 refusals, 1127 unknown eligible (no contact, voicemail), and 4467 ineligible controls (quota filled, deceased, out of area, neurological disease, tech barrier). A total of 243 blood draws have been completed to date. A total of 280 cases were identified from the National ALS Biorepository with survey data and blood specimens for organic pesticides. A total of 268 age, gender, and county matched controls completed surveys (96%) and there is a total of 243 controls with complete survey and blood specimens for organic pesticides (91% of controls).

In terms of demographics, the most important thing the investigators realized they had to adjust for because of the pesticides was that the controls were somewhat older. Even though they were matched to cases by birth year, the cases were an average of 62.7 years of age and controls were an average of 67 years of age. The information from the surveys permitted the investigators to self-report industry work by using the North American Industry Classification System (NAICS) Supersector for ALS cases. The following 2 tables show the self-reported occupational and at-home exposures and lifetime prevalence of hobbies by cases status that will be used in the final analysis:

Table 2. Self-reported Occupational and at-Home Exposures by Case Status

Work Exposure	Cases (n=267), n (%)	Controls (n=267), n (%)
Handled insecticides	18 (6.9)	20 (7.5)
Handled herbicides	19 (7.3)	22 (8.3)
Handled fungicides/rodenticides/fumigants	11 (4.12)	19 (7.12)
Used glues or adhesives	27 (10.6)	22 (8.2)
Used solvents and degreasers	60 (22.7)	53 (19.9)
Worked with unleaded gasoline	28 (10.7)	30 (11.3)
Worked with leaded gasoline	29 (11.1)	26 (9.8)
Used unleaded paint	17 (6.6)	17 (6.4)
Used lead paint	12 (4.6)	7 (2.6)
Used formaldehyde	11 (4.3)	13 (4.9)
Soldered	30 (11.5)	21 (7.9)
Welded, brazed or flame cut metals	27 (10.3)	23 (8.6)
Metal dust or metal fumes	46 (17.5)	34 (12.7)
Any other chemical	56 (22.2)	48 (18.1)
Pesticide Exposure at Home	**Cases (n=267)**	**Controls (n=267)**
Handled insecticides	167 (65.0) in home 153 (60.0) on lawn/garden	204 (76.4) in home 146 (54.7) on lawn/garden
Handled herbicides	180 (70.0)	186 (69.7)
Handled fungicides	42 (16.4)	44 (16.5)
Applied chemical soaps, shampoos, dips, powders (kill fleas, tick, other insects on pet)	122 (47.3)	118 (44.4)

Definition of Self-reported Exposure at Work: Pesticides or chemicals that the interviewees have personally handled at work during at least 100 days or more during your lifetime. "Handle" could be referred to preparing, applying, or cleaning up the pesticides/chemicals.
Definition of Self-reported Exposure at Home: Home pesticides or chemicals that the interviewees have personally handled in or around any house or apartment where they lived, at any time in their life. "Handle" could be referred to preparing, applying, or cleaning up the pesticides/chemicals.

Table 3. Self-Reported Lifetime Prevalence of Hobbies by Case Status

Hobbies [1]	Cases (n=267) N (%)	Controls (N=267) N (%)
Leather work (such as making belts, purses, etc.)	17 (7.3)	6 (2.3)
Lead glazed pottery or other ceramics	20 (8.9)	23 (8.6)
Paint pictures or furniture with oil-based paint	54 (24.9)	54 (20.3)
Home remodeling projects that involved scraping, stripping, burning and sanding paint (count only houses built before 1960)	71 (31.4)	64 (24.2)
Woodworking	78 (34.2)	64 (24)
Painted, repaired, or restored old cars, other than fixing a flat tire or changing oil	30 (12.9)	38 (14.2)
Built wooden or plastic models using glue	94 (40.9)	90 (33.7)
Developed photographs	41 (17.7)	33 (12.4)
Gardening	170 (73.9)	176 (65.9)
Soldering, welding, or metal work (such as sculpting, garden structures, etc)	33 (14.4)	34 (12.7)
Outdoor hunting or shooting with guns, including animals, skeet, trap or targets	105 (45.1)	84 (31.5)
Gun shooting in an indoor pistol or rifle range	38 (16.4)	25 (9.4)
Casting of bullets or reloading of	16 (6.9)	16 (6)
Fishing using lead weights or sinkers	101 (44.9)	107 (40.7)
Any other hobby such as knitting, making jewelry (do not consider physical activity, electronic games, writing as hobbies)	71 (31.3)	78 (29.2)

[1]Performed on a regular basis, for at least one hour per month for at least one year since the age of 10 years

In terms of Aim 1b, associations between air neurotoxicant exposures and ALS, an EPA NATA is done every 3 years that looks at neurotoxic and carcinogenic elements of which there are 187. About 34 of these are deemed to be neurotoxic. Data will be used for 2011 and 2014 in order to assign exposure levels based on residence at the time of blood draw for ALS cases and controls. NATA offers data on model-estimated ambient air concentrations of air toxics at state, county, and Census tract levels. Estimates are based on data sources (point, non-point, on-road, and non-road source groups) and monitored data, reports, models, et cetera. NATA data have been applied as an exposure estimate in research settings. The Pittsburgh investigators modeled quartiles for NATA with cut-off points decided by the distribution among controls. The 34 selected neurotoxic compounds include the following 5 classes:

- **Aromatic (6)**: 2,4-Dinitrotoluene, Benzene, Ethylbenzene, Styrene, Toluene, Xylenes
- **Chlorinated (11)**: 1,1,1-Trichloroethane, 1,1,2,2-Tetrachloroethane, Carbon Disulfide, Chloroform, Cresol and Cresylic Acid, Ethylene Oxide, Methylene Chloride, Vinyl Chloride, Tetrachloroethylene, Trichloroethylene, Hexane
- **Metal (7)**: Arsenic, Cadmium, Lead, Manganese, Mercury, Nickel, Selenium
- **Pesticide (6)**: 1,2,4-Trichlorobenzene, 1,4-Dichlorobenzene, Ethylene Dibromide, Ethylene Dichloride, Hexachlorobenzene, Hexachlorobutadiene
- **Other Hazardous Air Pollutants (HAPs) (4)**: Allyl Chloride, Cyanide, Hydrazine, PCBs

The investigators conducted an unconditional logistic regression stepwise selection. Each person was assigned levels of these 34 elements and quartiles were created by using the controls. The unconditional logistic regression was adjusted by education, smoking, sex, and birth year. These data were presented the previous week at the 2022 National Ambient Air Monitoring Conference (NAAMC). A larger proportion of ALS cases were male (62.9% compared to 37% female). Significantly more cases were ever smokers (42.7% vs 34.8%) compared to controls ($p < .05$). Odds Ratios (ORs) and 95% CIs: Unconditional Final Stepwise Logistic Regression Model Adjusted by Smoking, Education, Sex, and Birth Year: 2,4-Dinitrotoluene was associated with significantly increased risk of ALS compared to controls in the first compared to the fourth quartile of exposure (OR: 2.54, 95% CI :(1.13, 5.70), as well as for Cyanide: 3.28 (1.35, 7.96). The investigators are still trying to figure out what the Cyanide aspect is and are looking at different parts of the country to see where plants that use Cyanide in various preparations might be located.

The second part of Aim 1b has to do with ozone and air pollution. There is a lot of interest in this with regard to Parkinson's, Alzheimer's, cognitive function, and many potentially neurotoxic substances. $PM_{2.5}$ may get in quicker because it is smaller. The investigators are currently looking at different lags. While 1 year and 3 years are interesting, they want to go back as far as they can. This table is an example of unadjusted levels showing the means and standard deviations for 6 to 10 years prior. It is important to point out that air pollution has decreased rather dramatically than 10 years ago when the daily average was 17.75 (cases) and 16.89 (controls) maximum. The standard daily average was about 12.00 and now is down to about 9.0. Therefore, it would behoove them to go back as far as they can with ALS because it is known that something someone breathes in one moment in one day is not going to be the tipping point as there is a cumulative time factor:

Table 5. Distribution of annual $PM_{2.5}$ level of 6 to 10 years prior to the year of diagnosis of ALS for cases, and 6-10 years prior to the matched case's year of diagnosis for controls. (unadjusted).

N=267 Pairs		Variable	Case/Control	N	Mean (SD)	Median	P25	P75	Min	Max
PM2.5	Annual Mean PM2.5, of # year(s) before the year of diagosis	PM25_6yr_PriorDX	Case	256	10.07 (2.24)	10.03	8.57	11.35	4.68	16.70
			Control	256	10.06 (2.21)	10.01	8.71	11.40	4.22	16.49
		PM25_7yr_PriorDX	Case	253	10.37 (2.39)	10.23	8.80	11.81	4.97	17.35
			Control	253	10.34 (2.32)	10.22	8.95	11.74	4.97	16.45
		PM25_8yr_PriorDX	Case	251	10.8 (2.43)	10.75	9.18	12.46	4.82	18.63
			Control	251	10.76 (2.37)	10.70	9.22	12.42	4.82	17.24
		PM25_9yr_PriorDX	Case	241	11.31 (2.48)	11.60	9.65	12.97	5.16	20.19
			Control	241	11.24 (2.42)	11.58	9.64	12.92	3.89	15.82
		PM25_10yr_PriorDX	Case	225	11.58 (2.52)	11.89	9.62	13.32	5.43	17.75
			Control	225	11.52 (2.53)	11.84	9.92	13.28	5.21	16.89

In terms of Aim 2, CDC provided serum pesticide results for 280 cases. The laboratory analyses were conducted in SGS AXYS laboratories in British Columbia. After recruitment of controls, survey and consent, the Pittsburgh team scheduled an in-home blood draw by ExamOne. Blood samples were then overnighted to Dr Donnelly's laboratory and forwarded in batches to SGS AXYS laboratory (n=243 controls). This was completed in May 2021. All final laboratory results were obtained by July 16, 2021. The investigators are currently in the process of evaluating and analyzing the results. They began with 29 pesticides but ended up with 16 that have a reasonable amount of detectability because many pesticides have been banned. Of the 29, there were 13 that were non-detectable given the small amount of serum. Multiple imputation and adjustment will be made for the detectable pesticides that are below 20% to 25%. The team was very concerned about validation. When they received surveys 8, 9, and 10 about duration of exposure, they wanted to know whether when someone reported a duration >0.0 whether they would see a correlation with the duration in years and the actual pesticide level. Not so much fungicides and herbicides, but the insecticides are showing oxy-Chlordane and other levels. Although the ratios are not 0.75, they are seeing a relationship with what people report and what people have in their blood.

In terms of preliminary analyses, unmatched multivariable logistic analyses for nonachlor cis and for chlordane oxy indicates a possible association of these 2 pesticides and increased risk of ALS after adjustment for smoking, education, and age at blood draw. These analyses revealed an increased risk of ALS associated with chlordane _oxy, (OR=1.038, p<.001) adjusted for smoking, education, and age at blood draw and a borderline significant effect for Nonachlor cis (OR= 1.072 , p=.056.). Analyses will be carried out with multiple imputation whenever possible in order to optimize the sample size for the case control analysis. There are plans to publish this manuscript shortly.

Dr. Tennant briefly described the genetic aspects and explained the C9Orf72 positive and negative individuals who they are looking at more closely so that hopefully they can hone in on some of their demographic and phenotypic characteristics. The reason for assessing C9Orf72 is because it makes up over a third of all known familial ALS (fALS) cases and 8% of all sporadic ALS (sALS). About 1 in 10 individuals who suffer from ALS is going to have this genetic condition. The C9Orf72 gene has a repeat expansion in ALS. In healthy individuals, the sequence can get from 5 to 30 repeats of GGGGCC. In the disease condition, it is multiplied many-fold. While the actual pathological mechanism is not completely understood, it is likely part and parcel of the 3 concepts of loss-of-function, RNA toxicity, and DPR toxicity.

Specific Aim 3 of the study is trying to get a better understanding of whether there is a link between pesticides/pollutants exposure and C9orf72 GGGGCC repeat and whether it affects repeat size and/or stability. There is a tenuous link between the size and severity of disease, with >30 repeats being the current pathological cut-off. However, the size and severity are not well-understood. There is inherent heterogeneity to this genetic loci between individuals, tissue types, and brain regions. Over the course of a patient's life, this repeat expansion size can change dramatically. The high GC content of this region makes it impossible to use PCR. Southern blotting is used as a way to visualize the repeat size in an old school way. Restriction enzymes finely break down non-repeat DNA. The idea is to digest away everything that is not the repeat region of interest and running this out on a gel electrophoresis to separate out the size of the bands. Then the separated DNA is transferred to the membrane via capillary action. In the interest of efficiency and given the limited number of samples, they are probing only for G4C2 repeat region. The band size and smear range will be used to get an estimate of the number of repeats within each patient sample.

The next step will be to use this information to determine a type of measure of stability of the repeat within the region. It will be interesting to track whether there is correlation between the stability of the repeat region in patients and all of the history that has been collected from them to see if it is possible to tease out something to indicate earlier onset if there is an increase in stability or exposure to a certain type of pesticide. The genes to be tested include KIF5A (2018) NEK1 (2016), GLT8D1(2019), ARPP21 (2019), C21orf2 (2016), CCNF (2016), TIA1 (2018), and ANXA11 (2017). They looked at familial ALS patient samples with no known causative mutation, tested for genes not present on neurochip, and performed whole exome sequencing. This provided a lot of information. One patient had a novel recently identified target, the NEK1 R261H mutation. ANXA11 R230C was found in 13 of 21 samples. The next step is to assess variant single-nucleotide polymorphisms (SNP) frequency within genetic background and populations. Two of the tested mutations fall within ANX domains. ANXA11 mutations in ALS cause dysregulation of calcium homeostasis and stress granule dynamics. The mutation R230C is untested but falls within an ANX region. Prolonged stress granules formation could serve as nucleation points for interaction with TDP-43.

Discussion Summary

Regarding a request to define "stress granules," Dr. Tennant indicated that stress granules are members of the organelle in the biological response to stress that is a collection of proteins that come together to shelter certain RNA transcripts during the stress response of a cell. The reason it is interesting in ALS is because some groups have suggested that the formation of these stress granules can create sites of nucleation for TDP-43. The stress granules persist and generate detrimental insoluble aggregates.

A Genome-Wide Association Study on the New England and Ohio Amyotrophic Lateral Sclerosis Cohort

Jiang Gui, PhD
Associate Professor
The Dartmouth Institute

Dr. Gui noted that Dr. Stommel charged him with providing a summary of the genetic component of his CDC grant, so this presentation covered the Aim 3 portion of that entire grant. The genetic aspect is arguably a potentially less important factor compared to environmental factors. The motivation to perform a genome-wide association study (GWAS) came from the dogma held that basically DNA transcribes RNA and RNA translates protein. That protein would represent the phenotype, which is the disease. The people in GWAS believe that a lot of the variation in the protein or in the disease status can be changed by the genetic factors. For background, GWAS is an approach used in genetics research to associate specific genetic variations with particular diseases. The method involves scanning the genomes from many different people and looking for genetic markers that can be used to predict the presence of a disease. Once such genetic markers are identified, they can be used to understand how genes contribute to the disease and develop better prevention and treatment strategies. GWAS is basically finding the genetic loci that differ among cases and controls. This can be identified by using WGS platforms to identify all of the SNPs. Eventually, this will result in a statistical path to identify the association between each loci and the disease status.[28]

In order to combine different studies, genotype imputation is an essential tool in the analysis of genome-wide association scans. The technique allows geneticists to accurately evaluate the evidence for association at genetic markers that are not directly genotyped. Genotype imputation increases the power of genome-wide association scans and is particularly useful for combining the association scan results across studies that rely on different genotyping platforms.[29] A study sample may have a lot of gaps and may have half a million loci, while the reference haplotypes have probably close to 100 million loci. The gaps can be imputed based on the reference haplotypes. It takes a lot of computation time to fill the gaps. For the merged dataset for the Dartmouth College study, blood samples were collected at various times from patients in New Hampshire and Ohio. Those blood samples were sent to the NIH laboratory to be phenotyped. Because of COVID, there has been a delay in getting samples back. In total, there are about 700 samples from the data. There are about a half million SNPs for each sample. These were merged together to run GWAS.

One problem that was encountered in the first GWAS run was that there was a huge genetic inflation, which motivated the Dartmouth investigators to institute a rigorous quality control (QC) step. The problem with admixture populations is that about 1% of the loci that are significant are very high, which makes it difficult to tease out a signal. Having 1 type of ethnicity group helps to reduce genetic inflation. There are methods to tackle the admixture population in GWAS. The QC steps are as follows:

[28] Tam V, Patel N, Turcotte M, Bossé Y, Paré G, Meyre D. Benefits and limitations of genome-wide association studies. Nat Rev Genet. 2019 Aug;20(8):467-484. doi: 10.1038/s41576-019-0127-1. PMID: 31068683.
[29] Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. Annual review of genomics and human genetics. 2009 Sep 22;10:387-406

STEP 1: Sample-Level Filtering
- ❑ Heterozygosity check (remove heterozygosity outliers)
- ❑ Population Stratification (ancestry check)
- ❑ Sample call rate check, maximum missing calls per sample
- ❑ Remove duplicates

STEP 2: Variant-Level Filtering
- ❑ Case/control nonrandom missingness test
- ❑ Haplotype-based test for non-random missing genotype data
- ❑ Hardy–Weinberg Equilibrium (HWE) check
- ❑ Variant missingness check

Before QC, there were 714 individuals and 472,662 variants. After QC, there were 675 individuals and 242,090 variants. Among these 675 individuals, 613 do not have a family history of ALS and 30 have an ALS familial history. The investigators focused on sporadic ALS using the 613 subjects without a family history of ALS. The sample size of the familial ALS cases is too small to get enough power. Covariate adjusted logistic regression was used to screen all of the SNPs' associations with the disease outcome. Covariates included sex, age at symptom onset, and PC1-PC10. The genomic inflation factor was λ = 1.016. In terms of significant SNPs in the Dartmouth data, below are 12 SNPs with p-values <1E-5. No SNPs passed the threshold level of 5E-08, but 12 passed the threshold level of 1E-5:

CHR	POSITION	ID	RS-ID	P Value	Gene : Consequence
1	62571675	rs35129900	rs35129900	4.73E-07	PATJ : Intron Variant
1	11082610	TARDBP:NM_007375.3:c.1144G>A:p.(Ala382Thr)	rs367543041	6.23E-07	TARDBP : Missense Variant
1	11082610	chia_chr1:11082610G>A	rs367543041	9.38E-07	TARDBP : Missense Variant
11	99035893	rs7949592	rs7949592	1.36E-06	CNTN5 : Intron Variant
5	178172446	rs79957207	rs79957207	2.21E-06	None
16	79356800	rs145767144	rs145767144	2.30E-06	MAF : Intron Variant
7	117368167	indel.97965	rs777499040	3.20E-06	LOC105375469 : Intron Variant
19	44739303	rs2279073	rs2279073	4.84E-06	ZNF227 : Missense Variant
7	51500101	rs12056030	rs12056030	5.28E-06	None
7	51520250	rs12540310	rs12540310	6.65E-06	None
3	174506772	rs7648550	rs7648550	7.83E-06	NAALADL2 : Intron Variant
17	52075605	rs1125370	rs1125370	8.39E-06	None

Rs367543041, which is a missense variant of TARDBP gene, was reported to be associated with ALS in 4 publications.

In order to minimize the large known mismatch, all of the SNPs from the 2 cohorts essentially will be imputed. This was done on the Michigan Imputation Server with a reference panel of 1000 genomes (Phase 3 v5). The number of SNPs went from a quarter million to about 14 million after imputation.

Moving on to MEGA-GWAS, this is ongoing work so Dr. Gui reported on what they have so far. Essentially, they have merged a lot of the database of Genotypes and Phenotypes (dbGaP) data to get to about a 24,000 sample cohort compared to the 600 they had, so this is a much larger dataset. Again, they used all of the same QC steps as before. Before QC, there were 24,205 individuals and 471,211 variants (10,067 cases and 14,138 controls). After QC, there were 22,419 individuals and 335,021 variants (9686 cases and 12,733 controls). For New Hampshire and Ohio, the investigators surveyed everyone, so they know the exact ages. For

the dbGaP data, they only know age group so they determined transformed age. They also want to make sure that the genetic factor is okay. Because this is a much bigger dataset that is about 20 times bigger than what the investigators have, the inflation is somewhat higher at r λ = 1.1012. The last time they adjusted for sex, age, and PC1-PC10, the genomic inflation factor was λ = 1.1272. These are the SNPs on Chromosome 9 with the lowest p values by chi-square test:

Chr	SNP	BP	P Value
9	rs2453554	27561800	5.50E-12
9	rs868857	27488906	3.70E-10
9	rs17779457	27488092	3.93E-10
9	rs774351	27516640	5.32E-09
9	rs1982915	27579560	4.39E-08
9	rs2453556	27586162	7.63E-08
9	rs10812605	27510360	9.79E-08
9	rs868856	27489251	1.41E-07
9	rs7046653	27490967	1.65E-07

The genomic inflation factor was λ = 1.1012. After removing 193 SNPs from 27.00 Mb – 28.00 Mb of Chromosome 9, the inflation factor was 1.1010. The imputation takes a very long time, so it is still ongoing. The UNC13A variant rs12608932 is associated with increased risk of ALS and reduced patient survival. Among the 9686 cases, there were 2 types of ALS, sporadic (85.2%) and familial (10%). First, they ran the 8252 sporadic ALS cases and 12,733 controls and removed familial and unknowns. When all of the C9orf72 was removed, the only thing left was Chromosome 19 Rs12608932, which seems to be an important factor that contributes to sporadic ALS. Next, they merged the 971 familial ALS cases with the 12,733 controls and 13 SNPs on Chromosome 9 passed the threshold level.

The next step is to run the genetic imputation on the large Mega-GWAS and then they will value each single finding from the New Hampshire and Ohio data. Another portion of this work will be done by Dr. Gui's colleagues at Dartmouth in which they are pooling the histories of all of the residents together and will match them to identify their environmental exposure data. This has been done using regional data, so they are trying to get individual exposure data, which involves getting the addresses for all of the residents. This is more tedious and time-consuming work, but once they have that, they can combine it with the genomic data to assess the gene-environment interaction to determine whether there is significant association of additive risk findings that can boost the research for ALS.

End of Day Discussion

Danielle Boyce, DPA, MPH
Session Participants

Dr. Boyce led the group in a general discussion at the close of the day. She first shared the very sad news of the passing of Sandy Morris, a giant in the ALS patient advocacy world. Sandy was her first friend when she came into the ALS community and was a valuable member of the ALS Stakeholder Engagement Team that would be presenting on the second day of the meeting. Dr.

Boyce learned of Sandy's death earlier in the day as she was presenting and moderating. Sandy would have insisted that they keep going. In Sandy's memory, Dr. Boyce read an excerpt from the "Morris ALS Principles,"[30] which she thought they all would find applicable:

ALS/MND is a devastating neurodegenerative disease discovered in the middle of the 19th Century. It has killed millions of people across the globe. Yet more than 150 years later, there are no cures, no effective treatments, no urgency. This is unacceptable. Today, we unite as leaders to end ALS/MND. We take ownership of our disease so that the road to cures is faster, equitable, and more humane. We are not subjects or victims and are only occasionally patients—a term which implies passivity. We are a relentless community working alongside researchers, policymakers, and clinicians to identify and cure this heterogeneous disease.

We demand a seat at the table before decisions are made in drug trial design, research, healthcare policy, or anything that affects our care. From this point forward, we will not tolerate silos, disorganization, or lack of urgency by any agency or organization that serves us. There will be nothing about us without us. We live on the ALS clock. ALS/MND is stunningly brutal and kills us quickly. Our political leaders must make ALS/MND research and therapy development a national priority. Our nation has rallied to confront HIV/AIDS, cancers, multiple sclerosis, and now COVID-19. We demand that the United States leaders make a commitment to end ALS/MND.

To the scientific community and ALS/MND research community, provide virtual and in-person, access to all ALS/MND conferences and scientific meetings for people living with ALS/MND. Include people living with ALS/MND on all clinical trial protocol teams, on advisory bodies to the National Institutes of Health, the Food and Drug Administration, and Centers for Disease Control and Prevention. Build Expanded Access Programs (EAPs) into drug development plans. EAPs for other diseases were key routes to extend experimental therapies to those not eligible for trials and to learn more about their therapies and the disease. Sponsors must design trials that adhere to the patient-centric trial design rating criteria. Share your data, designs, failures, and best practices. Your duplication of work is deadly.

Dr. Boyce emphasized that Sandy was very proud of the work that is being done, and she would be very proud that this meeting was being done virtually and included people living with ALS. She said it was really something to see the outpouring of support from ALSA, Les Turner, and

[30] https://morrisalsprinciples.org/about/

other organizations who knew Sandy and what a difference a person living with the disease can make in such a short period of time despite their suffering.

Discussion Summary

Dr. Mehta expressed condolences for the passing of Sandy Morris. While he said he did not know her personally, she was very well known for her opinions, comments, and support of the National ALS Registry and Biorepository. This is not an easy disease. Every year, people they know and have befriended are lost to ALS. In memory of these friends, progress has to be made to figure out causes and treatments for ALS.

Regarding a question pertaining to the heightened prevalence of ALS in a community in Ohio, with 21 people with ALS in a population of 4900, Dr. Mehta indicated that several of the cases are juvenile onset living on the same street. Only 5 cases have C9 mutations. The town has 1 case of PLS and 3 cases of MSA, and innumerable cases of MS. ATSDR did some research on the back end and has the information regarding the MS investigation that was done in 2005, for which he offered to share. He has not heard anything about a request from the State of Ohio Health Department about looking into cases for ALS. They do receive requests on occasion from other states across the country. This has included North Carolina, Florida, New Mexico, and West Virginia. He will look further whether they have received a request from Ohio.

In terms of a question about the method for the public to report data and get investigations started, Dr. Horton indicated that ATSDR is divided into different disciplines. His group conducts a lot of studies and runs surveillance and registries. Another group deals primarily with health consultations and public health assessments that involve going into communities, listening to community concerns, performing environmental sampling where necessary, and so forth.

Dr. Mehta added that in the hierarchy of public health in the US typically state investigations are done first, and then CDC is asked to help later in the process. They cannot just go into a state without actually being invited. Once invited, CDC technically can go into a community. Their team is ready to provide information and so forth on an investigation in conjunction with the EPA. Typically, the state health department would assess the situation and then make a request.

Dr. Zarus added that in several instances when ATSDR is petitioned and they have a state program that is authorized, they take on a certain list of work. In this case, there are some documents that are reviewed by ATSDR. The document posted on the website was reviewed, at least for clearance, and so that is why it is posted on the ATSDR site. It appears that a follow-up is needed as there are 2 recommendations. Once the state has sufficient data, ATSDR would meet with them and discuss the follow-up options.

Dr. Finger asked whether ATSDR has a sense in a state like Massachusetts if all patients are being reported to the Registry. Dr. Mehta indicated that since ALS is reportable in the state, the state does not notify the Registry. From what they have been told by Massachusetts, they feel they have a pretty good handle on what is being reported to the state itself. They have indicated that they have 90% case ascertainment from the state since it is law in the State of Massachusetts to report ALS. However, he was not sure of the exact figure being reported to the state.

Regarding a question about whether patients receiving care outside of the state are being missed, Dr. Mehta said cases being seen outside major centers certainly could be missed. Boston has a huge ALS facility, which is a great resource and individuals could be presenting there from other states who may have registered elsewhere initially and then moved over to Massachusetts who potentially could be missed.

In terms of an inquiry posed about how ATSDR guarantees a mix of socioeconomic backgrounds in collection of data, Dr. Mehta emphasized that this is always a challenge. They are always trying to make sure that the Registry includes a diversity of patients, but sometimes it is just not feasible. Every disease has that issue in terms of trying to capture as diverse a population as possible. The portal data skews toward a higher SES and White non-Hispanics. ATSDR wants to work with their partners to make sure they get cases from California, Texas, New York, and Florida because those are very diverse states that would make the Registry more diverse. It is challenging, but ATSDR is doing what they can to address those challenges. Dr. Stommel added that he thought that was more of a role of the multi-disciplinary ALS clinics.

Dr. Dave inquired as to what percentage of people in the Massachusetts Registry who also are in the National ALS Registry.

Dr. Mehta indicated that this is a question that ASTDR is working on now through the analyses that are currently being done, with the hope of getting that reported in the next 3 to 4 months.

Dr. Siddique noted that the clusters have been of great interest to the ALS community for a long time. He did not catch the details of this particular cluster. He pointed out that many clusters have been studied by investigators at the Mayo Clinic. Epidemiologists indicate that the issue of mobility of populations and denominator of where the population has been for the last 50 to 70 years becomes a problem. Many of these clusters have turned out to be people who are genetically related in some form. That does not automatically mean there is something environmental going on. He asked whether Massachusetts has normalized their cases to each county of some unit rather than just pinpointing the cases. Population centers are going to have more cases and more pollution. A study was conducted at the Mayo Clinic that looked at all of Minnesota that did not find any difference in the incidence of sporadic ALS after removing familial ALS, suggesting that if there was something in the environment, it was pretty uniform.

Dr. Mehta indicated that it was a cluster of ALS patients in Ohio and that the MS area was in Lorain, Ohio. Regarding the Mayo Clinic study was interesting in that the investigators did not find any commonality for the sporadic ALS cases. He emphasized that clusters are extremely difficult to investigate because it requires figuring out exactly where the patient lived, their history before living in a particular area, obtaining medical records, determining whether there is familial ALS, et cetera. In one cluster investigation that ATSDR was asked to investigate, they found that a lot of the patients moved into the area from different parts of the states, so in that particular area, it was not clear where the exposure potentially could have happened.

Dr. Zarus added that the cluster of ALS patients in Ohio was in Wellington in Lorain County. It is difficult to investigate cancer clusters or any clusters. In this case, ATSDR was limited to the environmental data that were available. The investigators said they saw no significance in that environmental data, so that was why the current recommendations were to look further.

Dr. Mehta indicated that when CDC performs outbreak investigations, sometimes outbreaks have probably an easier way to kind of figure out exactly what the index case was only because infectious diseases are much more rapid in terms of evolving, getting the prodromal symptoms,

and getting the actual infection. In outbreak investigations, it is possible to see what happened, where the vector was, the exposure, where a person sat on an airplane such as next to a person with tuberculosis, et cetera. More information is available for researchers in outbreak settings.

Dr. Siddique emphasized that the cause is unknown for sporadic ALS. With the microbiome and bacteriome, it is a pretty straightforward task to figure it out if it is a microorganism. It may take a little time, but it is possible to get. People make assumptions that people can be sent to the moon, it should be possible to fix ALS or have a cure for the common cold. There are 400 viruses for the common cold. The common cold is not a bad disease in terms of mortality, but the mechanism of disease in sporadic ALS is not known, so there is hand waving in terms of what to look for.

Returning to the Massachusetts question, Dr. Dave said that assuming that 60% of people in the Massachusetts Registry also are enrolled in the National ALS Registry, that means potentially 40% are missing. His worry would be that if people sign up for their state registry whether they would assume that they have given their name to a registry, so they do not have to put the name into the National ALS Registry. Others may have enrolled in one registry and feel like that was enough. When Vermont and Maine set their registries up, he wondered whether data are rolling into the National ALS Registry.

Dr. Mehta indicated that no data are coming into the National ALS Registry at this time from any states. OMB approval is required before ATSDR works on any DSAs. This also is a resource-driven task.

Dr. Stommel indicated that if data from state registries go to the National ALS Registry, patients would not need to sign up for 2 or 3 different registries.

Dr. Dave pointed out that the issue would be that if they sign up for one registry, that would take care of only one component of the registry to count people. However, the National ALS Registry also is collecting samples and epidemiological information, connecting people to trials, providing a prevention research program, et cetera. CDC is the only entity that is really focused on all of that. If the state level takes precedence over the National ALS Registry, those other components of the National ALS Registry may be compromised.

Dr. Stommel said he did not think they were being compromised because once patients have been identified, they are encouraged to go to an ALS clinic or their own clinic. As a neurologist, he talks to patients about clinical trials, biobanks, and all of the research opportunities available. He assumes other neurologists do the same thing. That is part and parcel of taking care of ALS patients.

Dr. Dave asked if Dr. Stommel was saying that someone at the state registries would be informing people about the trials or research in their areas and/or the National ALS Registry when registries are set up at that state level. He wondered if there is a dedicated person who is paid to do this.

Dr. Stommel said he and other neurologists who work in ALS clinics are given information on patients and then can educate them. He does this every day. He did not think this would be an issue. The idea of the registry is to make sure all cases in a state are identified. As Dr. Mehta mentioned earlier, if 10% of patients in Vermont or Maine go to Massachusetts General because they want to get care there and it is closer, at least there will be a way to identify those

patients because their primary care physicians or their neurologists within the state of Maine or Vermont will have to report them to the Registry.

Dr. Mehta said that one way they could talk about doing this, which is getting into the weeds, would be to consent patients for ATSDR to contact them after their information has been passed on to the agency. Then ATSDR could contact them about the Registry, signing up, taking the risk factor surveys, asking whether they already have been added to their state registry, et cetera. This model has been used for newborn screening and cancer, for instance. There are ways for ATSDR to circle back to make connections.

Dr. Horton added that a state-based registry like Massachusetts is not set up like the National ALS Registry with a web portal where people enter and can enroll. The Massachusetts ALS Registry is pretty much set up like a cancer registry in that healthcare professionals who diagnose someone with ALS have to report the information to the state health department by law. The patients in Massachusetts do not enroll themselves or contact the state health department. He thought that would be the way Maine and Vermont would be set up as well.

An inquiry was posed about whether Massachusetts has mandatory reporting, Dr. Mehta clarified that reporting in Massachusetts is mandatory. Physicians have to complete and submit a 1-page form to the state health department. There is not self-reporting in Massachusetts.

Regarding a question from Dr. Finger about the racial or ethnic breakdown of patients in the biorepository, Dr. Mehta indicated that in Dr. Talbott's sample, 98.9% of the patients were white. Dr. Ostrow reported that 75% of researchers said those data are important. Currently, the biorepository mirrors the Registry. The portal data is somewhat socioeconomically more white non-Hispanic as opposed to Medicare, which tends to be more diverse. There is a recognition of the need to increase diversity. There is diverse geographic representation, meaning that all patients are not coming from Montana or Idaho. They try to sample equally across all states based upon the population size as well.

Dr. Anne Quinn, a physician with ALS, said that unfortunately, no one ever mentioned the National ALS Registry to her at her own clinic. She discovered it through her own research and imagines that she is not the only patient who does not know about the Registry. She has discovered 7 other people in her town of 15,000 MPA. She reported this cluster to the state health department. The issue is that the state indicated that they only have access to death data. They wanted to look at all-claims data to get more accurate information, but do not have access to this or patient addresses.

Dr. Mehta acknowledged that this is a common complaint that they hear from patients. ATSDR recognizes that they must do a better job working with their partners to inform and educate patients about the Registry. ATSDR works very closely with the ALS Association, MDA, and Les Turner to get the message out. It is important to keep in mind that they cannot force anyone to join the Registry per the IRB, but they certainly want to make sure that patients have the information available to them on how to enroll. To add to the response to Dr. Finger's question, the portal is not 99% white. It previously was 96% white and currently is 94% white, so they have done a better job in increasing diversity. They must do a better job increasing diversity going forward as well. In terms of Dr. Quinn's comments about access only to death data and the claims data that ATSDR receives from Medicare and the VA systems through the DUA, they make sure that people are aware that there is an ALS Act that was passed that charges ATSDR to work with other organizations, such as CMS and the VA, to establish a relationship to obtain their claims data. From there, ATSDR runs the algorithm to determine who has ALS. If OMG

gives them approval to release state-level data, it will be at the state-level at this time. It will not be below that level because there is a major concern about the ability to identify the patients. Regardless, having data available at the state-level is a first step to determine what is happening in states.

Dr. Talbott added that the particular population to which Dr. Finger referred was an early group when the Registry was just taking off. The early response rate was more SES-driven in terms of people who had computers and high-speed internet and could complete the surveys. Hopefully, that will improve over time. Regarding Dr. Quinn's comment, Pennsylvania does not currently have an ALS Registry and there is no current initiative there.

Dr. Ostrow said he heard a great idea from Phil Greene that it would be beneficial to have a national accepted global unique identifier for ALS for clinical trials, research, biorepositories, et cetera that would be administered by a governmental organization like the CDC National ALS Registry. If every patient is issued a GUID at the time of diagnosis to be used by every trial, protocol, et cetera, that instantly would bring traffic to the website and there would be an instant catchment of more people filling out the surveys. At a minimum, a national global unique identifier would allow linking of all of the mature and amazing efforts. While it would not be perfect, it seems like a pretty "low hanging fruit" to improve linking and catchment and ensure that neurologists and others are talking about the Registry when talking to patients about ALS.

A comment was made that it would be helpful to publish demographics for people entering the portal, and the specific demographics of the subset of portal patients who are in the biorepository.

Dr. Mehta indicated that they will determine with the IRB whether these data can be reported out. When ATSDR reports data, it is an amalgamation of both the portal, biorepository, CMS, Medicare, VA, and VHA data.

Regarding patients not hearing about the Registry, Dr. Boyce encouraged everyone to attend the second day of the meeting to hear the conversation with the stakeholders who are working to address getting the word out and helping to recruit.

Pat Dolin, a GIS expert and person living with ALS, asked whether the Registry information will be mapped. While he said he understands the concern over privacy, the information can be rolled up to the county-level similar to the CDC Wide-ranging ONline Data for Epidemiologic Research (WONDER)[31] database that he mapped to help with awareness.

Dr. Mehta indicated that ATSDR published 2013 data years ago in which they mapped all of the cases of ALS in the US to the county-level and looked to see how far they were from the nearest multidisciplinary ALS clinic. They found that almost half of the patients lived 50 miles or more from the closest clinic. While this has not been done for more recent data, he did not believe this would be different.

Dr. Horton indicated that they could map out additional years, which is a good use of the data. New clinics have come online since the first paper. Some patients had to drive 500 to 600 miles to the closest ALS clinic. Regarding Dr. Quinn's comment, he emphasized that ATSDR funds ALS Association, MDA, and Les Turner in Chicago to conduct outreach at the ALS clinics and through their chapters. Like any organization, some chapters and clinics seem to be better at

[31] https://wonder.cdc.gov/

promoting the Registry than others. He raises this issue every year. The goal is to get to the point that all clinics, chapters, and organizations are promoting the Registry at the same rate so that everyone hears about it the same way. That has been a challenge, but ATSDR has monthly calls with ALSA, MDA, and Les Turner. However, they still have not found the "magic bullet" for engaging all clinics, chapters, and support groups mentioning the Registry to newly diagnosed patients.

Dr. Ostrow indicated that Dr. Terry Heiman-Patterson is conducting a study about how the distance from large multidisciplinary ALS clinics impacts the cost and quality of ALS care.

Dr. Mehta added that ATSDR is working with Dr. Heiman-Patterson on that and is awaiting IRB approval. Dr. Heiman-Patterson will be using the Registry and other avenues throughout the country to recruit for that study.

The National ALS Registry Annual Symposium and Meeting stood in recess until 8:00 AM ET on August 30, 2022 at which time updates on the funded research resumed.

National ALS Registry Funded Research Presentations: Part 2

ALS Risk in Latin Americans: A Population-Based Case-Control Comparative Study with Three European Population Based Cohorts

Orla Hardiman, MD, PhD
Professor of Neurology
Trinity College (Dublin)

Dr. Hardiman provided an update on the Latin American Epidemiology Network of ALS (LAENALS) epidemiological study of ALS. The rationale for this study was that they have been interested for years in the polygenic risk for ALS and differences in clusters and populations. There was quite a bit of evidence to suggest that this was the case, but the vast majority of studies in ALS have been conducted in Northern European and ancestor populations. Looking at the population in Cuba many years ago, they found that the risk for ALS was lowest in populations admixed within Cuba and the rates were higher in those of primarily Spanish ancestral origin. They looked at the data across the US where the populations are admixed and those that had been published in Latin America around that time, which also suggested that this was the care. However, there had not been any systematic epidemiologic study conducted within these populations. The LAENALS was set up to do this. The LAENALS is comprised of 5 teams of researchers undertaking population-based studies. This includes clinical evaluation, ALSFRS, an appropriate neuropsychological battery, family studies, exposure studies, regular follow-up for survival, and DNA collection. This was based on a proposal that was submitted for which funding was received for 2 years. The study commenced around 2017-2018.

The first aim of the original study was to determine the incidence and clinical phenotype of ALS in 3 genetically distinct Latin American populations. These populations were chosen advisedly. Cuba is currently admixed with a good health system and Dr. Hardiman had a lot of collaborations already within the Cuban Neurological Network. Uruguay was selected because they already had published a study of Uruguay's primarily Northern European ancestral origin. Chile was selected because it is mixed, but in a different way. It is mixed between Native, ancestral, and American populations—mostly Spanish. They also are different in latitudes. For Aim 1, the plan was to train investigators and sub-investigators, ascertain cases using existing

infrastructures supported by trained investigators, establish Latin American dataset, and report incidence and detailed clinical phenotype of ALS in 3 Latin American countries. These are all achieved or partially achieved. The EuroMOTOR database was adapted to allow data collection from Chile, Cuba, and Uruguay. The goal was to generate the first population-based comparative studies of incidence, prevalence, and clinical phenotypes of ALS in 3 different Latin American populations of diverse ancestral origin. They ran into some difficulties with unrest in 2 of the populations and COVID-19 delayed things as well. The clinical phenotyping is underway and has been partially achieved.

The objectives of Aim 2 pertaining to the exposome are to establish the quantitative exposome in population-based cohorts from South American and the Caribbean, and identify environmental risk in 3 Hispanic populations of different ancestral origin and compare them with risks in European populations using standardized methodologies. They knew when submitting this proposal that the study would be significantly under-powered to do a comprehensive exposome study, so the alternative was to set up questionnaire and establish how best to collect the control population. Training, standardization, translation, and validation of JEM-based questionnaires have been achieved. The database has been completed and data entry is underway. The rest of the deliverables for Aim 2 have proven more difficult to achieve and include quantitative lifestyle data collection, comparison of life-style risk factors for ALS within Latin America, comparison of life-style risk factors for ALS between Latin America and Europe, and development of a rich dataset for interrogation of gene-environment interaction and for extrapolation to the US population. These aims were delayed for a number of reasons. For instance, the administration in the US changed and the administration and relationship between Cuba and the US changed. Therefore, it became more difficult to execute these deliverables and identify the control populations. These are currently in process now that the implications of COVID-19 are waning to some extent. Admittedly, the 2-year time period for the study was excessively ambitious.

In terms of the results to date, the aims for Uruguay were worked to some extent as a project grant. The Uruguayans set themselves the task of developing the epidemiologic study to conduct a comparison study between research done in 2002 and the current dataset and to do a genetic profile of ALS in the Uruguayan population, which was not part of the original project proposed. They also were interested in validation of ECAS for the Uruguayan population. The Uruguayans identified a small cluster of ALS in a small town of Uruguay (Empalme Olmos) and sought to identify whether this was a real finding or an epidemiologic phenomenon or statistical aberration. From the epidemiologic point of view, the total population of Uruguay based on the 2011 Census was just over 3.286.314 inhabitants and was estimated to be 3.505.985 in 2018. This included slightly more women (52%) than men (48%). A total of 92 patients were identified during the study period, which was comprised of 52 men (55%), 41 women, and a sex ratio of 1.27. The vast majority were of European extraction. The mean incidence is 1.31 per 100,000 persons for total population. Incidence for the population over 18 years of age is 1.7 per 100,000. That is lower than in Northern European populations at between 2.6 and 3.0 per 100,000. The further South, the lower the rates as well.

A study was conducted of the comparative epidemiology of ALS in Uruguay between the 2 incident cohorts from 2002-2003 and 2017-2018.[32] The numbers were very similar, with only slight variation depending upon the province. For 2002-2003, incidence was 39 (42, 8%) and for 2017-2018, it was 41 (45, 5%). Thus, they were satisfied that they had good case ascertainment. Cohort 1 was comprised of incident patients diagnosed between January 1,

[32] Perna A, Vazquez C, Logroscino G, Hardiman O

2002 and December 31, 2003 and Cohort 2 included patients diagnosed between October 1, 2016 and September 30, 2018. This table depicts the ages of onset, diagnostic delay, percentage of women, use of riluzole, dementia, and bulbar forms in the 2 cohorts:

	Cohort 1 (N=88)	Cohort 2 (N=90)	Total N= 178	p
Age at diagnosis (mean , SD)	60,8 (12,6)	62,3 (13,8)	61,5 (13,2)	0,38
Age at Onset (mean, SD)	59,4 (12,2)	60,1 (13,6)	60,2 (12,9)	0,42
Diagnostic delay (median in months)	10	10	10	0,87
Women (%)	34,1	44,4	39,3	0,16
Riluzole (%)	11,4	30,7	21,0	0,01
Dementia (%)	2,2	1,1	1,6	0,7
Bulbar forms (%)	33	21	27,3	0,1

The median survival from symptom onset for Cohort 1 was 36.9 months for Cohort 2 was 35.7 months. Median survival time after diagnosis was 22 months for Cohort 1 and 18 months for Cohort 2. The Uruguayans have begun a genomic study, but there is not much to report thus far. While there were some variants in 2 cases, but the total number is very small with only about 40 patients.

Moving to the preliminary results from Cuba, the original plan was to ascertain the entire population of 11 million within Cuba. While travel between the different parts of Cuba is challenging, they have a good health system. Given the difficulties that arose during the period, the investigators refined the plans for full ascertainment in 3 pilot regions in Havana (2,117,343), Cienfuegos (405,823), and Guantanamo (515,898) for a total study population of 3,039,064 out of the total Cuban population of 11,167,325. These have slightly difference ancestral origins. The Cubans also were interested in the cognitive profile, so the investigators validated the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) in Spanish. Assessment of the genomics of the Cuban population also has been started. The Crude incidence rates of ALS in the population over 15 years of age are 1.37/100.000 in Havana (25 cases at 12.5 per year; 12 patients with surveys), 2.04/100.000 in Cienfuegos (7 cases at 3.5 per year; 3 patients with surveys), and 1.93/100.000 in Guantanamo (8 cases at 4 per year; 8 patients with surveys; 8 controls with surveys). Havana is mostly admixed, Guantanamo is more African, and Cienfuegos is about equal. While the rates are slightly different, these numbers are very small and the confidence interval is very wide.

In terms of cognition, a comparison of the Montreal Cognitive Assessment (MoCA) and ECAS tools demonstrates that a significant proportion of patients experience cognitive change. A paper for this study is currently underway. The comparison included 21 patients and 42 controls matched on age and sex, with the comparison shown in the following table:

Tools	ALS (21)		Controls		T - value	P - value	D [1]	E.S [2]
MOCA	M	DT	M	DT				
Name	2.76	0.7	2.92	1.09	1.37	0.174	0.3	0.15
Short-term memory	3.95	1.02	4.45	0.8	2.12	0.037	0.55	0.26
Attention	4.8	1.66	5.07	1.21	0.71	0.48	1.86	0.92
Language	2.28	0.95	2.47	0.7	0.89	0.374	0.23	0.11
Abstraction	1.5	0.76	1.9	0.37	2.83	0.006	0.67	0.32
Long-term memory	2.14	1.52	2.66	1.66	1.21	0.23	0.33	0.16
Orientation	5.76	0.53	5.88	0.5	0.86	0.39	0.23	0.12
Total	22.24	4.8	25.29	3.73	2.77	0.007	0.7	0.33
Non specific ALS	27.14	5.57	29.07	5.11	1.36	0.176	0.38	0.19
Specific ALS	61.19	18.68	76.02	11.53	3.88	0.000	0.96	0.43
ECAS total	88.33	22.13	105.09	14.6	3.59	0.000	0.89	0.4

For the genomics study, the investigators have access to a larger DNA databank, the ALS Clinical Register in the Institute of Neurology & Neurosurgery. The databank has 222 patients over 15 years spanning from January 1, 2006 – December 31, 2020. Among these patients, 77 (34.6%) have Familial ALS, 28 (36.3%) have a positive family history, 17/77 (22%) have a Mendelian pattern, and 145 (65.31%) have no familial history. Of interest with the inheritance pattern is that 9 (12%) are autosomal dominant, 8 (10%) are autosomal recessive, and 60 (78%) are multifactorial. Mutations were found in a sample of Cuban patients with ALS diagnosis. In terms of skin color classification, rates are driven primarily by those of European ancestry. A high number of ATXN2 patients were identified, which the investigators will follow-up on with further investigation. There may be a significant enrichment of ATXN2 expansion within the Cuban population. Also of interest is that the number of patients with C9orf7 are much smaller at about 3% in total. There were no SOD1 or TDP-43. Age of onset was as expected at about 51 years for fALS amd 56 years for ALS.

In terms of pending tasks and future directions, the Cubans plan to expand further and the infrastructure is in place. The members of the National Group of Neurology (250 members) are now well-connected to a national network. Provincial groups of neurology are ready to cooperate in Holguín, Las Tunas, and Pinar del Rio in addition to Cienfuegos and Guantámano. An Android App was set up for mobile devices based on the Irish ALS registry and the LAENALS survey. An online version will be also available. There is a national network of telemedicine based at the INN to exchange information with local groups reporting patients with ALS, so the investigators are confident that they will be able to expand and enhance the numbers over the next months to years.

The Chilean group had undertaken a series of mortality studies in 2014 looking at 1994–2010 in Valenzuela, Lillo, and Zitko that that suggested that the mortality rates was 1.13/100,000 among an estimated population of 17 million. This was significantly lower than those of Northern European extraction. It is known that mortality rates approximate incidence rates. The initial aims in Chile were to estimate the incidence of ALS at the Metropolitan Region in Chile and to establish survival rates in ALS cases. This is a cohort study of incident newly diagnosed cases of ELA carried out in the Metropolitan Region of Chile between October 2016 and October 2019. The cases included were defined as "definite" or "probable" according to El Escorial Criteria.[33]

[33] Brooks et al. 2000

For case collection, patients with ALS in the Metropolitan Region are affiliated to Corporación ELA, which is the national association for patients with ALS and their caregivers. Patients were invited to participate in this study through a public announcement made by the same association. A total of 219 patients from the Metropolitan Region accepted to be contacted by the research team and to share essential demographic information. Basic demographic information from the Corporación ELA registry included date of birth, sex, dates of onset and diagnosis, and address. There was a capture-recapture methodology put in place here, which suggested that there was relatively good ascertainment. A paper is in preparation for this. Neurologists working in private and public hospitals in the Metropolitan Region were invited to join the study by email from the Sociedad de Neurología, Psiquiatría and Neurocirugía de Chile (SONEPSYN).

The median time of follow up was 2.1 years, there were 455.8 person-years of observation, and 148 deaths. The overall incidence of ELA diagnosis was 0.97 cases per 100,000 inhabitants. The overall ration male:female is 1.23, and the incidence tended to increase in the extent of older ages till 70-79 years. The median time to death from diagnosis was 2.3 years [95%IC 1.9 – 2.5] (men: 2.3 [95%CI 1.9 – 2.7]; women 2.2 [95%CI 1.5-2.6]). For survival from the onset of symptoms, the median time to death was 3.1 years [95%CI 2.8-3.5] (men: 3.3 [2.8 – 4.2]; women 3.0 [2.6-3.5]). Men showed higher risk for dying than women (HR 1.73 [95%CI 1.23-2.43]). Older ages showed higher risk for dying. Higher time for diagnosis was associated inversely to the risk of dying (HR 0.97 [95%CI 0.95-0.98]).

The Chileans are working on looking at the cognitive profile and are working with Uruguay for comparison purposes. These data are very small at the moment and are not really valid enough to report in any great detail other than to say that it is underway. They have looked at only 12 controls at the moment. Within Chile, the investigators ran into 2 problems. The first was social crisis beginning in October 2019 and continuing to date and the second was the COVID-19 pandemic beginning in March 2020 and continuing to date. The pandemic and made it impossible to evaluate patients clinically. There was a lockdown, and for the reports on incidence, the investigators do not have a very good phenotype at the moment. This is a function of the pandemic, unfortunately, but they do have the wherewithal and methodology to do this going forward.

In terms of dissemination to date, the investigators have published the rationale and methodology behind the LAEMALS group. That was published in the *ALS Journal* in 2022.[34] They also conducted a comparison study of ALS within 3 clinic-based populations from Cuba, Uruguay, and Ireland to determine the impact of known ALS-associated genetic variants on phenotypic manifestations within the Cuban population.[35] The Irish population is very similar to European populations and there was a slightly younger age of onset in the Uruguayan population, which is seen across the board within the South American populations. The age of onset is typically younger. In terms of genetic features, there is very little C90rf72 in the South American population.

[34] Hardiman, Orla & Heverin, Mark & Rooney, James & Lillo, Patricia & Godoy, Gladys & Saez M, David & Valenzuela Torres, Daniel Alejandro & Hughes, Ricardo & Perna, Abayuba & Ketzoian, Carlos & Vazquez, Cristina & Gutierrez, Joel & Arias Morales, Asdrúbal & Lara-Fernández, Gloria & Zaldívar Vaillant, Tatiana & Horton, D. Kevin & Mehta Md, Paul & Logroscino, Giancarlo. (2022). The Latin American Epidemiology Network for ALS (Laenals). Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 23. 1-6. 10.1080/21678421.2022.2028168.

[35] Ryan M et al. Comparison of the clinical and genetic features of amyotrophic lateral sclerosis across Cuban, Uruguayan and Irish clinic-based populations. J Neurol Neurosurg Psychiatry. 2019;90(6):659-665. doi:10.1136/jnnp-2018-31983

The funding is complete on this part of the study, but the network is set up and the database is up and running in Dublin. There is considerable interest in Limoges, Barcelona, Columbia, Ecuador, Argentina, Brazil, and Costa Rica. They invited Dr. Mónica Povedano from Barcelona, who is a member of the Treatment and Research Initiative to Cure ALS (TRICALS) and has done a lot of work looking at populations outside of Europe and has some work underway in South America and Africa. Dr. Povedano has agreed to join to lead the group. They also have linked with a Limoges group in France that has very good linkages with Uruguay and also have set up a new epidemiologic study of ALS in Equador. Equador has been brought into the LAENALS group as well. They have a young neurologist from Honduras who has been working in Ecuador who is going to participate. Dr. Povedano has introduced the investigators to a group in Bogota that has an impressive dataset comprised of clinical and genomic data. The Columbian group in Bogota is very keen to participate in the LAENALS network as well. The LAENALS network also now has links with Argentina, Brazil, and Costa Rica. Each of these centers has been invited to join the LAENALS consortium as well.

The LAENALS consortium is very grateful to the CDC for the support that has been involved. This has been a difficult project because of the political situation in the countries with whom they have been collaborating and the unfortunate impact of COVID-19. They had to move most of their consortium meetings to an online format, which was challenging with the different time zones. It has been very difficult to engage with colleagues and services, particularly in Cuba, because of the difficulties within that country. Nevertheless, Dr. Hardiman believes this has been a successful initiative. The network and infrastructure are in place, everything is ready to go, all of the questionnaires are appropriately translated, and there is a good scientific rationale from an epidemiologic point of view. This is an effort that is anticipated to grow over the next few years.

Discussion Summary

Dr. Boyce noted that in separate discussions, they had heard a lot about the importance of branching out into underserved regions, which Dr. Hardiman and colleagues are doing. She stressed that this is valuable work in terms of documenting some of the challenges as others try to perform this work as well. There probably is a lot that people do not think about when embarking on a project like this.

Dr. Hardiman said she wanted to comment on a discussion from the previous day regarding the value of registries to advocate in favor of registries, particularly longstanding registries. She believes there is huge value in understanding the wide range of phenotypes and the learnings that can be obtained from long-term evaluation of registries. Their registry has been running since 1995, so they effectively have 3 generations of ALS in their registry. They can detect variabilities across phenotypes, sub-phenotypes, or extended phenotypic history in addition to things like clusters for which very large numbers and long-term surveillance are needed to be sure that clustering studies are valid. There is a huge amount of added value, particularly in population-based registries. These are often undervalued. They need to be very long-term, need to have very good ascertainment, and capture-recapture methodology. The first couple of years in registries are replete with biases. They published a paper a few years ago in *Neurology* looking at the hidden pitfalls of registry analyses.

Ms. Collette asked whether it is possible that a good job has not been done in terms of finding the ALS-related gene jeans themselves that might be present in populations with darker skin than in European populations.

Dr. Hardiman said she thinks that ALS has a combination of single genes, an area in which Dr. Siddique is the expert. There is definitely a polygenic component to ALS as well, and GWAS has suggested that is the case. Heritability is probably polygenic, but the polygenic risk is probably going to be different depending on the ancestral origin from which the person emerges. One might speculate that in the more admixed populations that there may be a washing out, so to speak, of at-risk genes or an enrichment of protective genes within these populations. She thinks it is a little arrogant to believe that all populations are the same. It is known that genetic backgrounds in animals change the risk and the profile as well, so why would that not be the case with humans?

Ms. Lorenz asked whether Dr. Hardiman and colleagues are interested in including people in her data who have immigrated from Cuba, Chile, or Uruguay; how their findings compare to the Latino population in Spain, Portugal, and Mexico; and as a Latina living in the US, she wondered if/how a similar study of the Latino population could be supported in the US.

Dr. Hardiman noted that as somebody who comes from a Spanish-speaking population, Ms. Lorenz would be aware that the "Latino" really just means that someone comes from a Spanish-speaking population. The genetic ancestral origin of the so-called "Latino population" is quite different depending on which part of Europe the ancestors migrated and then the interactions within the populations in the New World. Quite a lot of work has been done on this already about the different populations and the different trading routes that happened. This is why they want to perform a comparative analysis between Cuba, which is African and Spanish admix, and Chile. A large proportion of the Cuban population is from the Canary Islands. Fidel Castro's parents were from the Canary Islands. A comparative analysis between Spain, Portugal, and Mexico, and these populations is definitely worth doing. She predicts that they will be different, because of the ancestral origins assigned to those different populations. Regarding the question about immigrants from these areas, particularly from Cuba and Venezuela, who have moved to the US would be interesting. Dr. Benatar may have much greater knowledge of this, but Dr. Hardiman's understanding is that the Cuban population in Florida would be primarily of Spanish ancestry and the admixture would be a little bit less, although the Cuban genomic enhancement suggests that even those who ancestrally define themselves as Spanish have some African. This would be similar to those who describe themselves as being primarily African having some European markers. It would be very interesting to look at the Cuban population in Florida. She visited Venezuela a number of years ago and it is a population that definitely would be worthy of study as well, but the political situation there would make that very challenging. It would be interesting and valuable to study a cohort of individuals within the US and regions of the Southern US who are from Venezuela. The third question about how to get a study of Latinos in the US underway is a question for Dr. Horton. The ALS Registry within the US is working very hard to include all individuals with ALS, so that is probably the best way to do that type of study.

Dr. Boyce noted that the last bullet on the list of action items for the Patient Engagement Session pertains to increasing diversity in the Registry. She offered to chat with Ms. Lorenz offline about how she could be a part of that.

Dr. Mehta added that ATSDR is always looking for ways to increase enrollment in the National ALS Registry, especially in terms of making the Registry more diverse. This is always a challenge because sometimes these populations do not want to join government-sponsored

studies. Focusing on targeted areas in the US such as Florida, Texas, and California that are much more diverse can certainly increase the portion of minority cases in the Registry.

Dr. Siddique asked whether the C9 patient had a European haplotype. He also inquired as to whether they are planning to do admixture analyses because with all of the ancient DNA studies done in in Europe, the ancestry is quite clear about the Anatolian farmers, the Western Steppe Yamnaya people, the migration to the New World with interactions with the Native American people, and then the African input. That could clarify the issue of skin color.

Dr. Hardiman indicated that the C9 patient self-identified as being of European origin. From a scientific point of view, they are very keen on doing admixture analyses. The difficulties are typically political. Looking particularly at the human population, it is a study that is waiting to be done. Within the Institute of Genetics in Cuba, there is a strong population genetics group. They already have looked at the admixture population and it is really interesting. The mitochondrial genotype is ancestral and the autosomal and markers are African and European. It is a study that should be done at scale by looking at the ancestral markers in the ALS population versus the non-US population and demonstrate that there are higher rates of European genomic signature within the ALS population. It is a study that she would love to do, but they need to scale up. They would need to collaborate with the Institution of Genetics in Cuba and provide some resources to do that within Cuba. Undertaking the analysis in this part of the world would be difficult. Other groups are very keen on doing this as well in Chile. Ecuador would be a really good population in which to this, but the numbers are very small. This would have to be a long-term study because it would need to be done at a scale that would result in a large number so that there is sufficient power. She and her colleagues have conducted a larger study with the Cuban DNA samples that they have, but they do not have sufficient controls to be able to publish that at the moment. In terms of clarifying skin color, someone living in Guantanamo who is somewhat less dark is counted as white and someone living in Havana would be classified as Black. They did look at family history in the studies she has done with her colleagues in Havana. People who self-report as white may have grandparents who are African. Genetic studies have demonstrated as well that even within the population where skin color determined someone's ancestral origin was not actually correct because they have mixed ancestral origin. If the hypothesis they have is correct (ALS is primarily a Northern European male disease) it would not be surprising that the rates are lower across the board in the Cuban population.

Dr. Siddique noted that he found it most interesting that in the European population, they found that the X chromosome goes from the original Anatolian farmers and the rest of the autosomes are more predominant with the Y chromosome. He wondered if ALS would be seen among those who have an African or Native American mitochondrial imprint.

Dr. Hardiman said she thought the question regarded whether the phenotypes of ALS within different ancestral populations are different. She suspects that they probably are. It is known that there is a high rate of SOD1 in Malaysia. A5B is a Native American mutation that may have become ALS as people migrated across from the North. She suspects that they will see that there are different sub-phenotypes in different populations. If looking at ALS as a disease that is recognized, one thing they see is that the age of onset in the population that is mixed is younger in the Cuban population. That would suggest that the risk is there and that the threshold is reached at an earlier age. There are some reports in parts of India of very regional phenotypes. If they look closely enough, they are likely to see disease that may be attenuated by the genomic risk and, in deference to the epidemiologists, environmental interactions as well. To her mind, the genomic factor is the more interesting and more important one.

Dr. Siddique recalled that there was a report from Argentina that settled a paternity case. There has to be a Northern European type of SOD population, but the numbers are very small.

In terms of the mixed group population being younger, Dr. Ascherio asked whether that took into account that the age of the entire population could be younger and whether the life expectancy would be the only relevant variable. There may just be more younger people in an age group.

Dr. Hardiman indicated that they published a paper a number of years again stating that the age of onset is proportionate to the life expectancy of that population. They have a paper in preparation that looks at this genetically as well, and it does seem to be the case. She presumes that it has to do with the factors that lead to aging. Poorer populations tend to be more prominent. The life expectancy in the Cuban and Uruguayan populations they have looked at, the life expectancy is similar to Northern European populations. It does seem to be an earlier age of onset notwithstanding the overall life expectancy of the population.

Identifying and Evaluating Potential Risk Factors for ALS in Sweden

Fang Fang, PhD
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Dr. Fang Fang described some of the ALS research work being done in Sweden by the Institute for Environmental Medicine at the Karolinska Institutet (KI). KI is located in Stockholm and was founded in 1810. It is Sweden's single largest center of medical academic research, offers the widest range of medical courses and programs in Sweden, and received the Nobel Prize in Physiology or Medicine. In terms of why the research is being conducted in Sweden, she explained that Sweden is one of the Nordic countries together with Iceland, Norway, Finland, and Denmark. Sweden provides a unique opportunity for research and care of ALS as a good complement to all of the other efforts that are ongoing in North America, Latin America, and Asia. The population size of Sweden is currently about 10 million, with 90% of the population being Caucasian. The capital city of Sweden, Stockholm, currently has a population size of about 2.2 million. The life expectancy is quite high in Sweden at about 81 years of age for men and 85 years of age for woman. Given this, neurodegenerative diseases, including ALS and MND, are increasingly important as healthcare and public health problems. Like many other European countries, Sweden has a universal health care system.
Sweden's healthcare system is nationally regulated and locally administrated and is funded primarily by general taxation.

Everyone in Sweden has a personal identification number (PIN) that is used broadly in all aspects of life by authorities, healthcare sectors, schools, universities, banks, insurance companies, et cetera. This specific number makes research relatively easy and less expensive. There are some unique research possibilities in Sweden, including 4 main components. The first is the national public authority registries that include a lot of information on population and socioeconomics, health, and population-based surveys that are conducted by the government regularly. The second opportunity is disease quality registries. Physicians in the country have initiated a lot of disease-specific quality registries over the last decades. Currently, there are more than 100 of these. These disease quality registries are partly or completely funded by the government and have a primary purpose to develop and ensure quality of care for patients who have different diseases. The third unique research opportunity in Sweden is research-generated data. These are maintained databases in research institutions and universities. Biobanks

represent the fourth research opportunity. These biobanks often have decades of history and from research-oriented activities and healthcare. In addition to various types of biospecimens, these biobanks contain data related to test results, clinical diagnoses, and self-report reporting through questionnaires.

Regarding the burden of ALS in Sweden, there are not a lot of research projects on this topic. Dr. Fang Fang described a paper that she published many years ago that looked at the burden of ALS from 1991 to 2005 using the Swedish patient registry[36] to calculate age and sex standardized incidence rates. On average, there was a 2% increase in the incidence rate during this 50-year period. Males had a slightly higher incidence rate of ALS compared to females, and there was a much later peak age for ALS compared to Latin America. The incidence rate of ALS peaked around 70 to 79 years of age and then declined thereafter. In 2015, she and her colleagues performed a retrospective medical records review in which ALS patients' medical records in Stockholm were reviewed for 2013 to 2014.[37] This study included definite, probable, and suspected ALS. In 2013, there were 59 new cases for an incidence rate of 2.75 per 100,000 persons. In 2014, there were 83 new cases for an incidence rate of 3.81 per 100,000. By the end of 2013, there was a total of 143 patients for a prevalence of 6.61 per 100,000. By the end of 2014, there was a total of 174 patients for a prevalence of 7.91 per 100,000. Even with a population of 2.2 million, the number of new cases can still change quite substantially between years, which is why Dr. Fang Fang agrees with Dr. Hardiman that a relatively long perspective is important when dealing with a relatively rare disease.

KI and the Karolinska University Hospital strive for multidisciplinary research and care for ALS. In terms of research, they have researchers dedicated to both pre-clinical and clinical research. Pre-clinical research focuses on cell lines, animal models, and population studies. Clinical research focuses on treatment patterns, patient outcomes, health economics, et cetera. Currently, there are more than 10 clinical trials actively recruiting patients in Stockholm in Karolinska University Hospital. At the major university hospitals and a few large regional hospitals in Sweden, there are specific ALS Care Teams. These are expert teams comprised of occupational therapists, curators, physiotherapists, psychologists, speech therapists, doctors, and nurses. There also is the ALS Center Karolinska.

Moving on to the research program, Dr. Fang Fang and colleagues were very fortunate to be funded by the CDC and the US National ALS Registry in 2020 to identify and evaluate potential risk factors for ALS. She lamented that while she did not yet have many findings to share at this point, she hoped to return in 2023 to disclose more exciting results. This study has 3 principal aims, which are to: 1) identify risk factors that are relevant in Sweden that hopefully can be generalized to elsewhere, including multiple risk factors individually or in combination; 2) evaluate how these risk factors function in terms of determining the risk for ALS, including effect modifications and genetics, chemicals, and gut microbiome; and 3) incorporate the first 2 aims and stratify patients using a novel patient stratification method based on outcomes and history of exposure. The long-term goal is to improve the understanding of disease etiology for ALS and to gain knowledge on potential preventive and therapeutic targets for this disease.

[36] Fang, Fang & Valdimarsdóttir, Unnur & Bellocco, Rino & Ronnevi, Lars-Olof & Sparén, Pär & Fall, Katja & Ye, Weimin. (2009). Amyotrophic Lateral Sclerosis in Sweden, 1991-2005. Archives of neurology. 66. 515-9. 10.1001/archneurol.2009.13.
[37] Longinetti E et al. ALSFTD. 2018

For Aim 1, the investigators will study the following risk factors:

- ❑ Military service, TBI, mental disorders, and infections
- ❑ Risk and prognosis of ALS
- ❑ Swedish population and health registers
- ❑ Swedish MND Quality Registry
- ❑ ALS patients vs. sibling controls, spouse controls, and population controls

The Swedish MND Quality Registry is a part of the web-based platform of the Swedish Multiple Sclerosis Quality Registry, which was launched in 2004. Different neurological disorders have been registered within the Swedish MS Quality Registry since the beginning in 2013. The Swedish MS Quality Registry was renamed the Swedish Neuro Registry 2013. In 2015, a retrospective study was performed of all prevalent MND patients during 2013-2014 in the Stockholm area. In mid-2015, the MND Quality Registry and Patient Own Reporting Portal was added to the Swedish Neuro Registry. A lot of information is collected in the MND Quality Registry and a minimum dataset is created at each clinical visit or contact every 12 weeks as shown in Table 1 and Table 2, respectively:

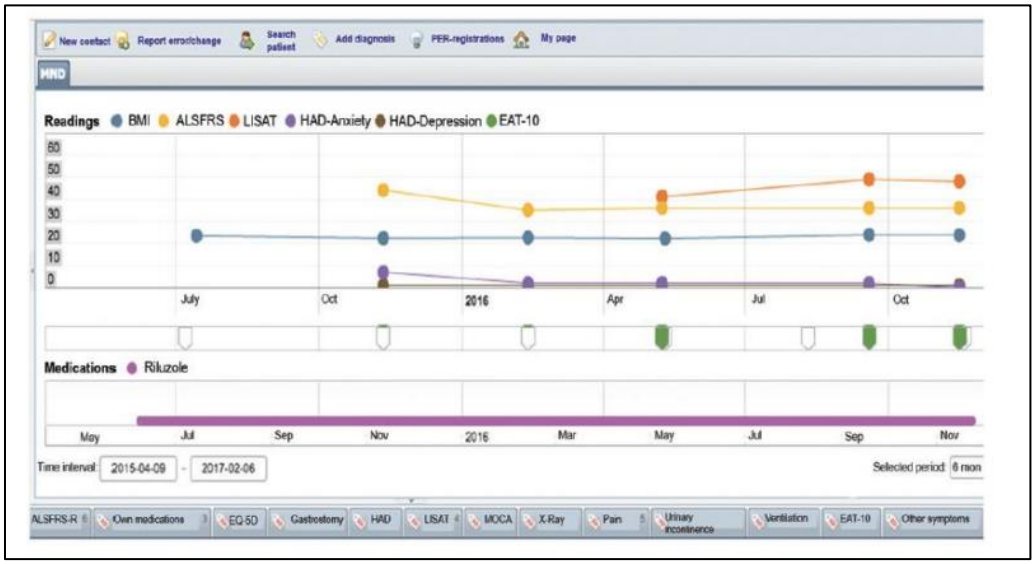
Table 1. Variables in the MND Quality Registry.

Groups	Type of variables
Diagnostics and follow-up	Neurophysiology, X-ray, laboratory, phenotype, clinical examinations
Neurophysiology	Electroneurography, EMG, tcMEP, biopsy
X-ray	CT, MRI, FDG-PET
Laboratory	Serum, CSF and DNA
Phenotype	Site of onset, place of onset, UMN, LMN, FTD, pseudobulbar
Spreading pattern	Site of onset, spreading pattern of paresis
ALSFRS-R	0-48 points
Life quality, depression, dementia	Questionnaires, clinical signs
HAD	Anxiety and depression
MoCA	0–30 points
EQ5D	0–100%
LISAT-11	0–66 points
BMI	Weight and height
Hypoventilation	Clinical signs, spirometry, laboratory
Clinical symptoms	Headache, dyspnea, nighttime sleep, coughing capacity
Laboratory	Bicarbonate
Spirometry	FVC%
Non-invasive ventilation	
Invasive ventilation	
Bulbar dysfunction	Clinical signs, questionnaires, BMI, laboratory
Clinical symptoms	Chewing capacity
Eating assessment tool-10	0-40 points
Laboratory	Albumin, Cobalamin, Homocysteine
Gastrostomy	PEG, RIG

Table 2. Minimal dataset created at each clinic visit or contact, every 12 weeks.

Mandatory variables	Registered as
Motor dysfunction and progression pattern	ALSFRS-R (scale)
Bulbar dysfunction symptoms	Clinical signs, 4 questions
BMI	Weight and height
Anxiety and depression	HAD (scale)
Cognitive decline, emotional incontinence	Clinical signs, MoCA (scale)
Quality of Life	EQ-5D, LISAT-11
Hypoventilation symptoms	Clinical signs, 4 questions, lab, FVC%
Pain	VAS scale 0-10
New or ended treatments	Medical treatments, PEG, NIV/IV

In addition, a patient reporting portal was created. Before each clinic visit, patients are instructed to complete information in this portal regarding various parameters (e.g., BMI, ALSFRS, HAD-Anxiety, HAD-Depression, EAT-10, medication, et cetera). This helps to document a patient's status and makes it easy for the physician and patient to see how the patient's functional status has changed over time as illustrated in this screenshot from the portal:



For Aim 1, 1600 newly diagnosed ALS patients will be identified according to the MND Registry between 2016-2021. These patients will then be linked to 1600 sibling controls through the Multi-Generation Register, which includes information on familiar links for everyone who was born in Sweden from 1932 onward. Then 1600 spousal controls will be identified by Statistics Sweden. From the Total Population Register, 5 controls will be identified for the 1600 ALS patients who are matched on age and sex for a total of approximately 8000 population controls. Once the approximately 12,000 patients, siblings, spouses, and controls are identified, they will be linked to the population and health registries mentioned to identify information on potential risk factors, as well as other co-variables that are of relevance in the studies on risk factors. For instance, in terms of military service, information will be identified from as early as the 1960s from household censuses every 5 years to 1990 and the annually updated data from 1991 onward. In terms of TBI, mental disorders, and infections, information will be gathered from the Swedish Patient Registry that has been collecting information on hospital discharge from 1964 onward. To understand how medication use can interact with potential risk factors in modulating the risk and prognosis of ALS, information will come from the Prescribed Drug Register. The Prescribed Drug Register has been collecting nationwide information on all prescribed medications in Sweden since July 2005. In addition to these main sources of information, sociodemographic factors will be obtained from the population surveys concerning SES, education, household income, and so on.

A series of analyses will then be performed as part of Aim 1 to understand risk factors and whether the risk factors are indeed relevant for the risk and prognosis of ALS. The analyses will compare ALS patients vs. sibling/spouse/population controls; assess timing, severity, and subtypes of the risk factors; examine individual risk factors and risk factor combinations; and assess disease characteristics, sex and age, clinical and self-reported outcomes, and medications (anti-inflammatory, antidiabetics, antibiotics, and statins). Comparing ALS patients with the 3 control groups separately will help to understand whether the results are similar or

different across these comparisons. Because they will know exactly when each of the risk factors occurred, it will be possible to study the timing, severity and subtypes of risk factors. Studying the risk factors individually and in combination, such as having a TBI and occupational history of military service, should help to better understand whether this introduced a higher risk of ALS rather than having them each individually. Because the information available is so rich, it will be possible to study whether patients have different disease characteristics and whether there are differences by age and sex. Patients are still followed up every 3 months, so it also will be possible to examine whether the risk factors are relevant to understanding the clinical and self-reported outcomes among ALS patients. The medications selected for assessment include those that have been proposed or have been shown to be related to the risk of ALS.

Aim 2 seeks to assess gene-by-environment and environment-by-environment interactions. The goal for this aim is to characterize interactions between TBI, mental disorders, and infections, and genetic susceptibility to ALS, chemical exposures, and gut microbiome. The ALSrisc Project will be used for this aim, which is a population-based case-control study of ALS in Stockholm. ALSrisc has been recruiting newly diagnosed ALS patients from the entire Stockholm area from 2016 onwards, as well as the sibling and spouse controls of these patients. A total of 360 patients and 220 controls are anticipated to be included in this aim. Clinical data and CSF have been collected from the patients. Blood, fecal, hair, and nail samples and risk factors have been collected from the patients and controls. Based on these biospecimens, biomarker data have been collected, including clinical chemistry, genotypes, C90rf72, proximity extension assays, fluorescence-activated cell sorting, and shotgun metagenomics. Hair samples are sent to the Icahn School of Medicine at Mount Sinai (ISMMS) for measurement of approximately 10,000 chemicals using a specific technology developed with the by the team there to quantify the dynamic exposure map of chemicals using hair samples. To summarize, for the 360 ALS patients and 220 controls, there will be information on disease phenotypes, including clinical characteristics and follow-up data. For patients and controls, there will be information on risk factors (self-reported and register-based), genotypes and C9orf72, gut microbiome data, and approximately 10,000 chemicals. The idea behind the statistical analyses is to create a genetic risk score, chemical risk score as a proxy for the chemical exposure effect and modification, and a gut microbiome health index for each case and control. Analyses will examine interactions with TBI, mental disorders, and infections; risk and prognosis of ALS; and will include a validation study using some samples available from the ATSDR National ALS Registry.

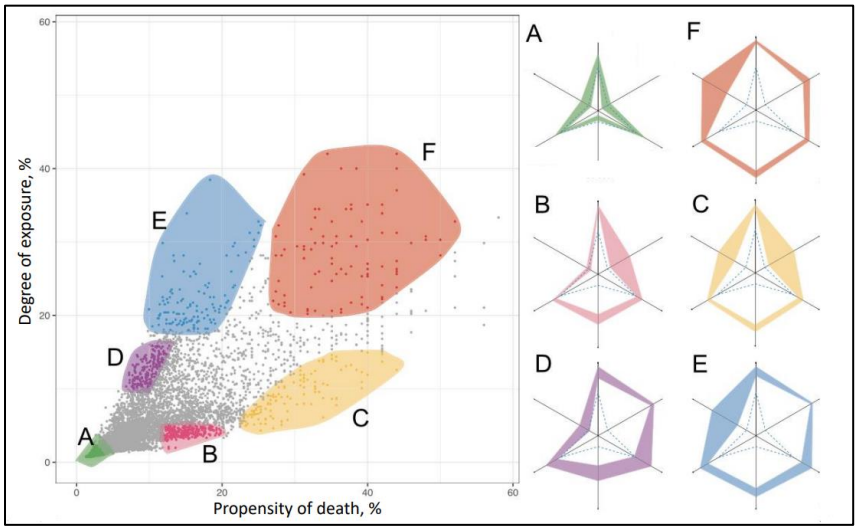
Using the findings from Aims 1 and 2, Aim 3 (clinical application) will focus on developing a stratification method of ALS patients based on the exposome, genetic susceptibility, and clinical measures. This aim will include the 360 ALS patients from the ALSrisk cohort with complete follow-up data. A variety of information has been collected across these patients. These variables will be classified into the 3 categories:

1. Exposure Variables: TBI, neuroinflammation, and infections; chemical exposome (hair); and gut microbiome (feces)

2. Outcome Variables: Survival (data/cause of death); self-reported quality of life (QoL) from ECAS, HAD, EQ5D, and LISAT-11

3. Indicator Variables: Clinical characteristics and chemistry; genotypes; C9orf72 (number of expansions; neurofilament light (CSF); neuroimaging (bran MRI); and immune responses (T-cells, et cetera)

Then ideally, these patients can be mapped with the Y axis being the degree of exposure and the X axis being the outcome variables, with the hope of identifying specific clusters or patients according to degree of exposure to potentially harmful risk factors and propensity of death as illustrated by this graphic:



While these 3 aims may not make the best or most unique contributions, what Sweden has to offer is at least slightly unique in terms of helping to improve the understanding of ALS and in finding potential preventive and treatment strategies for patients and people at high risk.

Discussion Summary

Regarding an inquiry about what a curator is or does in the multidisciplinary center, Dr. Fang Fang explained that this is a support-providing function.

Dr. Siddique observed that this seems to be a very ambitious study that probably will generate a lot of data. He asked whether Dr. Fang Fang could describe the change in population over time in terms of whether there has been a shift in the mean age of onset of the disease. He also noted that based on Figure 2, incidence was modestly rising over time and wondered whether that reflected the change in mortality statistics or the prediction for survival in terms of the average survivability of a person in Sweden.

Dr. Fang Fang said she should have checked the life expectancy in the 1990s, but this is precisely the kind of problem they want to address in such analyses. That is why they standardized all of the calculations by age and sex of the total population in Sweden in 1991. Through that method, they made sure that the change in the general population in Sweden does not contribute to the trends that they observe or claim. She agreed that although the life expectancy had always been slightly high in Sweden, that has been increasing over the last 2 or 3 decades.

Dr. Siddique pointed out that this matches the Japanese life expectancy. In many places, the peak incidence of ALS is among persons 60 to 69 years of age. He wondered whether that jived with the Japanese statistics in terms of the shift of the mean age of onset in the majority of patients seemed to be in that 1 or 2 decades she described. In other words, was the 1 shown in the ≥85 age range just because there were too few people there.

Dr. Fang Fang agreed that it seems like Japan has one of the highest life expectancies in the world. In terms of the ≥85 age range, no one has a clearcut answer. This is not only seen in Sweden. There was a similar age distribution in Germany. If they work exclusively hard on the oldest old, perhaps they will identify a lot more cases of ALS there as well.

Dr. Siddique asked whether she thinks it is an ascertainment issue. In Alzheimer's and Parkinson's disease, there is a continuous increase over time with age as one would expect. This information suggested that they had culled out the patients that were vulnerable in some way to ALS and are left with those who are more resistant. He stressed that Dr. Fang Fang raised many interesting questions. While the D90A-SOD1 mutation by Beckman and Beckman is identified in about 2% of the Swedish population, he did not hear her mention whether that is a risk at all in either the sporadic or non-familial ALS or in the familial ALS, because that seems to be quite constant. The question regarded whether, if 2% of the general population has that marker, whether it shows up in 2% of the ALS cases.

Dr. Fang Fang said she did not think so. They have seen about 10% C9, but very few have the other mutations. Most of the genetic research on ALS is done from the Northern part of Sweden where the genetic architecture of ALS patients is slightly different from the rest of Sweden. Their material is mainly based on Stockholm, so they are hoping to perform more sera analyses using their patient materials in Stockholm. Then they would perhaps have a better picture, C9 is most prevalent the other ALS risk genes are very uncommon.

Dr. Hardiman indicated that they also see the shift Dr. Siddique noted in their registry as the population ages. She does not think it is an ascertainment or bias problem. They see all patients with ALS. It is more likely that people with multiple morbidities are misdiagnosed with ALS rather than ALS not being picked up. She thinks it is a real change that is telling them something important about ALS that is different.

Dr. Fang Fang added that about 10 years ago, the care of ALS was not up to the standard that it is now, which is much better. The data she presented was from more than 15 or 17 years ago. They are trying to perform follow-ups from 2006 onward to determine whether this trend holds.

Dr. Oskarsson from Mayo Clinic in Florida noted that he suspects that case ascertainment is many-fold higher than in the National ALS Registry where capture-recapture methodology showed a significant under-count. Ethnic and genetic diversity have changed in Sweden, which also may impact the population being captured in this study. Confirming that there is not an undercount would be nice to know in terms of the high age group question.

Dr. Fang Fang indicated that because they have the registries, they are being somewhat less diligent than other investigators. They have not used a capture-recapture approach specifically. They want to make sure that identified patients are real ALS patients, so they have performed validation studies in which they have seen that the vast majority of the ALS patients actually have ALS. They do not know how many might be missed. In terms of population structures, about 10% of the Swedish population is comprised of migrants. The rest are almost all Caucasian populations. There has been some migration influx due to wars and so on, but otherwise Sweden's population is not as mixed as the US and UL populations. The numbers she presented from 1991 to 2005 came from a national study. This is not only from the neurologist who are seeing patients. These data also are from patients seen in every clinic in Sweden. There is a possibility that they miss some patients, but she agreed with Dr. Hardiman

that perhaps there is a declining rate surpassing a certain age. She hopes they will see the same in the next validation study that they are currently conducting.

A Novel Innate Immunity Risk Factor for ALS

Teepu Siddique, MD, PhD
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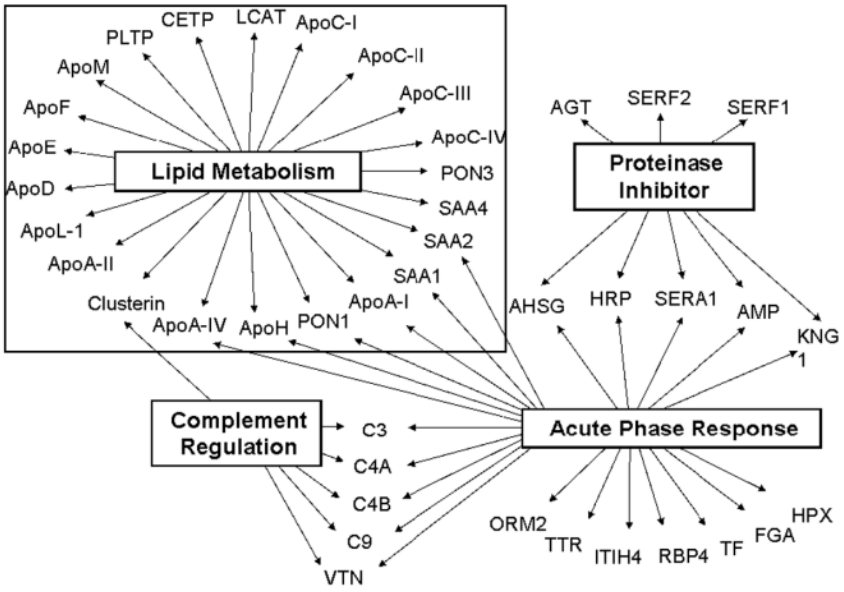
Dr. Siddique noted that when he and his colleagues worked with the National Institute of Environmental Health Sciences (NIEHS) many years ago, the idea arose that perhaps there are other gene variants in humans that are associated with xenobiotic-responsive genes that may have variants that are specific for certain types of input. The input of new compounds that have been released in the atmosphere, water, or elsewhere has been about 80,000 or so since the Second World War in the US. While it is not possible to go chasing every compound and its mechanism, one can look at the variants in the genes that are responsive because those are limited. They conducted that limited study many years ago and came across a number of variants that seem to be of interest. They published the first one, which was the structure around Chromosome 7 that had more of a signal coming from intronic locus. There were a couple of other studies and then a meta-analysis, but it never included their data, so he did not know what the value was of the meta-analysis. This still is an intriguing possibility because peroxidase is one of the most powerful antioxidants in the body.

This presentation focused on HDL particles, plasma concentrations of APOL1 that may vary between sporadic ALS patients and controls, the APOL1 gene variants and risk of ALS, Chromosome 22 eQTLs affecting APOL1 expression, and selected trans eQTLS. HDL is more than a cholesterol transporter. The core proteins involved in lipid metabolism and protection from oxidation are enclosed in the large box below. APOL1 is a subset of particles:[38]



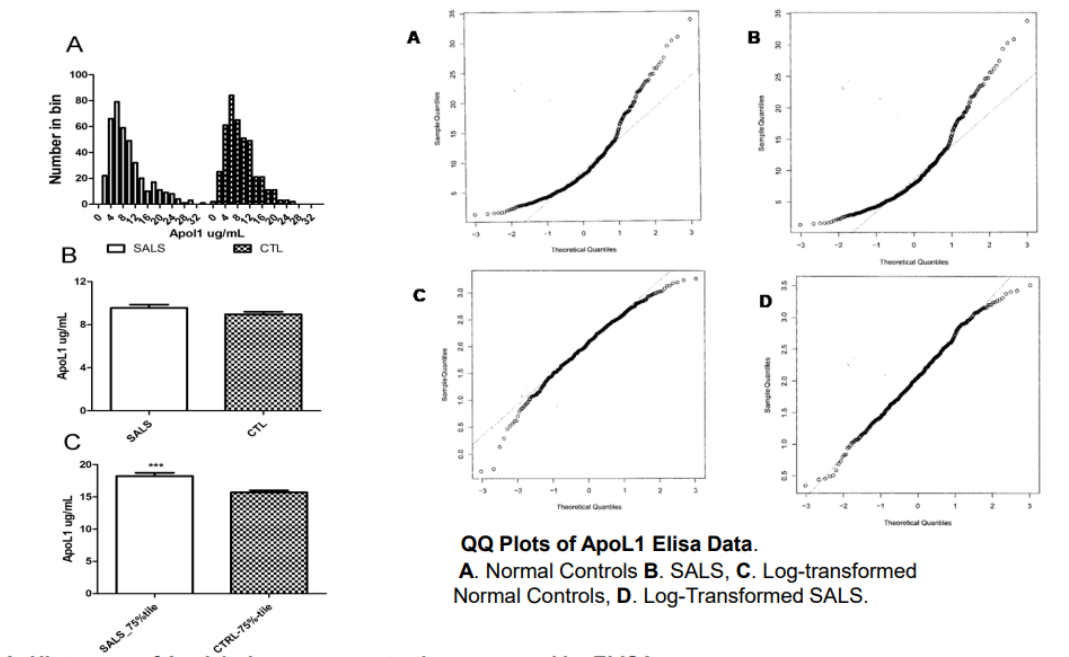
[38] Saeed et al.2006 associated PON1 with ALS in trios; Heinecke,J.W 2010 J.Clin.Lipidol. 4:371-375

This intrigued Dr. Siddique and colleagues, and they realized that HDL carries many interesting things and it affects important functions in the liver and the kidneys. APOL1 is on dense HDL3 particles. Most of the secreted APOL1 comes from the liver. Tyrpanosome lytic factor 1 (TLF-1) includes APOA1, APOL1, and Haptoglobin Protein Related (HPR). APOL1 kills trypanosomes by disrupting lysosomal and mitochondrial membranes. Expression of selected APOL1 variants can disrupt cellular trafficking in susceptible cells such as podocytes, endothelial cells, and HEK293 cells.[39] But that is not the whole story. They decided to dice APOL1 out to determine whether it has any relevance to ALS. They looked at the gene and protein because it is secreted by the liver. Initially, they had some nominal scores in terms of p-values, which made them think about looking further. They studied about 500 cases and controls who were gender, ancestry, and age matched. That held up in the nominal p-value range. APOL1 is unique to certain groups of animals and humans. It is not present in rodents or in all primates. For example, it is present in humans, gorillas, and baboons. However, it is not present in the chimpanzee. It has been deleted in the orangutan and the macaque. It is interesting that one has a gene and then gets rid of it.

APOL1 does many things, such as providing innate immunity that is silent and protects the organism from environmental challenges that are present all around. APOL1 also is related to production of cytokines and so forth. However, it is not part of the usual innate immunity system. It is exceedingly effective against certain kinds of parasites, such as trypanosomes in Africa. There is a continuous molecular war going on between the gene and the trypanosome in Africa. There are many trypanosomes in the world, they have different hosts, and they are all around. They are found in pigeons, fish, chickens, birds, camels, cattle, sheep, domestic animals, horses, monkeys, dogs, et cetera. In Africa, there is a specific type of trypanosome that causes a huge number of losses in cattle and causes a neurological disease called "sleeping sickness." There are 2 mutations in the APOL1 gene that are protective that are intrinsic to Africa. While those 2 mutations are not present outside of Africa, they are found in the African American population in the US. That is a problem because as people with these mutations age, they start to have kidney dysfunction. If a kidney from such a person is transplanted into another person, the transplant recipient also will have the genetic vulnerability of these 2 variants.

The investigators looked at the serum concentration of APOL1 protein in their 500 ALS cases and controls using an enzyme-linked immunosorbent assay (ELISA). It was a difficult ELISA assay before they finally mastered it and got good results that will be reproducible. As seen on the left side of the following graphic in A, there is a difference in terms of the structure of the distribution of the concentration of APOL1 in the serum. When taken all together, no difference is seen, but when looking at the quartiles, the difference becomes significant between the controls and sALS patients. It does not seem to normalize fully, even with the log-transformation. There is, indeed, a difference between controls and the upper quartiles in terms of concentration:

[39] Davidson et al. Arterioscler Thromb Vasc Biol. 2009 June ; 29: 870–87



A. Histogram of ApoL1 plasma concentration measured by ELISA. for SALS and control samples. B. ApoL1 SALS compared to control concentrations. C. 75%-tile comparison of SALS and controls. N=391 SALS, 409 CTL A&B, N=97 SALS, 103 CTRL for 75%-tile.

The question arises regarding whether very high amounts of this protein stay or go up and down depending upon the environment and whether there is an intrinsic genetic mechanism that controls it. It is very difficult for people who are not trained as epidemiologists to venture out into the world of epidemiology and think that one can capture full ascertainment of the various insults that exist inside and outside the body. The genome is much easier because it is finite and a lot more is known about it. Conditions associated with variable expression of secreted APOL1 include coronary artery disease, high triglycerides, hyperglycemia, striking kidney penetrance, and potentially ALS. APOL1 has been found to be present in the CSF of patients and that it is successfully made in an animal model. It is very difficult to make this generic model because people make it in specific tissues or transiently through viruses, but they were able to make a stable one in the liver and that particular animal has APOL1 in the CSF. It also is part of the cascade for autophagy and can have an effect on that. In preliminary studies, they saw that it was present in motor neurons, possibly in the lysosome because that is how it kills the parasite by destroying the membrane of the parasite, both at the mitochondrial and lysosome levels. Questions being addressed by the current work supported by the CDC include the following:

1. What other components of HDL are dynamic (vary in concentration and distribution)?
2. Do genetic variants in APOL1 serve as risk elements for ALS?
3. Does disease state correlate with changes in APOL1 mRNA or protein concentrations?
4. How does intracellular (retained) APOL1 protein contribute to disease state?

This table is part of the original data from screening of genetic variants in APOL1 among 750 controls and 750 cases, with adjusted p-values:[40]

SNP	BP[1]	A1	MAF SALS	MAF CTLS	A2	CHISQ	P-adjusted FDR=0.05	ODDS RATIO
rs136161	36261386	G	0.370	0.414	C	6.071	0.04755	0.8188
rs136168	36264796	G	0.174	0.206	A	5.107	0.05837	0.8008
rs136175(M228I[2])	36265520	G	0.174	0.206	A	4.927	0.02974	0.8113
rs136160	36261073	C	0.159	0.190	G	4.926	0.04622	0.8053
rs136148	36256885	C	0.301	0.338	T	4.549	0.03536	0.8380
rs136176(R255K[2])	36265600	G	0.175	0.205	A	4.277	0.03723	0.8186

This is not hugely effective, but when looking at third-level mechanisms in terms of how to influence a factor that influences the disease, the Mendelian randomization (MR) concept becomes clearer.

In terms of mechanisms of expression, Cis eQTLs variants act on the same gene. Trans eQTL variants have effects on distal genes. pQTLs are gene variants that affect plasma expression of a protein and can be cis or trans. Through data mining of their whole genome association studies and APOL1 ELISA correlations, Dr. Siddique et. al discovered several potential pQTLs and eQTLs. Trans eQTLs affecting other genes also are being investigated. In terms of measurements for APOL1 genotypes for rs136176 and rs5756115, neither of these exhibited changes consistent with a pQTL. APOL1 concentration of the 3 genotypes of ZPR1 SNP rs12286037 was significantly different, suggesting that rs1228603 is a pQTL. However, the variant allele frequency is only 0.04. It is possible that there are eQTLs within the APOL1 cluster and nearby genes. The problem is that it is a minimal frequency, so they had to move on.

They analyzed the Chromosome 22 region for eQTLs. Regulation of gene expression involves a complex interplay of genetic and epigenetic factors. cis-eQTLs are genetic variants relatively close (within ~2Mb) to a target gene that alter its expression. The mechanism of action is generally thought to be related to altering of transcriptional machinery for gene expression. Trans-eQTLs are genetic variants far from a target gene, often on other chromosomes, that alter gene expression. The mechanisms of action for these are more complex and are thought to involve 3D chromatin conformation changes affecting enhancer or silencer regions.

In terms of the study methods, they performed WGS and whole transcriptome RNAseq in lymphoblasts of an initial set of 52 individuals. Variants in the APOL1 region (chr22:35-37Mb on GRCh38) were extracted and assayed for eQTL potential. The software package QTLtools[41] was used to assess cis-eQTLs for APOL1 and trans-eQTLs on the Chr22 region for selected

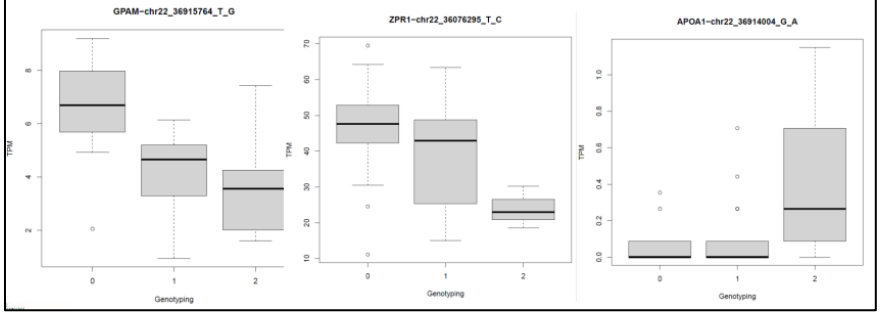
[40] Siddique, T et. al (unpublished)
[41] https://www.nature.com/articles/ncomms15452

targets. In total, they identified 44,571 single nucleotide variants in their regions with frequencies in their data ranging from 0.01 to 1.0. A total of 41,877 of these variants had existing dbSNP RS ids and 43,396 had existing frequency information in the gnomAD database. Only variants with a greater than 0.1 alternate allele frequency in their data were used for eQTL analysis, which was a total of 17,040 variants. There were 3568 variants assayed for cis-eQTL regulation of APOL1. Of these, over 30 were significant. The 5 most significant are shown in the table below:

ID	Gene	name	rsID	p-value	slope	data_freq	gnomAD_freq
ENSG00000100342.21	APOL1	chr22_36855435_A_G	rs1015775	1.96E-06	-58.1826	0.86538	0.8362
ENSG00000100342.21	APOL1	chr22_36143804_G_A	rs80577	0.00125	34.4395	0.18269	0.1662
ENSG00000100342.21	APOL1	chr22_37090225_G_A	rs2019848	0.00144	-37.7375	0.21154	0.152
ENSG00000100342.21	APOL1	chr22_37101553_G_A	rs228907	0.00214	32.1135	0.25962	0.325
ENSG00000100342.21	APOL1	chr22_37097138_G_A	rs2160906	0.00239	-35.9663	0.22115	0.1734

They selected 4 genes (APOA1, GPAM, HPR, ZPR1) to identify potential trans-eQTLs from the APOL1 region. Two forms of GPAM (glycerol-3-phosphate acyltransferase) exist for this enzyme, 1 in the mitochondria and 1 in the endoplasmic reticulum. It prefers saturated fatty acids as its substrate for the synthesis of glycerolipids. This metabolic pathway's first step is catalyzed by the encoded enzyme. HPR-haptoglobin related protein complexes with APOL1 in HDL3 particles generate trypanosome lytic factor (TLF-1). ZPR1 was previously identified as a trans pEQTL for APOL1 plasma levels. APOA1 encodes the main lipoprotein for HDL particles. The investigators identified 65 putative trans eQTLS for APOLA, 85 for GPAM, and 44 for ZPR1. HPR did not have detectable expression in lymphoblasts, so it could not be analyzed. APOA1 expression was low, but GPAM and ZPR1 had robust expression. None of the Chr 22 eQTL variants were within the APOL1 locus. The most significant variants are tabulated below:

Name	chr	start	chr	position	snp	rsID	p-value	slope	freq_in_our_data	gnomAD_freq
GPAM	chr10	1.12E+08	chr22	36915764	chr22_36915764_T_G	rs4821560	2.30E-05	-0.55106	0.471154	0.4189
ZPR1	chr11	1.17E+08	chr22	36076295	chr22_36076295_T_C	rs2413378	0.000966	-0.44439	0.240385	0.3754
APOA1	chr11	1.17E+08	chr22	36914004	chr22_36914004_G_A	rs2075726	0.001675	0.425243	0.519231	0.4217



In 4 gene regions, variants were collected to identify trans-eQTLs for APOL1 expression. A total of 92 of ~11,000 variants were significant trans-eQTLs for APOL1 in these regions (14 APOA2, 59 GPAM, 13 APOA1-ZPR1, 6 HPR).

To summarize, APOL1 protein in plasma exhibits skewed expression toward higher levels in SALS compared to controls. APOL1 variants are associated with disease risk. Most are protective. APOL1 plasma levels are influenced by variants in another gene, ZPR-1, indicating a potential pQTL. RNAseq reveals several loci on Chr 22 that influence APOL1 expression in lymphoblasts from SALS patients. Variants that affect other genes also were found. Genomic

variants are being integrated with their survey data containing external risk factors such as LDL cholesterol, exercise, exposure to pesticides and other chemicals, and the presence or absence of other comorbidities. Selected variants that have been previously identified as risk factors for ALS using Mendelian randomization are the focus of the studies being conducted by Dr. Siddique and colleagues.

Results from the Registry Engagement Session & Future Priorities

Danielle Boyce, DPA, MPH
Stephen Finger and Cathy Collet
Engagement Sessions Team

Dr. Boyce shared a video of the results from the Registry engagement sessions and their future priorities that was recorded on August 25, 2022 so that they could share it with people living with ALS and others affected if they could not attend the Annual ALS Research Symposium and Meeting. It was recorded before Sandy Morris passed, so she was listed as a member of the group. Dr. Boyce emphasized that she wanted to mention that and honor Sandy's work on this.

In terms of why she entered the advocacy world more than a decade ago, Dr. Boyce indicated that she is a mom of a child with a catastrophic and rare form of epilepsy. She would give her right arm for the CDC to make a registry for her son's incurable disease in rare epilepsy. Parents are doing bake sales to raise money to design and run their own registries with no help from the world class epidemiologists at the CDC. When she came into the ALS community, it really saddened her to see so much legitimate concern and negativity in the community surrounding the Registry. She knows as an advocate and a researcher that the Registry is incredibly valuable, so she wanted to do something about this. To that end. She could not resist the urge to talk about citizen engagement in research instead of just patient engagement, which is very important to this conversation. Sometimes there is a tendency to be dismissive of patient engagement, because it comes in so many forms in the private and nonprofit world. It is almost an afterthought sometimes.

Citizen engagement is "the effective and systematic involvement of members of the general public or persons from affected, community groups such as patients, caregivers, advocates, and representatives in the research processes. In this case, patient engagement is really taxpayers who happen to be patients. That framing is very important. The benefits of citizen engagement include the following:

- ❑ Improving the relevance of study findings
- ❑ Encouraging the representation of diverse groups in research studies
- ❑ Minimizing waste by facilitating stewardship over resources
- ❑ Promoting mutual learning and understanding
- ❑ Allowing broad dissemination of research findings beyond traditional academic audiences

In terms of how to do this well, first the issues have to be identified. This was done through qualitative interviewing, use of a modified Delphi process, and "member checking" that resulted in a reorganization and reprioritization of themes. Bi-weekly meetings were held with advocates and ATSDR staff to discuss issues that were sticking points or dilemmas that different groups were having to help make the larger meetings more productive. They also had rules of engagement that included a centering exercise at the beginning of each meeting emphasizing civil discourse and consensus building. It was not always easy, but she thought everyone would

be impressed with the results of these conversations. The team is comprised of Stephen Finger, Sandy Morris, Becky Mourey, Tyler Gaetamo, Nadia Sethi, Allison Bulat, Emily Lowrey, Tim Lowrey, Cathy Collet, and Gwen Petersen. Stephen Finger and Cathy Collet presented.

Dr. Finger began by stressing that patients really value and understand the importance of this project and do not take it for granted. Unlike rare epilepsy, they do have this great Registry resource. Understanding the epidemiology of ALS will help when advocating for more government funding or in attracting more investments from the government and pharmaceutical companies. Most importantly, gaining a better understanding of who does and does not get ALS hopefully will lead to additional potential treatments for this disease. Patients also recognize and believe in the tagline that the registry has been using, "ALS Research Counts on You." Patients recognize that this project will only be successful through their participation, and patients really take pride in that. Patients understand that this is a difficult project that will never be able to find every person with ALS given all of the difficulties that were laid out in the previous day's presentations. However, patients want to make sure they are moving toward the goal of better understanding the epidemiology of the disease and want to make sure that their participation really matters. A couple of ways patients want to see that is through timely reporting and signals that they are moving toward a common goal of better understanding the epidemiology of ALS.

There are several reasons why the current 5-year delay between when patients enroll and when their data are recorded is such a big issue in the community. It is difficult to motivate patients to enroll and try to improve how ALS patients are identified when they only see data from 5 years ago. Of the patients who enroll this year, over 80% of them will likely die before the first report using their data is published. Additionally, the 5-year delay means that patients and the portal are only compared against the NDI. Patients also continue to receive notifications up to 5 years after they pass. This means that most patients actually receive more notifications after they pass than when they are living. This is not an effective way to run the notification process and it sends a negative signal to patients about how their participation is respected. Patients understand that there may be reasons for this and that there always will be a delay in the research process. However, they are confused as to why that delay has been extended over time.

Initially, they were told that there would be a 3-year delay. Until 2018, that was the cadence with which reports were published. During the 2019 meeting, they were told that the report for that year would be further delayed because they were waiting for the results of the capture-recapture methods. During the 2020 meeting, they were told once again that the report was delayed because they were waiting on a citation for the capture-recapture methodology. Now they are being told that the 5-year plan is due to delays in receiving administrative data. These mixed messages make it hard for patients to understand what is going on. If the hold-up truly is delays in getting data from other parts of the government, these data need to be requested as soon as possible. The 2018 and 2019 datasets have been in-house at the CDC for multiple years now, so there is no reason why those reports cannot be produced in an expeditious manner. If CDC is waiting for additional data for some of these years and they want to include Medicaid once again because that is important, they should try to get the reports out for 2018 and 2019 and then add on the additional data for the 2020 report that should be published in 2023.

In terms of the work of the past decade leading to a better understanding of the epidemiology of ALS patients and using the example of prevalence, patients are seeing a lot of different numbers that make it hard to understand whether they are moving toward the goal. Articles in the popular press, from the FDA, from NIH, from the *Today Show*, from the *ICER Draft Scoping Document,* et cetera report incidence numbers that are all over the place. Perhaps those

numbers come from lazy interns who go on the web while making grocery plans, come up with a number, and move on. It is very concerning to look at the websites of CDC and paid partners who should be intimately aware of the latest research, but also have numbers that are seemingly all over the board and are not reflective of the work that is being done by the Registry. While the CDC updated their page in the past couple of weeks, it is still representative of some of the messaging failures that have occurred. This is a shame because over the past decade, the Registry has done admirable work with regard to providing a better understanding of what is occurring. Last year, they did a good job of coming up with an estimate of the number of patients in the US living with the disease. But in order for these numbers to be meaningful and achieve the purpose of providing a better understanding of the disease, it is important to understand their context. For example, the 31,000 estimate is the estimate of the number of patients living with ALS in the US in 2017. That number is made up of the 17,800 patients individually identified in the Registry, for which they should be commended. Finding this number of patients for a disease this debilitating in a healthcare system as crazy as the one in the US and finding 17,000 patients in private and public health care, rich patients, poor patients, patients from all different demographic backgrounds is quite an accomplishment.

But this number it is recognized that this is number of patients who have been found and that other research done by the Registry over the past decade has found that there they are still missing over 40% of patients. For the report to achieve the goal and for the dashboard on the CDC website to help people understand the prevalence of ALS, it is important to be crystal clear about what these numbers mean. For instance, it is important to be clear that 31,843 is not an estimate of an upper bound. After waiting for a citation of the capture-recapture methodology, nowhere in that paper does anything suggest that this method was used to estimate an upper bound. Along the same lines, the 17,800 number is not a lower bound. It is the number of patients that have been found by the Registry. Through their clinics and chapters, the ALS Association has identified over 20,000 patients, which conflates the numbers without bound language and does a disservice. The "mean case rate" should not be referenced. Discussing the 24,821 number and calling it a "mean case rate" is misleading and this number is not a meaningful statistic. Calculating an average of the number of patients found with the number of patients who are believed to be living with is not meaningful. It is like saying, "I have 2 children and 6 bananas, so the average is 4." While the average of 4 is a correct calculation, it does not help to understand how many children or bananas there are.

With regard to the difference between who has been identified and how many people are estimated to be living with the disease, it is known that 44% of patients who are being missed through the hard work of the Registry do not look like the people in the Registry. The 44% who are missing are more likely to be non-white, Hispanic, and under 65 years of age. When demographics are reported, given the goal to better understand the epidemiology of ALS, the limitations of the data must be noted clearly. Otherwise, it will not help people learn about the epidemiology of ALS. Finding patients from disadvantaged groups is difficult and patients recognize that this is not a failure on the part of the Registry. It is more a failure on the part of the entire healthcare system. This needs to be taken into consideration in terms of trying to improve methods. Patients were disappointed to learn in the latest paper that the algorithm that tries to identify patients in administrative data did not consider that sensitivity and specificity were different across different demographic groups. Recent papers have suggested that the Biorepository is made up of over 95% white patients. This issue must be addressed. Having patients from all 50 states is not a substitute.

With all of the reports the Registry is producing, it is important to be focused on how to improve the understanding of ALS. For years patients were told that based on how the Registry identifies patients in the administrative data, it was not possible to calculate incidence because of not having date of diagnosis for over two-thirds of the patients in the Registry. Now the Registry is producing papers and posting on the dashboard an estimate of new people with ALS in the US in 2016 of about 4800. Dr. Finger does not believe these numbers and the way they are presenting help to understand the epidemiology. These numbers are not contemporaneous. These are not patients who have been recently diagnosed. They are not representative. It is known that the cases being missed do not look like the patients the Registry is finding, and it is known that the 4800 number is missing over 40% of cases. In order for a paper and table like this to be informative, it must be made clear that these are new patients *identified* by the Registry and not new patients diagnosed in a given year.

Enough about numbers. Everyone knows and believes that the Registry is more than a nose-counting exercise and that a lot of the real power of the Registry comes from giving patients a chance to enroll and complete detailed surveys. Everyone recognizes that with a limited budget, a devastating disease, and patients across the country, it will never be possible to get everyone, but that is not an excuse to not make sure they are doing their best. It is concerning that even prior to COVID-19, there was a steady decline in enrollment and survey completion. In 2015, over 9500 surveys were completed. That declined by over 25% to 6845 in 2019. When COVID-19 struck, survey completion declined to 5099 in 2020. This is not just about how many emails are sent or how many patients are talked to. It is about how many people enroll and how many surveys they complete. On average, about 3 new patients enroll and 15 surveys are completed every day. If an action by a partner is effective, there should be increases seen in these figures. It is important to assess any increases and then try to duplicate the efforts that led to them.

Patients also have concerns about the Clinical Trial Notifications. This is an area of the Registry that patients believe in and recognize as a benefit, but communication about notifications is vital. It is important to make sure that patients know that not all trials use the notification system. Similarly, patients need to believe that the notifications are relevant to them. When sponsors send out notifications to all patients, many of them do not qualify for trials and this makes patients more likely to stop opening and responding to emails. Sponsors should be encouraged to limit emails to patients who meet inclusion criteria. Again, patients are receiving notification up to 5 years after they die. This sends a negative signal to the community about the timeliness of the project, it is not effective in filling trials, and most importantly, it is disrespectful. This is a very positive project, which they need to make sure that it is operating as efficiently as possible and that it takes into consideration how it impacts patients.

Cathy Collet indicated that as a result of some of the conversations they had talking about the things Dr. Finger discussed, the team decided that they would like to try to put some data around some of these ideas, even though they had to do it in a couple of weeks. To accomplish this, they developed a very short survey that was administered in a huge hurry. The survey was open August 9-12, 2022 with minimal questions For People in the United States Living with ALS or Caregivers Answering on Their Behalf (PLWASL). There were 132 respondents, about which they were very happy. The survey itself was online. While they realized that this was not ideal for some of the questions they wanted to get answered in this process, it worked given the parameters. They used social media and email requests from individuals on the team and in their networks to seek people to participate. The survey was structured very simply. The main question at the beginning of the survey was, "Have you enrolled in the CDC ALS Registry using the online portal?" That was the main piece of information gathered. Then there were questions for which respondents who answered "yes" were routed through the survey to provide more

information about the experience. Respondents who answered "no" were further probed to try to find out why not. The people who answered "no" were of the most interest in terms of trying to find out why they had not enrolled. The survey also attempted to capture anyone who was "unsure" because that could be telling as well. At the end of the survey, everybody was brought back together for some general impressions of the Registry questions.

In terms of responses, 39 (25.9%) of the 132 respondents indicated that they had self-enrolled in the National ALS Registry, 55 (41.7%) said they had not enrolled, and 38 (28.8%) were not sure whether they had enrolled. People who responded that they had enrolled were then asked, "Why did you enroll (check all that apply)?" The top 2 responses were "Clinic or organization staff member asked me to" (22, 54%) and "I thought it was important" (20, 51.3%). There were statement questions for which people were asked to respond to 1 of 5 answers (Agree, Strongly Agree, Disagree, Strongly Disagree, No Opinion). To the statement, "I found it easy to enroll," 85% responded "Agree" or "Agree Strongly." The responses to the statement, "I find the supplemental surveys easy to take" were more mixed. The most troubling was that 12 (33.8%) of respondents to this question answered, "No Opinion." This was a similar finding that Rob Tyson, a wonderful and smart guy with ALS, got to a survey he did himself and presented during the National ALS Registry Meeting 10 years ago. In response to the statement, "Clinical trial notifications I believe are relevant to me," only 21% answered "Agree" or "Agree Strongly." It is of concern that that 79% answered "Disagree," "Disagree Strongly," or "No Opinion."

Moving now to the people who said they had not self-enrolled in the Registry, the top 2 responses were "I wasn't aware of it" (44, 80%) and "Nobody asked me to" (15, 27.3%). People were then asked, "What might motivate you to self-enroll?" The top response was, "More information about what my data would contribute" (44, 80%). This is consistent with the altruism seen when people with ALS are willing to participate in studies, but they want to know that their participation is meaningful and worthwhile. The next 2 top responses to what might motivate someone to self-report were "More results from the registry" (18, 32.7%) and "More timely reporting of results" (14, 25.5%). There were mixed results to the statement, "I think that the CDC ALS Registry supplies important information to the public." Among the respondents, 34 (30.6%) answered "Agree." In response to the statement, "I think that the CDC ALS Registry has been a good investment," 25 (22.7%) answered "Agree." However, 22 (20.0%) answered "Disagree Strongly" and 30.0% answered "No Opinion."

Regarding the demographics of the respondents, 64% were female. While the survey was open, they were running about 50/50 male and female. Then Gwen Petersen asked her ALS team to fill out the survey on the last day and female participation increased considerably. While people responded from 30 states, California and Illinois had the highest participation rates. This was helpful in terms of getting a mix of locations.

At the end of the survey, there was an open-ended question asking, "Is anything else we should know?" This was not a required question, but people did volunteer information. Sample comments included the following:

- ❑ I know nothing about this ALS registry it's new to me.
- ❑ Why are the reports so delayed? Why does ALSA receive funding? Why isn't their mandatory reporting so we can get an accurate count? Just too many questions.
- ❑ I feel like it was started with the best intentions but has failed the community. It must be improved upon or it is just a waste of money benefitting a few researchers.
- ❑ I'd like to see research done, which questions ALS patients about their past medical history, head injuries, military service, etc. to try to determine possible triggers/causes for ALS. It

> doesn't seem like anyone cares about this information . . . .why is that? I think every ALS patient should be sharing that info with researchers.

Becky Kidd, an ALS patient who attended 4 annual meetings (2014, 2014, 2016, 2017), died in 2018. Her mantra at every meeting was, "You can't fix what you don't measure." She was right and this continues to be right.

In terms of clarity, the idea of a dashboard on the Registry website is wonderful. People yearn to go to one place to find out how many people have ALS. While the dashboard is a good start, it is not that clear. As Dr. Finger noted, the site needs to make clear what the numbers being shown actually mean. The numbers are more valuable if people understand what they represent. Perhaps something like, "The model estimates that 31,843 people were living with ALS in 2017" and then include a simple breakout in graphic form explaining how that number was calculated (number self-enrolled + data resources + capture-recapture model = 31,843). Then describe the demographics of the people who self-enrolled to show who the data actually represent and explain that those who self-enroll are not representative of everybody with ALS. Likewise, describe the demographics of the people who were identified through administrative databases (Medicare, VA, Medicaid) who did not self-enroll in the Registry. The incidence issue is significant. Perhaps instead of calling this "incidence" the dashboard could state, "We encountered new people with ALS in 2017 whom we had not seen in prior years" and identify where they came from in terms of the number self-enrolled and the number found in administrative databases. Include their demographic information as well. It is important to tell people how the Registry process works instead of reporting a surrogate of incidence.

To summarize, the ALS Registry plays an important role in combating ALS. Patients recognize that "ALS Research Counts on Us." CDC can count on people with ALS to participate in research enthusiastically, but they have to understand the purpose and that their information will be used for a greater good. Much has been accomplished, but efforts to date must be shared in a clear and effective manner. Statistics are only useful if people understand what they mean. Results need to be produced in a timely fashion and resources must be utilized as efficiently as possible.

In terms of action items for 2022-2023, Dr. Boyce indicated that in order to accommodate all of these great plans in the projects they are trying to tackle, the bi-weekly Stakeholder Engagement Team meetings will continue with the CDC Registry Team, volunteer patient advocates, and affected folks. One of the action items is new materials, including the updated dashboard, press kit, and infographic. There also will be a review of CDC and partner websites for out-of-date statistics and removal of those so that people do not Google and accidentally stumble upon the wrong number. Another action item is clinical trial notification improvements, which needs to be explored further, discussed, and planned. There also needs to be troubleshooting of the timeliness of data releases. While that is not going to be solved overnight, it is an action item for the coming year. Common language for all stakeholders is something that the team felt was a "low hanging fruit," such as, "There were an estimated 32,000 people living with ALS in the US in 2017." That is easy for partners to remember, it is easy to put in a tweet, and it is easy to put in social media campaigns. Common language will make it easier for reporters and others who are caught up in not knowing which statistic to use. Another action item is to maximize enrollments in the Registry and survey completions through better understanding of the barriers and incentives to enrollment through surveys and looking at the data. Representation matters in the portal and Stakeholder Engagement Team. Therefore, the last but not least action item is to increase representation on the Stakeholder Engagement

Team. The Stakeholder Engagement Team is not nearly as diverse as it should be, so they hope to expand.

Discussion Summary

Dr. Ascherio said he thought that the CDC ALS Registry has been a good investment.

Andrea Pauls Backman indicated that the Les Turner Foundation is one of the partners. They report to the Registry each month on how many discussions they have with people to encourage enrollment in the Registry and inform them of other promotional efforts. However, they do not get feedback on how effective their efforts are. Knowing how many patients enroll each month by state would be very helpful, especially as a trend analysis, if the Registry can produce this data.

Dr. Mehta indicated that the OMB approval must give them approval. They can release state-level data for Illinois to show exactly what the trend is going to be for enrollment. That by itself certainly is going to be very helpful to see exactly what the measurements are for each state. He thanked Cathy Collet and Stephen Finger for their constructive comments. The Registry Team knows that improvements need to be made. They appreciate all feedback, but there are things CDC can and cannot do. They can update the dashboard and materials, ensure that information is uniformly distributed, make sure the partner websites have that the number 32,000 across the board so that the same message is being communicated across all sites, improve clinical trial notifications, and review the NDI on a more frequent basis to remove patients who have passed away so that emails and notifications do not continue. In terms of data release, they do their best to publish data. There are times when they have the data in-house, but something is missing or outstanding. They received approval the previous day for additional data from CMS, which will include Medicaid and other data. They want to make sure they have the most complete dataset before releasing reports. He agreed that common language is certainly important. They are always trying to maximize enrollment and survey completion and will continue to do so. With the updated portal, the surveys should be much more manageable with a reduction from 18 to 5 major categories and a measurement tool that shows patients how many surveys they have completed. In terms of representation, they certainly want the Registry to be as diverse as possible. As noted earlier, engaging Texas, Florida, California, and New York will help to improve the diversity of the Registry as these are the most diverse states in the country. He emphasized that ATSDR is the caretaker, while the Registry belongs to the patients. They certainly want to improve the Registry based on patient feedback.

Regarding Andrea Pauls Backman's comments, Cathy Collet said she had a simple related question. While she understood the rationale about publishing/not publishing certain data, it seemed that because the Les Turner Foundation has a contract with ATSDR to complete certain services, the agency should be able to discuss data with them privately for their geography. This would be in terms of their performance under their contract as a confidential conversation, not as a conversation about publishing Illinois data. She emphasized that Ms. Beckman seemed to be simply asking for feedback on how they are doing in terms of how they are doing with increasing enrollment in the Registry and survey completion.

Dr. Mehta indicated that ATSDR sends all partners, including Les Turner Foundation, a table that shows the states within Tiers 1, 2, and 3. This includes the number of expected and observed cases for each state. Enrollment data are sent to every partner every month, which partners can share with their chapters.

Cathy Collet emphasized that there seemed to be an interesting disconnect between what Ms. Bachman was saying and what Dr. Mehta was saying, which she thought was worth further conversation to clarify what Ms. Bachman actually needs.

Dr. Mehta said he interpreted the question from Ms. Bachman to be that she would like to get state-level data. They do not give metrics in terms of hardcore quantitative data. They will give them the table with qualitative information about which Tier each state is in. If OMB gives approval, ATSDR will be able to share more state-level data, which by itself will give much more quantitative data. Right now, it can only be expected and observed cases.

Lauren Webb, from Les Turner Foundation, indicated that she prepares the monthly reports that are submitted to ATSDR. It would be helpful and valuable for her team to have a better understanding of whether what the Les Turner Foundation is doing to increase enrollment and survey completion is making a difference. The Stakeholder Engagement Team just outlined ways the partners can help drive the conversation forward. What helps motivate people living with ALS to participate in research like this is clarifying some of the misunderstandings around the Registry in terms of what it can and cannot do.

Ms. Collet said she thought they got a small start on that with their simple survey and that there is room here for conducting a much better survey in clinics to talk to people to determine from a much broader base of people why they are not enrolling. It was clear from the online survey that people do not know about the Registry. This suggests that something is not working if people do not even have a basic awareness of the Registry.

Ms. Webb agreed that having those conversations is important. There are many places where the Registry is not being talked about, as the survey showed. Her team struggles with this as well as how to keep making the conversation fresh. Helping motivate her own team to keep talking about it in a fresh way is challenging.

Dr. Boyce suggested that this is something they should think through as part of the action items she described. Perhaps the Stakeholder Engagement Team can engage with the partners to think about how to make this more patient-centered and useful.

Ms. Webb indicated that the Les Turner Foundation would love to work on this with the Stakeholder Engagement Team.

Dr. Finger said he thought what the Les Turner Foundation is doing is great. On a repeated basis, having some sort of updates from the Registry could be helpful in terms of providing the people with "boots on the ground" with fresh messages would be great. While providing the tables with tiers is good, it has to be translated to current actions. Even though there are limits on what can be explicitly said at the state-level, perhaps there is a way of saying something like, "Hey, in the past week, the Les Turner Foundation deserves a gold star because there was a big number seen for California."

Tim Lowrey, who is on the Stakeholder Engagement Team, commented that he was told about the Registry at the beginning. So much information is given to patients at the time of diagnosis, it could get lost.

Ms. Lorenz expressed gratitude to everyone for doing an amazing job and emphasized how impressed she is with all that has been done, recognizing how much effort goes into this work. A lot of people want to use the Registry. To get people to enroll, they need to get a benefit back. Because there is not a searchable database, patients are not interacting on the website. Offering people a reason to interact on the website more often likely would increase enrollment in the Registry. She suggested during previous annual meetings that it would be great to integrate the CDC data with EPA data from the Toxic Release Inventory (TRI) and Superfund Sites. People keep asking what is occurring in their county or state, but there is no place for them to find that information. She did a poll on a Facebook group that has about 13,000 people. Admittedly, this is a very biased poll with a very secular community, but she wanted to share some of the feedback from the approximately 270 responders. When asked where they found information on clinical trials, 30% of responders found trials through their own research on the web, 20% got information from clinicaltrials.gov, about 16% got information from that Facebook group, and 12% got information from a neurologist. The rest of the numbers dropped down to 1%. This should be very concerning. Hopefully, ATSDR could build that into a future poll that reaches the entire community. It concerns her when people say they need to look for Hispanics and people of color in New York, Texas, and California. Admittedly, those states have large populations. However, the density of Hispanic populations is in New Mexico and Colorado. Some of those places do not have ALS Centers of Excellence. This is similar for African American populations. There is no outreach to Historically Black Colleges and Universities (HBCUs), but there are some gaps in clinics in the South as well. Some people have to travel 12 to 15 hours to get to a Center of Excellence, so data are missing from those communities. She expressed her hope that they can do a better job on this. There are databases from the ALS Act with about 15,000 people in one database and about 20,000 people in another, separable by states. They have a large population they can hit but have ignored that. She asked Dr. Mehta to address what hurdles ATSDR has in terms of publishing the data for the community so that the advocacy community can try to impact that from a legislative perspective in order to speed this up for the community.

Dr. Mehta said that right now, OMB approval is pending. If they receive that approval, they can release state-level data. There has been a considerable push by Dr. Walensky, CDC's Director, to release more CDC data in general in a timely fashion. There are changes afoot at CDC that could impact the dissemination of data that could affect the Registry program as well. They are still awaiting guidance on that. He assured everyone that they do not sit on these data. They want to get the information out, but they are a small team that does not have unlimited resources. Working with partners like CMS can be very challenging. They go back and forth with CMS, which sometimes can take over 12 months. ATSDR has to pay CMS for their data and uses its resources to try to get those data in a timely fashion, but there are hurdles and delays. ATSDR's team of epidemiologists work on the data all of the time and they want to get it out. They know the pressure they are under to get it out. It does them no good to sit on these data. However, they also want to ensure that what they have and publish is complete and accurate. If the data are incomplete and/or inaccurate, they will be scorned for that as well. There are challenges in science, unfortunately. The premise behind that is that ALS is not a reportable disease, with the exception of 3 states. It is not notifiable to CDC. Public health is not an exact science. While 1 + 1 = 2 in public health, there are other factors involved as well. They are committed to improving areas that need improvement.

Dr. Horton added that another hurdle is the OMB, which has the authority to pull the plug on any kind of study if they do not believe it is being done correctly and through the terms of clearance. Currently, the terms of clearance from OMB state that CDC cannot release partial data. There is a 2-pronged approach to data collections through the portal and through large national databases. When those 2 sources are merged, duplicates must be removed to ensure that people are not being counted more than once. The terms of clearance state that data from both data sources must be merged and deduplicated. They could not release just portal data alone under these terms, which is what people are asking for. The rationale for this is valid. The OMB wants to ensure that if CDC releases portal data, that someone does not use those data to calculate incidence or prevalence on their own and come up with incorrect rates. This is an OMB restraint that prevents them from releasing data, which is a major hurdle. While their hands are currently tied, they are engaged in ongoing conversations with OMB. One of those conversations involves releasing state data.

Ms. Collet stressed that the feedback loop is important. FasterCures published a white paper long ago about the characteristics of effective disease registries, one of the key takeaways of which was that people have to see something back that makes them feel like they can learn something. In terms of the diversity issue, she agreed 100% that if they go to big urban areas and a couple of big states to add people of color, that will not solve the diversity issue. Data are needed that are reflective and representative of people living with ALS and these people need to be found where they live. As Dr. Boyce noted, Sandy Morris said, "We can do hard things." In memory of Sandy, Ms. Collet said that some of the things that need to be done are not that hard. While they may be procedurally difficult—they are not that hard.

Dr. Boyce indicated that Becky Mallory, an ALS leader and one of the Stakeholder Engagement Team panelists, asked her to make the following statement, "We want to contribute, so the more we show people how enrolling in the Registry contributes to advancing the science, the better."

Aditi Narayan Minkoff, Director of Community Support at I AM ALS, said she was curious to hear from current partners about what strategies they are using to identify and reach Hispanic/ Latino, African American/Black, and other ethnic minorities living with ALS to inform them about the Registry and get them support to enroll in the Registry.

Dr. Mehta said that ATSDR can only do so much. The partner organizations are the "boots on the ground" and have tremendous power and sway with people living with ALS. Their words have impact and are like a feedback loop. The premise behind this is that if people with ALS say something is not good, it is a feedback loop. It has been said that a lie can cross the world 10 times before the truth even gets out. The partner organizations are working with patients, seeing patients, talking to patients. Working with partners is incredibly important for ATSDR, because the agency is at the 30,000 feet level rather than "boots on the ground" seeing patients in ALS clinics, speaking to them in social media groups, engaging with them at events, et cetera.

Lauren Webb indicated that Les Turner Foundation has an approach and framework for reaching communities. Outreach work must be intentional and consistent, such as trying to recruit more Hispanic, Black, and other minority populations. It is important to involve people living with ALS who are members of this community to help them figure this out together collectively. I AM ALS has done a great job with some of their different groups. There is still a long way to go in order to achieve health equity and equal access to the Registry and show those numbers. That is definitely an area in which they need to be more intentional. Over the last 2 years, the Les Turner Foundation has focused on health literacy and the need to engage with different communities in a more meaningful way.

Dr. Finger agreed with the importance of intentionality. It was concerning to him to see some of the presentations the previous day regarding the Biorepository, which is 99% white. That is never mentioned in terms of future directions or acknowledged as an area that intentionally needs to be improved. It is a known problem that is important to address. The algorithm needs to be improved to capture different socioeconomic groups. This has to be intentional and future actions have to reflect priorities. There are ways beyond partners to do a better job.

Dr. Boyce agreed and emphasized that the Stakeholder Engagement Group is planning to address this, but she realized that a framework must be developed for how this will be done and what the best practices are in this type of engagement.

Patricia Stanco, with the ALS Association, indicated that this is a priority for the ALS Association. In the Spring, they had a roundtable meeting discussion to brainstorm on ways to be inclusive, not just for the Registry, but for all of its programs and services so that they can do better at reaching underserved populations. One of the things that they have learned is that it depends on the population they are trying to reach. Different approaches will fit different populations, and that is important to recognize. The common thread through all of those approaches is establishing trust, whether trust is established by doing a home visit or partnering with other organizations such as hospice organizations. Stakeholders must be included. They cannot just sit around and surmise how to do this.

Dr. Ostrow said this discussion and how everyone works together was warming his heart. He suggested that a framework for this could be pretty simple and some of it already was being done. The first step is to figure out what topics are of interest by surveying the right people. The next step is to prioritize the topics that are identified. Some specific steps can inform brainstorming around those topics. Some brainstorming and discussion are underway, one point of which is to identify the barriers that are keeping this from happening and another of which is to determine what resources are available. Then it is important to establish relevant objectives, timelines, and metrics to evaluate whether the objectives are being met. Outside expertise may be helpful and could be leveraged for input to avoid "reinventing the wheel." Everything does not have to be perfect right away. When someone wants to design something, they end up spending a lot of time talking about each individual thing and end up talking in circles trying to achieve perfection. At some point, it is a good idea to look at the potential "low hanging fruit" and start someplace. The Stakeholder Engagement Group could develop a framework to approach each topic.

Dr. Boyce said that they did identify some "low hanging fruit," like the stakeholder language that everyone can use to make sure everyone is using the same prevalence. She acknowledged all of the legwork that Dr. Ostrow did in the early days interviewing all of the stakeholders and helping them initially with that qualitative work. That set them up for success and enabled them to work quickly to turn this around over the Summer.

Peyton Navarrete, MDA, expressed appreciation for all of the time and effort that went into the Stakeholder Engagement Panel. It spurred valuable conversations and a new way forward about how and why they speak about the Registry. She agreed with the importance of intentional outreach to the populations who are being missed. It is clear that they are not reaching everybody they want to reach. MDA typically reaches out to any registered individual who has been seen at one of their Care Centers across the nation to speak to them directly about the Registry. Given some of the limitations on information, they do not necessarily have information about race or ethnicity on file, so they cannot directly target individuals of certain races and ethnicities when doing outreach. However, the more and better they talk about it, the

more they will be able to reach the missing populations of interest. One of their hurdles pertains to how to get participation and buy-in from individuals who either do not attend Care Centers or are in rural populations who are not already connected to a partner organization or to the National ALS Registry. This must be done through community groups, Facebook groups, and other ALS outlets so that everyone is working together. They just need to find the correct path forward.

Dr. Ostrow pointed out that in addition to stakeholders, it will be important to involve researchers depending on what topics are prioritized. When the Registry was conceived many years ago, the world was not as data heavy as it is currently. He thinks the Registry should be a beacon for open science, data sharing, and collaborative research. There are experts and consortia already working with the Registry who have done a lot of work on the regulatory hurdles involved in making patient-level data available and using genomic data. Surveying the researchers once the topic areas are identified to leverage their knowledge and experience in overcoming some of the barriers could be invaluable.

Ms. Collet added that she would like to see more researchers actually using the Registry data and for consideration to be given to how to improve the database to make it more useful to them.

Dr. Boyce said that as someone who uses the Registry data herself and has run research programs, she could identify some of those hurdles. As with the patient community, awareness about the Registry needs to be raised with the research community. In her own member checking exercise, she realized that people did not know that the Registry collected certain information or that there even is a Registry. Learning why researchers are not using the Registry more could be beneficial.

Patrick Dolan, a person living with ALS, asked for the top 3 things the ALS community could do to help. This work is incredibly important to the ALS community, who want to be its champions.

Dr. Horton pointed out that there are critically important things that ATSDR staff cannot do as federal employees. They are the only federal agency that funds ALS studies that look at etiology and risk factors. The NIH funds more preclinical and basic science research for ALS, but ATSDR is the only agency that funds ecological studies. As federal employees, they cannot go to Congress to lobby for more funding. However, they tend to receive more applications than they have funding to support. Many great studies are proposed that they cannot support. The ALS community can take this to the decision-makers. As Dr. Mehta mentioned, the Registry Team is very small and is positioned in Atlanta. They do not have "boots on the ground" throughout the country. They rely on partners, stakeholders, and persons living with ALS and their caregivers to help promote the Registry. Though the Les Turner Foundation has a much smaller geographic area, they represent the gold standard in taking a concierge type of approach in which they go out to meet with newly diagnosed patients to explain the Registry and its benefits. MDA has a much larger footprint to cover, so they are not able to take the same approach. MDA has chapters and clinics throughout the country, some of which have high enrollment rates such as Alabama, that could serve as models. This is not "one and done." People are diagnosed with ALS every single day, so they have to keep the message going constantly and have to get in front of people who are who are newly diagnosed. Partners, advocates, patients, and caregivers can help with this.

Dr. Mehta emphasized that they are a small group with limited resources. Each year, they have to try to figure out exactly what to do with what they have in terms of funding research, paying fees to obtain data, et cetera. If they had more funding, they could spread their wings and umbrella size. They do the best they can with what they have and are always willing to learn and make improvements.

Dr. Boyce pointed out that she analyzed those 3000 comments for free, which others could do as well or they could seek funding someplace else. The point is that the data are rich and should be used. She also noted that Patrick Dolan is a master at GIS and cartography. People who are living with ALS have an astounding matrix of skills and talent. That is why it is so important to engage all stakeholders, particularly people living with ALS because they have so much knowledge and so much to contribute. Sandy Morris was a brilliant master at Project Management. They could get so much more done tapping into the skills of additional stakeholders.

Ms. Collet shared her honest opinion about the funding issue. While she realized that this is the government, $10 million a year is a lot of money—especially in the disease registry space. However, she would not be comfortable asking for more without having the functional accounting breakout of where the money goes by function. This is important to understand before "adding anymore fuel to the fire."

Dr. Mehta said that while he was not trying to be defensive, their partner receives $40 million that goes to pre-clinical researchers to find compounds and molecules that may or may not work. They do not publish anything or disseminate any science. CDC receives $10 million to do research organically. They get criticized because the numbers are low, so they make fixes and the numbers go up. They still get criticized that it is not good enough. They do what they can with what they have. While $10 million is a lot of money, they have to do a lot with it. These resources go a long way and they fund some of the best institutions on earth to find out what causes ALS. Nobody else is doing that. ATSDR has the background and publication record to demonstrate what they have done. Scientific publications are a validation that the science works. People may not like that it is a slow process sometimes, but they are a small group doing what they can with what they have.

Ms. Collet clarified that her point was that she is not comfortable asking for more money until they understand the functional breakout of how the money is being spent in terms of research grants, the Biorepository, the Registry, et cetera. That is a perfectly reasonable and basic request. Partners reported on best practices in self-enrollment, but there are also other disease spaces from which they can learn best practices.

Ms. Webb, from Les Turner Foundation, noted that peer-to-peer is a very effective outreach tool. She expressed her gratitude to all of the people living with ALS who joined the meeting.

Ms. Lorenz, ALS Advocate, requested that they discuss the process for reporting ALS clusters to ATSDR. She emphasized that ALS advocates are passionate about what ATSDR is doing and want to help make the job easier by giving the agency more data. There are over a dozen significant populations where there are clusters. There are a couple of clusters in some states with 100 times the expected prevalence rate, but they cannot get the state to investigate. In part, this is because the states have local business interests and it is not a priority for them. There must be a way to overcome that hurdle, whether it is direct reporting to ATSDR or fixing it legislatively. They are sitting on massive amounts of data and Biorepository data that would be so valuable for ATSDR and ALS patients. This is a missed opportunity because nobody is

responding to them. They have approached Senators from Michigan and Ohio where there are massive problems and the EPA and are still not getting any responses to very large clusters.

Dr. Mehta indicated that as a federal entity, ATSDR must follow a hierarchy for investigations. A local or state health department would conduct an initial investigation and then from there, they could ask for resources from CDC. Once CDC is invited, they can conduct a cluster investigation or provide resources. Health consultations can be done through ATSDR as well.

Mr. Zarus pointed out that as part of the 1886 legislation when Superfund funding was reauthorized, a clause was added that any resident could petition the agency for a "Health Assessment." It is actually a health assessment process that also includes health studies, although there is criterion which must first be met because it has to have an environmental link. There has to be a source of pollution that is a high emitter of certain constituents. Once those data are available, they proceed with the health assessment process. They accept any tool. It might be a consultation just on the pollution data available and whether there are adverse effects likely, or it could involve pivoting to one of the other two programs, the Health Study or Surveillance Program, where they would look at the health outcome data. There are 25 states currently funded. Often the states will choose to accept it on their behalf and ATSDR will review their results. In terms of the question about where the money goes, there is a lot of volunteer work that goes one. ATSDR has a panel of volunteers who review the proposals for allocation of grant funding. Those external panelists evaluate for all parts of the CDC, so it is not internal to the ALS program. An overwhelming number of very worthy grant proposals are submitted, which are all reviewed by the external expert panelists for funding allocations based on merit. Ms. Lorenz and Mr. Zarus agreed to touch base offline to further pursue the discussion on clusters.

Dr. Finger clarified that he did not want the point of the presentations that he and Ms. Collet made earlier to be lost. They are not concerned that the data are not 100% complete. They are concerned that it is not presented in that way, so it is important to always be clear on what the data represent.

Reflecting back to Dr. Ostrow's point, Dr. Boyce emphasized that this is easy and "low hanging fruit." Dr. Mehta used Ms. Collet's easy visual, which was very helpful in understanding how the Registry works. They should focus on those messages and the things that they can do to clarify the understanding of the statistics as they identify other priority areas and work on these very tough challenges. In wrapping up this session, she offered her heartfelt thanks to the ALS community who participated in these meetings and gave their trust and to those who were in attendance, for many of whom this might be their first scientific meeting. The ALS community contributes so much and brings so much value to the work. In addition, she expressed gratitude to Dr. Mehta, Dr. Horton, and the rest of the ATSDR staff for attending the engagement sessions all summer, committing to continuing to attend these sessions, and recognizing that "Rome wasn't built in a day." They are going to "keep pounding at the rock" as Sandy Morris used to say to fix what is broken and improve access to the Registry.

Persons Living with ALS: Perspectives on the Registry

Introduction

Danielle Boyce, DPA, MPH
Moderator
Johns Hopkins School of Medicine

Dr. Boyce moderated this session in which persons living with ALS were invited to offer their perspectives. She asked presenters to introduce themselves and say whatever they would like about their lives, children, work, and so forth.

In Their Own Words

Joel Shamaskin, MD
Rochester, New York

Joel Shamaskin is a retired Primary Care Physician and Teaching Professor at the University of Brighton who currently lives in Rochester, New York. He is 67 years old and has had ALS since 2016. Dr. Shamaskin has been involved in ALS advocacy since 2019.

I am a retired Primary Care Physician. In the 30 plus years that I practiced, I was lucky enough to care for several people with ALS. Like most doctors, we remember the patients who stood out for various reasons—either you like them on a personal level, their case was challenging to manage, you got to know their families, and you saw the resilience with which they approached their diagnosis and life. All of those were characteristics of the patients I took care of. After I was diagnosed in 2016 when I was 60, I returned shortly thereafter not knowing how my disease would progress. I was alerted to the Registry 3 years later in 2019 and enrolled at the ALS Association National Advocacy Conference in Washington at that time. I went to their booth and gave my saliva sample for the Biorepository, and that really launched my interest in advocacy in not just the ALS Association, but the broader network of organizations like yours that help support people with ALS and their caregivers. I estimate that in the 3 years between my diagnosis and when I enrolled in the Registry, I easily saw my neurologist a dozen times. I saw other providers probably 10 to 12 more times, either in Boston for second opinion/confirmatory visits, enrolling in studies, and follow-up visits and in Hershey, Pennsylvania. Considering the 25 times when I was face-to-face with a provider and whether I was given a pamphlet about the Registry or not, honestly I don't recall. I certainly don't recall anyone saying to me, "Enrolling in the Registry is important. Please do this." With the presentations yesterday and today, clearly I can see the very sophisticated infrastructure and network of people and organizations that make a gigantic effort to get the word out about the Registry and the systems that are in place to enroll people.

In preparing for my talk today, I also reached out to my local ALS Association Chapter that services me. This is the Upstate New York Chapter, which is very ably led, well-organized, and administered. The Care Services Director there said that like many other chapters, there are many things in place to disseminate information about the Registry whether it is direct mailings, emails, contact through ALS walk events, and face-to-face. These have been set up with the Registry that that chapter has done in, I believe, Rochester and Buffalo and many of the ways that there is outreach. I began thinking, "What then is the breakdown? Why are there patients like me?" I believe I heard one or two other patients yesterday say that they hadn't heard much

about it. I began thinking that I believe the breakdown may be at the doctor/patient interface in the exam room. When I was in practice, when I wanted a patient to do something that I thought was important that wasn't right on their radar, like stopping smoking, exercising, checking their blood sugar, I would take the time to literally text them and say, "Look, I think this is important." I was very old-school. I would write things on a pad rather than leaving it to be buried somewhere in their printed after visit summary. I think the direct communication between providers and their patients is really paramount. I think, like most of you, we believe you will do what your doctor asks of you. Clearly, I think we all know how busy our neurologists are and our primary care doctors. Also lost in the business of a visit is something that maybe the doctor assumes the patient knows about, or they may even know that the ALS Association or the social worker in the multidisciplinary clinic has contacted them.

I think working on mechanisms to make the Registry something more front of mind to the providers could really be, I think, a very important supplement to all of the other things that have been talked about the last 2 days from the 30,000-foot level. Certainly, the important organizations are the "boots on the ground" to do this, but when it comes down to it, I really believe that my neurologist or yours said to you, "Look, I think this is really important. Please do it," I think it's much more likely to happen. Now we all know that many times, instructions given to us as patients aren't always followed. The high degree of non-compliance, not intentionally, but just is human nature. There is very good evidence in the clinical psychological literature of the power of what is termed "motivational interviewing" where the provider figures out what it is that the patient views as an important motivator that makes them change or do something. We all know that we as a group are highly motivated. We're engaged. As many people said yesterday, we want to be involved in moving things forward. If the providers can tap into knowing that we want to not only effect change for ourselves, but for the broader community, I think that will go a long way to helping the doctors remember to mention that in the course of a visit.

During the last years of practicing, at least in my network at the University of Rochester, there were times when a doctor had to close out and sign an office note for billing progress. We were required to attest to that in that certain very important things were done like screening for depression, or screening for fall risk, or screening for abuse. It did not matter whether the doctor did it or a medical assistant did it, but embedded within the EMR system that I know at least half the country uses is called Epic, are ways that you can create hard stops that won't allow the neurologist to close their notes until they have discussed something like the Registry. In closing, nearly 50 years ago, I was a student at Emory in Atlanta, literally right next door to the CDC. Obviously, when I was walking by there or riding my bike by there all those years ago, I couldn't know that I would be here now. But, that's kind of the whole point of this, right? We want to make efforts to improve things for the people who will come after us. On that note, I want to thank Danielle and the whole team for the opportunity to bring this perspective. You're doing an amazing job and I thank you very much for the chance to talk.

Brad Dusek
Temple, Texas

Brad Dusek is 72 years old and was diagnosed in 2018 and also was diagnosed with frontotemporal degeneration (FTD). He played football his entire life, including high school football in Temple, Texas A&M in college, and the Washington Redskins for 10 years. Though he has the lumbar version of ALS and cannot move any of his limbs, he can still speak fairly well. Brad's wife, Marta, presented on his behalf.

Brad was diagnosed in 2018 at the Mayo Clinic in Minnesota. One of the first things that we were told—I mean, they were very thorough—we were told about the ALS Registry with the CDC. We were told about ALS organizations in our neighborhood and who to reach out to about where to get resources. That was wonderful, but very overwhelming I have to say. What you were saying, Joel, was really profound in that it is nice to have a doctor tell you about those things up front and let you know which direction to kind of head in, because these are waters that we've never been on and how to navigate those was kind of scary at first. We received all of this information and a follow-up about a week or maybe two weeks later to remind us about things that were happening in our area and to say, "Have you done this?" We only got one reminder. It would have been nice to have been on a checklist to where we could have gotten something like that. We did go to the Registry immediately and registered with that. I'm an accountant by trade, so I always check lists and do things. I've got Excel spreadsheets everywhere with lists of things on them. Bradley, of course, was overwhelmed by the whole thing. Like Diane, his started in his left arm and then he couldn't do anything with his left hand. Then it went down to his left leg. Once we got into an ALS clinic, he got a chair, which was really nice because we were having lots of falls and that sort of thing—just having that resource available to checklist our way through things. We do get reminders from the ALS Registry that there's things out there. Once we finally got a handle on everything, it's nice to get the reminders. A lot of what we do is, again, overwhelming. We end up doing our own research trying to find help in some way.

Diane Mummaw
Longwood, Florida

Diane Mummaw was diagnosed in November 2015 and is progressing surprisingly slowly. All of her issues have been in her arms and hands. People frequently ask her what she is doing that differs from others with ALS, but there is no magic pill or surprise. Her voice is good, she is still walking, she is still eating on her own, and she is still able to use the keyboard with one hand.

I would say that when I was diagnosed, I'm in Florida, by the way, I was diagnosed at University of Florida and then changed my care to Mayo Clinic in Jacksonville, which is about a 2-hour ride for me. I go every 6 months. I will say that because I went to so many neurologists while I was being diagnosed, I think I went through 6 different ones, the Registry became important when I got the final diagnosis. Two weeks later, I attended my first ALS Association meeting. I tend to be that person to jump on it, do whatever I can do, you know, who knows what this is going to look like in a year or six months. Very quickly in the beginning, the ALS Association support group was tremendous help for me. It was a resource. I will say, my first meeting scared the daylights out of me. One of the patients noticed it and approached me afterward and told me, "You know, it's not all bad news." Almost 7 years later, it isn't all bad news at this point. I think the Registry keeps getting promoted through the ALS Association support group. I know that the person that runs ours, Marissa, is fantastic and she tends to put it in her emails, "Just a reminder. Here's how you do it" and gives you the link. I did it very early on. I do think that

COVID changing everything to being online certainly helped the ALS groups to kind of get together a little bit better rather than being in-person. Although, then there's also the problem that some people are not as technology equipped as I might have been.

I will say, the Registry was pushed very early on and then reminded through the through the meetings. I've been active the whole time, but now I'm doing advocacy for them with the public, the Congress, and Senate in the in the Spring every year. This is something new to kind of get involved in this today. I will say my process of it was very quick and easy. I was still able to just do it myself. I didn't need anybody to do it for me. Every time I do something like that, I think about the patients who are much further along, and their symptoms are much more devastating than mine. I am amazed when I used to go to Washington at the people that were travel from all over the country to be there to advocate. I think that the online ability in some ways is great and other ways, you lose some things with it. My ability to sign into the Registry and gather information—it was really me doing it and disseminating the information to my children, who are adults and have taken care of me and moved into a different situation where I was the mom taking care of everything and little by little as my abilities go away in my arms and hands, I rely a lot more on them and a caregiver that comes into the house. I can never say enough about the ALS Association between the home visit I originally got, to the meetings, to the speakers that come into the meetings, to the group of people that are patients—unfortunately many of whom have already passed. I've learned so much and I think it helped me to prepare early for the things I could and then kind of wait and see what happens as I go on this journey.

It certainly changes your possibilities for the future. I was only 54 when I was diagnosed, so I certainly wasn't looking at leaving work and I certainly wasn't expecting all of the challenges that come with this. Mine did go slow enough that I felt like I could kind of stay on top of it. The fact that I'm the organizer—I'm the one who runs the show in my house, my business, my family—everything about that. I'm that person and it takes something from you when you have to change and become the dependent person. When you've been your whole life the one that other people depend on, it changes your own view of yourself and it changes your day. I had to leave my job. I feel grateful for the fact that I had a good job, and I had good disability insurance, and things like that in place for the unexpected that I never thought I would use. I've had to use it and it just changes you. You have to learn to get help, to take help, to ask for help—even though my changes have come slowly. For everybody in my family, it's just a whole different dynamic now. Just last weekend, not this past, but the weekend before, I started having chest pains for 2 days and I've got a lot of heart problems in my family. But immediately, chest pains don't make you think it's ALS at all, because that's just a whole different thing. Well, emergency room, admitted, test, test—not my heart but an inflammation in the muscles of my chest, which may be ALS-related. There is never a solid answer. It's always a gray area. You just live in this gray area.

Discussion Summary

Dr. Boyce emphasized the importance of efforts such as motivational interviewing and the incentive of giving back to the larger community that Dr. Shamaskin mentioned. She also was struck earlier when Tim Lowry said that a lot of information gets thrown at patients, which raised the issue of when the best time is to receive information and reminders. She was thinking about what Marta Dusek mentioned about being overwhelmed. The diagnosis changes the family dynamics and all of a sudden, the family is talking about this all of the time. This is distracting and can take time away from the time people might spend at the computer filling out surveys, in addition to the impairments that might make it difficult for the person living with ALS to complete them without assistance. Something they have done in rare epilepsy is just sit with someone to

have them enroll live with specific time set aside. As a caregiver to her 12 year old son who has multiple and very profound intellectual and physical disabilities, the best thing someone can do for her is make her MRI appointment for her because she cannot do one more phone call.

Mrs. Dusek said that when Bradley was first diagnosed, they were looking for places where he could get into the research, be a test subject, and do all of that. As his disease has progressed, they have not been able to travel as much to go to places. Surveys are great since they can do those at home.

Dr. Boyce observed that one of the things she picked up from Diane Mummaw's presentation regarded the many people who have become her trusted advisors within the ALS Association and other support groups. In terms of recruiting people to participate and keeping them involved in an ongoing manner, she also emphasized how overwhelming life can be with a diagnosis like ALS. She stressed that she did not think there is enough discussion about the "bomb that goes off" in someone's life when they are diagnosed and the lack of control, vulnerability, and how busy it makes someone. It is overwhelming and Dr. Boyce said she felt strongly that this needs to be heard, especially in terms of asking people to do something that may not feel worth it. It is critical to understand the value in the task someone is being asked to add. It also is difficult to ask one's children to do things for them, given this new relationship that is being forged.

Ms. Mummaw said she is not comfortable living in the gray area. She has had to learn to depend on other people, to ask for help to, and do what she can on her own. However, when it becomes dangerous to even go out to water her plants on the patio when she is home alone for example, she has to remember that she is no longer the person who could just handle it. She has to give those reins over and downsize her life to many less activities because she has to have somebody with her or have someone drive. Just paying bills online is a new transition. She was trying desperately to not have to get another laptop. She has the Tobii Dynavox, but the usage of it is daunting and difficult, so she had to get a new laptop. Now she is out of her wits because she does not feel comfortable on it yet and does not feel like she can maneuver her way around the keyboard quick enough.

Dr. Shamaskin added that another change in someone being diagnosed with ALS is the lack of confidence in whether one is able to still do things well and accurately. That goes hand-in-hand with the physical changes, but when someone is then asked to do something new like signing up for the Registry, it can be daunting. A person's self-worth and self-confidence has been shaken right from the beginning, so understanding that aspect can help inform how people with ALS are approached and engaged given their complicated new life.

Mrs. Dusek added that it is a lot and it is daunting. There is so much to do and learn. As Diane Mummaw mentioned, someone had to figure out whether her chest pain was ALS-related. The Duseks have a list of 8 doctors that they were going to, trying to coordinate, and trying to get in to see. Bradley developed a wound on the bottom of his foot because he cannot move his feet and it was a pressure sore. She had a 6-month appointment to get into wound care. She was super overwhelmed by all of this 2 months ago. She talked to the ALS clinic and told them that she was overwhelmed with his dementia, ALS, and his inability to do things and that he is very frustrated. He does not even remember that he has ALS and has to be reminded of this. He cannot physically do anything. In trying to get wound care, they ended up calling hospice. Now they have a nurse who comes in once a week to help them with everything. They still have to keep everything organized, have certain supplies, et cetera. It is overwhelming like a storm and depends on how the disease affects the person. Bradley's ALS will be slow, then fast, then slow again. He now is unable to drive his chair, so they have to figure out what to do next. He is now

in hospice, so she has to figure out how to get a letter of medical necessity. She is constantly trying to stay ahead of everything to make sure that everything flows nicely. Being able to have the children come over and help would be tremendous, but she thinks some of them are embarrassed by some of the things that they would have to do. Bradley has 4 wonderful children who love their daddy beyond the moon, and it is absolutely wonderful that they will spend time with him, call him, and things like that. However, the minute she has to do something that would embarrass them, they are out the door. She is thankful that they have caregivers who come to the house and do these things. But it is literally overwhelming just trying to navigate these waters. His son took over his home building business and they are trying to assist him in certain ways. Some people they thought were their close friends have stayed with them and some have not. Bradley has a dear friend who cries when he leaves their home because he feels so sad. Bradley is still there, he is still enjoying life, and he is still having a good time. They watch the cows and do all kinds of things, but their lives have changed in dramatic ways.

Related to the impression that children are embarrassed, Dr. Shamaskin noted that he has 3 adult married daughters and 4 grandchildren who all live nearby. He really believes that exposing children and even grandchildren to someone with a disability normalizes that for them. Without realizing it, the Dudeks are teaching their children something important that will make them much more sensitive to the needs of anyone different from them going forward, whether it is a disability or any other features that may make someone different from themselves. He thinks it is natural for the children to be uncomfortable around their father, but the more they do, the more they will become more sensitive to others who are disabled.

Mrs. Dudek added that they have normalized their 9 grandchildren. They love their grandpa's chair and stand on the feet and drive around with him. They hook the wagon to the back of it and he drives them around the yard, which they think is pretty cool.

Dr. Boyce pointed out that a common thread was vulnerability. People like to be a certain way in front of their friends, neighbors, and family. She never let anyone in her house when she was not in full make-up and the house was not neat. That goes right out the window when someone wants to fold your laundry now, but it is hard to give up. Vulnerability can be humiliating for everyone. Circling back to friendships and the community, someone once told her that the reason they were standoffish was because they did not know what to do or how to help. They thought if they were in her shoes, they would not want someone to say the wrong thing to them. She asked Ms. Mummaw to speak further about her community and the new community of support that she touched on earlier.

Ms. Mummaw indicated that she had a large network of people through her job—hundreds of people who she saw on a regular basis. When she was diagnosed, it was traumatic for an entire industry of people who have known her for so long. Her daughter was only 15 and she did not know if she would be around for high school graduation. Now her daughter is graduating from college and has turned into a caregiver. That is a complete change as she did not really have that type of personality before ALS because her mom was in charge, running everything, and she did not have to do any of that. She does it now, is capable, and is good at it. If on her diagnosis day she had been asked what this would look like in 7 years, she would never have thought this is where they would be. Her son is 35 and he is also taking shifts with his sister living with Ms. Mummaw for a year or two, and then the other trades out. They cover weekends and then she has caregivers during the week. In terms of her friend community, she has been very lucky in that she has at least 25 close friends she still hears from at least every other week. Beyond that, there is still a community of people who do not want to say the wrong thing.

Someone may reach out to shake her hand and she says, "Oh, I'm sorry, my arms don't work. It doesn't hurt though, so you can give me a hug. I just can't hug you back." She sees a lot of people at conferences every 3 months or so who have rallied a support system. They have a golf tournament for her once a year to which about 400 people show up. While they are not close in her circle and she sometimes thinks they must be sick of hearing about her after all of these years, they are still encouraged by and rally for her. There were definitely people who told her right from the beginning that they could not handle this and she has never heard from them again, but she realizes that is on them—not her. If they called her after 5 years, she would be happy to have them visit. The people she knows will be there with her until the end are why she thinks she is still doing well after 7 years. She thinks the sooner she can put someone in her community at ease, the better. She might not be able to pour someone a glass of wine when they visit, but they can pour her one, put a straw in it, and she can join them. That gives people the chance to be a part of her life while either her children or caregivers cover things like showers, changing clothes, and other things that are difficult for her to do. There are layers of people who are close who will do anything she needs, those who will sit with her to visit for an hour, those who will donate to GoFundMe or show up at a golf tournament, et cetera. There have been many people diagnosed since she was who already have passed, which was very difficult for their families. She feels lucky to still have some control, but she knows the days are coming when she will have to give up that control. She can still use the computer, get around the house on her own, and other things. She does not know what the future looks like, and that is very hard on her emotions. Will her children be caring for her for 2 more years, 10 more years, what does that look like? No one can answer that. Not one person in the emergency room even knew what ALS is. She was grateful to still have her voice so that she could tell them that she has ALS while they were trying to put the blood pressure cuff on and they could not get the IV in. She had to explain to every single person walking in the room that she has ALS, her arms do not move, and they would need to help her get out of the bed. It seemed very odd that this group of people did not know this disease even though they were medical professionals. She was so desperately trying to make them feel comfortable, she was explaining what they could and could not do.

Ms. Dusek added that in terms of friendships and community, Bradley has got some of the best friends. He still has high school buddies who show up every time. He has some college football buddies who are planning a trip to visit with him. He also still has Washington Redskins buddies who call and email regularly just to communicate what is going on. There are probably over 150 people on email, which is amazing. He randomly gets calls from players he played with in high school, college, and the NFL. They text back and forth and there is a lot of sharing going on. They are good friends who love each other and stick together. While he cannot remember a lot of short-term things, his long-term memory is intact and it is delightful to see him light up when friends call or visit.

Dr. Shamaskin noted that in his work as a primary care doctor, he led a pretty busy life, so they did not have a bigger circle of friends. However, the depth of caring and understanding that even a small group of friends has had has been of great value.

Dr. Boyce asked Dr. Shamaskin to talk about some of the medical experiences that surprised him within the medical community when he became the patient.

Dr. Shamaskin said he thought that even someone who understands medical terms and the process must advocate for themselves and ask questions. He learned quickly not to be embarrassed to ask his own colleagues questions.

Regarding a question about how the Duseks learned about the Registry, Mrs. Dusek indicated that at the time of diagnosis, the Assistant Nurse went through a checklist of things they needed to do, the first of which was to go to the CDC website to enroll in the National ALS Registry. The second was to find an ALS clinic.

Dr. Mehta recalled that when he was giving a talk in October 2018 in Houston, Texas at Methodist Hospital, he met Marta and Brad. Brad Dusek was newly diagnosed and he remembered him saying that he went to the Mayo Clinic to speak with Eric Sorenson who was a neurologist there. Eric Sorenson is a huge supporter of the Registry and Minnesota is the number one state in the entire country, so he was not surprised that he told them about the Registry. Unfortunately, the pandemic has limited the ability to conduct outreach in person. He emphasized how amazing the patients with ALS and their families are. Dr. Mehta, Jamie Raymond, and Brunet-García visited the Dusek ranch in Temple, Texas. Marta and Brad were gracious hosts and they met their families and grandchildren. They shot the video of Brad and his family there. He expressed gratitude to all of the patients and their families for sharing their experiences.

Andrea Pauls Backman asked what the best and worst advice was that patients were given, which she noted she was asking as a professional advocate and as a caregiver to her mother who died of ALS and she remembered well what they went through. She also thanked the patients for their contributions during this meeting.

Ms. Mummaw said that the best advice she was given was from another ALS patient at her first support group who said, "It's not today. It's not that bad. Just breathe and take things as you can. Don't try to do everything right away." She thought that was really good advice. She is also on a lot of ALS Facebook groups and has found sometimes that she has to separate herself from it, because there is a lot of needed conversation about where people are when they are further advanced, but she is not there yet. At first, she wanted to know everything about what that looked like down the road, but she found that it was too much and was overwhelming to her. As it turns out, it was probably good she did not spend a lot of time on what it looks like in the last 6 months. Since she is not a medical person, a feeding tube is scary. That was overwhelming. While it is good to be prepared, she knew she would be involved in falling, being in a chair, and so forth. The early advice to take a breath and deal with the things as she could was very good and helped her segregate in her mind where she was, what the next couple of months might look like, and the next, and so forth instead of trying to look at the whole plan and impact at once.

Dr. Shamaskin said the best advice he received early, despite the desire of the care team locally to do everything for him, was that he would need to pursue studies and other broader activities on his own. When someone told him he would have to decide what he would have to cut back on soon, he fortunately ignored them. He has been fortunate that his disease has progressed slowly and he has a lot of family around him, so he has been able to keep doing basically everything other than work in the office.

Mrs. Dusek said the best advice was probably when they met Dr. Mehta in October at a symposium. The best advice there was being able to see everybody and see how they always were so happy and joyful. They realized that it was not the end of the world and that they should take one day at a time and be patient. The worst advice was to sign up for everything. They do get slammed with a lot of stuff and it is just too much. She has learned to pace herself better. She agreed with what was said about social media. She has to stick with the positive people

who are uplifting, and they have not limited themselves and allowed others to plan their next moves.

Dr. Boyce emphasized that social media could be an incredible blessing, but also a curse. People need to learn social media literacy rules, health literacy rules, et cetera. People like to give a lot of advice that applies to them in their life on medications, supplements, and things to try. It can be overwhelming to sift through. However, if not for social media, a lot of them would not know each other and have the connections they do.

An inquiry was posed about whether CDC provides an educational webinar explaining the Registry for multidisciplinary teams that could be used by any staff member (e.g., physician, nurse, social worker, physical therapist, office staff, pharmacists, et cetera).

Dr. Mehta indicated that they do have a training webinar for chapter clinic staff that is available on the partners' link on the website. This will be updated some time in 2022, given that the information is about 2 to 3 years old. A lot of the information is still relevant, but some needs to be updated. The webinar does not specifically talk about the clinics because that is typically done by the ALS Association, Les Turner, or MDA that they sponsor or support. The CDC webinar talks more about how to enroll in the Registry and so forth.

Gudjon Sigurdsson expressed gratitude for the time and willingness to speak on behalf of everyone with ALS. Doctors need to learn more about communicating with persons in general. People with ALS are human beings—not just numbers or things. He said that Marta is one of the angels they have, but they need to do better to help their helping angels.

Mrs. Dusek indicated that she completed the enrollment in the Registry for Brad because he was confused by most of it, but she did not find it daunting at all. Most of it was pretty easy for her to slip in between all of the other things they were doing.

Ms. Mummaw said it was so long ago that she did not even remember doing it, so it must have been easy at the time since she did not recall having a problem.

Dr. Stommel said he thought it would be useful to have a printable pamphlet that patients could take home with them with information about the National ALS Registry. Most medical centers have color printers. It would be useful to have some kind of pamphlet to give patients each time they visit the clinic. Most ALS clinics have a social worker who could do this and bring up the subject with patients while they are in the clinic.

Dr. Boyce said that having worked with Epic quite a lot in her career, she was thinking about clinical reminders in the health record.

Dr. Mehta noted that all of the materials on the Registry can be easily ordered by physicians or clinics free of charge. This includes high quality glossy information, infographics, brochures, and so forth. They also are developing a folder that will include all of this information. The plan is to assemble a folder and mail 50 folders to each clinic.

It was noted by an attendee in California that it had been interesting to attend and see so many representatives and researchers tied to the Midwest and East Coast. It seemed that the Registry champions (Minnesota, Illinois, Michigan) are located in these regions. The West Coast had to tune in at 5:00 AM to even attend this symposium. California is also a low tier state. This raised a question about how this regional disconnect is experienced and explained by the Registry.

Dr. Mehta responded that California has been an enigma for a long time. Oregon and Washington State are Tier 1 states that do a very good job. California is a large state and there are 2 or 3 chapters of the ALS Association and a number of MDA clinics that cover California. It is a challenge to do the outreach there. ATSDR is working with ALS Association and the MDA to make a more concerted effort in California. There is discussion underway about more roadshows, webinars, and so forth. It is unfortunate that California has been such an enigma, particularly given that it is a very diverse state.

Regarding a question posed about whether there has been any consideration about tracking gene-positive but asymptomatic patients, Dr. Mehta indicated that there is a study currently underway by Michael Benatar at the University of Miami called the ATLAS study. This study is sponsored by Biogen using their SOD1 drug, tofersen, to determine whether it can delay onset or slow progression of ALS when initiated in pre-symptomatic SOD1 mutation carriers.

Wrap-Up, Adjourn

Paul Mehta, MD
National ALS Registry, Principal Investigator
Registries and Surveillance Section, OIA
Division of Toxicology and Human Health Sciences
Agency for Toxic Substances and Disease Registry

Dr. Mehta thanked everyone for their attendance and contributions. He emphasized that ATSDR is listening to everyone's suggestions and will make changes for the better. He expressed gratitude to Dr. Boyce for serving as the amazing moderator, thanked her for her time and effort, applauded her for having a pulse on the community as well as their respect, and expressed hope that she would serve as moderator for the 2023 National ALS Annual Research Symposium and Meeting.

Danielle Boyce, DPA, MPH
Moderator
Johns Hopkins School of Medicine

Dr. Boyce emphasized that it was her great pleasure to participate and that they should all move together to make this beautiful Registry as great as it can be. She said she felt that they had forged many bonds with the ALS community, heard many great comments and suggestions, and accomplished what they had set out to do during this meeting.

D. Kevin Horton, DrPH, MSPH, CPH
Chief, Chief, Environmental Health Surveillance Branch
Agency for Toxic Substances and Disease Registry

Dr. Horton stressed that this is a collective effort among ATSDR, the National ALS Registry, researchers, organizations, patients, and others who can talk about and promote the Registry. It is more challenging in the US to find cases, given that there is not socialized medicine as in Sweden, Denmark, and other European countries. When the US National ALS Registry was first launched, ATSDR quickly realized that Registry promotion is difficult, people will only come to the Registry if they know about it, and that promotion efforts must be scaled up. ATSDR has worked on promoting the website through social media, webinars, road shows, and working with partners. He welcomed input via email to him or Dr. Mehta with other ideas for reaching people. He added his gratitude for a great meeting and expressed appreciation for everyone's efforts.