Ph.D., Associate Deputy Director, National Institutes of Health (NIH) Diana Bianchi, M.D., Director, NICHD

Meeting co-hosts Dr. Parisi and Dr. Schramm opened the meeting at 10:00 a.m. ET. Dr. Parisi welcomed the attendees to the two-day meeting, which was sponsored by the Office of the Director (OD), NIH, in conjunction with the Trans-NIH INCLUDE Project Working Group (WG). Dr. Parisi introduced Dr. Bianchi, Director, NICHD; Dr. Schramm, Program Officer, NHLBI; and Dr. Schwetz, Associate Deputy Director, OD, NIH, Co-

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Dr. Schramm provided additional details regarding INOLUDE funding and awards. Funding for INOLUDE was

other clinical trials in DS. Several key outcomes resulted from the meeting, including the need to identify the appropriate endpoints and biomarkers to measure for the development of AD in DS and a time frame during which efficacy of those trials can be assessed. The meeting also pointed to the need for greater emphasis on harmonizing measures across studies to maximize productivity, discussion of infrastructure to support clinical trials in adults with DS, and strategies to address barriers to recruitment and retention.

Another workshop, Planning a Virtual Down Syndrome Cohort Across the Lifespan Workshop, was held September 23 24, 2019. The goals of this meeting were to bring together clinicians, researchers, advocates, self-advocates, and data scientists to learn from existing DS cohorts in order to create new cohorts and to inform the DCC. Sx cohort WGs were formed as a result of this workshop, including four focused on data standardization and harmonization needs (learning from existing cohorts, looking at Global Unique Identifiers [GUIDs]/linkages, defining a minimal common data set, and establishing biospecimens and biorepository linkages). The focus of the other two WGs were community outreach efforts, including ways to incorporate underrepresented populations, and clinical trial readiness. The WGs included members of the clinical, basic research, and DS communities. Dr. Schramm noted that the groups worked diligently from September of last year until May of this year and developed several valuable products that would be presented and reviewed during Day 2 of the current meeting.

A third workshop, Ginical Trials in Down Syndrome for Co-Occurring Conditions Across the Lifespan: Virtual Workshop, convened May 7 8, 2020. The discussions at this meeting focused on co-occurring conditions from the pediatric population through the aging population, considerations for participation of people with DS in clinical trials, non-pharmacologic and lifestyle interventions in DS and highlights of NIH funded trials and dinical awards made under the INCLUDE project. Some of the outcomes from this workshop included the need to establish clinical guidelines for aging and dementia in DS Dr. Schramm noted that a set of guidelines has already been published; this effort was spearheaded by the Gobal Down Syndrome Foundation (GLOBAL), a U.S-based nonprofit dedicated to improving the lives of people with DSthrough research, medical care, education, and advocacy. Participants at this workshop also concluded that greater emphasis is needed on the importance of collaboration, outreach, engagement, and very importantly, trust in the research community, and that further discussion is needed on the infrastructure and tools to support dinical trials, including existing resources and trial networks, Some of these resources include DS-Connect® The Down Syndrome Registry (DS-Connect®) and PTN, which are funded by NICHD, and the Alzheimer's Clinical Trial Consortium, which is funded by the National Institute on Aging (NIA). These resources are existing support mechanisms designed to help facilitate clinical trials, specifically for DS

The current workshop addressed INQLUDE Components 1 and 2. Component 1 focuses on basic science studies and model systems in DS and had not been a main focus in the INQLUDE workshops to date. INQLUDE leadership agreed that Component 1 should be a central theme of a workshop that addresses co-occurring conditions in people with DS and that the workshop should explore the dynamic interplay between basic science and cohorts formed to generate new basic science questions. This dynamic reflects an iterative cycle between the basic science and the clinical aspects of DS A workshop planning committee was formed, and these complementary aspects of DS research were the foundation for the development of the agenda and title of the current workshop.

Dr. Parisi provided an overview of the workshop agenda and meeting logistics. Day 1 would include keynote presentations on research study participation by people with DS and their families, brief presentations from WGs on the state of the science and gaps in basic science and cohort development, breakout sessions, and a report-back session from the breakout groups. Day 2 would include two concurrent discussion sessions one focusing on basic science and one focusing on cohort

development a report-back session from the breakout groups, and a panel discussion with six experts who have extensive experience working in the DS community.

A workshop summary will be published, and the outcomes and recommendations from the meeting will be incorporated into the NIH Research Plan on Down Syndrome, as noted by Dr. Bianchi. Dr. Parisi wrapped up her presentation by thanking the members of the <u>Down Syndrome Consortium</u>, which includes the 18 NIH ICs that are part of the INLCUDE project and 16 advocacy and professional organizations with an interest in Down syndrome. Dr. Parisi also thanked members of the workshop planning committee and the INCLUDE Steering Committee, NIH and contractor staff, the IT support team, and all of the speakers, breakout session leaders, and panelists whose commitment and involvement are essential to this work.

Megan Bomgaars, Self-Advocate, and Kris Bomgaars, Parent Nora Chesnut, Self-Advocate, and Emily Chesnut, Parent

Drs. Bianchi and Parisi introduced the family members and self-advocates with DS who were the keynote presenters for the meeting: Emily Chesnut and her 9-year-old daughter Nora and Kris Bomgaars and her 27-year-old daughter Megan. Megan and Nora have enrolled in a number of clinical research trials and shared their experiences as study participants.

Emily lives in Oncinnati, Ohio, with her husband, Brian, and their four children, including Nora and her twin sister, who does not have DS Emily is an IT project manager for Oncinnati Children's Hospital, and in her free time, she advocates for people with special needs through a variety of eds *n7 Tm0 g0 0 1 148.94 488.11 Tm6(

- Is there invasive testing?
- Are blood samples taken? Collection of blood samples can be distressing and affect decisions about future participation.
- Are urine or saliva samples taken? These specimens are easier to collect and usually do not present the same concerns as blood draws.
- For any study with blood samples, Emily will agree if they are in conjunction with other required samples and if Nora or her siblings (when they are study participants) understand and consent on their own.

Time commitment:

- With full-time work and the kids in school, it can be difficult to fit in a 2-hour round trip to the study site plus the time for a study visit.
- Studies where tests and activities can be done remotely or partly at home are easier to participate in.

Sharing of results:

Some of the studies Nora participates in involve cognitive testing. The investigators on these
studies are often able to share the test results, which, in turn, better inform her
individualized education plan (IEP) team.

Emily said that as a family of someone with DS, they have benefitted from the many who journeyed before them to pave the way and that it is now their responsibility to improve the path for future families.

Kris continued the presentation by noting that many of the experiences she and Megan have had parallel those of Emily and Nora. Kris added that, with the additional years of being a family of someone with DS and as an educator, she has learned and come to appreciate how current research not only will benefit kids in the future but also benefits Megan and other young people with DS today. That is one of the reasons for the strong commitment she and Megan have to this research.

Megan said that her favorite subject is science and that she loves to talk with the 10012 reW*nBT/F1 11.04 Tf100172

without requiring invasive procedures. It is helpful to be able to talk with the investigators or study coordinators about what a study involves and to see which parts of the research are required versus optional.

Kris and Megan noted how fortunate they are to be so close to GLOBAL. In addition to participating in clinical research, Kris and Megan have also been able to help with raising money for DS Just one of the Be Beautiful Be Yourself fashion show, the single

addition to DS-specific Facebook pages, the following hashtags were suggested for Twitter, Instagram, and other outlets: #DownSyndrome, #DontLimitUs, and #DownSyndromeAwareness.

An attendee commented that the feedback from the keynote presenters suggested the need for scientists and researchers, including those at NIH, to try to make the results of research understandable and clear so that families can appreciate some of these scientific advances and what is being learned from all the research that is being pursued.

The families were also asked about lessons they have learned that they would suggest researchers not do. All agreed that it is important to not to lose sight of the impact on the individual outside of the lab or the dinic. A gap area that researchers should consider is to provide individual results to those who participate in a study if it helps their medical care, as well as some kind of summary on the overall findings of that research project. Study results and information should also be shared across social media after being translated for the general population. Media is the most powerful form of advocacy, and the families have learned from their experiences that if you want to impact the world, use of media is how it is going to be done. A range of formats should be considered. Video presentations in particular are engaging, but written materials and media campaigns are also means of conveying important information to broad audiences.

In response to a question about availability of her book, Megan said the launch is planned for next summer, after which the book will be available everywhere.

The following questions were directed to Dr. Bianchi: Where do you see the future of NIH INCLUDE funding in the next 5 to 10 years, and what are some of the goals of INCLUDE that have not yet been realized? In response, Dr. Bianchi noted that is it difficult to anticipate funding that far out and that the focus at this time is on the current fiscal year. She hopes that the productive relationship with members of Congress who are very supportive of research in DSwill continue. NIH will continue to work with families and advocacy groups and also continue to communicate the progress being made under INCLUDE and other programs with Congress. She pointed out that NIH cannot ask Congress for money, but that leadership can convey how productive NIH has been with the money appropriated to the s. In terms of

gaps, Dr. Bianchi said the NIH is looking in part to participants in workshops such as the current one to inform the research going forward. She noted her research laboratory at NIH, which focuses on treatments in animal models and in stem cells to identify interventions that can safely be given prenatally. She also serves as Co-chair of the INCLUDE Steering Committee. Dr. Bianchi pointed to the complexity of DS and that the three main goals of INCLUDE are designed to address multiple gaps in the research. She asked participants to review the NIH research plan on DS, which will be updated at the end of this year.

One of the working group presenters, Christine Seidman, M.D., said that scientists and clinicians greatly appreciate all that the DS community does by participating in research. She pointed out that through their participation in research studies, people with DS and their families contribute to important discoveries that, in turn, help investigators understand conditions in people both with and without DS, such as celiac disease and other autoimmune disorders.

Dr. Parisi closed this session by thanking the presenters, particularly the keynote speakers. Putting faces to the work that many investigators do, especially in the basic science realm, helps make it more meaningful and easier to appreciate what the research community is doing and to understand and appreciate the contributions that people with DS, their families, and the advocacy community at large are making to help advance understanding in DS.

Eight topic-based WGs convened before the current meeting to discuss and identify key issues, advances, and gaps in the following areas of DS research with respect to basic science and cohort development:

Neurodevelopment structure, cognition, and language Behavior autism, ADHD, and regression CVD and pulmonary hypertension Respiratory and airway conditions (including OSA) Cancer risks for leukemia and resilience to solid tumors Autoimmunity and infections Endocrine, metabolic, and skeletal conditions Aging and AD

Each of the WGs was asked to address the following two questions during their pre-meeting sessions:

- 1. What is the current state of the science and the research gaps with regard to basic science?
- 2. What is the current state of the science and the research gaps with regard to cohort development in this domain?

some extent, but the largest number of studies on language peaked in childhood. The highest number of

The prevalence of ADHD in persons with DS ranges from 10% to 45%, based on varying methods of ascertainment. In contrast with ADHD in TD people, which is more common in males and typically worsens with age, ADHD in DS is not related to gender or age. Various methods and sources of information are used to diagnose ADHD, including rating scales; clinical interviews; the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5); and chart reviews. Reports suggest significant disparity in medications used to treat of ADHD in DS In a small within-subject study of guanfacine in children with DS and ADHD, clinically important target behaviors (e.g., irritability, hyperactivity) were reduced, the medication was generally well tolerated, and the incidence of treatment-emergent side effects remained low.

Based on case reports, the prevalence of regression in DS is low. Diagnosis is made using a 28-item symptom checklist developed by GLOBAL through interrogation of literature comparing clinical indicators in people with and without regression. Data suggest that regression is certain stressors, particularly in the younger population with DS, but that onset in middle-age adults is more idiosyncratic. There is some evidence for natural recovery of symptoms of regression.

NIH has several funding opportunities in support of cohort development for the DS and behavior domain. A series of R01-funded projects are working together to phenotype children and young adults with DS to try to harmonize and provide linkages for constructs and measures when appropriate. U grants are similarly focused on harmonizing measurements in the aging population with DS. Efforts are also underway to expand TD cohorts to include people with DS (e.g., PTN, the Infant Brain Imaging Study [IBIS]).

Primary research gaps and needs in cohort development across conditions included the following:

Multi-site natural history studies that follow participants from early childhood through young adulthood and include milestones of development are needed to inform regression or loss of skills in DS

Consistent methods of diagnosing conditions and evaluating the validity of these methods are needed. This effort needs to include validated methods that allow for differential diagnoses of co-occurring conditions in DS and clinical expertise to make these diagnoses.

More data are needed to better understand the prevalence of each disorder, developmental emergence, and symptoms in young adults, along with how family history might contribute to these conditions.

More studies need to focus on identification of developmental risk factors and their impact on diagnosis and treatment.

Harmonization among cohorts across the lifespan include developing/recommending standards for diagnosis or screening, using shared data points and shared measures, and establishing data linkages and linkages to omics studies.

More data are needed on the impact of these co-

life, aging process, and risk and onset of dementia.

Mental health conditions (e.g., anxiety and depression) should be considered when using the ehavior e with DS

Efforts should be made to ensure that a rare form of DS mosaic DS is included in study cohorts.

The WG also identified the research gaps and issues for each of the three co-conditions in DS. ASD: Efforts should be made to include people with DS in autism cohorts that currently do not enroll persons with DS Comparisons of ASD in DS with other genetic syndromes (e.g., FXS) may inform whether or not autism is present in those syndromes. Clinical assessments such as the Autism Diagnostic Observation Schedule (ADOS) may capture other aspects of DS and lead to inflated scores and a potentially higher presentation of autism. It is also important to be able to differentiate between overall low functioning and higher functioning with lower social and communication skills.

ADHD: There are gaps in understanding how children with ADHD and DS compares with TD children with ADHD. Studies are needed on whether poorer outcomes are seen in ADHD +DS versus

Of interest is evidence showing that while many patients with DS have risk factors for common atherosclerotic disease, including elevated triglycerides, obesity, diabetes, and a sedentary lifestyle, they have a lower incidence of coronary artery disease and vascular and atherosclerotic diseases, which predispose them

of established PH in subjects with DS

Gaps in clinical research include:

Limited understanding of disease-specific mechanisms in many pediatric pulmonary vascular disorders, including DS. The following issues warrant further investigation.

- o Risk based on issues related to lung airspace and vascular growth
- Specific roles of hypoxia, hemodynamic stress, inflammation
- Impact of diverse co-morbidities associated with DS

The need for comprehensive multi-site database and biorepository to more fully characterize the natural history and outcomes of PH in children with DS.

A lack of well-validated endpoints for assessing risks and response to therapy. More data are needed on the use of PH drug therapies for PH in children with DS

Development of infrastructure for performing multicenter trials in children with DS and PH. The need for enhanced and interdisciplinary training in clinical care and research, especially as applied to PH.

In summary, improving outcomes of PH in neonates, infants, and children with DS presents many challenges due to persistent gaps in understanding of basic disease mechanisms, especially as related to cardiopulmonary development; insufficient characterization of disease-specific phenotypes and biomarkers of disease risk, mechanisms, and progression; and the need for sufficient infrastructure to promote multi-site, interdisciplinary registries to optimize clinical care and research, including multicenter trials.

Ignacio Tapia, M.D., Children's Hospital of Philadelphia Emily DeBoer, M.D., Children's Hospital Colorado

Dr. DeBoer noted that animal models present good opportunities for basic and translational science for this domain. Several animal models, including three mouse models that mimic some of the traits of DS, such as abnormalities in lung and pulmonary vascular development, are currently available. One model is particularly relevant for studies of pulmonary vascular disease, PH, and airway and lung development. A rat model is in development for similar investigations of the airway and lung abnormalities in people with DS.

The WG identified a series of opportunities related to gaps in the area of basic science:

Use induced stem cells from people with DS to grow specific cell lines of the airway.

Use mouse and other animal models to look at aspirations and the effects on lung inflammation.

Look at molecular signals governing airway and airspace development.

Collect and preserve pathology samples either from surgeries or deceased patients, particularly lung tissue.

Pursue information on gastrointestinal (GI) tract function at the cellular level in DS Pursue related immunology questions, including research questions focused on bone marrow swap and signatures of interferon expression or blocking and whether expression or blocking differs based on stimuli (e.g., respiratory syncytial virus [RSV], other viruses, bacteria). Explore cellular immunity differences and their relationships with pulmonary disease. Explore gene expression differences in pulmonary disease in people with DS versus people without DS

Dr. Tapia summarized the current state of the science regarding clinical research and cohort

development to address the array of problems facing this population. Epidemiologic studies indicate that pneumonia is a significant cause of morbidity and mortality at all ages in people with DS and that underrepresented groups (Blacks and Hispanics) have worse outcomes. Risk and severity of pneumonia can be increased with co-infections from RSV, influenza, and COVID-19. Several cohort studies are underway, but most are still single-site trials. Assessing pulmonary outcomes in people with DS is are difficult, and spirometry (the gold standard) may not be feasible in this population. A study by Dr. evaluating measures of pulmonary morbidity for clinical trials. Seep studies show

promise and are recommended for people with DS. Ongoing investigations are exploring which sleep measure and outcomes are feasible for this population. Novel therapies being studied include a hypoglossal nerve stimulator trial, a feasibility study of home sleep

The Intersection of Basic Science and Clinical Cohort Development

Dr. Parisi thanked the presenters and opened the meeting to questions and comments about the first four WG presentations.

One question for the Neurodevelopment WG was whether there have been any studies of the brain and executive function tasks in infants with DS Dr. Bhattacharyya noted the importance of being able to observe in real time what is happening in the brain as a person carries out a task or executive function. However, only a few small, older functional studies have been conducted in this area. Many of the new imaging projects funded by INCLUDE will conduct functional imaging studies to expand this knowledge base. Dr. Lee agreed that the literature on functional imaging in DS research to understand neurodevelopment is limited. However, some progress is being made. Functional imaging studies to date in DS have used magnetic resonance imaging (MRI) to look at brain functioning at rest, when people are lying in the scanner and looking at patterns on a screen. A handful of other functional MRI (fMRI) studies have looked at tasks such as language comprehension and object recognition. Other studies have used EEGs to assess brain activity during functional tasks. Dr. Lee s team has received a grant to use an alternative neuroimaging technology, fNIRS, to look specifically at executive dysfunction in DS and its neural correlates in this population.

Other INQLUDE-funded research is using resting state fMRI to understand more about brain function in infants and very young children with DS. One team at Washington University is able to obtain neuroimaging results in infants by scanning babies during their normal napping or sleeping times. The group is following the changes in brain structure in infants and toddlers from 6 months to 24 months of age to better understand neurodevelopment early in life.

A follow-up question was why the study of executive function has lagged for the DSpopulation given that this is an area where there is increasing recognition of the importance of study in children without DS and those with ADHD. Dr. Lee noted that functional imaging is a newer area of research. In addition, original studies of executive function focused on adults who had brain injuries; over time, tasks have been developed that can be used to measure executive function in increasingly younger children. How

cited studies showing less prominent carotid interval thickening in people with DS compared with the general population, regardless of age or other co-morbidities. Other studies show that blood pressure is lower in people with DS than in those without DS. These and other factors contribute to vascular disease, including vascular disease associated with AD. Whether there are other cardiovascular factors contributing to AD in people with DS or there is a more neurocentric component of the AD phenotype in DS is not clear and warrants further investigation.

A question for the Behavior WG was how is regression distinguished from accelerated aging in DS, and is there an age point that can help separate these two phenotypes. Dr. Esbensen said this question points to an important gap in knowledge of these conditions, in particular, in being able to categorize and define regression. She pointed out that regression in DShappens very quickly and is manifested clinically, with sudden loss of language, motor, and self-care skills character and personality. It is seen in teens and young adults, and is therefore different from accelerated aging. Trying to quantify this phenomenon has been difficult. Some researchers in the field have taken the approach of looking at regression in DS as a type of disintegrative disorder as distinct from catatonia.

Another participant noted that many conceptions with T21

The co-occurrence of DS and leukemia has been known for decades. A link between DS and leukemia was initially reported in 1930, and the first systematic study of the risks for leukemia and persons with DS was conducted in 1957. Data show that people with DS are 20 to 150 times more likely to develop leukemia than their peers without DS, with an cumulative risk of about 2% by the age of 5 years. About 2% and 10% of all cases of pediatric acute lymphoblastic leukemia (ALL) and pediatric acute myeloid leukemia (AML), respectively, are in children with DS.

Multistate linkage registries show that development of AML in children with DSstarts early in life, continues to increase, and then levels off after about age 5. The estimated relative risk (RR) in DS children using these data is 136, reflecting very strong risk of developing AML in this population. The type of AML that occurs most often in people with DSis acute megakaryoblastic leukemia (AMKL), also referred to acute myeloid leukemia associated with DS(DS-AML). People with T21 frequently have a *GATA1* mutation that leads to transient myeloproliferative disorder (TMD), which is found in 5% to 30% of neonates with DS About 20% of TMD cases in infants with DS spontaneously go into remission, leading to a state of non-leukemia. Outcomes for children with DS-AML are excellent; pediatric patients with DS often do much better in terms of relapse and survival than their peers without DS. These outcomes, however, decline with increasing age.

Data from cancer and congenital condition registries show a pronounced risk for ALL in pediatric patients with DS, with children with DS about 25 times more likely than those without DS to develop ALL. In contrast with DS-AML, however, the risk for ALL in patients with DS (DS-ALL) is low in infancy, starts to take off after about age 2, and steadily rises throughout childhood and adolescence. DS-ALL has a distinct immunophenotype and a distinct spectrum of genetic alterations, most commonly alterations in *CRLF2* and *JAK*. Unlike children with DS-AML, children with DS-ALL have poorer outcomes than children without DS Data show that children with DS-ALL have an increased risk of both relapse and treatment-related mortality and are more likely to have treatment-related toxicities such as severe infections, mucositis, and hyperglycemia compared with children without DS. The increased likelihood of treatment-related is also associated with higher rates of induction-related mortality and death in remission.

Further data show that people with DS are much less likely to develop a third set of cancers, solid tumors, compared with their peers without DS A Danish study of people with and without DS (from birth to more than 60 years old) reported a standard incidence ratio of 0.45, suggesting that people with DS are much less likely to develop solid tumors than people without DS Analysis of data in the linked cancer and congenital condition registries showed a similar risk of developing solid tumors in children regardless of whether they had DS

Key research gaps are reflected in the following basic science questions:

How does T21 contribute to increased risk of leukemia?

Why do some children with DS develop ALL or AML while others do not?

What is the DS-associated tumor microenvironment? What is unique to this population in relation to cancer risk?

What is the role of immune system in the background of DSon leukemia development? Does T21 lead to the rapeutic dependencies?

What is known about toxicity outcomes during and after therapy?

Is there truly a resilience to solid tumors, and if so, why?

What are the best animal models for evaluating cancer risk in a DSbackground?

association study (GWAS) using a sample of approximately 500 patients with DS and close to 1,200 controls. No significant loci unique to the DS population were identified. A limitation of the primary genotyping technology was that no variants on chromosome 21 were assessed. The investigators addressed this shortcoming using other methods and still found no variants or loci of note. Given these results, it is not clear how chromosome 21 increases the risk of ALL

therapies, DS and multidrug resistant infections, potential long-term interactions between infection, autoimmunity, and cancer, and long-term interactions with other co-morbidities (e.g., OSA, lung disease, OHD, thymectomy). What is going on in the immune system provides scientists with important information regarding when co-morbidities in DS might develop and which features have

Clarifying the role and effectiveness in patients with DS of treatments used in patients without DS

Better understanding the impact of supplements on the immune and metabolic systems in patients with DS

A growing body of evidence points to emerging broad interactions of the immune system with neurological and metabolic

and bone strength. Bone mineral density (BMD) tends to be underestimated in this population because of short stature. Although overall risk of fracture is higher in older versus younger people with DS, sexbased differences have been observed. Boys and men with DS appear to have low BMD, raising the risk for fractures. Girls and women with DS appear to preserve BMD and are at lesser risk of fracture than their male counterparts. birth much earlier in life than in the neurotypical population which shifts the time curve for amyloid accumulation to much younger ages in the population with DS Tau pathology begins in the 40s and is a better predictor of cognitive function than amyloid.

In contrast with the general population, in which the onset of clinical signs of AD typically occurs at a median age around 75, onset of symptoms in people with DS can start in the early 40s. AD pathology in people with DS is similar to that of both LOAD and

o Involve university/hospital

assessed with respect to the immune system as well, possibly with cross-comparisons and through monitoring of aging and the immune system. Dr. Khor agreed that this in an important issue that deserves further investigation, including in connection with COVID-19, and specifically whether there is evidence of advanced immune-related

studies

groups. Despite evidence that DS youth can start to lose skills during this phase of their lives, studies have not explored whether such changes are related to a specific neurodevelopment trajectory or, for example, the relationship between the facial features and oral structure in the DS population and their impact on speech and speech and language development.

Studies also need to consider social aspects and development during adolescence, including selfadvocacy and language that supports self-advocacy, changes associated with puberty and sexual development, and whether young people with DS are more susceptible to inflammatory processes during this important transitional period than adolescents without DS. Studies that look at inflammatory markers and the impact of hormonal changes during puberty and longitudinal assessments of brain development in people with DS and TD people could provide answers to many of these questions.

Additional issues that need to be addressed include how cortical and subcortical pathways are expressed phenotypically and expanding neuroimaging studies to look beyond microcephaly to obtain more specifics regarding brain structure and identify which regions of the brain are activated in people with DS. The relationship between facial features and oral motor structure and impact on speech should also be studied.

Studies of non-psychiatric co-morbidities are needed to characterize inter-subject differences and variations within the DSpopulation and define the b

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The group suggested collecting samples following fetal demise and demise of children with DS when possible. Fetal loss early in pregnancy is not well understood, and the factors that impact loss of a fetus with DS versus pregnancies that reach full term warrant further study. One area of interest is the role of placental development and whether subtle deficits in this tissue lead to fetal loss.

The role of physical activity in people with DS was also discussed, with a focus on collaborating with Special Olympics in designing studies that access DS datasets. From a bioengineering perspective, data are needed on how blood flow and pressure affect tissues, including at the subcellular level, and function.

There was considerable interest in taking advantage of some of the ongoing COVID-19 vaccine trials. Efforts are underway to try to coordinate with CDC to include the DS population in those studies. In more general terms, there is a lack of data regarding immune evaluation and vaccine responses in DS

Clinicians in the breakout group identified ways to overcome barriers in terms of pulmonary testing. For example, the 6-minute walk test, which is used to assess heart, lung, and other health problems and treatment for those conditions, is not always practical for people with DS Shortening the test to a 1- or 2-minute walk might be feasible while still allowing for clinical assessment. Spirometry is considered the gold standard of pulmonary function tests bu

breakout groups and workshop participants for their thoughtful ideas and recommendations that reinforce the core components of INQLUDE. He pointed to the multi-disciplinary longitudinal observations from DS studies that promote data sharing and comparisons that resonate across organizations and with the mission of NHLBI. With advances in technology, this work can be done at scale for both common and rarer disorders. Dr. Gibbons closed his remarks by saying he was looking forward to Day 2 of the workshop and delving further into the coordination efforts that are needed to establish the infrastructure and collaborative expertise environment to realize the goals of this workshop.

Charlene Schramm, Ph.D., NHLBI

Dr. Schramm welcomed the attendees and gave a brief recap of the Day 1 session, which began with Dr. Bianchi providing an overview of the INCLUDE program, followed by a presentation by Dr. Parisi and Dr. Schramm describing both current INCLUDE funding opportunities and INCLUDE projects that were funded over the past 3 years.

Two families participating in DS research offered a personal perspective about the importance of engaging with participants throughout the course of the dinical trial, making the experience personal

The meeting broke into two concurrent sessions, one to discuss basic science and the other to discuss cohort development.

Dr. Schramm welcomed the attendees to the basic science session, co-chaired by Anita Bhattacharyya,

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Roger Reeves, Ph.D., Johns Hopkins University

Dr. Reeves commented that animal models are simpler than working with humans, but they are not simple. The possibility of controlling and measuring subtle phenotypes is a strength of animal models, but it needs further refinement.

DS mouse models have phenotypes that include hippocampal and forebrain deficits in learning and memory, craniofacial anomalies, congenital heart defects, and bone formation deficits. However, the prenetranation deficits. However, the prenetranation deficits. However, the prenetranation deficits are say destruction of the prenetranation deficits. However, the prenetranation deficits are say destruction deficits are say and the prenetration deficits. However, the prenetration deficits are say and the present deficits are

Gaps in mouse models include incomplete penetrance; that is, the DS phenotypes appear in some of the mice within the model but not all. However, this reflects what happens in human DS, where there is a high degree of phenotypic variability among individuals.

Other gaps include not having complete information about the genetic background and the sex of the mouse models used in studies and not having enough genetic information such as the gene dosage and genome topology.

Anita Bhattacharyya, Ph.D., University of Wisconsin Madison

Dr. Bhattacharyya discussed how iPSOs have been used in research, the challenges of using them, and future directions of research with iPSOs.

Among the advantages of iPSOs is that it is possible to obtain the somatic cells of people from different racial and ethnic backgrounds and different clinical characteristics to produce stem cell lines that are representative of the population. Also, iPSOs can be induced to develop into all types of cells including cells of the nervous system and other organ systems. Using stem cells, it is possible to study cell interactions and differentiation, work on the prevention and treatment of birth defects by using gene editing, and generate cells for drug testing.

Researchers can use patient-derived T21 iPSCs to study a variety of conditions for

Other newer approaches are also being used to study DS. One example is using X-inactive specific transcript (XIST) to silence the extra chromosome 21. Single-gene manipulations are being used such as with *APP* and *OLIG2*. Another approach is to use clustered regularly interspaced short palindromic repeats (CRISPR)—associated protein 9 (Cas9) to manipulate specific genes. Yet another approach involves subjecting cells to stress to reveal more robust phenotypes.

The challenges of research using iPSCs include the following:

There is limited availability of iPSCs, which are time consuming and resource intensive to produce.

The iPSOs that are available are limited in terms of donor ethnicity and clinical data about the donor.

There is too much technical variability in the methods used to produce the iPSOs. iPSOs are limited in their maturation and aging.

Ways to address these challenges include

The Machairaki laboratory studied the roles of EVs in AD pathology using EVs secreted from AD iPSCderived neurons. They found that, although there was a relatively low amount of A in the EVs, there was an increased ratio of A -42 to A - -42 to

-40 is a better biomarker of AD than A -40 or A -42 alone.

AD is a complex disease that presents differently from person to person. Dr. Machairaki s group is using their methodologies to characterize different subtypes of AD patients. The group uses the patient s blood to generate the different brain cells to discover pathways that can be used for precision drug therapies. Their methods could be used to bring a precision medicine approach to DS

Co-chairs: Melissa Parisi, M.D., Ph.D., NICHD; Joaquin Espinosa, Ph.D., University of Colorado

Dr. Parisi welcomed the attendees, saying this session would focus on the INCLUDE DCC and parameters for future cohorts.

Joaquin Espinosa, Ph.D., University of Colorado

Dr. Espinosa described the three components of the INQLUDE project: conducting targeted, high-risk, high-reward basic science studies on chromosome 21; assembling a large study population of people with DS; and including people with DS in existing clinical trials. The creation of the DCC is focused primarily on the second component, with the goal of facilitating the work of the other two components. Funding for the INQLUDE project has risen steadily since FY 2018. NIH responded to this increased funding with a request for applications (RFA-OD-20-007) to support the development of the DCC for the INQLUDE project. Dr. Espinosa said some of the research that could be made possible through this coordinated effort to standardize, harmonize, and aggregate DS data includes studying TS 21 in underrepresented groups, looking at rare co-morbidities and mosaicism, and assembling large sample sizes for studies that require them, such as genome-wide association studies (GWAS).

NIH s RFA resulted in funding a team of world leaders in data coordination centers and data portals to create the DSDCC and the portal for data sharing. The initiative, which consists of three cores, is led by three scientists: Dr. Espinosa for the Administrative and Outreach Core (AOC), which will focus on INCLUDE data sites and the public website; Justin Guinney, Ph.D., for the Data Management Core (DMC), which will focus on identifying clinical data and creating an INCLUDE virtual biorepository; and Adam Resnick, Ph.D., for the Data Portal Core (DPC), which will focus on the INCLUDE portal user interface and offer features such as a cohort builder and a biospecimen request system. The overall mission is to provide the data access and analysis tools required for evidence-based transformative action for DS Dr. Espinosa credited the work of the NIH INCLUDE working groups, which gathered important information and produced specific recommendations that will be incorporated into the design of the INCLUDE DCC. The DCC team will also build upon the pioneering efforts of the Crnic Institute Human Trisome Project and the GMKF Data Resource Center.

Next steps include querying the community with DS about how the INCLUDE DCC and its data portals can best be of service to them and procuring and harmonizing data from key major cohorts such as DS. Connect® ABC-DS, the Ornic Institute Human Trisome Project, and the GMKF DS Cohort. Dr. Espinosa urged everyone to dream big in thinking about how the INCLUDE DCC can foster a collaborative, multidisciplinary, and holistic research in DS. He thanked all the scientists, project officers, research participants and their families, and advocates for their involvement in this team effort.

Dr. Brower reported on the findings of a REDCap survey of existing cohorts and databases related to DS research, which was conducted to highlight gap areas, help guide prospective data collection (i.e., cohort building), and facilitate sharing and linkages across datasets to foster new collaborations.

Survey participants, which included Data Standardization and Harmonization (DSH) WG members, funded INCLUDE investigators, recent INCLUDE workshop attendees, and professional organizations and contacts, were contacted in June 2020. Responses were compiled in mid-July 2020. Survey fields included cohort name and contact information, general cohort information (size, age range, whether NIH funded, availability of data, sharing restrictions, design, sites, recruitment methods, and subject identifier types), and whether biospecimens and genetic or genomic data were collected. Sxty-one surveys describing 57 cohorts were returned from 39 institutions across the United States. Dr. Brower noted that because study participants are recruited from across the U.S., the 39 institutions actually provided a representation of participants from nearly all the states. Responses were also received from seven international cohorts in Spain (2), the United Kingdom, the Netherlands, Italy (2), and Argentina.

The survey found that NIH funded 56% of the cohorts. The majority of cohorts (46%) had fewer than 100 participants. Only 19% of cohorts had enrollments of more than 500 participants. The largest cohort was NICHD DS-Connect® with 5,038. The next largest cohort, Giorgio Albertini s study at the Istituto di Ricovero e Qura a Carattere Scientifico (IRCCS) San Raffaele Pisana, recorded 2,235 participants. Dr. Brower identified the institutions that participated in the study and provided enrollment numbers for all cohorts at those institutions with more than 100 participants.

The REDCap survey was designed to capture information in three areas: descriptions of the institution; descriptions of the cohort, including data sharing policies; and basic information on genomic and biomarker collection. Dr. Brower said that while this was not an exhaustive compilation of cohorts, these data could be useful as a starting point for the INCLUDE DCC.

Russ Waitman, Ph.D., University of Missouri Kansas City

from clinical research networks (CRNs) and insurance claims from health plan research networks (HPRNs) to create a national infrastructure for people-centered clinical research. Users can access these real-world data, which are collected from the everyday medical encounters of more than 66 million people across the United States. The PCORnet Common Data Model standardizes data across systems into a single usable language, including data that are ready for research and data that are available or linkable but still evolving.

Datavant (<u>https://datavant.com</u>) is a de-identified record linkage vendor, which is used by PCORnet, the National Center for Advancing Translational Sciences (NCATS) National COVID Cohort Collaborative (NC3), Pharma, and Invitae, the industry contractor for DS-Connect® Datavant is similar to the NDAR GUID but uses more common data and does not require a

can be linked to other data held by industry, state, or nonprofits.

The referral code model is similar to affiliate programs on the Internet where users on a website are referred to, for example, Amazon to buy a product and the payment goes back to the website that made the referral. It is used to streamline workflow for specific trials. This could be valuable for DS-Connect®in attracting projects that have a validated instrument but do not want to reconfigure it in DS-Connect® An example of the use of the referral model is the DS-DETERMINED project, which will recruit participants for DS-Connect®based on their electronic health records (EHRs) from five health systems in PCORnet with a link to REDCap. REDCap obtains consent for using EHR data, generates a referral code, and provides a link to DS-

Dr. Head said that blood volumes are limited by the age and weight of the donor and blood collection is dependent on the availability of resources, such as centrifuges and freezers, at the collection site. The BWG reviewed a number of protocols using blood specimens and found significant variability across studies, likely because of different study hypotheses. Dr. Head presented a decision tree approach

Attendees suggested collecting the following additional biospecimens: liver, heart (myocardium), vascular specimens, autopsy specimens, amniotic fluid from prenatal diagnoses to look at possible environmental exposure and their effect on health outcomes, and placenta. Dr. Seidman said that refrigeration or freezing is not wanted for iPSC derivation. Shipping is preferred, because the samples are viable for two to four days, albeit with declining efficiency of cell survival.

Dr. Seidman said it appears that fixating tissue is prioritized over freezing. She strongly suggested a small frozen specimen as a priority.

Javier Blanco, Ph.D., observing that basic donor demographics and clinical information are essential, asked whether there are plans for working with tissue procurement resources such as

the participants EHRs, it would be possible to know which DS-Connect®participants have been seen in other systems. Then, on a project-by-

The organoid model, which is expensive to maintain, is very good for obtaining molecular information and for surveying cell populations. The availability of different cell types could present an opportunity to study cell interaction and cell signaling, but issues about how to analyze the data and whether organoids can recapitulate complex cell interactions or cell matrix interactions must be addressed. One strategy for developing a better organoid is to add vascular cells into organoids to reduce the necrotic core. Organoids sometimes stop growth at a certain point because of their necrotic core and lack of an outside matrix. Bioreactors or spinning orbital shakers can be used to maintain organoids for about 6 months.

The monolayer culture is also good for obtaining molecular information. The ability to get a larger number of neurons and glial cells in high purity allows for a deeper sequencing of given cells types with this method. Mixture cultures can also be done with a monolayer culture by adding different types of cells.

The group discussed the potential of developing intestinal or cardiac organoids for studying DS. There are also exciting data for modeling DS for a monolayer culture. This would be easier to manipulate but would lack the complexity of organoids. A combination of the two models could also be considered for certain research questions.

The group identified problems to be addressed: Small sample numbers and particularly in development so that there can be more certainty that in vitro models are actually modeling the actual human developmental change. That is a weak link right now. More clinical data on diseases such as cancer and leukemia

IPSCs from different populations and ethnic backgrounds. More diversity among participants with DS should be considered.

discussed how the molecular phenotype is reproduced and considered various mouse models in terms of RNA-Seq and proteomics to enhance rigor and reproducibility, asking what measurement of these models across labs might be done to maintain the base level and consistency across models.

The group made the following suggestions:

Consider minimum colony sizes.

Compare phenotypes of different mouse models. Members noted this can be difficult, because

More frequent dialogue with clinicians would be helpful to better understand the data and specimens that are needed.

The group suggested other research initiatives:

Encourage collaboration between the community with DS and communities that represent rare diseases similar to DS and might have shared common phenotypes, such as mitochondrial phenotypes.

Explore the relationship between DS and fetal alcohol syndrome.

Consider transcranial magnetic stimulation (TMS) for people with DS TMS is a well-developed, safe, minimal-pain therapy that could be used in mice studies.

Maria Stanley, M.D., University of Wisconsin Madison Nicole Vasilevsky, Ph.D., Oregon Health & Science University

Dr. Vasilevsky reported on behalf of Group 7.

The group suggested collecting the following data:

Data across the lifespan, which may differ by age group (e.g., infants and toddlers; young adults and adolescents; adults, including older ones). Phenotypes across different age groups should be included.

Basic medical history across the lifespan from EHRs and EMRs

Behavioral and cognitive metrics. The challenge is harmonizing behavior and cognitive phenotype data from individuals and populations with DS in a standardized way. Domain-

specific phenotypes, such as for ADHD and regression, are needed.

Quantitative measures of behavioral phenotypes

DS-Connect®health history surveys

Minimal Common Data Element REDCap survey data, which is organized by system (Gl, immunity, neurodevelopment) and collects clinical phenotyping data (<u>https://redcap.ucdenver.edu/surveys/?s=NHLDPD48R</u>)

The group suggested harmonizing data with the Human Phenotype Ontology (HPO) (<u>https://hpo.jax.org/app/</u>), which provides a standardized vocabulary of phenotypic abnormalities encountered in human disease. Best practices for data collection should be developed to achieve better consensus.

The group suggested collecting the following biospecimens:

Blood, tongue swab, saliva, and skin tape biopsy. The group acknowledged how helpful the blood draw decision tree was from Dr. Head s presentation at the concurrent session. Tie26 Tm0 g0 G[()] TJETQ EMC / PAMOD 30 BDC q0.000a9B2I.6426 Tm0 g0 G[()] TJETQ EMC / PAMOD 30 BDC q0.000a9B2I.6426 Tm0 g0 G[()] Determine risk factors that lead to ∞ -morbidities and protective factors that prevent some people from developing ∞ -morbidities.

Include both a discovery cohort and a validation cohort to validate new and existing measures.

influence gene expression); racial, ethnic, and gender diversity; and broad consent for future use by the larger stakeholder community.

The group determined that to understand the risk factors and progression of AD in people with DS, samples that address specific, targeted questions, such as oxidative stress, immune dysregulation, and inflammation, must be collected. Untargeted omics studies are also needed. The implementation of longitudinal studies that collect multiple tissues specimens (e.g., blood, CSF, postmortem) creates an opportunity for clinical trial-ready cohorts for studies of preventive medications. Large, diverse cohorts are the way to address bias in sample collection.

There are unique questions relevant to families of people with DS that keep the research grounded. Clinical scenarios have a lifespan perspective on DS A deeper understanding is needed about:

Both risk and protective factors (e.g., exercise, diet, lifestyle interventions that may be protective against AD)

Prevention strategies, which requires knowledge of ages of onset

Co-occurring conditions that develop with age, which will require conducting longitudinal research studies

Network gene analysis to determine which genes cause which phenotypes.

A systems biology approach combining genomics, proteomics, and metabolomics to generate the datasets needed to understand fundamental biology in people with DS across the lifespan and drive precision medicine approaches to improve

Nicole Baumer, M.D., Boston Children's Hospital

Dr. Baumer, whose sister has DS, called this an exciting time for DS research as it moves into a new era focusing on neurological biology and mechanisms. She suggested that researchers must realize that people with DS and their families have diverse views. Dr. Baumer recalled the Roche DS clinical trial, which angered families of people with DS participating in the study, because the people with DS were

was striking and led the investigators to acquire a more in-depth view of the way families and individuals perceive research. They learned that while many families and individuals were in support of efforts to ameliorate disability and improve functioning, the goal of cognitive enhancement was not universally accepted. The investigators learned that the way they communicate about DS research matters and that their efforts must be portrayed in a way that does not make the participants feel devalued. The heterogeneity of people with DS in terms of medical conditions, neurodevelopment, and function, must be considered. People with DS who have more severe functional deficits are often not included in research studies because they are not able to participate in extensive neurophysiological assessments. Because of this heterogeneity, interventions will not be one-size-fits-all, and investigators must better understand the wide range of personal challenges that people with DS face. Dr. Baumer concluded by noting that biomarkers are needed to identify subsets early so that treatment interventions can be targeted and positively influence the developmental trajectory.

George Capone, M.D., Kennedy Krieger Institute

Dr. Capone said he would speak to the natural history and longitudinal trajectory of certain medical comorbidities, developmental, and aging issues. As cohorts are assembled, although they may be staggered by age, participants often undergo repeat testing and repeat collection of biospecimens. Researchers should be mindful about how to incentivize these experiences for the participants and their families. Researchers are making a long-term commitment and investment in these families, and the families are doing the same. Researchers should build tangible benefits into the research interaction itself as a short-term takeaway. Families are focused on where they can find specialized health care for people with DS, especially adults, people in rural communities, and underrepresents minorities. Researchers should provide them with something tangible, such as medical recommendations, a guidance plan, or some other kind of group experience, to keep them engaged.

Stephanie Sherman, Ph.D., Emory University

Dr. Sherman said she would add to the points about what clinical researchers and basic scientists need to know. All involved need to communicate better about every aspect of the research—both the clinical and basic science aspects, because all are part of the team working together to enhance the research. It is important to ensure that investigators are using the right samples, interpreting data the right way, and linking clinical assessments and model systems. This can be expensive, but it should not deter investigators from using the resources of people in the community. Communication is critical. Dr. Sherman recognized the research efforts of people with DS and their families, noting how many things they are asked to do that take up much of their time. Investigators—both clinical and basic scientists—should do their best to minimize that time commitment by evaluating how many samples are really needed and whether there are other biomarkers that can be used that are not so invasive.

Roger Reeves, Ph.D., Johns Hopkins University

and organoids into people is the hard part of the process and whether seeing people as patients or interacting in clinical trial settings investigators must think prospectively about what kinds of biomaterials would be helpful in the future. IRB structures must be ready for this. The more tissues correlated with a detailed description of the people in the studies, the faster the goal of having people reach the potential they would have without T21 will be achieved. This will allow them to live the most independent life possible.

Christine Seidman, M.D., Ph.D., Harvard University

Dr. Seidman said that as a cardiologist and geneticist, she has always wanted to understand why certain similarities and differences occur in people. DS is a collaborative endeavor, because the investigators learn so much from the people with DS and their families. It is still not known why so much heart disease occurs in a subset of people with DS However,

An attendee asked whether basic science researchers should focus primar

EEG electroencephalography EHR electronic health record EMRs electronic medical records ESDM Early Start Denver Model EVs extracellular vesicles FDA U.S. Food and Drug Administration FHIR Fast Healthcare Interoperability Resources fMRI functional magnetic resonance imaging fNIRS functional near-infrared spectroscopy FOAs Funding Opportunity Announcements FXS Fragile X syndrome FY fiscal year **GI** Gastrointestinal GLOBAL Global Down Syndrome Foundation GMKF Gabriella Miller Kids First **GUID** Global Unique Identifier GWAS Genome-wide association study HPO Human Phenotype Ontology HPRN health plan research network IBIS Infant Brain Imaging Sudy ICs NIH Institutes and Centers IDDs intellectual and developmental disabilities IDDRs Intellectual and Developmental Disabilities Research Centers IEP individualized education plan IFN interferon INCLUDE INvestigation of Co-occurring conditions across the Lifespan to Understand Down syndromE iPSC induced pluripotent stem cell IRB institutional review board IRCCS Istituto di Ricovero e Cura a Carattere Scientifico IVF in vitro fertilization JAX Jackson Laboratory LOAD Late-MRI magnetic resonance imaging MTA material transfer agreement NACC National NAFLD non-alcoholic fatty liver disease NC3 National COVID Cohort Collaborative NCATS National Center for Advancing Translational Sciences NDAR National Database for Autism Research NDRI National Disease Research Interchange NfL neurofilament light chain NHLBI National Heart, Lung, and Blood Institute NIA National Institute on Aging NICHD Eunice Kennedy Shriver

NOS Notice of Special Interest

OD Office of the Director

OSA obstructive sleep apnea

PBMCs peripheral blood mononuclear cells

PGCG Pediatric Cardiac Genetics Consortium

PCORI Patient-Centered Outcomes Research Institute

PET positron emission tomography

PFT pulmonary function testing

PH pulmonary hypertension

PPI protected personal information

PTN Pediatric Trials NetworkeoBT/F1 12 Tf1 0 0 1 2k