

Down Syndrome Research: The Intersection of Basic Science and Clinical Cohort Development

November 9–10, 2020
NIH-Sponsored Virtual Meeting¹

DAY 1: November 9, 2020

Session 1: Welcome and Introductions

Welcome Remarks from INCLUDE (INvestigation of Co-occurring conditions across the Lifespan to Understand Down syndromE)² Leadership

Melissa Parisi, M.D., Ph.D., Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

Charlene Schramm, Ph.D., National Heart, Lung, and Blood Institute (NHLBI)

Tara Schwetz, 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-chair of the INCLUDE Steering Committee.

[The INCLUDE project](#) involves 18 NIH Institutes and Centers (ICs) to increase research specific to people with Down syndrome (DS) and encourage inclusion of people with DS in all research projects.

INCLUDE has three components:

- Component 1: Conduct targeted high-risk, high-reward basic science studies on chromosome 21.
- Component 2: Assemble a large study population of people with DS across the lifespan.
- Component 3: Include people with DS in existing and future clinical trials.

The current workshop focused on Components 1 and 2 and brought together researchers, data scientists, self-advocates, and other members of the DS research community to give presentations on the current state of the science and gaps with regard to basic science and cohort development. The meeting included breakout sessions for participants to discuss these topics in greater detail.

Dr. Schwetz continued the opening remarks in welcoming everyone to this workshop on the intersection of basic science and the clinical cohort development in DS research. She noted that more than 300 people had registered for the meeting. This is the second virtual workshop for INCLUDE, following the Clinical Trials in Down Syndrome for Co-occurring Conditions Across the Lifespan workshop held in May 2020. An in-person workshop convened in September 2019 was titled Planning a Virtual Down Syndrome Cohort Across the Lifespan Workshop.

¹ All presentations from [Day 1](#) and selected presentations from [Day 2](#) can be viewed via the [NIH VideoCast](#) site.

² A glossary of terms and acronyms used in this report can be found at the end of the document.

Dr. Schwetz thanked all involved in the planning and coordinating of the current event, including NIH program staff, primarily staff from NICHD, NHLBI, and the NIH OD, as well as the INCLUDE WG co-chairs, who are experts from the extramural research community who led presentations and discussion groups. She looked forward to the presentations on updates in basic science and cohort development in DS research and dynamic, interactive discussions among attendees.

Overview: Summary of INCLUDE Initiative History and Updates

Diana Bianchi, M.D., Director, NICHD

In fiscal year (FY) 2018, Congress directed NIH to develop a trans-NIH research initiative to improve the health and neurodevelopment of people with DS and typically developing (TD) people at risk for Alzheimer's disease (AD), cancer, cardiovascular disease (CVD), immune system dysregulation, and autism. As a result, NIH established INCLUDE, a project that now involves 18 ICs and covers all major co-occurring conditions in people with DS. Congress has directed NIH to continue to invest in DS research projects, fund early-stage DS investigators, and develop the trans-NIH initiative. The ICs have also been encouraged to increase overall funding for research on DS from their own budgets. All of the projects must share their data and use DS-Connect®: The Down Syndrome Registry (DS-Connect®) whenever possible.

The INCLUDE project was launched in June 2018. Initial funding of research through INCLUDE began with the FY 2018 appropriation, which increased NIH funding for research on DS by \$23 million to a total of \$60 million. With that funding, 49 existing NIH-supported projects received supplemental funding. Congressional support of INCLUDE continued through FYs 2019 and 2020, with funding increasing each year to a total of approximately \$113 million in FY 2020. Because all federal funding is currently under a continuing resolution, a full budget for 2021 is not yet in place. Given the progress made to date for INCLUDE, it is hoped that Congress overall and the congressional DS caucus in particular will continue to support this initiative. Dr. Bianchi noted that several INCLUDE Funding Opportunity Announcements (FOAs) are expected to open in FY 2022.

Dr. Bianchi described some of the programs and projects funded by and/or collaborating with INCLUDE. NICHD's Pediatric Trials Network (PTN) is working with INCLUDE to support drug studies in people with DS and train investigators to conduct research involving people with intellectual and developmental disabilities (IDDs). NICHD supports PTN through the Best Pharmaceuticals for Children Act (BPCA), which mandates drug studies in children to enable Food and Drug Administration (FDA) labeling. Many of the drugs that children with DS receive are neither labeled for children at all nor specifically meant for children with DS. PTN consists of more than 100 clinical research sites across the United States. Funds were recently allocated to PTN to partner with INCLUDE to specifically support drug studies in children with DS. Dr. Bianchi noted that in the coming year, PTN will promote a clinical trial of guanfacine for attention-deficit/hyperactivity disorder (ADHD) in children.

A virtual meeting was held earlier this month with PTN investigators to discuss challenges, lessons learned, best practices, and upcoming trials. Issues discussed included treating heart defects, sleep apnea, AD, leukemia, and infectious diseases. As always, the meeting included a panel of self-advocates, parents, other advocates, and physicians to discuss considerations for participation in clinical trial. Dr. Bianchi said one of the main points she took away from that meeting was that parents are finding it easy, and in some cases preferable, to participate in clinical trials or clinical studies remotely. One of the interesting research questions posed was whether triplication of interferon genes on chromosome 21 predisposes people with DS to complications of COVID-19 infection.

Dr. Bianchi cited results of a [recently published survey](#) conducted by the Trisomy 21 Research Society (T21RS). The survey included 577 respondents who were asked about complications of COVID-19 in people with DS. Preliminary results indicate no evidence for additional risk of contracting COVID-19 with regard to the level of intellectual disability or any co-occurring medical conditions, including hypothyroidism, congenital heart defects, or additional behavioral or psychiatric conditions. The only category for increased risk was for people living in a residential care facility.

Another trans-NIH initiative that includes a focus on people with IDD as an underserved population is a program for testing for exposure to COVID-19 called [Rapid Acceleration of DiagnosticsSM Underserved Populations \(RADx-UP\)](#). NICHD supports this program and the study of the impact of COVID-19 on people with IDDs through its [Intellectual and Developmental Disabilities Research Centers \(IDDRCs\) network](#). To date, one project specifically looking at IDDs in children and young adults has been funded in collaboration with the school system in the greater St. Louis area.

NICHD has a [Notice of Special Interest \(NOSI\)](#) to fund administrative supplements for COVID-19 research in people with DS for the INCLUDE project. The goal of this research is to improve understanding and treatment of COVID-19 in people with DS, as opposed to the RADx-UP program, which is more focused on testing. Sixteen NIH ICs and Offices are participating in this initiative. The most recent application submission date has passed, but additional application dates will be available in 2021.

Dr. Bianchi reported that the 2014 DS Research Plan is being updated and combined with the INCLUDE Research Plan for 2021. [Responses to the Request for Information \(RFI\)](#) on the updated plan have been posted, and the draft plan for 2021 is expected to be posted by the end of this year.

As a lead-in to the keynote session of this meeting, Dr. Bianchi pointed out that the voices of advocates, particularly people with DS and their families, are included in all INCLUDE meetings and other NIH committees and advisory panels. She noted two strong voices, Kathleen Egan and her son, David, a self-advocate for DS. David has participated in prior INCLUDE workshops and numerous meetings around the country and the world and has given Congressional testimony in support of DS research. The entire Egan family has participated in research studies, and David, now in his 40s, was recently given a certificate of appreciation in recognition of his integral contributions to research in DS at NICHD and NIH over the course of his lifetime, from when he was a baby. Continuing her advocacy work, Kathleen is now a member of NICHD's National Advisory Child Health and Human Development Council.

Dr. Bianchi closed her presentation by thanking all the families and advocates for their hard work and input and ongoing commitment to DS research. Perspectives from research participants provide crucial and invaluable input for researchers and decision-makers.

Overview: Summary of INCLUDE Funding for Basic Science and Clinical Cohort Development

Melissa Parisi, MD., Ph.D., NICHD

Charlene Schramm, Ph.D., NHLBI

Dr. Parisi opened this session by citing a news release that NIH had awarded \$60 million in INCLUDE funding for DS in FY 2020, noting further that this is the third year of funding for INCLUDE. NIH and its INCLUDE partners see this project as an opportunity to move toward personalized medicine and to continue to engage and improve the health of people with DS and their families, through the three components of INCLUDE.

Dr. Schramm provided additional details regarding INCLUDE funding and awards. Funding for INCLUDE was initially awarded in 2018 in response to a congressional directive; however, because of time limitations in FY 2018, it was possible to issue administrative supplements only to existing grants. In FY 2019, a suite of Requests for Applications (RFAs) for clinical trials, clinical trial readiness, and transformative R01s was issued, along with announcements for supplements and competitive revisions. Funding opportunities were expanded further in FY 2020, with reissued RFAs from FY 2019 in addition to new RFAs for a data coordinating center (DCC), small R03 grants for data analysis, and several NOSIs for fellowships, career development awards, resource and animal model development, R01 projects, and supplements. The RFA receipt date for FY 2021 has already passed, but a number of NOSIs remain active. The next receipt date for RFAs is November 3, 2021, for FY 2022 awards.

Funding of awards through the INCLUDE project has steadily increased over the past 3 years. In FY 2018, \$23 million was awarded to 49 supplements. In FY 2019, \$35 million was distributed among 43 awards. In FY 2020, the total amount of funds increased to \$60 million for a total of 63 awards, of which 42 were new. Each year, projects have been funded in all three components on INCLUDE, with some awards overlapping the different components.

INCLUDE-funded projects that fall under Component 1, basic science, include studies involving the role and/or impact of induced pluripotent stem cells (iPSCs), organoids, immune dysfunction, epigenetics and genome organization, gene function, animal models, and COVID-19 on people with DS.

Examples of projects funded for Component 2, cohort development, include the DCC; studies focused on the discovery of susceptibility genes for DS-associated congenital heart defects using whole genome sequencing (WGS), the impact of COVID-19 on the mental health of people with DS, and the molecular epidemiology of acute lymphoblastic leukemia; and the development of a cognitive test battery for intellectual disabilities.

Dr. Schramm noted that funding for the DCC was awarded September 2020 to a multi-institution consortium including Children's Hospital of Philadelphia, the Crnic Institute at the University of Colorado, and Sage Bionetworks. The award includes an administrative and outreach core, a data management core, and a data portal core.

To date, six clinical trials have been funded through INCLUDE. The aims of these trials are to prevent AD in DS, study JAK inhibition in autoimmune skin conditions in DS, evaluate medication treatment of ADHD in children with DS, assess the use of positive airway pressure for the treatment of obstructive sleep apnea (OSA) syndrome in children with DS, assess the effectiveness of medications for OSA to improve cognition in children with DS (MOSAIC DS), and study the effects of hypoglossal nerve stimulation on language and cognition.

One of the charges from Congress was to increase the pipeline of DS investigators through the INCLUDE project. Since 2019, the program has supported a total of 24 new trainees: 12 predoctoral candidates, 1 postdoctoral fellow, 4 Clinical and Translational Science Award (CTSA) KL2 scholars (i.e., K awards to M.D. or Ph.D. scholars affiliated with clinical and translational science awards), 5 M.D. scholars who are part of the PTN with a focus on cardiology and related studies, and 2 mentored career development (K) awardees.

Dr. Schramm summarized the workshops and some of their outcomes that have been sponsored over the past 3 years of INCLUDE. Participants attending the Alzheimer's Disease Clinical Trials in the Down Syndrome Population Planning Meeting, held November 7, 2018, reviewed lessons learned from NIH-supported clinical trials of AD in DS, genetically at-risk populations for AD (with and without DS), and

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. Six 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, Clinical 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 clinical 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 Global Down Syndrome Foundation (GLOBAL), a U.S.-based nonprofit dedicated to improving the lives of people with DS through 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 clinical 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 INCLUDE 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 INCLUDE workshops to date. INCLUDE 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 [Down Syndrome Consortium](#), 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.

Keynote: Perspectives from Research Study Participants

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 Cincinnati, 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 Cincinnati Children’s Hospital, and in her free time, she advocates for people with special needs through a variety of activities. She is a board member on the Clermont County Board of Developmental Disabilities. She is also a member of the Research Review Committee for DS-Connect, through which she has an opportunity to review all of the protocols that are proposed for support through that registry. Emily uses her voice as a mom and as an elected member of her local school board to speak of on behalf of kids of all abilities. Nora often joins her mom as an advocate for DS.

Megan and Kris, a special education teacher, live in Colorado. In high school, Megan became the first cheerleader with DS in the state and worked with her teachers to compose a popular video, “Don’t Limit Me.” She attended the University of Colorado in Colorado Springs in pursuit of a degree in film studies. With her mother’s help, Megan founded and became a co-owner of the tie-dyed clothing company Megology. She is one of the stars of the A&E docu-series “Born this Way,” which details 4 years in the lives of young adults with DS. Megan has developed her skills as a public speaker and travels across the country and globe giving speeches and presentations on a variety of topics.

Emily opened her presentation by introducing her family, noting that Nora engages well with others, including new people. All six members of the Chesnut family have participated in research studies through Cincinnati Children’s Hospital on diverse topics, including vaccines (including for COVID-19), allergies, cognition, hearing and listening, and kidney function. Nora has participated in five DS-specific studies. She loves research and “her doctors,” who include everyone on the research team and especially Dr. Hanna. Emily noted an unexpected benefit of Nora’s participation in these studies: She has become more comfortable in a clinical setting, because the visits are not only for medical appointments that involve a lot of poking and prodding.

Before deciding whether to enroll Nora or her siblings in a research study, the family considers the following:

- What the study involves:

- 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 doctors and researchers about DS and the studies she is in so that she can learn more about how to improve the health and lives of herself and others. Kris added that having a young adult whose favorite subject is science and being part of a research study make a nice blend of those two passions. She pointed out that Megan has had many incredible experiences in her life, including being on an Emmy Award–winning television show that has provided a way for people with DS to share their lives and dreams with millions around the world. Kris and Megan are also part of several strong advocacy organizations, such as GLOBAL, which is headquartered in Denver. Kris pointed out that being able to have a voice—and a platform—that people listen to also carries responsibility and an obligation, as to Emily’s point, to educate and advocate for people with DS.

Kris and Megan talked about Megan’s medical issues, which are one of the biggest challenges in Megan’s life. Megan has been diagnosed with hypothyroidism, arthritis, psoriasis, and celiac disease. Megan and her mom discussed a situation in the past year in which Megan started experiencing significant, debilitating pain. The pain was affecting Megan’s daily living skills to the point where she could not get out of bed and had to move back home. Kris and Megan were having a difficult time getting in to see the specialists Megan needed. They turned to the Denver Health and GLOBAL Adult Down Syndrome Clinic and were able to enroll Megan in a clinical trial being conducted in collaboration with Joaquin Espinosa, Ph.D., and his team at the Linda Crnic Institute for Down Syndrome at the University of Colorado. Within 2 weeks of being part of the clinical trial, and with the support of the clinic and research teams and the medication Megan started, Megan was signing up for and was able to

join a Zoom class independently. With this intervention and participation in the clinical trial, Kris and Megan said they were able to return to a mostly normal life, COVID-19 notwithstanding.

Being part of a clinical trial and having the expertise and support of the teams at the Adult Down Syndrome Clinic and the Linda Crnic Institute for Down Syndrome have enabled Megan to live her life as independently as she wants to and also have supported Kris, a solo parent, in being able to live as independently as she needs.

Megan's latest accomplishment involves the upcoming release of her book, *Born to Sparkle*, with the themes of not limiting oneself, following one's dreams, and not giving up on those dreams. It includes a shout-out to the scientists and health professionals for all their hard work and ongoing support.

Both Megan and Nora and their mothers enthusiastically support participating in clinical trials and praised the teams conducting the studies they have joined. They all expressed gratitude for the opportunity to contribute to the research enterprise and recognized the importance of commitment to these studies. They remain encouraged and inspired by continued and increased funding for research on DS.

Dr. Parisi thanked the keynote presenters for sharing their life experiences and their perspectives about what it has been like to be part of research studies.

Q&A

An attendee asked what Megan and Nora like best about participating in research. Megan said she finds it fun to learn about herself, including details such as her blood type, and to learn how to be healthy through results of research studies. Nora likes playing the "games" that are part of her clinical trials and now loves going to the doctor and seeing her whole research team. Emily added that through participation in clinical trials, Nora has learned not to be intimidated by medical visits.

Another attendee commented that the keynote presentations speak to the fact that there are some benefits to participating in research that families may not have previously appreciated, such as becoming more comfortable with the medical community at large and individual care providers, as well as being comfortable in a hospital or a clinical setting.

A follow-up question focused on what might encourage or help individuals and families who are not enthusiastic or are not sure about volunteering to be part of a research study to be more comfortable about participating, for example, if there any particular strategies that investigators have used that have helped Megan or Nora or their moms to feel more comfortable and at ease with some of the procedures that may have been part of those research projects.

Emily noted that Nora's research team has done a very good job of getting to know her, and from project to project, they can read Nora well enough to understand her limits and comfort level. In some cases, for example, a lengthy series of tests scheduled for a single day might be split into two days, if needed. This approach reflects the willingness of the team to work with Nora and Emily, and visa versa, rather than requiring everyone to follow a rigid schedule. Emily added that early-morning appointments also tend to work better for Nora, when she has more energy, as opposed to after-school appointments.

Emily continued by pointing out the importance of understanding what is involved in a research study and communicating with the research team. Some procedures such as blood draws and other invasive components of a study can be uncomfortable or daunting, and it is preferable to not put children through these steps unless necessary. There usually are other parts of a study that can be completed

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 events Megan has participated in is GLOBAL's annual "Be Beautiful Be Yourself" fashion show, the single largest fundraiser for DS research in the world. Through both research and fundraising activities, people with DS, their families, and the larger DS community are impacted around the globe. Kris and Megan said that bringing everyone together, and seeing the outcomes of all of the work of the researchers and scientists, is both energizing and powerful. These efforts, in turn, reinforce the full spectrum of commitment to DS research.

The keynote speakers were asked for their opinion on important research goals and what they think scientists should be focusing on now and in the near future. Kris and Megan said autoimmune conditions and aging, with Megan specifically noting celiac disease. Emily said primary concerns are aging, AD, and COVID-19, the last of which poses a greater risk to people with DS than the general population. The potential long-term effects of COVID-19 in people with DS are not known and need to be studied.

A participant asked how people with DS can give feedback to scientists about the research studies they have participated in or that have been published. Kris said that after being part of the research for so long, they are able to have very open, honest unfiltered conversations with the investigators about what they consider is important. She recognized, however, that approaching researchers can be intimidating. She and Megan pointed out that interacting on a one-on-one basis or a more personal level can be helpful; they noted, for example, that at some of the events they have attended, Dr. Espinosa has danced with Megan, which has provided a nice bridge to their interactions about the research. Nora noted that she likes "playing games" with the researchers, especially Dr. Hanna, and that Oreos and chocolate can sometimes help get through those games (i.e., the research tests). Emily added that while she, too, has open dialog with their research team, she has not considered giving specific feedback about the individual studies Nora is in. She noted that she receives results of Nora's cognitive tests but has not read the results of the overall studies.

Dave Egan submitted his comments on the meeting so far, noting "a great opening" to the workshop. He also thanked the families who spoke and stressed the importance of attracting as many families as possible to participate in research. He conveyed his commitment to this effort and asked if the keynote speakers have any other ideas on how to bring more families into the research community and participate in research studies.

Other participants similarly asked Megan, Nora, and their mothers about strategies to reach out to other people with DS and their families to promote and encourage volunteering in clinical studies. One approach is to publicize some of the really "cool stuff" that has come out of the clinical research and highlight that to the non-scientific community. Megan added that even if it is difficult to understand the science, sometimes understanding "the why" is more important than understanding "the how." All agreed that personal contact with peer groups and use of social media are key ways to connect with other families and increase awareness of and share experiences about clinical studies. Having a cadre of self-advocates and parents working with the scientists and co-presenting at meetings and over social media could facilitate educating individuals and families about ongoing studies and research needs. Kris and Emily both found local DS organizations to be foundational touchstones. As for social media, in

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 clinic. 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 it is 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 DS will 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 agency, what has been learned, and the difference the research is making in people's lives. 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.

Overview of Pre-meeting Working Groups

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?

The co-chairs of each WG presented their group's findings in Session 2 below.

Session 2: Current State of the Science and Gaps with Regard to Basic Science and Cohort Development

Neurodevelopment—Structure, Cognition, and Language

Nancy Raitano Lee, Ph.D., Drexel University

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

Dr. Bhattacharyya provided a brief background and described the basic science for this domain. Dr. Lee's presentation focused on cohort development.

The life expectancy for people with DS has increased significantly over the past several decades, from 12 years in 1949 to 60 years in the 21st century. The extended life expectancy for this population has increased awareness that information on different stages across the lifespan is needed to optimize development and set the stage for aging in people with DS.

The WG focused on the state of the science and gaps in structure, function, cognition and language, and behavior and looked at neurodevelopment as a continuum of brain structure that leads to behavior. Deficits in any one of these areas, in turn, can affect the other areas. These different domains are present in different stages of the lifespan. Brain structure is established during the prenatal phase, and deficits in behavior and function emerge in childhood and young adulthood. More recently, research has increasingly focused on later developmental time points within the context of aging, AD, and associated decline in functional abilities, including cognition, in the population with DS. One of the primary gaps identified by the WG was the need for more foundational information about neurodevelopment before middle age and into young adulthood.

In the area of brain structure and development, people with DS have microcephaly, which is established during prenatal development and continues postnatally. What is not known is what accounts for microcephaly in this population. Outstanding questions include when microcephaly emerges (i.e., whether early or later in brain development) and whether microcephaly develops in the absence of

certain types of neurons and, if so, when does this deficit occur. Investigators also do not have a clear understanding of the detailed biology or molecular pathways underlying microcephaly in people with DS. In addition, more information is needed about structural connectivity in the brains of people with DS. Understanding the fundamental biology of early DS is important not only to understanding brain function and function but also to identifying and addressing priorities in support of new treatments and genetic pathways that are responsive to therapies.

The same issues and themes apply to behavior, cognition, and language: as people with DS age, what kind of shifts in ability and skills occur across the lifespan, when do these changes occur and how do they unfold over time, and what co-occurring conditions impact the function of the brain to impact behavior, cognition, and language.

The WG supported longitudinal studies to define the developmental trajectory and to define or describe maturation differences that may occur as people with DS develop.

The main basic science questions to guide this research domain include the following:

- When do deficits emerge?
- How do they change across developmental time/over the lifespan?
- How can investigators distinguish phenotypes in DS from those in co-occurring conditions?
- How do the answers to these questions inform interventions?

To fill in these gaps, basic research studies need to provide fundamental information about early brain structure in DS, which can be acquired in different ways, including through use of human stem cell models and an array of molecular analyses. Imaging studies can provide comprehensive detail about the structure of the brain. Scientists are working on developing expanded functional measures to relate function to structure and behavior and connectivity in the brain.

The WG recognized that phenotypes in DS are not static. To gain a better understanding of DS phenotypes, studies across the lifespan need to integrate biological measures (e.g., neurophysiology, imaging, omics) to obtain a much richer and deeper picture of what neurodevelopment looks like in the DS population. Addressing these issues will require development, refinement, and use of more functional measures of people with DS in the early part of the lifespan, including neurophysiology measures (e.g., electroencephalography [EEG], functional near-infrared spectroscopy [fNIRS]), expansion of imaging measures (i.e., functional imaging) and molecular studies (“omics”), and longitudinal studies to define developmental trajectory and maturation differences that may occur in people with DS compared with TD people. These biological measures need to be incorporated into behavioral studies going forward.

In preparing for this workshop, Dr. Lee conducted PubMed searches of DS across different stages of development. The searches included terms such as cognition and behavior and yielded a large body of literature. To assess the search results, the studies were broken down by 9 age brackets from birth and infancy to age 80 and older. The age brackets in early childhood were much smaller than for later years because of the active changes in and development of the brain and in cognition and behavior during that period of time. The overall number of publications per year was relatively small through adolescence and increased considerably starting in the young adult years (beginning at age 19). In contrast, the number of studies focusing on behavior was highest in childhood, while the number of studies focusing on cognition was relatively similar across all ages.

The studies on cognition were broken down further into language, executive function, and memory to assess possible trends in domains being evaluated. The DS cognitive phenotype drove the results to

some extent, but the largest number of studies on language peaked in childhood. The highest number of studies on memory was for the childhood through adult years (ages 6 to 44). The curve was flatter curve for executive function, which is a newer area of study for DS.

For cohort development, the WG was encouraged that knowledge is advancing regarding different stages of development and different domains of cognition and behavior, particularly in infancy and early childhood. However, many existing studies are characterized by small samples (i.e., lots of little “cohorts”) and use of heterogeneous measures, making synthesis across studies difficult. More work is needed to disentangle aspects of neurodevelopment that are more strongly associated with T21 versus the sequelae of co-occurring conditions and to describe trajectories for different subsets of people with DS.

Progress is being made through NIH Outcome Measures grants, efforts to adapt existing measures to DS (e.g., Stanford Binet, NIH Toolbox Cognitive Battery), and development of measures to use across a wider range of ages and stages of life to study the unfolding of neurodevelopment in DS.

Strategies to address these gaps include consortium development to gather larger cohorts with similar measures and inclusion of Fragile X syndrome (FXS) or other neurodevelopmental disorders as a models for DS. The WG also recommended creation of a “common core” of measures to be used across studies, with the caveat that the duration of core measures be short to minimize subject fatigue. A precautionary note from the WG was to keep in mind that one size does not fit all for human development, measure development, or study development. The group also stressed the importance of bringing together people with diverse backgrounds and who study diverse aspects of DS to discuss active research projects.

Behavior—Autism, ADHD, and Regression

Anna Esbensen, Ph.D., Cincinnati Children’s Hospital

Tarik Haydar, Ph.D., Children’s National Hospital

Dr. Haydar reviewed the basic science aspects of this domain, and Dr. Esbensen addressed cohort development.

The WG identified several key issues that define the current state of the science within this DS domain. The WG noted that investigators continue to try to define what is needed to assess basic science so that mouse models and human characteristics can be better linked. Stem cells and organoids may provide insights to understanding the association between co-occurring or similar conditions and DS, especially during early development. For example, autism spectrum disorder (ASD) is understudied in the DS population, and ADHD has a distinct impact on DS neurophysiology that could influence pharmaceutical interventions. A third condition, regression, is not well understood, particularly with regard to how it can be measured in basic science. Mouse models demonstrate decline in function by 1 year of age, but it is not clear whether this decline represents aging or indices of ASD and ADHD. Additional studies are needed to determine whether the current assessments and tests can accurately capture these different features of DS. Animal studies that might be able to correlate biological or emotional challenges (e.g., foot shock, injection of lipopolysaccharides to induce depression- and anxiety-like behaviors) with regression should be considered. Other studies of mouse models could explore preliminary data suggesting that regression in DS may be linked to DNA methylation.

The WG recognized the challenge of evaluating complex behaviors and co-conditions in DS and identified several general conclusions on gaps in basic science regarding these three cross-conditions in people with DS, as follows:

- Differences at the anatomical and cellular levels need to be studied to determine what distinguishes DS from autism, ADHD, and regression.
- The research should focus on molecular, cellular and behavioral components found in humans first to better use and evaluate animal models.
- Biobanking and linkages to phenotypic cohorts should be established. More biological samples are needed for sequencing.
- Assess through omics studies if the risk factors for DS are similar to and/or correlated with these other conditions. Study the intersection of gene and protein expression in DS, typical development (TD)+ASD, and TD+ADHD.
- Studies are needed to better understand the underlying mechanisms of these co-occurring conditions to inform pharmaceutical interventions specific to DS. Such studies would mirror similar efforts in FXS.
- Stronger connections and collaborations between basic and behavioral scientists are needed to inform research questions.
- Further examination of the relationship between immune function and in DS, especially in the brain, is needed.
- The need for post-mortem analysis of brain tissue is critical and would benefit understanding of cognitive outcomes in DS and any connection to ASD, ADHD, and regression. This type of analysis is commonly done for AD.
- Interface with other cohort studies. For example, the WG suggested connecting with the Alzheimer's Biomarkers Consortium of Down Syndrome (ABC-DS) to target questions for older adults with respect to ASD, ADHD, and regression and to boost recruitment of younger study participants. The ABC-DS collection post-mortem samples, which would benefit this domain.

The primary research gaps and recommendations by co-condition were delineated as follows:

- ASD
 - There should be clinical recommendations for genetic testing and markers of ASD in all people with DS.
 - It could be very useful to use pluripotent stem cells to identify early risk factors for autism.
 - Data suggesting a decrease in vasopressin expression in the CSF in autism may be a useful biomarker to look for in people with DS.
- ADHD
 - Human studies are needed to understand what to model in basic science.
- Regression
 - Studies are needed to characterize this condition in DS so that basic science models can be used to more fully evaluate those characteristics. Identifying or developing animal models that mirror this type of regression in middle age is critical.

The WG broke out the current state of the science with regard to cohort development for this domain by co-condition, as follows.

The prevalence of ASD in people with DS ranges from 2% to 60%, based on varying methods of ascertainment, with a 3:1 male-to-female ratio. Available data show that diagnosis of ASD in people with DS is often delayed by 2 years compared with TD children (at age 6 versus age 4). Cross-sectional comparisons in toddlers indicate that repetitive behavior does not differentiate DS plus ASD from DS alone. Associated characteristics and behaviors of ASD in DS are often related to low cognitive development. Methods of diagnosing ASD vary, and there are concerns that ASD diagnoses are missed, particularly in young adults with DS, if such behaviors are not captured.

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-occurring conditions on individuals' quality of life, aging process, and risk and onset of dementia.
- Mental health conditions (e.g., anxiety and depression) should be considered when using the label of "behavior" for people 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 TD+ADHD and the long-term impact of ADHD in DS over the lifespan.
- Regression: The etiology of regression in DS is not well understood. To address this gap, the WG recommended development of a clinically validated diagnosis, use of checklists for diagnosis, and determination of whether regression is a distinct condition or related to catatonia. Once a validated diagnosis for regression is in place, the trajectory for regression versus catatonia, including onset of each in people with DS, needs to be assessed and evaluated in intervention studies.

Cardiovascular Disease and Pulmonary Hypertension

Christine Seidman, M.D., Harvard University

Steven Abman, M.D., Children's Hospital Colorado

Dr. Seidman opened the presentation by noting that congenital heart disease (CHD) is a major feature of DS. Approximately 40% of persons with DS have CHD, compared with 1% of the general population. Predominant lesions in patients with DS include atrioventricular septal or canal defects (AVSD/Canal) (40% of cases), 40% have atrial or ventricular septal defects (ASDs/VSDs), and 6% have tetralogy of Fallot, a type of heart defect that is a combination of four congenital abnormalities. Epidemiologic studies show that the rates of CHD and types of lesions differ among DS patients. The rate of ASDs/VSDs is increased among Asians, American Indians, and Alaska Natives, and the rate of AVSD/Canal, which are more serious malformations, is twice as high among Whites versus Hispanics, Blacks versus Whites, and females versus males.

Numerous studies have sought to understand the precise genes that are responsible for these lesions. Results show that there is a CHD critical region on the distal end of chromosome 21 that contains genes that are important for these heart malformations. However, epidemiologic and clinical studies and mouse models of T21 demonstrate that T21 is not sufficient for the malformations to occur. For example, more than half of patients with DS have the genes in this CHD critical region, but they all have T21.

Considerable effort has focused on looking at other potential contributors to these defects, including common and rarer genetic mutations. To date, only modest influences have been found and are beginning to point to the roles of the Notch signaling pathway, ciliome genes, and other specific genes, particularly those related to the vascular endothelial growth factor (VEGF) signaling pathway. Development of heart malformations in T21 mouse models increases with addition of mutations in other genes (e.g., *Creld1*^{+/-}, *Hey2*^{+/-}). *CRELD1* is associated with AVSD in people with or without DS, and *HEY2* appears to be a transcription factor for *CRELD1*. About 40% of one strain of T21 mouse model (Dp3Tyb) has CHD, including AVSD. Additional evidence suggests that disruption of the dorsal mesenchymal protrusion by hedgehog-dependent genes (e.g., *Fox1a*, *Fox2*) during embryo development contributes to malformation of the atrial septal canal.

Heart disease in adults with DS is often related to their congenital malformations, including progressive deterioration of heart function, development of heart failure, Eisenmenger syndrome, and arrhythmias. Unrecognized and subclinical CHD includes mild to moderate valvular regurgitation, which can contribute to ventricular dysfunction over time.

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 to heart attacks and stroke compared with the general population. People with DS tend to have lower blood pressure and changes in arterial stiffness and in the thickness of the media of the arterial vessels that may contribute to these protective events. Better understanding of the protective mechanisms for the reduced risk in the population with DS would be an important advance in this field.

Despite a large body of knowledge of heart disease in DS patients, there are many gaps to fill, including the following:

- CHD: Why do some but not all DS patients have congenital heart malformations, and why is there such a prominence of a very few select malformations?
- Atherosclerotic disease: Why do some but not all people with DS develop atherosclerotic heart disease despite having CVD risk factors, and what accounts for improved vascular risk parameters?
- Limitations of models:
 - Why isn't DS with CHD recapitulated in T21 models?
 - What genetic, maternal, or environmental factors are missing?
 - How can those additional factors be used to improve long-term deleterious outcomes such as PH, Eisenmenger syndrome, and heart failure?

The WG identified the following strategies with potential to accelerate answers and address these gaps:

- Large cohorts with DS: It would be beneficial to have large cohorts with DS, including adults and children with and without CHD and longitudinal follow-up.
- Multipronged tissue analyses: Studying these cohorts by requiring biospecimens such as blood and heart tissues upon repair of a heart malformation that could be sequenced at the whole exome and genome levels and looking at the single heart nuclei transcriptome and epigenetic factors that affect cardiovascular (CV) changes in these groups.
- Better models: Add more tissues and cell types, genotypes, and environmental factors, including iPSC-derived cardiomyocytes and organoids (for epigenomics, proteomics, and transcriptomics studies) and trisomy and partial-trisomy mice, rats, and larger mammals.

Heart-lung interactions are very important in understanding the clinical course of many children with DS. Dr. Abman noted that many of the gaps in science and clinical research for heart disease in DS also apply to the issue of pulmonary hypertension (PH). Children with DS have a high incidence of PH, with 25–30% affected by this condition. The presence of CHD increases the odds of developing PH. Respiratory conditions outside the setting of CHD, such as pulmonary hypoplasia, asthma, chronic pulmonary hypoxia, and OSA, can also induce or exacerbate PH. PH contributes to high morbidity and mortality in children with DS and CHD and impacts post-operative management, resulting in longer duration of mechanical ventilation, inotropic support, and days in the ICU.

Gaps in basic science research for this domain include:

- Lack of sufficient genetic models of DS to specifically examine basic mechanisms of impaired growth and development of the upper and lower airways, distal airspace and vasculature;
- Limited understanding of basic developmental mechanisms underlying pulmonary vascular growth and maturation, including the coordinated growth of alveoli and distal vessels;
- Insights into the increased susceptibility of the lung circulation for PH due to co-morbidities that contribute to hemodynamic stress, intermittent or chronic hypoxia and inflammation; and
- Identification of specific signaling pathways as therapeutic targets for the prevention or reversal

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.
 - 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.

Respiratory and Airway Conditions (Including Obstructive Sleep Apnea)

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 difficult, and spirometry (the gold standard) may not be feasible in this population. A study by Dr. DeBoer's team is evaluating measures of pulmonary morbidity for clinical trials. Sleep 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 apnea testing, and a trial to assess use of positive airway pressure for the treatment of OSA in children with DS. Racial/ethnic disparities including prevalence, parental/family perceptions, and outcomes remain understudied and represent an important opportunity for investigation.

People with DS are at increased risk for anatomic and functional diagnoses affecting the lung and airway, which place this group at increased risk of pneumonia and poor lung development and pulmonary health. Other related conditions include pulmonary vascular disease, CHD, and increased risk of dental problems and immune dysfunction. People with DS also have structural and endocrine abnormalities, low muscle tone, and impaired neurocognitive development. This population has structural gastrointestinal (GI) tract abnormalities, including duodenal atresia, pyloric stenosis, esophageal atresia and stenosis, and malrotation. In addition, people with DS are at very high risk of OSA, with a prevalence ranging between 55% and 97%, compared with 1% to 4% in the TD population; the risks associated with OSA include complications from anesthesia and other sedating medications, recurrent pneumonia and hypoxemia, and feeding and swallowing problems. These co-occurring conditions and risks lead to an increased number and length of hospitalizations and increased need for intensive care when admitted to the hospital.

Dr. Tapia reviewed specific gaps and opportunities the WG identified in the clinical area of this domain. The primary clinical research gaps, including gaps in treatment, included the following:

- Dysphagia and its associated risks and outcomes need to be defined specifically for the DS population.
- Another condition, airway malacia, is also prevalent in this population. The condition needs to be defined for DS. Airway imaging can be used to study central airways non-invasively and better understand the effects of this condition on the breathing cycle and growth.
- CT studies of the lung parenchyma should be conducted.
- The safety of sedation and anesthetics in DS need to be better understood.
- Studies of the effect of ICU stays in DS, including respiratory failure and ventilation, need to be conducted.
- Consistent with increased risk of OSA for this population, assessment of feasibility and effectiveness of novel sleep diagnostics such as home sleep apnea testing and wearables is warranted.
- Another area to explore is the association between sleep and dementia, and whether sleep could be a biomarker of cognitive decline.
- Studies of circadian rhythm disorders would also be informative.
- Insurance companies do not always cover medications, as in the palivizumab trial for children with DS.
- Effective treatments for dysphagia, including outcomes to assess with treatment, need to be identified in people with DS need to be identified.
- Evidence-based data to create a diagnostic and treatment algorithm for dysphagia are needed as well.

- Treatment options for OSA and the effects of treatments on other outcomes (i.e., behavioral, developmental, dementia) need to be studied.

The WG identified additional clinical research gaps associated with specific conditions and diseases.

The relationship between pulmonary disease and sleep and immunity and infections with different viruses and bacteria is unknown. The microbiome is open to be studied in people with DS in relation to GI and airway abnormalities. The impact of dental health on pulmonary outcomes also needs to be better understood. Other important issues that are understudied in the DS population include pulmonary hypertension and pulmonary vascular disease, and growth, obesity, and failure to thrive. Feeding and eating disorders, such as ARFID (Avoidant/Restrictive Food Intake Disorder), have similarly not been sufficiently studied, and a specific definition of obesity should be considered for DS.

A lot of work is needed in terms of GI and aerodigestive conditions. For example, people with DS may have different GI anatomy and function than persons without DS, and further research is needed to clarify the interplay between the GI and respiratory systems. Dr. Tapia noted that approximately 50% of children with DS appear to have aspiration problems. However, this group is not well defined, and there is concern that subtler issues may be missed in at least some of the other 50% who do not have overt symptoms. Children with DS have decreased airway sensation, but it is not clear whether this is a primary deficit or secondary to another condition such as chronic aspiration. The interplay of decreased airway sensation with OSA also is not well understood. Studies to characterize airway sensation in DS would help answer these questions.

In terms of gaps in research over the lifespan, one area that needs study is how dysphagia changes and evolves from infancy to adulthood and how it affects AD. Further longitudinal characterization of lung function using novel techniques (e.g., forced oscillation) because use of routine pulmonary function testing (PFT) may not be feasible in many people with DS. Additional studies need to assess the risk of cancers such as Barrett's esophagitis that may be related to co-occurring conditions or effects of ionizing radiation.

The WG identified a series of gaps and needs regarding large data repositories and biobanks:

- Increased efforts should focus on mining of big medical/health datasets such as outpatient visits (e.g., in the MarketScan database) and hospital discharges (e.g., using Kids inpatient database)
- Aggregate of sleep study results within and across health systems would help identify phenotypes, as has been done in adults.
- Wearable technologies such as watch apps can provide insight into sleep and circadian rhythms in people with DS.
- Sample collection should be expanded to include bronchoalveolar lavage/other airway samples and biobanking of blood, nasal swabs, induced sputum, and pathology samples. Protocols should include samples collection from parents and siblings. Aerodigestive clinics could coordinate specimen collection and banking.

Dr. Tapia closed the presentation by pointing to the need to address racial and ethnic disparities, including family perceptions and health outcomes, which continue to be understudied. He noted, for example, that African Americans with DS may have worse cardiovascular outcomes than other groups, but better data are needed to inform this health measure and to improve and target evaluation and treatment options. Mixed methods research should be pursued to identify the patient/family experience and important outcomes/areas of research. These may differ by race, ethnicity, gender, and socioeconomic status (SES).

Q&A

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 INCLUDE-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 DS population 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. However, some of the existing tasks done with MRI are challenging for people with DS. Tasks for fMRI studies in children with DS have been developed or adapted recently and are being tested for their feasibility and appropriateness in this population. INCLUDE-funded projects will explore and compare executive function in the population with DS versus the general population.

Drs. DeBoer and Tapia responded to a question about how much of the dysphagia in DS is due to reduced muscle tone versus other factors. Dr. Tapia said one theory is that it could be secondary to reflux but the primary or underlying cause is not clear. Dr. DeBoer added that guidelines recommend a swallow study in patients who are on the lower end of the muscle tone spectrum, which might contribute to this condition in people with DS. Feeding problems are seen in about half children with DS, and true dysphagia with aspiration occurs in about half of those children, or approximately 25% of this population. The group with true dysphagia does not have the lowest muscle tone, however. Drs. DeBoer and Tapia agreed that more longitudinal studies are needed. One of the questions would be to identify ways to predict who has dysphagia to better understand and treat the condition and also to be able to prevent or mitigate risk of and progression to pneumonitis, bronchitis, and pneumonia in this group.

Another attendee noted that in the general population, co-morbid atherosclerotic diseases of the great vessels, particularly those of the intracranial vessels, seriously contribute to neurocognitive decline in aging people. In contrast, in the population with DS, there is far less atherosclerotic disease despite several risk factors, such as elevated lipid profiles. The question was what mitigating factors contribute to this difference in DS and what is the impact of co-morbid CVD on AD in DS. In response, Dr. Seidman

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 DS happens very quickly and is manifested clinically, with sudden loss of language, motor, and self-care skills and changes in the individual's 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 are thought to result in spontaneous miscarriage and asked what is known about when and why this occurs, and whether it can inform broader understanding of DS. Dr. Seidman said that serious cardiovascular malformations that occur, irrespective of DS, significantly increase risk of early fetal demise. Because there is a higher risk of such abnormalities in DS, they comprise one factor that may contribute to spontaneous miscarriage in this population. Confirming this type of event is complicated, however, given how early in fetal development spontaneous miscarriages usually occur. Other potential factors that might lead to spontaneous miscarriage in DS are not well understood.

There was universal support for a concerted effort to collect tissues from individuals with and without DS and conduct single cell omics studies to advance understanding of DS and co-occurring conditions. One strategy to achieve this goal would be to collect research samples in conjunction with clinical procedures and visits for a tissue repository. Dr. Parisi conveyed her long-term support of this concept and said it should be a research priority. She noted how technologies have advanced to the point where all omics studies can be done with frozen tissue, in contrast with prior requirements of fresh tissue, which was very complicated from a logistical point of view. Any discarded material can be frozen and analyzed as needed. A key issue in this process is obtaining consent for collection and subsequent analysis of samples.

A follow-up question was whether it is possible to include a funding stream for this work in awarded contracts, or if this is already being done. Dr. Parisi said it is an option to consider and that additional information and strategies would be presented and discussed during this workshop, in conjunction with sessions about cohort development and enhancement of specimen collection and integrating collection of data with data and biospecimen repositories. The mechanism by which this work would be done (e.g., grants, contracts) has not yet been determined.

Because of time constraints, several additional questions could not be addressed. After a break, the meeting continued with reports from the co-chairs of the remaining four WGs.

Cancer—Risks for Leukemia and Resilience to Solid Tumors

Soheil Meshinchi, M.D., Ph.D., University of Washington

Philip Lupo, Ph.D., M.P.H., Baylor College

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 DS starts 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 DS is 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 DS on leukemia development?
- Does T21 lead to therapeutic 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 DS background?

Through collaboration with the Children's Oncology Group, Dr. Lupo's team has been investigating the genetic underpinnings of why there is higher risk of ALL in DS. The group conducted a genome wide

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 in this population.

Cohorts and strategies that need to be developed to better understand cancer risks in people with DS include:

- Generating a richer and larger birth cohort that follows people with DS from birth to cancer onset
- Following cohorts after cancer diagnosis
- Including patients with DS in cancer survivor cohorts
- Having well-annotated clinical datasets
- Cohorts that collect or link information on DS as part of the Surveillance, Epidemiology, and End Results (SEER) Program or other cancer registries
- Better assessments of cancer risk in adults living with DS

Sample collection needs to include rigorous protection of subjects, tumor samples and corresponding non-malignant tissue, neonatal blood spots, cord blood, isogenic cells from people with mosaic DS, and human stem cell-based models.

Some of these issues and questions are being explored through INCLUDE projects addressing:

- Mechanisms of leukemogenesis in DS
- Hematopoietic progenitors in DS
- Whole-genome sequencing of DS-AML
- Pan-omics and deep phenotyping of DS-ALL
- Systems biology analysis of DS-ALL
- DNA methylation and immune development in DS-ALL

Establishing and strengthening collaborations will advance these efforts.

Autoimmunity and Infections

Joaquin Espinosa, Ph.D., University of Colorado

Bernard Khor, M.D., Ph.D., Benaroya Research Institute

Immune regulation in DS underlies many of the co-morbidities in this condition. Infections contribute to up to 50% of deaths among people with DS, with pulmonary infections predominant in these cases. Approximately 30% of people with DS have an autoimmune condition, which is about twice the rate in the general population. These illnesses are usually chronic and can significantly affect quality of life and a wide variety of organ systems.

Key questions in the fields of basic and translational science that will improve understanding of and ability to address infections and autoimmunity in DS involve further study of primary clinical features of immune conditions in people with DS, cellular and functional immune landscape, genetic features and interactions, mouse models, therapeutic selection, and broader interactions of the immune system.

Research under the category of key clinical features of immune dysregulation in DS should pursue better understanding of the spectrum of disease, autoimmune contributions (e.g., encephalitis), specific clinical features (e.g., age, prevalence), the sequence of autoimmune and/or infectious events, the relation of features to natural history of the immune landscape, the clinical response to specific

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, CHD, 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 prognostic or diagnostic value.

The WG also underscored the importance of interrogation of the cellular and functional immune landscape. The issues that warrant further investigation include better understanding of how the immune landscape in DS is different not only at baseline but in relation to age, co-morbid diseases, and treatment; the effect of antigen-specific T and B cells; the immune response to defined perturbations; vaccine response and durability (e.g. flu, pneumococcal), including different classes and routes of administration; preclinical autoimmunity, including identifying the events preceding autoimmune onset and the role of B/T cell responses; tissue-resident immune cells; and the role of the microbiome/virome/fungome. The mechanistic interactions between infection, autoimmunity, cancer are also relevant to cellular and functional immunity. Emerging omic technologies (e.g., TCR/BCRseq, citeseq, autoAb profile, iPSCs) need to be enabled to differentiate cell subsets including immune cells.

Understanding genetic features and interactions in the setting of DS is another area of interest. Studies should focus on identifying the key genes that drive immune dysregulation within chromosome 21 beyond those that affect interferon receptors and describing how those genes interact with other genes (e.g., single-nucleotide polymorphisms [SNPs] and polymorphisms linked with autoimmunity). Dr. Lupo noted that these efforts will complement a project in which 2,600 DS genomes in people with DS have been successfully sequenced. This project is being supported through INCLUDE, the Gabriella Miller Kids First (GMKF) Pediatric Research Program, and the Trans-Omics for Precision Medicine (TOPMed) Program. The plans for this project include expanding the sample to 20,000 people with DS. The WG also recognized the importance of mouse models in understanding the immune system and how it is dysregulated. Key issues in this area of research include:

- Identifying which mouse models best recapitulate key immune feature
- Interrogating immune cell—intrinsic and —extrinsic roles
- Interrogating organ-specific immune dysregulation
- Referencing results from preclinical and animal studies against human findings, in part through enhanced tissue banks

Another topic of discussion focused on how despite evidence that the immune system is different in people with DS than people without DS, very few studies have assessed how this difference affects the selection of therapies. Clinicians usually follow a hierarchy of medications that have been successful in modulating immune disorders in the general population (e.g., steroids, methotrexate, hydroxychloroquine). However, it is not clear if the same ranking is applicable to people with DS or whether the priority of drug selection and dosing regimens need to be re-ordered to allow for better therapeutic outcomes in people with DS. Other questions that need to be addressed is whether people with DS show increased resistance and/or escape from efficacy to any of these therapeutics. Having a good clinical understanding of how these interventions work in the DS population will, in turn, help shape basic and mechanistic research questions.

Strategies to address immune modulation and therapy in DS should focus on:

- Developing DS-specific therapeutic selection
- Considering an up-prioritized role for specific agents, including JAKi, IL-6/17/1b/IFNAR, and tumor necrosis factor alpha (TNF α)
- Better understanding and targeting dosing, resistance, and escape in people with DS
- Conducting integrative work to identify and subcategorize patients for treatment selection

- 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 systems. Neurological links show a potential immune impact on AD, autism, regression, behavioral disorders, and cognitive ability. Immune cells are found in the brain, and inflammation affects the activity of glial cells, which are present in both the central and peripheral nervous systems and perform all kinds of functions in the brain and the nervous system throughout the body. An interferon (IFN) signal in AD has also been identified. Clarification of the roles of specific immune and neurological cells is needed.

Several metabolic links between the immune system and DS have been discovered and are being investigated. For example, people with DS are predisposed to nonalcoholic fatty liver disease (NAFLD) independent of obesity, which can lead to more serious complications such as cirrhosis of the liver. Emerging evidence from mouse models of DS shows that immune perturbation exacerbates liver disease. DS also predisposes people to celiac disease and other conditions that affect the intestines, type 1 diabetes (T1DM), and hypothyroidism. A person's metabolic state, in turn, affects the immune system.

Diverse cohorts are needed to address the complexity of autoimmunity and infections in people with DS. Key factors to be considered in building these cohorts include studying different age groups across the lifespan; including siblings, people without DS, and people with and without immune conditions; developing definitive epidemiology that increases inter-hospital data connections, enhancing clinical metadata sets and analyses; enhancing immune-relevant tissue banks and targeting biospecimen collection; integrating noninvasive data (e.g., imaging); and increasing inclusivity and diversity of study cohorts.

Endocrine, Metabolic, and Skeletal Conditions

Randall Roper, Ph.D., Indiana University–Purdue University Indianapolis

Andrea Kelly, M.D., M.S.C.E., Children's Hospital of Philadelphia

Dr. Kelly and Dr. Roper presented side-by-side comparisons of clinical or human versus animal data, respectively, in summarizing the findings from this WG. The major themes of their presentation can be applied across conditions and were as follows:

- Scarcity of longitudinal, deeply phenotyped diverse cohorts across the lifespan limits the ability to describe the emergence and progression of endocrine co-morbidities, their contribution to non-endocrine conditions, and their impact upon quality of life, morbidity, and survival. This is especially relevant for clinicians who are trying to determine what needs to be treated and when.
- Animal correlates of endocrine, skeletal, and metabolic co-morbidities in DS have received limited attention.
- Studies of metabolic differences and bone health must consider the short stature and alterations in body composition and proportions that are characteristic of DS as well as sex and ancestry differences.

One area of interest and concern is the high proportion of adults with DS—up to 75%—who are overweight or obese. An overabundance of overweight and obesity is also seen in children and young adults with DS. This characteristic of DS has been attributed to low resting energy expenditure, but the

mechanism underlying this reduced resting energy expenditure is unknown. DS-specific body mass index (BMI) curves for children and adolescents show that the 50th percentile for adolescents with DS correlates with the 85th percentile on the Centers for Disease Control and Prevention (CDC) curves for TD individuals. These data also indicate that obesity (per BMI) tends to worsen with age for females, while it plateaus for males. A limitation of BMI for this population, however, is that the measure is based in part on height, and people with DS, particularly females, are shorter on average than the TD reference population. Thus, BMI may be inflated because of this trend toward short stature in adolescents and adults with DS. When body fat is taken into account, people with DS have lower body fat than the matched reference population at all BMI measures.

Mouse models have been used to understand a number of phenotypes associated with DS. Data show that Ts65Dn mice—the most commonly used mouse model of DS—weigh significantly less than their littermates without DS when they are young but then become obese later in life. Few studies, however, have investigated factors that might contribute to excessive weight gain in these animals. Information from animal models that could inform the human condition include having longitudinal correlations of weight changes with phenotypes to determine how T21 affects metabolism in these phenotypes and data on body composition and resting energy expenditure. A starting point would involve indexing all phenotypes to size (weight) in DS mouse models.

Another area of research involves cardiometabolic risk in DS. Data show that approximately one-third of adults with DS and one-third of adolescents with a normal BMI have abnormal levels of lipids in the blood (dyslipidemia). Prevalence of dyslipidemia increases markedly with increased BMI. Data show that approximately 70% of adults and adolescents with DS with a BMI in the 85th percentile have dyslipidemia. Abnormal glucose tolerance is seen in 25% of adolescents with DS, while up to 9% of adults have type 2 diabetes, with the higher rates reported in females. Rates of fatty liver are high in both non-overweight (45%) and overweight or obese (82%) children with DS.

In contrast with the general population, however, DS has traditionally been associated with little to no atheroma (abnormal fatty deposits or plaques in the arteries); as a result, people with DS are at low risk for atherosclerosis and are not expected to develop heart disease. This population has lower rates of myocardial infarction in males (but not females), lower rates of cerebral atherosclerosis, and lower left ventricular mass and end-diastolic function. A caveat regarding these outcomes is that some of the DS cohorts were 25–30 years younger than the comparator groups.

People with DS are at increased risk for T1DM, but the role of T1DM and other co-occurring conditions, including overweight and obesity, inflammation, glucose intolerance, dyslipidemia with OSA, AD and dementia, and macrovascular and microvascular diseases, in cardiometabolic risk in people with DS is unknown and warrants further investigation. The aging of the population with DS and other demographic factors, notably people at increased risk for diabetes based on ancestry (i.e., African Americans/Blacks and Hispanics), have largely been underrepresented in most studies of cardiometabolic risk and DS and need to be taken into account going forward.

Some clinical cardiac phenotypes have been replicated in DS mouse models, but generally not to the extent as those found in humans. The TcMAC21 DS mouse model seems to have cardiac anomalies that most closely replicate those seen in humans, but correlative studies of cardiac phenotypes to other phenotypes in these and other relevant models are currently lacking.

A third area in this domain involves skeletal health. Few studies have looked at fracture risk in youth and adults with DS. Data on the relationship between skeletal health and bone fragility, gross motor deficits, and vision issues are also lacking. Little information is available on bone geometry/microarchitecture

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, sex-based 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. Studies should be extended to determine if there are any additional skeletal deficits in males and when deficits start to occur in females, and to identify any underlying mechanisms to explain why skeletal deficits appear to occur much earlier in males than in females. In contrast with risk of fractures, at adulthood, women typically have earlier emergence of osteopenia and osteoporosis than men do. A key question for this domain is whether skeletal deficits are a threat to well-being of young and older people with DS.

Many of these issues can be explored in mouse models, in which bone geometry/microarchitecture and strength can be measured. Recent data indicate sexual dimorphism between DS model male and female mice, mimicking findings in humans. Another research question being pursued in DS mouse models is whether exercise influences bone health.

Other endocrine conditions in people with DS include autoimmune hypothyroidism, hypothyroidism, and hypogonadism. Increased levels of gonadotropins as markers of ovarian and testicular dysfunction present as early as infancy and toddlerhood in both males and females with DS. Puberty tends to be of normal onset and progression in this population, but females with DS are more than twice as likely to undergo premature menopause than females without DS, based on self-reported data. Gonadal hormone deficiency is associated with increased visceral adiposity, osteoporosis, skeletal muscle, and cognitive function. To date, however, there have been no longitudinal studies of gonadal function in the DS population, which is a clear gap in this research domain. The intersection of hypothyroidism, hypogonadism, and diabetes needs to be studied in DS mouse models, but there currently are no good models for these endocrine disorders in mice.

In closing, the presenters pointed to the complex interrelationships and phenotypes that contribute to a range of co-morbidities and conditions in DS, including overweight and obesity, excess adiposity and reduced skeletal muscle, reduced BMD and skeletal fragility, increased risk of fractures, OSA, dementia, diabetes, and hypogonadism. These conditions, in turn, can lead to reduced quality of life.

Aging and Alzheimer's Disease

Ben Handen, Ph.D., University of Pittsburgh

Mark Mapstone, Ph.D., University of California, Irvine

Dr. Mapstone reviewed basic science issues for this domain, while Dr. Handen addressed issues related to clinical care and cohort development.

Investigations of risk of AD in people with DS have been undertaken only recently within the scientific community as life expectancy for this population has increased. However, the basic science of AD in people with DS is driven by more than a century of research on AD in the neurotypical population on what is referred to as late-onset AD (LOAD).

The amyloid/tau/neurodegeneration (ATN) framework for LOAD is useful for understanding AD risk in people with DS. Accumulation of abnormal tau and beta-amyloid proteins is associated with AD-related brain changes. In general, the risk for people with DS comes from the extra copy of the amyloid precursor protein (APP) gene on chromosome 21. The APP gene provides instructions for production of APP, and the additional copy results in overexpression and an increased rate of amyloid accumulation in people with DS. The APP gene is present from conception. Production of this abnormal protein starts at

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 autosomal dominant Alzheimer’s disease (ADAD) or “familial Alzheimer’s,” depending on the stage of AD. Early deposition of amyloid is detected by PET imaging as early as the 30s in people with DS, while evidence of changes in brain glucose metabolism and accumulation of tau may occur as early as the 40s. In contrast, these changes tend to occur much later in people with neurotypical AD, usually in the 50s to 60s.

Deposition of amyloid in the part of the brain known as the striatum, which is a critical component of the motor and reward systems, is unique to ADAD and DS. However, with progression of the disease, the distribution of amyloid in people with DS looks more like the LOAD pattern. Other biomarkers of AD pathology in people with DS include amyloid and tau levels in the cerebrospinal fluid (CSF) and plasma levels of a structural protein, neurofilament light chain (NfL), all of which begin to diverge from normal as early as the 30s.

There still are many gaps in knowledge and outstanding questions regarding the basic science of AD in DS, including the following:

- How do critical life events affect amyloid and tau pathology? What constitutes typical brain development from both structural and cognitive standpoints, including during adolescence and adulthood?
- What are the neurodevelopmental trajectories of DS?
- Should we be studying children and adolescent biology for AD risk?
- How do complex co-morbidities influence risk for AD?
- How do other genes on chromosome 21 affect risk?
- What work is being done to develop better models of AD in DS (e.g., animal models, iPSCs, brain organoid)?
- What protective factors might mitigate AD in DS?
- Should we be studying childhood and adolescent biology for AD risk?
- What are the roles of other mechanistic pathways (e.g., inflammation, immune function, oxidative stress)?

Many of these basic science and clinical research questions are being addressed in the numerous cohort studies already underway. These studies include a broad range of groups—toddlers and preschoolers, school-age children, adolescents, and adults—and sample collections (brain, tissue, and bio-fluid donations). Some examples include investigations of language development in young child cohorts, OSA in adolescent cohorts, and dementia in adult cohorts (e.g., ABC-DS, LuMIND, H-21, LunDowns, TRC-DS). DS-Connect® alone has supported more than 50 research studies. Data-driven clinical cohorts collect and analyze information from patient mailing lists from child and adult DS clinics, large medical providers in the United States, nationwide medical databases, and regional and nationwide organizations (e.g., Special Olympics, The Arc). The “LIFE Group” from the May 2020 INCLUDE meeting was tasked with surveying existing cohorts in approximately 60 studies.

The WG divided opportunities into the following major categories:

- *Collaborations* should:
 - Leverage resources available through Aging and Disability Resource Centers, the National Alzheimer’s Coordinating Center (NACC), and IDDRCs;

- Involve university/hospital-based child and adult DS clinics;
- Develop a minimal dataset for visits; and
- Use GUIDs to pull together various registries.
- *Communities* should:
 - Involve missing subgroups (e.g., minority/underrepresented/underserved, severely cognitively impaired);
 - Work with advocacy groups, such as Special Olympics and The Arc.;
 - Work with large providers and national medical data bases; and
 - Expand the LIFE Group survey to develop a complete list of cohorts and registries.
- *Service-based efforts* should:
 - Expand the number of tertiary medical care programs for children and adults with DS;
 - Address transition issues (e.g., child to adult medical care), such as assisting families to identify adult providers;
 - Provide greater support to community practitioners to meet the medical needs of the population with DS (e.g., the consultant model, Environmental influences on Child Health Outcomes [ECHO] workshops for providers); and
 - Support continued updating of [medical guidelines for the care of people with DS](#).
- The *research enterprise* should:
 - Continue to support research in DS across the lifespan,
 - Expand research registries and means of contacting families and individuals about research opportunities,
 - Develop a complete list of DS cohorts including contact information for principal investigators,
 - Broaden opportunities to collaborate internationally, and
 - Include more racial and ethnic groups and underserved populations.

Q&A

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

A participant asked whether the raw sequencing data from the project that included 2,600 DS genome samples will be publicly available and, if so, when. Dr. Lupu confirmed that the complete genome dataset will be available to all in an estimated 6 months. Sequencing is underway and will generate a rich resource of genomic data coupled with phenotypic information. Dr. Parisi pointed out that one of the requirements of the INCLUDE program is that investigators share their data publicly as soon as possible, consistent with the goal of ensuring that genotype, phenotype, and all other data generated through INCLUDE funding are available broadly to the research community.

Another participant asked what researchers mean when they say they do not have “sufficient data” and how this problem can be resolved. For example, is the lack of data related to too few volunteers with DS, or is it due to other factors? Dr. Capstone said a stated need for more data is not necessarily related to whether sufficient-size cohorts are in place but may refer to investigators not being able to follow a group or groups long enough to answer the questions of interest. He added that the keynote presentations earlier in the meeting from the two families show there is clear interest within the community with DS to help researchers achieve this goal. Dr. Handen noted further that one question usually leads to another, leading to multiple follow-up questions that need to be addressed in additional research studies or experiments.

It was noted that biomarkers of the aging process are well developed for the immune system and that biological aging in DS, while typically thought of within the context of the brain and AD, needs to be

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 is an important issue that deserves further investigation, including in connection with COVID-19, and specifically whether there is evidence of advanced immune-related aging in DS and/or whether there is a point where risk of infection increases in this population.

A participant asked what is known about the onset of the autoimmune conditions in people with DS relative to the general population. Dr. Khor said the best evidence for accelerated onset of autoimmune conditions is from studies showing that 17% of children with DS age 2 and under are diagnosed with T1DM, compared with only 4% of the same age group in the general population. Larger, definitive epidemiological studies in different cohorts are needed to address this question.

Another question for the Aging and AD group was what factors are currently thought to be protective from the development of AD in people with DS. Dr. Mapstone noted that although certain factors, such as diet, exercise, aerobic exercise, and preventing head trauma and traumatic brain injury, all lessen the incidence or occurrence of AD, this is still a very open question. Dr. Handen added that cardiovascular risk factors appear to be less of a risk factor in DS and could be protective. The E2 variant of the apolipoprotein E gene (APOE2) is protective in the general population and may be in the DS population as well. Scientists do not know whether higher cognitive abilities or the extent of lifestyle and co-morbid conditions may be protective. Flipping the question to ask about protective factors rather than risk factors of the disease itself is an interesting approach, and several longitudinal cohort studies that are planned or have started are addressing some of these issues. Once definitely protective factors have been identified, researchers and clinicians can begin to develop therapies and intervene in ways to possibly slow or prevent the disease in different populations.

In a follow-up comment, a participant inquired about the differences in risk between atherosclerosis and AD and solid tumors and AD in the general population versus the DS population. In response, it was noted that the question as to why children and adults with DS are protected from co-morbid conditions such as atherosclerosis and certain types of tumors remains open. Data show that obesity and inflammation in general are associated with cancer risk. An environmental factor that is often overlooked is that people with DS typically do not smoke, which is protective for respiratory and cardiovascular conditions and certain cancers. Other phenomena that need to be studied further are the nature and scope of immune dysregulation and metabolic dysregulation in DS, which differ from what is seen in the general population, and the impact of co-occurring conditions.

A question for the Endocrine, Skeletal, and Metabolic Team concerned the mechanism contributing to loss of bone mass in people with DS. Dr. Roper stated that the mechanism is not well known, but current human and mouse model studies that are investigating cellular and molecular mechanisms may shed some light on this question. Possible mechanisms include a problem with osteoblasts that leads to reduced building of bone, abnormal activity of osteoclasts that leads to breaking down of bone, and the presence of a low bone turnover phenotype. Research has shown that the mechanism of loss of bone mass in people with DS may vary over the lifespan.

The last set of questions in this session focused on a rare form of DS—mosaic DS—which occurs in about 2% of all persons with DS. In mosaic DS, there is a mixture of cells; some cells contain the standard 23 pairs of chromosomes, and some contain the extra copy of chromosome 21. Babies born with mosaic DS may have similar features and health issues as those born with the more common T21 DS, but overall, they tend to have fewer of these characteristics. There currently is little research comparing the effects of mosaic DS with the more common form of the condition. A participant asked about research into cell types with active proliferation in mosaic DS and plans in place to include mosaic DS in future longitudinal

studies. In response, Dr. Handen noted, for example, that ABC-DS, which is exploring the connection between DS and AD, does not exclude people with DS mosaicism or partial trisomy; however, because the proportion of participants with mosaic DS is approximately the same as in the overall DS population, the total numbers in the cohort are relatively small. It may be necessary to combine several cohorts to get a large enough group of individuals with mosaic DS to obtain definitive answers to these questions.

Another limitation of current research is that mosaicism can only be estimated in the peripheral immune compartment. A lot of the phenotypic variability seen in mosaic DS may be in the tissues, but there is no good way at this point to estimate the extent of mosaicism in, for example, the brain, heart, or gut. Innovative approaches are needed to assess mosaicism in DS through non-invasive biopsies or other methods.

As with the Q&A session following the first set of WG reports, there was not enough time to answer all the questions submitted during this session. Some questions, however, likely were answered during the breakout group report-backs or during Day 2 of the meeting. The co-chairs noted that any additional questions were recorded and will be made available in the future.

Charge for the Breakout Groups

For the next session of the meeting, the eight WGs above were consolidated into breakout groups:

- Breakout Group 1: Development and Behavior
- Breakout Group 2: Heart and Lung
- Breakout Group 3: Cancer and Immunity
- Breakout Group 4: Aging and Metabolic Conditions

Each breakout group was charged with addressing the following questions:

1. Are there additional gaps and barriers that have not yet been raised?
2. How can these gaps and barriers be overcome?

Session 3: Breakout Sessions

The breakout groups met separately via Zoom. Workshop participants could join any of the breakout groups. At the end of the breakout sessions, the co-leaders of each group returned to report on their findings during Session 4 of the meeting.

Session 4: Breakout Reports Joint Basic and Cohort Development Session

Breakout Group 1: Development and Behavior

Lina Patel, Psy.D., Children's Hospital Colorado

Lotta Granholm, Ph.D., D.D.S., University of Denver

Are there additional gaps and barriers that have not yet been raised?

The group identified several areas in this domain that warrant further investigation.

Gaps and barriers regarding language development include the need for assessment of hearing loss and hearing health across the lifespan, improved communication between basic scientists and clinician-scientists, and understanding of the role of motor control within the body in hypotonia and speech, physical development, feeding, and related functions.

Increased focus is needed on adolescence, when significant developmental changes occur. Large studies in TD, depressed, and anxious adolescents document changes and differences in brain development in these

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 self-advocacy 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 DS population and define the biological basis of phenotype to guide and determine treatment interventions. Use of neuroimaging, EEG, and eye tracking are some of the approaches that can inform these questions. Additional research that targets the underlying biology of cognitive deficit progression beyond birth is also needed. Although IQ scores decrease over time in people with DS, this measure is based on comparison with TD children. Novel ways to measure these changes in people with DS over time are needed (e.g., looking at raw scores versus standard scores to capture gains in skill sets that occur in this population).

The group also supported research that advances understanding of what motivates people with DS at different stages of life. The group noted that much more longitudinal data are needed and that long-term studies need to follow younger, middle-aged, and older adults with DS.

How can these gaps and barriers be overcome?

The group divided its response to this question into what data, technologies, and treatments already exist and can be built on (current) and what is needed going forward (future). Efforts are currently underway to identify strategies to integrate data from biological samples, genes, and clinical presentations and between groups of scientists. Investing in existing cohorts and quantifying interventions being used (e.g., the Early Start Denver Model [ESDM] and other behavioral therapies and assessments) over the lifespan are two approaches that should be considered.

The following types of data and processes are missing and need to be explored going forward:

- Molecular, cellular, imaging, and hormonal timelines
- Neuroimaging data and functional imaging for language acquisition and processing and to inform behavioral interventions
- Collection of data across the lifespan, from the prenatal period to older adulthood
- Ways to improve recruitment for treatment intervention and assessment via public health approaches for outreach within the research community and expanding the pool of participants and diversity

These gaps and barriers can be addressed by integrating clinical care and research visits, developing more appropriate measures for the population with DS, identifying tests that are appropriate within and

across age groups, establishing larger cohorts to validate appropriate tests and measures, determining which measures have predictive ability for future use across ages, and harmonizing those measures.

Breakout Group 2: Heart and Lung

Michael Yeager, Ph.D., University of Colorado Denver

Beverly Rothermel, Ph.D., University of Texas Southwestern Medical Center

The group's report-back addressed the two questions posed—gaps and barriers and how to overcome them—together.

Are there additional gaps and barriers that have not yet been raised, and how can these gaps and barriers be overcome?

An overarching theme from this group was to promote and increase collaboration and data sharing not only between basic/translational and clinical researchers but also to take advantage of and leverage public–private partnerships. To facilitate these efforts, an integrated network and global dataset should be developed, along with mechanisms that connect data to people who can use this network and access these data (i.e., matchmaking for DS data). The group also supported longitudinal/life-course approaches and long-term follow-up of cohorts to address many of the research and clinical issues in cardiovascular and pulmonary health and disease in people living with DS. The group then identified specific priority issues and concerns for this domain.

The group discussed the high incidence of stroke and cerebrovascular events and thrombosis in adults with DS. To date, however, most studies of these conditions have been small, and larger studies in adults with DS, who are now living well into their 50s and 60s, are needed to better understand these co-morbidities in this population.

Studies should obtain non–cardiac-focused data from sleep clinics to more clearly define these types of co-occurring conditions in the DS population (e.g., blood pressure super dippers). Intake forms and electronic medical records (EMRs), including questionnaires for family members, should be revised to capture common exposures such as secondhand smoking that contribute to CV and respiratory problems in people with DS. The mechanism for pulmonary hypertension in people with DS is not well understood, and connecting the dots for this co-occurring condition would be beneficial for therapeutics. Another area of concern for people with DS is their increased risk for sleep apnea. One contributing factor may be degree of hypoxia, but better methods to examine that factor clinically need to be developed.

More robust genetic studies that take advantage of existing genetic models and use iPSCs are needed. The group was especially interested in studies that compare models with and without T21 to isolate change in genetic background. In addition, large genomic sequencing and computational studies should be conducted to advance this knowledge. Approximately 20% of children with T21 have atrioventricular (AV) canal defects, but many of the animal models, including the more newly developed animal models, do not have this or other CV abnormalities. Having a wider variety of genetic tools in animal models, including in rodent species other than the mouse, was highlighted during the group's discussion.

Collection and coordination of biospecimens and repositories, similar to what is done for pulmonary hypertension and thoracic aortic disease, should be supported. Repositories should include samples from children that do not have heart defects and isogenic cell lines created from those specimens. The Pediatric Cardiac Genetics Consortium (PCGC) is a key resource for this effort.

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 but can be challenging for people with DS. Some data show a success rate of only frequently done 10% to 15% when used with this population. Innovative alternatives to this testing are needed.

Breakout Group 3: Cancer and Immunity

Dusan Bogunovic, M.D., Icahn School of Medicine at Mount Sinai

Kelly Sullivan, Ph.D., University of Colorado Denver

The group’s report-back addressed the two questions posed—gaps and barriers and how to overcome them—together.

Are there additional gaps and barriers that have not yet been raised, and how can these gaps and barriers be overcome?

One of the main questions raised was when screening for TMD should begin, whether it should be mandatory, and what types of samples (e.g., cord blood for research) and testing (e.g., T cell/B cell receptor [TCR/BCR] sequencing, clonal hematopoiesis) should be included for this screening.

Another issue that needs further consideration involves immunosurveillance for solid malignancies. One question in this area of concern is whether to focus on individuals who develop solid tumors, which are rarer in the DS population compared to the general population; this effort would require multiple collaborations across the country to have a sufficient cohort to begin to understand the lower incidence in DS. Other areas of interest are the connection between autoimmunity and malignancies (including for the typical population) and linking the T21 status of tissue samples across cancer databases. Better understanding of differences in presentation of autoimmune conditions in DS and expanding clinical definitions and biomarkers (i.e., arthropathy) should be part of these investigations.

Differences in treatment modalities, including response/toxicity in patients with DS of all ages, and strategies for risk mitigation and early intervention also need to be investigated.

Clinical trials focused on people with DS should include cohort development and biobanking, expand clinical data capture and sample type diversity, maximize sample collection and biobanking efforts,

study the genetics of immune conditions in people with DS, and further develop and adapt methods for studies in DS populations.

Studies of the genetics of immune conditions in people with DS should explore sensitized backgrounds and differentially pathogenic alleles and exploit the use of mouse models, which have been underused in the study of malignancies.

The group acknowledged that although there are very clear differences in the presentation of autoimmune conditions in DS compared to the general population, a lot more that is not known about the immune system DS than is known. Whether the language and terms used to describe clinical presentation of immune conditions in the typical population also apply to DS or all new language is needed was also raised by the breakout group. Expanding biomarker studies that are specific to DS and then developing biomarkers that are suggestive of immune diseases in people with DS is one possible approach to address this issue. Trials that focus solely on people with DS may be needed to assess and compare different treatment modalities in this population also should be considered. More diverse cohorts and sample types are needed across studies, including those investigating the immune system in DS. As discussed by other groups and in other presentations, collection of samples for biobanking purposes, with the consent of the participant, should be pursued for this domain.

Breakout Group 4: Aging in Down Syndrome

Ann Cohen, Ph.D., University of Pittsburgh

Sheela Magge, M.D., M.S.C.E., Johns Hopkins University

Are there additional gaps and barriers that have not yet been raised?

There is a paucity of longitudinal data on aging in people with DS. Because people with DS are living longer and experience some important effects of aging earlier than the general population, longitudinal studies are needed to follow the aging process across the lifespan. Challenges with following individuals for decades could be addressed by using estimated age-of-onset models, which allow for studies of shorter duration. There also is a need for more diverse cohorts of historically underrepresented groups and better recruitment strategies to achieve more balanced study populations. Little is known about the underlying reasons for health disparities across groups, but both social determinants and biology may play a role in relation to aging in DS. The group also stressed that resources are needed to create and coordinate a nationwide infrastructure across clinics to develop a common U.S.-based dataset.

More data are needed on the important transition from adolescence to young adulthood. Given the changing demographics of the population with DS, there should be enrichment of cohorts and datasets for CHD and brain-related aging. In addition, variability in types of lesions compared with the general population should be studied. Differences related to race and social determinants of health also need to be investigated, which will require addressing gaps in recruitment and diversity in cohorts. Biomarkers need to be defined within the context of age of onset and use in clinical intervention trials and for individualized precision medicine. Better understanding of sensory deficits in aging could provide insight into changes in hearing and vision in older people with DS.

Results of studies on CVD in people with DS are mixed and reveal contradictions about mortality and morbidity from heart disease. Smaller studies suggest that adults with DS do not develop atherosclerosis, while data from the early 2000s show increases in ischemic heart disease in some cohorts. Results from the ABC-DS cohort show no evidence of increased prevalence of heart attack, atherosclerosis, or type 2 diabetes and a lower rate of obesity than in other cohorts. Younger people with DS have more dyslipidemia than their non-DS peers, but it is not clear if pharmacological interventions are worth pursuing over lifestyle changes if there is little to no heart disease later in life.

Questions about microvascular complications in relation to type 1 diabetes and other factors also were raised. Short stature in general appears to be related to increased risk of hypertension and stroke, driven by pulse pressure. Studies of adolescents and young adults have shown that people with DS had significantly lower levels of A1c, a marker of diabetes risk, than typically developing individuals.

The group identified studies of AD biomarkers in late adolescence/early adulthood as a key area of research to pursue. Markers that can predict the age of onset of AD present the opportunity to intervene before the onset of any pathological cascade. Evidence suggests that children with DS have higher CSF levels of amyloid than children without DS. Other markers of interest include blood-based beta-amyloid, tau, and NfL and mechanistic pathways of inflammation and glucose metabolism. Imaging may not be useful in people younger than 30. The trajectories of changes in biomarkers and how they relate to the TD population need to be studied and distinguished with regard to what is AD related versus what is neurodevelopmentally related. Key behavioral and cognitive markers in people with DS also need to be clearly defined and assessed for sensitivity.

How can these gaps and barriers be overcome?

The group identified several strategies to address basic science and clinical gaps and barriers for this domain:

- Develop an infrastructure system that has a uniform dataset and standards for biomarkers.
- Foster familiarity/trust relationships with providers to enhance participation in clinical studies.
- Take advantage of DS-Connect[®], which has extensive data that are waiting to be utilized and can be leveraged as a recruitment tool and to promote additional research in DS.
- Look into creating a resource for differences in consent laws from state to state, especially for adults with DS and people with DS people transitioning from adolescence to young adulthood.
- Develop a more comprehensive, uniform co-morbidity evaluation that better relates to risk factors for disease. For example, for CHD with AD, such an evaluation would include more detailed questions and inquire about the type of CHD (and surgical repair, where applicable) the patient has and tests that have been done or are needed to assess their individual risk.

Q&A

Dr. Schramm thanked the breakout groups for their thoughtful discussions and feedback. She noted some common themes across the four groups, particularly the need to coordinate research and care across the lifespan. The groups addressed the complex nature of DS and the role and interplay of multiple organ systems that manifest in a range of co-occurring conditions. The co-chairs and meeting participants were invited to offer any further comments or questions. Hearing none, Dr. Schramm noted that Day 2 would include topic-driven discussions and an additional series of breakout groups within the domains of basic science and cohort development. She then introduced Dr. Gary Gibbons, who made closing comments for the day.

Closing Comments, Day 1

Gary Gibbons, M.D., Director, NHLBI

Dr. Gibbons welcomed attendees and said he shares Dr. Bianchi's view that it is a privilege to work alongside people living with DS, who generously offer their unique perspectives, including their experiences participating in clinical studies. He noted that input from people with DS and their families enriches meetings such as the current INCLUDE workshop and make a real difference both in the lives of individuals with DS and for the larger DS population as well. Their participation and contributions underscore why the research community continues to pursue the science that is being done, to able to provide meaningful support and interventions to individuals living with DS. Dr. Gibbons also thanked the

breakout groups and workshop participants for their thoughtful ideas and recommendations that reinforce the core components of INCLUDE. 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.

DAY 2: November 10, 2020

Session 5: Joint Basic and Cohort Development Session

Welcome Remarks from INCLUDE Leadership

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 clinical trial, making the experience personal and relevant, and sharing the outcomes of the study. Participants said their research experience gave them a familiarity with hospitals and health providers, which made going to see the doctor a more positive experience. They asked that researchers try to schedule invasive research procedures such as blood draws to coincide with the participants' routine health care visits. The participants emphasized the need to keep participants informed using social media and understandable language. They suggested that more information about the transition from adolescence to young adulthood is needed.

Presentations on co-morbidities associated with DS followed, with discussions on development, behavior, CVD, PH, and respiratory and airway conditions. Additional presentations were given on cancer; autoimmunity and infections; endocrine, metabolic, and skeletal conditions; and aging and Alzheimer's disease. The meeting then divided into four breakout groups. The breakout groups identified the need for longitudinal cohort studies with well-validated endpoints, better animal and cellular models for preclinical data, more cohort diversity, integration of adult and pediatric cohorts into single cohort across the lifespan, collection of "samples of convenience" from routine surgical procedures, and better harmonization and linkage of databases. On the basic science side, the breakout groups discussed the need to bring together information on phenotypes of various mouse models, provide more funding opportunities for model development, and develop induced stem cells lines to generate lines from people with DS. Dr. Schramm announced that WGS data on 2,600 people would soon be available to be shared with the community. All presentations from Day 1 and selected presentations from Day 2 will be accessible on the [NIH VideoCast site](#). Dr. Schramm then outlined the agenda for Day 2.

Session 6: Concurrent Working Sessions

State of the Science: Basic Science and Cohort Development

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

Basic Science: Model Systems and Tools to Advance Down Syndrome Basic and Preclinical Science

Dr. Schramm welcomed the attendees to the basic science session, co-chaired by Anita Bhattacharyya, Ph.D., University of Wisconsin–Madison, and Roger Reeves, Ph.D., Johns Hopkins University.

Introduction

Roger Reeves, Ph.D., Johns Hopkins University

Dr. Reeves outlined the upcoming topics and introduced the speakers.

Current state of mouse models and phenotypic drift

Randall Roper, Ph.D., Indiana University–Purdue University Indianapolis

Dr. Roper said that one of the difficulties with mouse models is that some of the genes found on human chromosome 21 are located on three different mouse chromosomes (chromosomes 10, 16, and 17). This is one reason that it is impossible to make a completely accurate genetic mouse model of DS.

More than 20 mouse models have been created to study the gene–phenotype relationship of DS. A 2019 paper, “[Mouse models of neurodegeneration: Know your question, know your mouse](#),” addressed the question of which mouse model is best for a particular research project. The paper describes the available mouse models, their phenotypes, and some of the ways they could be used.

- **Transgenic mice** have multiple copies of exogenous DNA. The phenotype severity often depends on the transgene copy number. There are possible artifacts from overexpression and from the insertion site of the transgene. These mice can be used to investigate later-stage disease mechanisms.
- **Knock-in mice** have one sequence replaced with another sequence. The phenotypes could be mild and may appear in mid- and late life. These mice may be used to investigate earlier-stage disease mechanisms.
- **Genomically humanized mice** have a mouse sequence that is replaced by an orthologous human genomic region. The phenotype produced may appear in mid- and late life and may be mild. These mice can be used to investigate earlier-stage disease mechanisms.
- **Chromosome engineered aneuploidy mice** are mice in which a chromosomal region is duplicated or deleted. The phenotype may be mild. Its uses include investigating dose-sensitive genes and mechanisms.
- **Transchromosomal mice** have human chromosomes added to the mouse genome, meaning that it expresses mouse and human genes. The phenotype may be mild. Its uses include investigating dose-sensitive genes and mechanisms.

Researchers should know the genetic content of their mouse including which genes have three copies, how the trisomic information is inherited, and whether there is a freely segregating chromosome (as is true in most people with T21). Researchers should also know whether the model can replicate human phenotypes, especially those seen over the course of human development, and whether there are non-

anticipated effects of genes in three copies on other chromosomes that are not orthologous to human chromosome 21.

- The Ts65Dn mouse model is the most frequently used mouse model in DS research. These mice contain the freely segregating extra chromosome, but has a centromere and 35 protein coding genes that are not orthologous to human chromosome 21. The model is not amenable to an inbred background, that is, they must be kept on a mixed background; most of the males are infertile.
- The Ts1Cje mouse model has fewer genes in three copies than the Ts65Dn. It is a translocation model, and there is a single copy of genes on mouse chromosome 12.
- The Dp16 mouse model has two subtypes: Dp16Yey and Dp1Tyb. It is a duplication model that contains three copies of all the homologous regions on mouse chromosome 16 corresponding to human chromosome 21. There is no freely segregating chromosome, the males and females are fertile, and the mice can be inbred.
- The Tc1 mouse model is a transchromosomal mouse model. There is mosaicism; that is, not all cells inherit the extra human copy of chromosome 21. Many genes are found in only two copies, and there are significant rearrangements, deletions, and duplications.

Dr. Roper presented a chart showing how the various DS mouse models were derived and their mouse strains of origin. For example, Ts65Dn derives from radiation-induced translocation from chromosome 16 to chromosome 17. Its origins are from B6 and C3H mice, but it also has remnants of the DBA/2j mouse. One study showed that embryos from a B6 background have a greater incidence of heart defects compared with mice from a mixed background. Another showed that different genetic backgrounds of the Ts1Rhr mouse produces significant phenotypic differences in the development of the pharyngeal arch.

There are two different lines of Ts65Dn mice: the 1924 and 5252 lines. The 1924 line has a propensity to retinal degeneration and blindness, leading to development of the 5252 line, which was bred to have a very similar genetic background to the 1924 line but without the propensity to blindness. Researchers have tracked the differences between the 5252 and 1924 lines and found that, over time, genetic drift has influenced the mouse phenotype both within and between the lines.

Researchers should use both male and female mice and disaggregate the results when doing their analyses. When the data for men and women with DS are disaggregated, men with DS show a significant reduction in BMD beginning in their 30s, and women show it beginning in their 40s. Dp1Tyb male and female mice show the same pattern of bone density loss with a similar difference between the sexes.

Dr. Roper made the following recommendations for authors of papers using mouse models:

- List the source and strain of mice (e.g., Ts65Dn 1924 or 5252) and how long they have been bred in the colony to estimate the genetic drift.
- Provide the genetic background of the mouse model.
- Use mice of similar ages when conducting a study.
- Use well-defined protocols and provide the order of tests.
- Describe how the animals were housed and handled, which can affect phenotype.
- Use male and female animals and in sufficient numbers.

TcMAC21 and upcoming rat models

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 penetrance and expressivity of the phenotypes vary significantly among all models.

There are about 20 DS mouse models, including a transchromosomal model developed by Elizabeth Fisher, F.Med.Sci., that contains a human centromere. One problem with the model is that only about half the cells in those mice retain the human centromere. It is not known when in the mouse development the centromere is lost. These random changes to the genotype mean that every mouse has a different developmental and functional environment, making it hard to draw conclusions from those mice.

Dr. Reeves described the development of the TcMAC21 mouse model. Researchers created a mouse artificial chromosome containing the long arm of human chromosome 21. The researchers who developed the mouse, Yasuhiro Kazuki and Mitsuo Oshimura, relied on a procedure called microcell-mediated chromosome transfer. The resulting TcMAC21 mice are not mosaic; all their cells have the human chromosome 21.

The TcMAC21 mouse retains 93% of the human chromosome protein coding genes on its mouse artificial chromosome. The mouse is not mosaic, and human genes are expressed at expected levels. WGS shows that there are no deleterious mutations in this model.

TcMAC21 mice grow slowly and are small, analogous to people with DS, who tend to be short. TcMAC21 mice have a small cerebellum. The cerebellum in people with DS is smaller and hypocellular. About 4% of Ts65Dn mice have a structural defect in the heart compared with 31% of the TcMAC21 mice. About 45% of people with DS are born with a structural defect in the heart. The TcMAC21 does not have a complete AV canal. This could be a shortcoming in these models. Craniofacial skeleton analysis of the TcMAC21 mice reveals a shortened face, reflecting what happens in humans with DS. But the craniofacial deficits are milder in TcMAC21 compared with Ts65Dn. Both the TcMAC21 and the Ts65Dn mouse models show learning and memory impairments compared with euploid mice. The hippocampal long-term potentiation is perturbed in both models. Comparing mouse models on learning and memory tasks is very important, although carrying out the experiments to compare them is costly.

The analysis above is the type of phenotypic analysis that researchers should do on mouse models. The first step is to know whether the model has characteristics that are parallel to what is found in humans.

Work has begun to insert the human chromosome 21 into rats. There are two regions of the rat genome that contain all the orthologs of the human chromosome 21 whereas in mice, the orthologs are spread across three regions. In an unpublished study, rats were found to tolerate the human centromere, and unlike mice, rats are rarely mosaic. The craniofacial skeleton and the cerebellum are more affected in the rat model compared with some mouse models.

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.

Current state of human stem cell research and induced pluripotent stem cell resources for Down syndrome research

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

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

Among the advantages of iPSCs 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, iPSCs 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 which people with DS are at higher risk, such as congenital heart defects, leukemia, GI disorders, intellectual disability, and AD.

The somatic cells of people who are mosaic for T21 can be induced to become iPSCs, which will also be mosaic for T21. The resulting mixture of trisomic and disomic cells will show differences in gene expression because of the extra chromosome in the trisomic iPSCs. The disomic cells can be used as isogenic controls for the trisomic cells. Isogenic controls are popular because they can be used to pinpoint trisomy-specific changes.

The classic way to grow and study cells is the two-dimensional (2-D) cell culture. But the 2-D cell culture does not reveal the complex cell-to-cell interactions that occur in the living organism. In recent years, there has been a lot of interest in using three-dimensional (3-D) cell cultures. The 3-D method involves allowing cells to self-organize into organoids that allow for more cell types and more cell interactions than the 2-D method. The 3-D model has challenges. The cells do not always organize in the same way every time, and the degree to which the cells self-organize can vary significantly.

Another challenge is that human iPSCs develop very slowly compared with mouse iPSCs and thus are less useful for researchers studying postnatal events. One way to overcome the problem of slow development is to transfer the developing cells into a mouse, which speeds the cell development. The human iPSCs integrate with the cells of the mouse.

Most of the work on DS using iPSCs has been done in the nervous system (e.g., neuronal and glial cell development), and most of the neuronal studies have been done using the 2-D model, but 3-D is emerging. There has also been work on oxidative stress and mitochondrial deficits, transcriptomics and epigenomics, and cell degeneration in the context of AD and aging. While this work has promise, researchers must be careful to ensure that the phenotypes that they are investigating have relevance to DS.

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 iPSCs 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 iPSCs.
- iPSCs are limited in their maturation and aging.

Ways to address these challenges include the following:

- Banking iPSC lines in a central repository
- Producing more iPSCs from diverse people
- Linking patient clinical data to the cells
- Developing rigorous and reproducible methods to enable cross-study comparisons

Patient-derived iPSCs will continue to be important. Future work is likely to include greater use of CRISPR and work should expand to other genes that may be modifying DS phenotypes. There will be more insight into the variability among people with DS. The power of the iPSCs could be harnessed to address more organ systems, not just the brain. And more attention can be paid to disease conditions such as AD.

Extracellular vesicles (EVs) and deposition of amyloid in organoids derived from iPSCs

Vasiliki Machairaki, Ph.D., M.Sc., Johns Hopkins University

Dr. Machairaki's laboratory does research on familial AD. The laboratory generates human iPSCs from patients with familial AD, producing neuronal cell lines that have the presenilin mutation, the most common cause of familial AD. Dr. Machairaki described the methods employed in producing the iPSCs.

The laboratory is using 3-D cultures to better mimic the complexity of human brain cells and how they interact. The cells organize in discrete regions within the organoid, much as they would in the development of the human brain. The cells also show increased synaptogenesis, and after two months, the glial cells begin to form. The laboratory compared the organoids with familial AD pathology to organoids of healthy control cells. The AD organoid showed evidence of amyloid beta (A β) and tau pathology.

Dr. Machairaki's work also focuses on how EVs are involved in the pathogenesis of AD and how they can be used in the discovery of biomarkers. EVs are secreted from nearly every cell in the human body, including brain cells, and are involved in cell to cell interactions. The EVs in brain cells can cross the blood–brain barrier and enter the bloodstream.

EVs have surface markers that are specific to the neurons from which they are secreted. The EVs contain cargo such as proteins, lipids, and microRNA. The cargo can be analyzed and used as biomarkers. MicroRNA and protein profiling of brain-derived EVs suggest that there are significant differences between AD and control brains.

The Machairaki laboratory studied the roles of EVs in AD pathology using EVs secreted from AD iPSC-derived neurons. They found that, although there was a relatively low amount of A β in the EVs, there was an increased ratio of A β -42 to A β -40. This was an important finding because the ratio of A β -42 to A β -40 is a better biomarker of AD than A β -40 or A β -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.

Cohort Development: INCLUDE Data Coordinating Center and Existing and Future Cohorts

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.

Introduction to the INCLUDE Data Coordinating Center

Joaquin Espinosa, Ph.D., University of Colorado

Dr. Espinosa described the three components of the INCLUDE 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 INCLUDE 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 INCLUDE 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 DS DCC 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 Crnic 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.

Overview of Existing Cohorts

Amy Brower, Ph.D., American College of Medical Genetics and Genomics

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. Sixty-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 Cura 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.

Considerations for Global Unique Identifiers (GUIDs) and Linkages for DS-INCLUDE

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

Dr. Waitman said the main reasons for linking or sharing data are to obtain a larger cohort, to combine data in a more elegant way, and to streamline workflows for participants. Linking data can also help overcome privacy and regulatory restrictions and facilitate secondary use of data. Dr. Waitman described the following options for linking data:

- The GUID approach, which was popularized by National Database for Autism Research (NDAR), uses software that identifies whether specific inputted protected personal information (PPI) on participants (sex, first, middle, and last name, date of birth, and city or municipality of birth) matches an existing GUID, or whether a new GUID should be created. The benefits of this system are that the PPI never leaves the researcher's computer, nothing about the GUID allows anyone to infer identity, and the same individual's information will result in the same GUID across time, location, and research study. The NDAR GUID is used by NIH's DS-Connect[®] registry. However, it is not always possible to use GUIDs. Some studies do not generate GUIDs, some GUID approaches used by NIH collect different pieces of PPI and therefore cannot harmonize with the NDAR GUID, and some valuable data resources, such as death records, lack required information on the city or municipality of birth.
- PCORnet is a resource funded by the Patient-Centered Outcomes Research Institute (PCORI) for linking existing health records to support observational and prospective research. PCORnet is

a “network of networks” that harnesses the power of partnerships by combining health records 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 (<https://datavant.com>) 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 “user” to generate codes. It is useful for tracking patients across multiple health providers and 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-Connect®. After consent and other forms are filled out, participants are streamlined directly from DS-Connect® to the DS-DETERMINED Self-Determination Inventory, which is exploring the relationships between self-determination for those with intellectual and developmental disabilities and their health. The referral code is stored in each dataset for linkage during analysis. REDCap tracks payment (a gift card) and recruitment.

In summary, Dr. Waitman said GUIDs are a well-proven approach and a core component of DS-Connect®. Other large studies and trials like NC3 that see the benefits of population linkage use PCORnet. For DS-INCLUDE, linkage to biospecimens supporting translational research plays a heightened role.

Biospecimen Collection and Associated Datasets

Elizabeth Head, Ph.D., University of California, Davis

Dr. Head listed the members of the DSH Biospecimens Working Group (BWG) and outlined the biospecimens that the group recommended, adding brief comments about some of them:

- Blood: DNA, RNA, plasma, serum, and peripheral blood mononuclear cells (PBMCs). Dr. Head noted that blood is very easy to obtain and provides considerable data.
- Brain
- CSF
- Saliva
- Cord blood
- Surgical specimens (e.g., bone marrow aspirate, thymus, heart and tonsillar tissue). Dr. Head said these specimens would be collected while the person is already undergoing surgery for a procedure unrelated to the research.
- Urine
- Stool. Dr. Head said this specimen was chosen because of ongoing work on the microbiome.
- Skin/fibroblasts

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 outlining steps to take after determining whether a blood draw is possible. If a blood draw is not possible, the alternative is to collect mouth swab, saliva spit, and skin tape biopsy and provide mail-in kits for urine and stool samples. These biospecimens would be stored cold or shipped to a third party. If a blood draw is possible, blood would be collected using PAXgene® RNA and DNA blood tubes. If no freezer is available the tubes would be shipped to a third party for RNA and DNA extraction. If a freezer is available, ethylenediaminetetraacetic acid (EDTA) and/or sodium heparin blood tubes would be collected. If a centrifuge is available, the blood would be fractionated into plasma, serum, and white and red blood cells. If no centrifuge is available, the blood would be frozen or shipped to a third party for DNA extraction.

The BWG suggested that for brain donation, where the protocols are well established, dissection and processing protocols should be consistent with the AD research centers and with protocols used by the NIH NeuroBioBank. Like blood collection protocols, brain collection protocols depend on available equipment at the collecting site. At a minimum, the brain should be bisected and placed in fixative. The best option would be to bisect the brain into left and right hemispheres, fix one half in formalin or paraformaldehyde, and coronally section and freeze the other half. Some sites prefer to subdissect certain brain regions before freezing. Long-term storage of fixed tissues must also be considered.

Since one of the most common co-morbidities in DS is AD, the BWG focused on aging and AD outcomes. Until recently, it was not feasible to upload neuropathology data from people with DS to NACC, because a clinical module for DS was not available. Now a brief form has been developed for DS, and the NACC database will include people with DS, which will present new research opportunities.

The BWG was also charged with assessing the efficacy of multiple biobanks compared with a central biobank. Central biobanks offer more standardization of protocols and procedures, with common datasets and the need for only a single material transfer agreement (MTA), but stakeholders might prefer to maintain their own biospecimens and not lose control or ownership of specimens. Multiple biobanks offer expertise in specific biospecimens, allow research-driven biospecimens to remain under the investigator's control, and could develop virtual biospecimen inventories. However, tracking of samples may be more difficult, investigators would need multiple MTAs, and the quality of associated clinical data may vary. The BWG concluded that, given the diversity of samples, a hybrid model might be feasible. But at a minimum, the goal is to develop a virtual biorepository that enables searches of specific sample types, their locations, and the PIs to encourage and facilitate collaborative research. NIH would be the ideal home for this repository. The platform should ensure uniform access by using a common sample request and investigators could indicate whether they are willing and able to share samples. Institutional review board (IRB) consents will need to be broad to address sharing. The problem of needing multiple MTAs when using multiple sites must also be addressed.

Dr. Head said other working groups are discussing what type of clinical data should accompany the specimens and opined that collecting just sex and age is not sufficient. More discussion is needed about GUIDs and how to link biospecimens back to clinical data, and a better understanding of existing cohorts and their willingness to share collaborative samples is required. Investigators must consider strategies to encourage families and self-advocates to donate samples and also find ways to give back to families and communities who are willing to make the sacrifices necessary to further research.

Discussion

- 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 National Disease Research Interchange (NDRI) or the Cooperative Human Tissue Network (CHTN). Dr. Seidman said the Pediatric Cardiac Genomics Consortium (PCGC) has experience in multicentric sample collections. She offered to share information about the PCGC, noting that a common IRB can overcome concerns about multiple consents.
- The mother of a child with DS asked how the DCC would reach out to families to have them sign up for studies. Dr. Espinosa said the DCC would prioritize working with DS-Connect[®] to increase outreach to families to find studies through both DS-Connect[®] and the data portal.
- Dr. Head, noting that community medical groups do not have the equipment, such as freezers or centrifuges, needed for collecting specimens, asked how to make it easier for these offices to collect and send samples for sharing. Dr. Esbensen suggested creating an infrastructure for researchers to obtain blood vials to give to participants, who would then take those vials to their physician for collection and shipping. This might require additional paperwork depending on where the blood was being drawn (e.g., at the physician's office or at a lab) and would implicate consent issues. Dr. Head cautioned that if researchers send a participant to a physician to get a blood sample, they must be sure the participant is not billed for research blood.
- Dr. Espinosa clarified that the DCC will not do biobanking. Various sites will collect specimens and biobank. The DCC will provide guidance about the preferred way to collect specimens for maximum collaboration but will not be a centralized biobank. Dr. Resnick added that although the DCC is not a repository, it must ensure that all biospecimens are data linked. This will involve working with biobanks on linking to an entity and a subject, which goes to the question of having central or multiple biobanks. Dr. Resnick suggested that it does not have to be an "either-or" choice; the key is to be able to interconnect across infrastructures to derive data from them.
- Dr. Seidman said the PCGC has collected more than 15,000 biospecimens with linked clinical information using well-defined strategies. Ensuring that a specimen from the doctor's office is a consented specimen could be accomplished by having the physician get the consent form online and have it signed before taking the sample. Another area to consider is having a mechanism in place, perhaps using FedEx, for the delivery of samples that require rapid transport but need not be immediately fresh. This will be important for getting broad involvement. Central IRBs will be needed to address these issues, particularly when consent involves existing specimens. Dr. Seidman suggested that a central repository is much more valuable than lots of little collections, and she cautioned that cost must be addressed, because this banking is very expensive. Dr. Espinosa said that he has prioritized discussions with the PCGC. He noted that centralized and multiple repositories are not mutually exclusive mechanisms. Dr. Head agreed, saying there is a two-pronged issue: how to deal with existing specimens and how to make the process easier going forward.
- Dr. Resnick said that in a decentralized biobank process, it is important to track and validate the consents being used across institutions to ensure that use of the data from the specimens is aligned across those consents. He noted that delays in cohort collection could be significant if there are not preexisting agreements across institutions to fast-track the request process. This is

a particular consideration for existing cohorts. The DCC repository could potentially create master agreements to expedite requests. Dr. Espinosa said areas such as how samples were prepared and how data are shared will be looked at more deeply and the DCC will be reaching out for more information to enable the assembly, even virtually, of cohorts across sites that are ready to be harmonized to expedite research.

- A family member of an individual with DS asked whether the data portal would show advances in research. Dr. Espinosa said the challenge is for the data portal to serve multiple stakeholders with multiple needs and he would expect to share discoveries on the portal. Dr. Resnick added the portal should be a place where people go multiple times for information and where they could receive notifications when something of interest to them, such as a new study or a new cohort, arises.
- Dr. Espinosa emphasized the importance of metadata attached to samples, saying that metadata makes the database more powerful. Dr. Mapstone suggested the importance of knowing in advance what type of analyses the samples will be used for so that they are collected properly for the method the investigator is using. There is a difference between the type of samples needed in transcriptomics or genomic analyses, where fasting is not imperative, and the type need in metabolomics analyses, where fasting is important. Dr. Espinosa said the role of the DCC is to be a good listener and get the best consensus about an emerging set of needs from researchers.
- Dr. Lupo said there are 30,000 children in the Childhood Cancer Survivor Study, which has been a paradigm for characterizing chronic health conditions in survivors of childhood cancer over time. He asked whether NIH would be interested in developing a bona fide cohort beginning at some point along the life course and following these participants over time. It would serve as a resource for recontacting participants, obtaining additional samples, and having a template to build on. Dr. Espinosa said the importance of a longitudinal cohort of the lifespan is undisputed. There are fragments of this in the field for adults and more will be learned in polling the research community. Probably no one single site could run such a large cohort, but many sites would likely be interested in being part of such a longitudinal study. This is part of what the DCC is tasked with identifying.
- Tracie Rosser, Ph.D., asked about having the participant portal be a part of DS-Connect®, suggesting that for consents to share data, especially from existing datasets, DS-Connect® could be leveraged to potentially consent participants to broad sharing from all past studies. Debbie Jae agreed and suggested also using DS-Connect® for “just-in-time” consents, which would address the problem of investigators not having anticipated other research avenues at the time of the initial consent.
- Dr. Parisi said work on data harmonization and back-end integration is being done in an effort to link the datasets. She elaborated on the idea offering online consent for research purposes. The online consent could be used when people with DS go to a clinic for a regularly scheduled blood draw and want an extra tube drawn for research. They could access their DS-Connect® account to show that they had consented for that particular research purpose. This may also be a way to utilize the registry. Dr. Head also endorsed the idea of online consent through DS-Connect®, saying it would enable people who are not directly involved with research studies to contribute, would reduce bias by adding an increasing number of people, and could encourage better recruitment of underrepresented minorities. She suggested that to be successful, it would require community outreach. Dr. Rosser observed that the online consent would cover the permission, but there must be a process for getting the appropriate collection kit to the participant. She suggested that collection kits could be ordered ahead of the appointment and sent to the participant, but she asked who would cover the cost.
- Dr. Waitman suggested considering the Datavant option, noting that Quest Diagnostics is now signed up with Datavant. If enough information were collected to allow for a reliable match with

the participants' EHRs, it would be possible to know which DS-Connect® participants have been seen in other systems. Then, on a project-by-project basis, data could flow from one environment to another to determine whether an upcoming appointment offered the potential for a research blood draw.

- Dr. Rosser said that if blood draws are done at labs such as Quest or LabCorp, research accounts with the labs could be established, and after draw orders were entered for the participant, the labs would know what to draw and would have the tubes there already. She suggested Apple Health as a good option for allowing people to share their health information and link to DS-Connect® as well.

Session 7: Breakout Sessions

A new set of four breakout groups met during this session:

- Breakout Group 5: Ensuring Robust iPSC and Organoid Systems as Preclinical Models
- Breakout Group 6: Ensuring Robust Animal Model Systems as Preclinical Models
- Breakout Group 7: Clinical Phenotyping and Minimal Common Data Elements
- Breakout Group 8: Biospecimens and Related Omics Datasets

Breakout Groups 5 and 6 each addressed these four questions:

1. How does one choose the best model system?
2. How does one ensure rigor and reproducibility?
3. What clinical and epidemiological data would be useful to guide basic science studies?
4. What biospecimens would basic scientists like to have access to and for what uses?

Breakout Groups 7 and 8 each addressed these four questions:

1. What clinical and phenotyping data should be collected?
2. What biospecimens should be collected and for what purposes?
3. What fundamental scientific questions are addressable by large cohort studies?
4. What clinical scenarios require a deeper understanding of the underlying science?

At the end of the breakout session, each group returned to report on their findings.

Session 8: Breakout Reports Joint Basic and Cohort Development Session

Breakout Group 5: Ensuring Robust iPSC and Organoid Systems as Preclinical Models

Peng Jiang, Ph.D., Rutgers, The State University of New Jersey

Jeanne Lawrence, Ph.D., University of Massachusetts Medical School

Dr. Jiang reported on behalf of Group 5.

How does one choose the best model system?

The model depends on the questions being asked and the analyses to be done. The group discussed the strengths and limitations of the organoid model and the monolayer culture of human stem cells derived, for example, from neural or cardiac cells. The goal is to examine the cells at the right time point in the right stage and recapitulate phenotypes or symptoms in the area of interest. It is also important to be open-minded about better protocols that become available for stem cell differentiation, because this field advances very quickly.

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.

How does one ensure rigor and reproducibility?

The group identified problems to be addressed:

- Small sample numbers
- Not knowing whether the phenomenon that is observed is caused by T21 or a variation among cell lines
- Common sources of variation in isogenic lines caused by reprogramming events, cell-of-origin effects, and epigenetic drift
- Organoid-to-organoid variation when modeling with cerebral organoids, making subtle differences difficult to discern

Strategies to overcome these problems include the following:

- Inactivate the extra chromosome 21 to generate an isogenic line that completely shuts off that chromosome.
- Pool cells from multiple lines to extract common phenotypes from different lines.
- Consider iPSCs for cardiac studies regardless of whether the person has cardiac defects because T21 is the driver of the disease. People with DS have a dramatically increased chance of developing cardiac problems. The goal is to see the same trajectory and phenotypes across different lines that can be modeled.
- Develop guidelines for data handling, sample size, blinding, and randomization.

The group also discussed conducting in vivo studies by transplanting human stem cells derived from cardiac cells or newer derivatives into mouse brains or hearts to generate human-mouse chimeric brain models. Organoids could also be transplanted into the mouse brain to vectorize the organoid to further differentiation. CRISPR/Cas9 could be used to normalize the extra gene and rescue the phenotype.

What clinical and epidemiological data would be useful to guide basic science studies?

The group discussed cardiac malformation and whether DS iPSCs can be modeled in vitro. Other useful data would include the following:

- More early clinical information on brain development to guide disease modeling. Dr. Lawrence emphasized the need for more data from actual human brains about changes in brain structure

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.

Because of the lack of meta-analysis in the field, it would be helpful to link data generated from cells from different iPSCs and correlate the findings with the person's pathology, symptoms, and phenotype.

What biospecimens would basic scientists like to have access to and for what uses?

The group suggested the following biospecimens:

- A bank of iPSC lines
- A central repository of iPSCs with clinical data. The T21 Research Society (T21RS) moves toward this. These high-quality cells could be distributed to researchers, but strict quality control measures are needed to ensure stable cells.
- Plasma biomarkers
- A registry of all available DS cells so researchers can compare and analyze omics data from different studies to identify both consistent findings and discrepancies
- Human fetal brain tissues. These are very hard to acquire. The group discussed the availability of banks of human tissues within and outside of the United States.

Breakout Group 6: Ensuring Robust Animal Model Systems as Preclinical Models

Benjamin Tycko, M.D., Ph.D., Hackensack University

Cathleen Lutz, Ph.D., M.B.A., The Jackson Laboratory (JAX)

Dr. Lutz reported on behalf of Group 6.

How does one choose the best model system?

The group focused on the various mouse models for DS, noting that the Ts65Dn mouse model is still a standard. Choosing the best system depends on the research question to be answered. If a phenotype of cardiac abnormalities is being studied, a model more robust in that area would be the choice. Current mouse models have overlapped, which is a positive development for the phenotypes they present.

The group discussed the phenotyping and genetic background of the models. Because the models cannot be maintained on an inbred background, the universal B6C3H genetic background was adopted. This solved much of the problem in terms of rigor but also introduced considerable variability in how the colonies are maintained, such as creating a "genetic bottleneck" when only a couple of breeder pairs are used. To address this problem, the group discussed using sperm for generating large cohorts of the animals to address the issue of small breeding colonies that require "rolling enrollment" into the study. The group discussed whether banked sperm should be distributed or made available and how this would "reset" the phenotyping community and the DS models. Dr. Lutz noted that there are always bottlenecks with mice, especially with the breeding colonies, and suggested there would be with sperm as well.

The group discussed creating guidelines or standards for using mouse models, including recommending a minimum number of breeders. It was noted that although working with a mixed genetic background automatically introduces variability, a certain amount of variability is necessary for genotypic penetrance. The group also discussed different genetic backgrounds and whether the TcMAC21 mouse model should be moved onto the same genetic background as the rest of the trisomy model. Members noted the possibility of minimizing the focus by staying with one genetic background. The group

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.

How does one ensure rigor and reproducibility?

The group made the following suggestions:

- Consider minimum colony sizes.
- Compare phenotypes of different mouse models. Members noted this can be difficult, because study sections are not always aware of this issue. An application for a cross-comparative phenotyping study when interrogating a particular area of interest in a grant was suggested.
- Address concerns about the TcMAC21 mouse model in terms of the B6/DBA of its genetic background and how to maintain this particular mouse model long term. For mitochondrial function and other components that would influence the trisomy model, the effect of the *Nnt* mutation in this particular mouse model must be tracked.
- Consider in vitro fertilization (IVF) technology as a possible solution to issues breeding inbred strains.

What clinical and epidemiological data would be useful to guide basic science studies?

Epidemiological data for DS are not well organized for translating to basic science. Clinical descriptions are vague and not easily mapped to the animal. The group recommended collecting data for the following:

- Clinical phenotype variations
- Differences between sexes (as a variable) within groups
- From the bone field, phenotypes from young and old populations. Studies are not big enough for translation from mouse to human and from human to mouse.
- Disease phenotypes that are ripe for impactful research
- Cognitive phenotype
- Regression clinical phenotypes, although it is difficult to model this in animals
- Clinical data on circadian rhythms and sleep. Sleep is important put into animal studies, especially in researching an older population and AD.
- Drug testing data, perhaps both neurological and cognitive
- Respiratory and infectious disease natural history data, because people with DS are more susceptible to these problems
- Biospecimens and samples from brain biobanks to study more components of omics data from mouse to human. More accessibility is needed for these samples; perhaps NIH could help with this.

The group also discussed encouraging more interaction among basic scientists, clinicians, and families of people with DS to address important issues, especially non-invasive approaches, and help bridge the gap in epidemiological data.

What biospecimens would basic scientists like to have access to and for what uses?

The group suggested:

- iPSCs with fully developed clinical history. A significant number of these cells lines is needed, at levels similar to those of other large efforts such as Project MinE, which collected hundreds of cell lines. These larger numbers would allow more profiling for omics and other organoid information across a larger population.
- Isogenic iPSC lines

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.

Breakout Group 7: Clinical Phenotyping and Minimal Common Data Elements

Maria Stanley, M.D., University of Wisconsin–Madison

Nicole Vasilevsky, Ph.D., Oregon Health & Science University

Dr. Vasilevsky reported on behalf of Group 7.

What clinical and phenotyping data should be collected?

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 (GI, immunity, neurodevelopment) and collects clinical phenotyping data (<https://redcap.ucdenver.edu/surveys/?s=NHLJDPD48R>)

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

What biospecimens should be collected, and for what purposes?

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.
- Tiers 1, 2, and 3 of omics dataset prioritization

The group discussed the need for data harmonization and mapping with other datasets so that data from a study collecting height, weight, and DEXA scans could be linked with a different study.

What fundamental scientific questions should be addressed by a large cohort study?

The group suggested that a large cohort study could be used to:

- Determine risk factors that lead to co-morbidities and protective factors that prevent some people from developing co-morbidities.
- Include both a discovery cohort and a validation cohort to validate new and existing measures.
- Allow cohort profiling to characterize the health trajectory of the participants.
- Enhance the statistical approach by determining what percentages of participants have various co-morbidities.
- Measure stressful life events and quality of life (e.g., living situations, employment support services).
- Compare determinants of health for diverse populations (e.g., urban and rural, high- and low-functioning, underrepresented minorities).

What clinical scenarios require a deeper understanding of the underlying science?

The group suggested the following scenarios:

- Regression
- Mechanistic studies for co-morbidities
- Co-morbidities (e.g., autism, autoimmune diseases, PH and infections, behavioral and neurodevelopmental issues across the lifespan but especially at an early age, feeding and swallowing problems, seizure disorders)
- Logistical considerations needed to improve sharing more uniform clinical data. It would be helpful to obtain input from the medical records vendor Epic Systems and Fast Healthcare Interoperability Resources (FHIR).

Breakout Group 8: Biospecimens and Related Omics Datasets

Adam Resnick, Ph.D., Children's Hospital of Philadelphia

Qiang Chang, University of Wisconsin–Madison

Dr. Chang began the presentation of the group's report.

What clinical and phenotyping data should be collected?

Samples that lack quality clinical phenotypic data are limited in their use. This is a critical consideration when collecting samples. It is very important for basic scientists to partner with clinicians in pairing clinical data with specimens. This pairing should be prioritized by the quantity of data needed and the domain; for example, when collecting brain tissue, CSF and neuroimaging could be key clinical data that should be paired with the specimens to help with interpretation. The group recommended collecting the following data to pair with biospecimens:

- Clinical EHR data
- Data on medications and supplements taken by people with DS. This information is collected through DS-Connect®.
- Neurobehavioral and other cognitive measures (there are limitations of common measures when applied to DS, and some measures may not have the best correlation)
- Environmental data. Environmental factors could influence epigenetic changes in cells and the nucleus to influence disease-phenotypic presentation. Categories of environmental data relating to the lifestyle of people with DS include diet, sleep patterns, and activities diaries. DS-Connect® collects this type of geographic and lifestyle information. The Undiagnosed Disease Network (UDN) is conducting an environmental survey, and the ECHO program is using wearables to collect activity data and home dust samples from participants' homes.

In summary, clinical data, additional neurobehavioral data, and environmental data are all highly significant.

What biospecimens should be collected and for what purposes?

The group benefitted greatly from Dr. Head's discussion of biospecimens in the concurrent session and recommended her working group's biospecimen suggestions supplemented by their own additions to the list:

- Blood: DNA, RNA, plasma, serum, and PBMCs. Blood also provides a source of cells to generate stem cells and for use in future kinetic work.
- Autopsy samples: brain, cardiac, and other vascular tissue (atherosclerosis) and liver (which could inform pharmacokinetics and pharmacodynamics in DS clinical trials in collaboration with the PTN)
- CSF
- Saliva and buccal DNA swabs
- Surgical specimens (e.g., bone marrow aspirate, thymus, heart, liver, tonsillar tissue). A surgical specimen would be most useful if it were a lesion or from a pathological site with specificity to the underlying clinical symptoms of interest to the investigators.
- Urine and stool
- Skin/fibroblasts
- Amniotic fluid, cord blood, and placenta (from a spontaneous miscarriage)
- Neurotypical controls and parental samples to compare with biospecimens from people with DS. Parental samples could also be used for WGS.
- Environmental samples relating to the research participants' exposures

NDRI could be used to collect postmortem samples. The CHTN would also be a valuable source of specimens, and partnerships with NeuroBioBank and Alzheimer's Disease Research Centers to collect brain samples from people with DS should be explored. These biosamples could support cell-based model development.

Dr. Resnick continued the presentation for the final two questions.

What fundamental scientific questions should be addressed by a large cohort study?

The group discussed questions that would inform a clinical impact, such as addressing susceptibilities and co-morbidities. Layered on top of co-morbidities were questions about the intersection of therapeutics within the DS context and whether therapeutic interventions should be applied in a precision-like approach that accounts for the DS setting and the metabolism of different therapeutics.

The group suggested the following questions:

- Why do people with DS seem less susceptible to atherosclerosis?
- Why do children with DS metabolize chemotherapeutic drugs differently from children without DS?
- Polypharmacy (the use of multiple self- and physician-prescribed medications) is common in people with DS. How does that affect well-being in people with DS?
- What are the genomic variants that may predispose people to (or protect them from) some of the co-occurring conditions in DS?
- What are the unique transcriptomic signatures in specific tissues of relevance to DS (e.g., heart, lung, vascular endothelium)?

The group emphasized again that specimens must be linked to phenotypic data. There is a role for large-scale capturing of biospecimens, but investigators must be intentional about their sample collection before adopting broad sample acquisition efforts. This requires uniformity in sample collection, accounting for pre-analytic variables (e.g., fasting); time of collection (e.g., circadian rhythms can

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.

What clinical scenarios require a deeper understanding of the underlying science?

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 people’s lives and health

Dr. Parisi thanked all the breakout group members for the useful and specific insights and information.

Panel Discussion: What Do Clinical Investigators and Basic Scientists Want Each Other to Know?

Dr. Parisi invited six esteemed DS investigators to answer one or both of these questions: What do basic scientists want clinical investigators to know? What do clinical investigators want basic scientists to know?

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 made to feel that they needed to be changed or “fixed.” The ethical concern about targeting cognition 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

Dr. Reeves noted how much the DS research field has evolved with new investigators and more resources. He said that his thoughts, from a basic science perspective interfacing with the clinical side, come back to variability. Intellectual disability can run the gamut. The basic science side needs to define the problems to be worked on and ensure that the clinical data are as detailed as possible and broken out into collective phenotypes in the clinical trials. Attention must be paid to that collection of basic information to learn what is important to the people and families. Moving from basic science stem cells

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, human genetics has illuminated much about how the heart works, and the participation of people with DS will keep the knowledge going. It is important to keep in mind the collaborative nature of the scientific work. Dr. Seidman said she is always struck by the graciousness of people with DS and their families who want to participate; it speaks to the generosity and altruism of the community with DS, which is so willing to help science discover not only why these medical problems occur in people with DS but also why they occur in people without DS.

Maria Stanley, M.D., University of Wisconsin–Madison

Dr. Stanley said much of the work during this workshop was built on the dialogue between basic science and clinical investigators, families, and advocates. People with DS are very complex and diverse and are challenged by co-occurring medical conditions and environmental and lifestyles factors. Models to capture this vast array of conditions are needed. This is an opportunity for critical dialogue. The research community is committed to working to advance knowledge through rigorous science and is hopeful about a continuing dialogue.

Discussion

An attendee asked about the potential for future meetings to ask families what they want from researchers to help both clinical and basic science researchers be responsive. Dr. Baumer suggested reaching out to families of people with DS not involved in research to understand their perspective as well.

An attendee asked how to measure the variability of phenotypes in terms of what is caused by environment as opposed to genetics. Dr. Seidman said that genotype is not sufficient to explain the diversity in people with DS. Also, the epigenome, the protein surrounding DNA, is influenced by aging. This is a new frontier, so looking at all three of these factors is critical. Dr. Parisi said a newly funded project is evaluating the influence of lifestyle factors and how they might modify the risk of developing AD. These modifiable factors are important, and families are likely to support research to promote brain health, longevity, and quality of life.

An attendee said one of the lessons of the Roche trial that was terminated prematurely was how families were alerted. Dr. Stanley agreed there is a lot to learn from that trial. Families were very generous to participate with no expectations except to contribute to science. There was also a powerful placebo effect that surprised researchers. Dr. Stanley said it is important for researchers, even when they believe they are doing something good, to make the effort to understand what participants want and need. Dr. Reeves added that the complexity of understanding basic science in the context of clinical research in the Roche trial introduced a galaxy of eye-opening issues. The pharmaceutical industry will play a critical role in research, and it would be good to begin incorporating that dimension into the discussion.

An attendee asked whether basic science researchers should focus primarily on identifying therapeutic targets or on the fundamental understanding of the biology. Dr. Sherman and Dr. Baumer suggested that two are intertwined. Understanding the mechanism informs therapeutic targets, so one cannot be prioritized over the other. Dr. Seidman said that the mechanism is important to predict target effect, not just to understand cause and effect. Until mechanisms are understood in full detail, there is the possibility of having unintended adverse consequences. Dr. Parisi observed that the question speaks to the purpose of this workshop and efforts to understand both basic science and clinical issues and how each can inform the other.

An attendee asked whether there was interest in continuing this dialogue between the basic science and clinical groups and, if so, how to organize it. Dr. Reeves suggested the T21RS as a possibility to facilitate that.

A family member attendee said that the researchers are amazing and families are grateful for their observations.

Wrap-Up and Next Steps

Dr. Parisi recalled an in-person meeting a year ago to plan a virtual cohort for DS research, which brought a wide variety of the community with DS together. The exchange of ideas at that workshop resulted in WGs with more than 100 participants. When NIH released the RFA to create a DCC, the award for the DCC went to a team of investigators whose collaboration came out of participation in that workshop. This illustrates the importance of people talking and sharing ideas and experiences about how to develop critical research projects. In addition, the INCLUDE initiative has resulted in more people joining the DS field and more RFAs that speak to the needs of the investigator community.

Dr. Parisi said the positive feedback from this meeting would inform the NIH DS research plan, which is to be published in 2021. She thanked all who have been part of the working groups that are so critical to this field and all who participated in this meeting and provided such important feedback and information.

Glossary

2-D two-dimensional
3-D three-dimensional
A β amyloid beta
ABC-DS Alzheimer's Biomarkers Consortium of Down Syndrome
AD Alzheimer's disease
ADAD autosomal dominant Alzheimer's disease
ADHD attention-deficit/hyperactivity disorder
ADOS Autism Diagnostic Observation Schedule
ALL acute lymphoblastic leukemia
AMKL acute megakaryoblastic leukemia
AML acute myeloid leukemia
AOC Administrative and Outreach Core
APOE2 apolipoprotein E gene
APP amyloid precursor protein
ASD autism spectrum disorder
ASD/AVD atrial or ventricular septal defects
AV atrioventricular
AVSD atrioventricular septal defects
AVSD/Canal atrioventricular septal or canal defects
BPCA Best Pharmaceuticals for Children Act
BMD bone mineral density
BMI body mass index
BWG Biospecimens Working Group
CDC Centers for Disease Control and Prevention
CHD congenital heart disease
CHTN Cooperative Human Tissue Network
CasAS9 CRISPR-associated protein 9
CRISPR clustered regularly interspaced short palindromic repeats
CRNs clinical research networks
CSF cerebrospinal fluid
CTSA Clinical and Translational Science Award
CV cardiovascular
CVD cardiovascular disease
DCC data coordinating center
DMC Data Management Core
DPC Data Portal Core
DS Down syndrome
DS-ALL acute lymphoblastic leukemia in patients with Down syndrome
DS-AML myeloid leukemia associated with Down syndrome
DS-Connect® DS-Connect®: The Down Syndrome Registry
DSH Data Standardization and Harmonization
DSM-5 Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
ECHO environmental influences on child health outcomes
EDTA ethylenediaminetetraacetic acid

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 Study
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-onset Alzheimer's disease
MRI magnetic resonance imaging
MTA material transfer agreement
NACC National Alzheimer's Coordinating Center
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* National Institute of Child Health and Human Development
NIH National Institutes of Health

NOSI 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 Network
RADx-UP Rapid Acceleration of DiagnosticsSM Underserved Populations
RFA Request for Application
RFI Request for Information
RR relative risk
RSV respiratory syncytial virus
SEER Surveillance, Epidemiology, and End Results
SES socioeconomic status
SNP single-nucleotide polymorphism
T1DM type 1 diabetes
T21 Trisomy 21
T21RS T21 Research Society
TMD transient myeloproliferative disorder
TCR/BCR T or B cell receptor
TD typical development/typically developing
TMS transcranial magnetic stimulation
TNF α tumor necrosis factor alpha
TOPMed Trans-Omics for Precision Medicine
TRC-DS Trial-Ready Cohort-Down Syndrome
UDN Undiagnosed Disease Network
VEGF vascular endothelial growth factor
WG working group
WGA whole genome sequencing
XIST X-inactive specific transcript