Eunice Kennedy Shriver **National Institute of Child Health and Human Development**
Division of Intramural Research
BOARD OF SCIENTIFIC COUNSELORS
MINUTES
December 5, 2014
Building 31, Room 2A48

Members Present: Dr. Michelle A. Williams (acting chair), Dr. Rita J. Balice-Gordon, Dr. Jeanne Brooks-Gunn, Dr. Barbara L. Hempstead, Dr. Laurinda Jaffe, Dr. James R. Lupski, Dr. Antonios G. Mikos, Dr. Tarun B. Patel, Dr. Lucia B. Rothman-Denes, Dr. Yoel Sadovsky (nominee), Dr. Lilianna Solnica-Krezel, and Dr. Richard C. Wasserman.

Federal Employees Present: Dr. Constantine Stratakis, Dr. Alan Guttmacher, Dr. Catherine Spong, Dr. Arlyn Garcia-Perez, Ms. Brenda Hanning, and at various times additional members of the NICHD staff participated in the meeting.

I. OPEN SESSION

The meeting convened at 8:05 a.m. Dr. Constantine Stratakis welcomed everyone and thanked the members of the BSC for their service. Dr. Stratakis then invited Dr. Guttmacher to provide an update on the institute to the BSC.

Director's Remarks

Dr. Alan Guttmacher presented several NIH-wide updates:

1. Dr. Story Landis has stepped down as the Director of the National Institute of Neurological Disorders and Stroke (NINDS). Her deputy, Dr. Walter Koroshetz, is serving as the Acting Director until the next director is appointed following a national search.

2. Mr. Patrick White, who served as the associate director for legislative policy analysis has left NIH to work for a new group that advocates for NIH on Capitol Hill.

3. Dr. Donald Lindberg, director of the National Library of Medicine (NLM) has announced his plans to retire in March 2015.

4. Other NIH news: A new initiative by the NLM, the PubMed Commons, is a forum that allows researchers to comment on each other's publications. The President of the United States was recently on campus to commend NIH on its role in handling the Ebola outbreak, notably the successful treatment of the nurse who contracted the disease while treating a patient in Dallas and Dr. Anthony Fauci's efforts to communicate accurate information to the public and dispel myths surrounding the disease. NIH, along with

GlaxoSmithKline, is developing a promising vaccine for Ebola, with a Phase I study just published in the *New England Journal of Medicine.* Dr. Hannah Valantine has been named the first-ever Chief Officer for Scientific Workforce Diversity at NIH.

Dr. Guttmacher continued his update to the Board with NICHD news:

- Following a national search, Dr. Catherine Spong was appointed as the new Deputy Director of NICHD, succeeding Dr. Yvonne Maddox. Dr. Spong was a member of the intramural program for a number of years before serving as a branch chief in the extramural program and, most recently, as the first-ever associate director for extramural research. Dr. Caroline Signore is serving as associate director for extramural research until a permanent replacement can be identified.

- A search was also recently concluded for a Director of the National Center for Medical Rehabilitation Research; announcement of the new appointment is forthcoming.

- The Human Placenta Project, a collaborative effort involving multiple researchers, institutions, and funding sources, was launched in May 2014 with a meeting of about 70 participants to help draft a plan, and included several intramural investigators. It will be a 10- or 15-year project with the goal of being able to monitor human placental development and function in real time. A second meeting focusing on future directions and approaches will be held April 27-28, 2015 on the NIH campus and will be open to all who are interested.

- A report on the National Children's Study (NCS) from the National Research Council and the Institute of Medicine (IOM) was released in June 2014. While the IOM was pleased by many components of the study design, they also had some concerns, which the NIH felt were substantial enough to halt preparation of the main study. A report in 2008 by the IOM had also noted concerns and, while NIH had responded to those critiques, the NIH did not want to continue funding the study only to find that there were still concerns. NIH Director, Dr. Francis Collins, has named a working group of the Advisory Committee to the NIH Director to look at the NCS and report at their meeting in mid-December. The group is co-chaired by Dr. Russ Altman (Stanford University), and Dr. Philip Pizzo, a pediatrician who was formerly a member of the intramural program at NIH and a former dean at Stanford.

- Another item for discussion at the upcoming meeting of the Advisory Committee to the NIH Director is the intramural research program (IRP), which includes the NICHD IRP. A separate working group has been tasked with looking at the IRP across NIH to provide an overall vision for the next ten years.

In legislative news, the Society of Maternal Fetal Medicine is organizing a congressional briefing sponsored by the Congressional Caucus for Women's Issues. Dr. Roberto Romero, an NICHD intramural investigator, will present research on vaginal progesterone. The NIH is currently funded through a continuing resolution until December 11, 2014 at which time another continuing resolution or an omnibus would need to be passed to avoid a government shutdown.

It is predicted that the NIH budget will be similar to FY14; however, NICHD is being conservative with grant pay lines in extramural and funding in intramural until the budget is known.

Scientific Director's Presentation

Dr. Stratakis began his presentation by noting that members of the Division of Intramural Research (DIR) would be presenting on their strategic planning efforts over the last ten months after a few remarks from him. He then went on to present his updates to the Board:

1. Significant personnel changes; new appointments, retirements:

- Dr. Michael T. Collins, an investigator in the National Institute of Dental and Craniofacial Research (NIDCR), has been recruited to be the new associate director for the Inter-Institute Endocrine Training Program at NICHD. Dr. Collins is an NICHD alumnus, having completed his endocrine training at NICHD prior to being recruited by NIDCR as a Tenure-Track Investigator. He will retain his lab within NIDCR but will have his new administrative role within NICHD under a recently signed NICHD-NIDCR agreement.

- In other personnel changes, during FY14, three DIR investigators retired: Dr. Kuo-Ping Huang, Dr. Greti Aguilera, and Dr. Judith Levin. Dr. Huang has been appointed as a scientist emeritus; the scientist emerita appointments for Drs. Aguilera and Levin are pending as their retirements were finalized only recently.

2. Updates from DIPHR:

DIPHR, the Division of Intramural Population Health Research, is part of the NICHD's intramural research program. The division, headed by Dr. Germaine Buck Louis, reports directly to the institute director; however, the BSC is responsible for the review of its science and, in turn, its tenure-track scientists.

- Dr. Louis announced a few recent awards to DIPHR staff including Ms. Uba Backonja who received a scholarship award from the Nurses Educational Fund, Ms. Katherine Sapra who received a travel award from the American Society of Reproductive Medicine, her own receipt of the Society for Pediatric and Perinatal Research President's Award, Dr. Karen Schliep who received a travel award from the Society for Epidemiologic Research, Dr. Sunni Mumford who won the Rising Star Award from the Society for Pediatric and Perinatal Epidemiologic Research, Dr. Denise Haynie who received the NIH Postbac Mentoring Award, and Dr. Tonja Nansel who was a finalist for the NICHD Mentor of the Year Award.

- Following the support of the BSC, Dr. Cuilin Zhang was awarded tenure in September 2014.

3. Budget, space & staff update

The FY14 DIR budget was approximately $175 million, about 14 percent of the total NICHD budget of approximately $1.28 billion and 2.5 percent less than the previous year due to the sequestration cuts. Of the $175 million, almost $60 million is allocated to salaries, and the lab consumables are only about $40 million, with an additional $5 million in animal care costs. The sequestration cut in FY13 to the DIR was 4.45 percent (compared to FY12) and a further cut was expected in FY14; however, the DIR ended up receiving an increase of about 2 percent over FY13, allowing for a final reduction over FY12 of 2.45 percent. Since cuts of 4.45 percent had been projected, the additional budget that came late into the year allowed for the first major capital equipment purchases in nearly four years, over $3.5 million worth. Additionally, $1.5 million was set aside and distributed in fifteen competitive awards to DIR investigators in an effort to incentivize collaborations and implement an important recommendation by the Blue Ribbon Panel review, also a wish that has been expressed repeatedly by the BSC. Renovation costs for FY14 were at $2.5 million, as we continue to implement our efforts to provide new or renovated space to more than 80 percent of the DIR labs by FY16-17. Several labs have now moved into the new Porter Neuroscience Research Center, FY14 being the first year of actually occupying new space.

Dr. Stratakis noted that personnel numbers have seemed to plateau and are not expected to drop below approx. 1050-1100 in total staff. In FY14 there were 66 senior investigators, 6 tenure-track investigators, 2 assistant clinical investigators (ACIs), and approximately 300 trainees. The number of staff clinicians is slated to increase, as the DIR continues its reorganization of the clinical programs.

4. Intramural-extramural collaborations: the first NICHD supported UO1 grants

An update was provided on the efforts to open up the NIH Clinical Center to extramural investigators through collaborations with intramural researchers. NICHD has been the lead institute in this effort. The third round of X-02 applications is due December 10, 2014.

Two grants were supported in 2013: (a) a phase I escalation study of vorinostat in Niemann-Pick C1 disease in collaboration with Dr. Forbes Porter, and (b) a study of Moebius syndrome and related facial weakness disorders, with Dr. Carlo Pierpaoli from NICHD and other clinical staff from NHGRI and NEI.

5. The NICHD reorganization in response to the recent review: report to the BSC

Dr. Stratakis then introduced the draft report by the NICHD DIR and proposed reorganization in response to the Blue Ribbon Panel (BRP) review of 2012-2013 for discussion and approval by the BSC.

The report of the panel was received in summer 2013 and, following several workshops and town hall meetings, working groups were formed. The staff generated a draft report-response with a suggested reorganization that has been circulated to the BSC members. Following input and approval from the BSC today, the draft will be finalized by the DIR staff and a final response-report will be submitted to the NICHD Director, the BRP members, and the DDIR.

The reorganization will become effective October 1st, 2015, the start of FY16. The new structure provides a much more flexible environment to foster inter- and intra-IC collaborations, one of the recommendations of the BRP. The cornerstones of the new structure are the creation of intellectual Affinity Groups (that have already been formed by the staff) and specific research "hubs," the 5 or so building complexes where most of the NICHD DIR investigators will be located by FY17. Renovation and relocation efforts have reduced NICHD's dispersal from more than 13 buildings to about 5 building clusters. Each will be served by an Associate Scientific Director who is housed in the building and works in the respective area of science.

Dr. Stratakis then invited the representatives of the staff, chairs of the respective working groups that drafted the report/response of the NICHD DIR to the BRP report, to present to the Board the main elements of the report.

1. Dr. Ben Feldman presented on the **mission and vision**. The BRP found that a number of the DIR laboratories had poor connectivity to the mission while many of these same labs were conducting scientifically excellent research. The working group pulled elements from the current NICHD DIR mission statement, the current NICHD mission statement, and the NICHD Vision Statement, which embraces basic research but focuses on translational outcomes, in developing a new DIR-specific mission statement. The group felt it was important to include the notion of seeking fundamental knowledge that is encapsulated in the NIH mission and ensure that the statement explicitly included the word "research." After some revision, 86 percent of the DIR PIs expressed their satisfaction with the new mission statement: "*our mission is to seek fundamental knowledge about the nature of development and behavior of living systems through basic and clinical research and to determine how to apply such knowledge to help ensure that women and men have good reproductive health, that children are born healthy, and that people develop to live healthy and productive lives.*" The group also set out to create a longer vision statement. While this job was not necessarily completed, a survey they conducted of DIR PIs provided good data that may be useful as the efforts go forward. For instance, labs were asked what themes their work is most aligned with from the NICHD-wide vision statement, and many labs did not feel that they mapped well to those themes, particularly groups working at the molecular and cellular level. One potential solution to this perceived problem is to make it clear that research with broad and general relevance to human development is just as important as research with specific translational relevance, perhaps by adding subcategories to the existing themes. Suggestions were made by the BSC that the group should consider including population

research in the mission statement as well as modifying the current language from "people develop to live healthy and productive lives" to "developmental origins of health and disease."

2. Presenting for the **administration** working group was Dr. Brant Weinstein. He noted that the group had a very diverse set of opinions and that while everyone liked something in the proposal, no one liked everything. The BRP had recommended flattening the DIR structure; however, the working group proposed something a little more complex to preserve things that have been working well scientifically and administratively while addressing those that do not. The group also sought to increase the perception of fairness and transparency, representation in the decision-making process, and diversity in leadership. The structure is a hybrid consisting of self-organized, scientific-based affinity groups and an administrative structure organized around buildings. The affinity groups cover the current scientific interests and major collaborative groups. Each affinity group includes at least two investigators and will have a group head serving a four-year term coinciding with the site visit cycle. Affinity group heads will not serve in a supervisory role nor will they have a budget. Administratively, everyone will be split into one of five buildings, each managed by an associate scientific director (ASD) since PIs in the same building are likely to share resources. These ASDs would maintain separate budgets for building-specific expenses, with complete transparency in spending to all members of the building. Additionally there will be two (or three) ASDs serving the DIR at-large to assist the SD in areas such as recruitment, budget oversight and transparency, and liaison with the administrative branch. All ASDs will be voting members of the Group of Senior Advisors (GSA), which will also include senior leadership: the SD, deputy SD, deputy director for clinical affairs, deputy director for administration, and deputy director liaison and training. The GSA will continue to serve an advisory role to the SD, particularly on issues such as PI budgets and salaries, access to cores, recruitment, and administrative procedures. A propos of how the structure was determined, Dr. Weinstein said that it was PI-driven within the framework of what was suggested by the BRP and advice by the OSD on administrative manners. The ASDs for management will serve as supervisors for all PIs and will receive input from the affinity group heads. All positions will have four-year terms. Affinity group heads will be elected by each group but ASDs will be appointed by the SD after a selection process that will involve the Institute's Director, Deputy Director, and senior management; these appointments will be made from names proposed through self-nominations and staff nominations, and other input. A propos of the effect of the new structure on personnel budget, Dr. Stratakis indicated that there should not be an impact in terms of leadership positions, though we are still looking at what effect it may have on secretarial support. The BSC applauded the group's efforts to come up with an administrative structure that combined the BRP's recommendations with the wishes of the staff, and approved in principle the proposed structure.

3. Dr. Paul Love then presented on behalf of the **site visit** working group. The group recommends standardizing the size of the reviews so that they are more equitable, establishing and implementing some minimum criteria for site visits such as requiring at least two expert reviewers for each PI, aligning the site visit schedule with the BSC meetings, standardizing budget reporting, reinstituting laboratory visits, and establishing

a formal process to contest site visits. Having two site visits per year aligned with the BSC meeting would allow more members to attend, making it easier to compare and rank labs across the institute. Since intramural PIs do not have the opportunity to resubmit proposals as they would with a grant application, having one-on-one meetings with the reviewers is seen by PIs as an important part of the site visit process to resolve any misunderstandings or questions that arise from the written submission. The group also proposes reporting budgets during site visits in a format modeled on those used in R-01 grant applications. Other items the group still hopes to address include the apportionment of retrospective and prospective work in the report; adding mentorship, citizenship, and mission relevance in the written submission; and developing a formal process for contesting a site visit; for the latter point, it was noted that as per the DDIR's Office of Intramural Research, site visits may only be contested on procedural grounds. Before it is finalized, the proposal will need to be aligned with NIH-wide guidelines. A propos of the site visit schedule, it was clarified that the agenda would be a standardized two-day format. The exact schedule still has to be worked out to align affinity group members together under the same cycle. The BSC supported the site visit proposal in principle.

4. Presenting for the **metrics** working group was Dr. Mark Mayer. The BRP had used H-Index to evaluate PIs and this group was asked to come up with an alternative metric for evaluation. The group met on several occasions and discussed using publications, citations, and impact factor, as in other institutes. They also felt it was important to give weight to service on committees, mentoring, training, clinical fellows, and so on, but could not agree on how to quantify these. It was unclear what the metrics would be used for but possibilities included resource allocation or monitoring career development. The group developed a productivity metric based on a PI's number of citations over the past five years, compared to the impact factor for all NICHD publications over the same period. The values were compared to site visit scores and there was a clear correlation. Among PIs with the highest site visit scores, however, there was a lot of disparity in productivity that might be attributed to being at different stages in one's career or to being in different fields. There was a strong correlation between the productivity factor and budget but it was not clear whether more productive labs were being rewarded with more resources or whether labs with more resources were just more productive. One drawback to the metric is that it does not account for differences across fields, and all of the extreme outliers in terms of high productivity were labs doing clinical research. DIR PIs felt strongly that the productivity metric should be weighted in terms of lab size and budget but did not feel that this metric should be supplied to site visit committees.

5. The **translational and clinical research** working group was represented by Dr. Alan DeCherney. The goal of this group was to increase efficiency and facilitate translational research as recommended by the BRP. The clinical programs will be reorganized into disease-focused units. Support personnel, such as nurses and staff clinicians, will be consolidated under the Office of the Clinical Director so that staff can be easily moved from one protocol to another as projects start up and wind down. Within the next year, two staff clinicians will be added to support internal medicine and a third to support pediatrics with a focus on genetics. Protocol proposals and maintenance will also change. PIs will now be required to submit a letter of intent to the scientific director and the

clinical director for each new project. After a proposal is scientifically reviewed, it will be reviewed by the BSC Clinical Protocol Review Committee to determine whether it represents a DIR priority. Once the structure is more formalized, secondary affinity group affiliations can help bring together basic and clinical scientists. The BSC Clinical Protocol Review Committee membership currently includes Drs. Brooks-Gunn, Sadovsky, Strauss, Wasserman, and Williams.

6. Next, Dr. Karl Pfeifer presented on behalf of Dr. Mary Lilly for the **recruitment and retention** working group. The NICHD DIR has been successful in recruiting six out of the last seven candidates it pursued. Historically, the promotion rate has been about 60 percent, accounting for those who leave and those unsuccessful in their bid for tenure. Following tenure, there have not been many departures and there has not been much hiring at the mid-career level. The NIH-wide Stadtman tenure-track investigator searches have been successful at bringing in top candidates in the basic sciences but are less successful at finding physician-scientists. A lack of diversity in the workforce continues to be a problem. The new associate scientific director for recruitment will be important in assessing the future priorities for the institute, rather than hiring decisions being made on the basis of politics and history, for example. All recruitment efforts will focus on scientific excellence, relevance to the mission, and increasing workforce diversity. In the discussion that followed Dr. Pfeifer's presentation, BSC members recommended finding new strategies to freshen things up, for example by increasing interactions across intramural programs and with extramural awardees, given the lack of turnover among mid- to senior-level investigators.

7. Dr. Mary Dasso then presented on behalf of the **NICHD Women Scientist Advisors (WSA)**, a standing group that advises the Office of the Scientific Director on matters related to female scientists within the DIR. Over the past twenty years, the number of female scientists in the intramural program has steadily decreased and, more distressing, women are dramatically underrepresented in the number of investigators making it through the tenure-track process. A propos the representation of women at the recruitment level, Dr. Dasso noted that approximately 35 percent of the Stadtman applicant pool is women. An open NICHD staff meeting was held in August 2014 and was attended by the majority of women in the DIR to discuss this issue. Suggestions made during the meeting largely fell into four categories: increasing the representation of women in leadership roles, increasing the recruitment of women at the tenure-track and mid-career levels, providing ongoing career support, and providing assurance of equitable resource distribution in an effort to retain women in the DIR. To promote women in leadership roles, the WSA has identified and nominated a number of suitable candidates for leadership positions within the new structure of the DIR and would like to see women filling about 30 percent of the voting positions on the GSA. In the discussion that followed Dr. Dasso's presentation, Dr. Solnica-Krezel pointed to a recent *Science* article that found that the biggest issue related to increasing the proportion of women in faculty positions is that there are fewer applying for faculty positions, even though women make up about 50 percent of the graduate school and postdoctoral trainees, and that this needs to be addressed more broadly.

Dr. Stratakis thanked the presenters and added that the final names for the ASD positions and the new structure with all affinity group heads will be reported to the BSC in the June 2015 Meeting.

The BSC endorsed unanimously the report-response of the DIR staff to the BRP report with the suggestions made during the discussion above.

Dr. Stratakis then finished his report to the BSC for the Dec 2014 meeting with updates on specific initiatives and related activities:

- NICHD participated in the NIH-wide review of the IRP, and Dr. Collins is expected to present the report to the Advisory Council to the NIH Director in mid-December.

- The NICHD DIR Director's Investigator Awards (that utilized some of the restored sequestration funds – see above under "budget update") used a modified R21 application and included 37 reviewers from 12 different ICs. From 33 applications, a total of 15 awards were made, providing more than $1.5M in funding in FY14 and in FY15. Dr. Stratakis was hopeful that another round of funding could be provided for FY16-FY17, depending on the budget situation.

- Another competitive funding opportunity was the Human Placenta Project awards funded by the NICHD Office of the Director. The DIR submitted eight applications, two of which were funded: Drs. Carolyn Ott and Jennifer Lippincott-Schwartz for "Monitoring Placental Cell Membrane Potential Fluctuations Using Live-Cell Light Sheet Microscopy" and Drs. Sergey Leikin and Peter Basser for "Extracellular Matrix Imaging in Human Placenta."

- NIH Director's Challenge Innovation Awards were made to three NICHD DIR investigators in 2014, Drs. Anirban Banerjee and Matthias Machner, both tenure-track investigators, and Dr. Roger Woodgate.

- Dr. Brant Weinstein was selected by fellows as the NICHD Mentor of the Year and Dr. Jack Yanovski was the recipient of the NIH Director's Mentoring Award.

- A few announcements were made regarding the Office of Education's efforts to promote mentoring and training. The NICHD Scholars program for underrepresented minorities in science is supporting two new scholars for 2014-2015: Gian Rodriguez (Porter lab) and Nicole Millan (Wolff lab). Sara Armaiz (Yanovski lab) and Presley Garrison (Baron lab) are physician-scientists receiving support through the NIH Medical Research Scholars Program.

- The Three-minute Talks (TmT) Competition for fellows and graduate students, developed based on the 3MT thesis talks conducted in Europe and elsewhere, provides a creative opportunity to communicate science clearly and dynamically. The three winners, announced in summer 2014, were 1st place: Alex Ritter (Lippincott-Schwartz lab); 2nd place: Eva Szarek (Stratakis lab); and the people's choice: Monica Gupta (Ozato lab). The three winning videos were then shown.

A short recess followed.

Scientific Presentations

Dr. Erin Wolff, Head, Unit on Reproductive and Regenerative Medicine, PRAE

Dr. Stratakis introduced the first speaker, Dr. Wolff, who is an Assistant Clinical Investigator.

Cellular Therapy Approaches to Treating Reproductive Disorders

Project 1: Depletion of T Regulatory Cells Impairs Embryo Implantation in a Female Murine Model, Which is Correctable with Adoptive Transfer

Maternal immune tolerance to fetal engraftment is critical for the establishment of pregnancy, but the exact mechanisms permitting this semi-allograft are not completely understood. T regulatory cells (Tregs), defined by CD4+CD25hi surface expression and the FoxP3+ transcription factor, appear to play an important role in pregnancy disorders associated with abnormal placentation such as pre-eclampsia and intrauterine growth restriction. In this study, we used the DEREG transgenic mouse (DEpletion of REGulatory T cells) to evaluate uterine implantation after conditional depletion of Tregs via Diphtheria Toxin (DT). We confirmed depletion of Tregs in blood, as well as documented consistent uterine Treg depletion. Circulating Tregs nadired 3 days after DT, while maximum uterine depletion occurred at 10 days post injection. Litter sizes after Treg depletion were significantly smaller compared to controls (7.3 vs 4.5; $P<0.01$) despite equivalent numbers of oocytes as well as embryos. The number of gestational sacs detected by early pregnancy ultrasound correlated with the number of pups born, indicating that post implantation embryo resorption could not account for the smaller litter sizes, further implicating a defect at the time of implantation. This defective implantation was reversed by adoptively transferring normal congenic Tregs back into mating females after Treg depletion, allowing litter sizes to return to baseline levels ($P<0.05$). These data suggest that Tregs are important for maternal tolerance of embryo implantation in this conditional mouse model. Low Treg levels could be a potential cause of infertility due to recurrent implantation failure, which may be amenable to cellular therapy approaches. These data have culminated in submission to PNAS, which is currently under review.

Project 2: Ovarian-derived Stem Cells Generate Mature Oocytes in a Rhesus Macaque Model

We have demonstrated for the first time the presence of oogonial stem cells (OSC) in adult non-human primates (NHPs), which can be isolated, cultured in vitro, genetically labeled, and transplanted in a healthy autologous and ovarian radiation injury model. Rhesus OSC exhibit stem cell and germline marker expression, as well as increased proliferative capacity in vitro. After autologous OSC transplantation, transplant-derived oocytes were retrieved from rhesus following exogenous gonadotropin stimulation. This

is the first report of a mature oocyte derived from any type of stem cell in primates. Next we sought to investigate if OSC are related to ovarian disease. Although there is not a good NHP model of spontaneous idiopathic premature ovarian insufficiency (POI), radiation is a known cause of iatrogenic ovarian failure. In a NHP disease model of ovarian injury, OSC could not be isolated from monkeys who had previously undergone high-dose radiation. Therefore, allogeneic OSC transplants were performed. Donor OSC were found to engraft in recipient ovaries, which readily re-generated OSC in culture. These data demonstrate that transplantation of OSC can generate mature oocytes, and suggest that OSC may hold potential as a novel therapy for infertility. These data are currently under review at Nature Medicine. We have also examined OSC in women with and without premature ovarian insufficiency (POI), where we have confirmed that radiation damage prohibits isolation and culture of OSC. However, in spontaneous idiopathic POI, OSC can be isolated and cultured, albeit at lower percentages compared to normal healthy ovaries. Although we cannot test oogonial potential in human OSC similar to our NHP transplant model to determine if human OSC can also generate mature oocytes, these cells were observed to undergo spontaneous differentiation into oocyte-like appearing structures in vitro as has been described for these cells in mice, rat, rhesus, and humans.

Multiple groups have confirmed that OSC can be isolated using a polyclonal antibody raised against DDX4. However, while studying OSC, we noted inconsistent DDX4 antibody performance. We have characterized DDX4 expression in mouse, rhesus, and human ovarian cells that label with the DDX4 antibody and in human ovarian cancer tissue and cell lines. We were unable to detect DDX4 expression by PCR, immunoblot, or mass spectrometry in mouse, rhesus, or human cells isolated using an antibody purported to recognize the DDX4 extracellular domain. Similarly, kidney cells enriched via the DDX4 antibody did not express DDX4. Additional analyses examining the subcellular distribution of epitope-tagged DDX4 protein in human ovarian cells support an intracellular localization of DDX4 and further suggest that the published antibody is not recognizing true DDX4 expression at the cell surface. These data are also under review at Nature Medicine in a separate manuscript.

Using this data-driven approach, we have found evidence that simultaneously 1) confirms the presence of cells in the ovary with the ability to make oocytes, but 2) refutes the idea that DDX4 can be expressed at the cell surface. Using our mass spectrometry and RNA sequencing libraries, we identified candidate marker(s) that are cross-reacting with the DDX4 antibody and have confirmed that these cells label with antibodies against these alternate markers by FACS. These preliminary data suggest that the antibody is cross-reacting with an alternate epitope that is recognized by the DDX4 antibody, for which we are currently filing a patent application.

Project 3: Female Germline Differentiation from Rhesus Macaque Induced Pluripotent Stem Cells (iPSCs)

While male germ cell differentiation from embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC) has been established for some time, female germline differentiation has typically been more difficult to achieve. However, in 2012, viable

oocyte differentiation from mouse iPSC was reported for the first time by Hayashi et al., and these oocytes could fertilize normally and give rise to offspring. Moving forward, we will focus on an iPSC approach to generating oocytes in NHPs. The clinical significance of this approach is that it could allow women with premature ovarian insufficiency to have their own genetic offspring. As a first step toward human trials, we have adapted the Hayashi mouse protocol to NHPs by testing multiple in vitro conditions. Our optimized protocol has resulted in primordial germ cell like cell (PGCLC) differentiation, which can be FACS purified by SSEA1. Purified SSEA1+ cells express DDX4 by RT-PCR as well as surface staining, while the SSEA1- fraction does not. Further, these differentiated cultures exhibit evidence of haploid cell formation by FACS ploidy analysis. In addition to the SSEA1 surface staining, a conditional reporter (PRDM1, DDX4, and DDPA3) approach will be utilized in order to enrich for PGCLCs. Purified rhesus PGCLCs will be co-cultured with neonatal mouse somatic gonadal cells (which were depleted of germline cells by MACS) to form "reconstituted" ovaries. These reconstituted ovaries will be engrafted under the mouse ovarian bursa in a xenograft in vivo differentiation approach to induce the penultimate meiotic maturation. Ovarian grafts will then be harvested and in vitro maturation performed on immature oocytes to obtain mature oocytes. Resultant oocytes will be assessed by single cell RNA sequencing to profile iPSC derived ooctyes and determine if expression approximates normal oocytes.

Questions on the science followed. The BSC cautioned Dr. Wolff on quality assurance issues related to the characterization of cells that may be used for the described experiments.

Dr. Harold Burgess, Unit on Behavioral Neurogenetics, PGD

Dr. Stratakis introduced the next speaker, Dr. Burgess, a tenure-track investigator in the Program on Genomics of Differentiation (PGD).

Neurons that express Gsx1 during embryonic development regulate performance of the startle response

Many neuropsychiatric disorders, including schizophrenia, have an important but poorly understood neurodevelopmental contribution. Disruptions in neuronal development that increase schizophrenia risk may manifest during adulthood in abnormal regulation of the startle response. Mechanisms regulating the development of functional neuronal circuits can be uncovered using the powerful genetic tools available in zebrafish, which share the same fundamental brain architecture as humans. Recently, cutting edge studies in invertebrates have exploited libraries of transgenic animals to conduct 'circuit breaking' screens to identify the neuronal pathways that control behavior. Using a novel method, we created a library of brain-specific transgenic zebrafish which express Gal4 in restricted groups of neurons. We then used this library of transgenic lines to perform the first circuit breaking screen in a vertebrate model. This screen revealed that neurons specified by the transcription factor Gsx1 are required for regulation of the startle response by a mechanism known as prepulse inhibition. These neurons directly connect to central neurons that trigger the startle response and surprisingly, despite their

functionally inhibitory role, are primarily glutamatergic. We found that in mice, Gsx1 is expressed in brainstem regions associated with prepulse inhibition. Prepulse inhibition was strongly reduced in Gsx1 knockout mice, indicating that the underlying neuronal circuitry is conserved across vertebrates. During development, Gsx1 is expressed in the medial ganglionic eminence, a region which gives rise to diverse interneuron types in the forebrain, including cortical interneurons which have long been suspected to develop abnormally in schizophrenia. Future work will identify Gsx1-specified forebrain neurons and address the possibility that these are at risk in schizophrenia. This study is the first molecular identification of neurons required for prepulse inhibition, and highlights the power of zebrafish as a discovery platform for neuronal pathways regulating behavior in mammals.

Questions and a discussion followed.

Dr. Matthias Machner, Unit on Microbial Pathogenesis, CBMP

Dr. Stratakis introduced the next speaker, Dr. Machner, a tenure-track investigator in the Cell Biology and Metabolism Program (CBMP).

Host cell manipulation by the pathogenic bacterium Legionella pneumophila during infection

Infectious diseases are a major threat to the health and lives of humans, and a significant economic burden. My group uses the bacterium Legionella pneumophila, the causative agent of Legionnaires' pneumonia, as a model organism to decipher the molecular mechanisms that allow pathogens to survive and replicate within human cells. During infection, L. pneumophila injects more than 300 virulence proteins, or effectors, through a type IV secretion system into the host cell cytosol in order to establish intracellular conditions favorable for bacterial growth. The main goal of our research is to determine the molecular function of L. pneumophila effectors, to identify their host targets, and to reveal how these host-pathogen interactions contribute to bacterial virulence.

In one project, we made the intriguing discovery that the effector protein VipD from L. pneumophila possesses robust phospholipase A1 (PLA1) activity, which was missed in earlier studies because it is triggered only upon VipD binding to host cell Rab5 or Rab22, small GTPases enriched on endosomes. Once activated, VipD hydrolyzed phosphatidylinositol 3-phosphate, a key component of endosomes, thereby altering the lipid and, consequently, protein composition of this organelle and protecting intracellular Legionella from endosomal degradation. These findings were the first example of a bacterial type IV-translocated effector with phospholipase activity and the very first case of a host cell Rab GTPase not being the target of a bacterial effector but, surprisingly, the trigger of its catalytic activity. The crystal structure of VipD in complex with host cell Rab5c, which we solved in a collaborative project, further supported our finding and provided a first look into the ingenious molecular mechanisms underlying allosteric activation of a virulence factor by a host protein. In a separate project, we investigated how AMPylation, the covalent attachment of adenosine monophosphate (AMP) to host

factors, is regulated during infection and if it can be reversed. We made the exciting discovery that Legionella pneumophila, in addition to secreting an AMPylation enzyme, also translocates a de- AMPylase into host cells. This finding represented the first bona fide de-AMPylation enzyme in any system, and provided a paradigm for understanding a post-translational modification of emerging relevance for bacterial pathogenesis. More importantly, our findings showed how a microbe, by encoding two opposing activities on adjacent genes, can precisely control host factor activity during infection.

In the future, we will continue to employ state-of-the-art approaches such as CRISPR-mediated gene silencing or protein-spotted microarrays to decipher which of the more than 300 L. pneumophila effector proteins have a target within human cells and are, thus, likely to contribute to infection. Obtaining a detailed understanding of the molecular mechanisms that allow L. pneumophila to manipulate human host cells and to cause disease is fundamental for the effective treatment and prevention of Legionnaires' disease and related illnesses.

Questions and a discussion followed.

Dr. Steven Coon, Staff Scientist, Molecular Genomics Laboratory (MGL)

Dr. Stratakis introduced the next speaker, Dr. Coon, to provide an update on the efforts to set up a core for molecular genetics and genomics in the DIR. Dr. Coon was recently appointed the acting head of the MGL under the direction of Dr. Forbes Porter.

The Molecular Genomics Laboratory (MGL) provides DNA and RNA sequencing services for genomic and genetic research to investigators within the NICHD. Our services include whole exome, targeted exome and gene-specific DNA sequencing, as well as whole transcriptome sequencing (RNA-Seq), microRNA sequencing, microbiome sequencing, bisulfite sequencing (DNA methylome), ChIP-Seq and ribosomal profiling. The MGL provides significant primary data processing and downstream bioinformatic support, and can assist in designing experiments or sequencing strategies (for example, optimization of targeted exome design). During calendar year 2014 the MGL has provided sequencing for 15 projects across the full spectrum of sequencing types; these projects involved 11 NICHD Principal Investigators. In addition to sequencing and providing our standard primary analysis of the resulting data, the MGL has delivered enhanced bioinformatic support for 13 NICHD investigators and 1 NICHD/NHGRI collaboration. Our mission is to offer accurate and innovative sequencing and bioinformatic tools to facilitate research into the diagnosis, counseling and treatment of hereditary disorders.

Questions followed. Dr. Stratakis noted that the goal is not to replicate the efforts of other ICs but to be part of a central genomics core service, should NIH decide to form one. Having the core in-house also provides an educational opportunity for trainees to better understand the sequencing process. The core will also be linked and supported by the new bioinformatics recruitment(s).

Dr. Julian Lui, Research Fellow, Section on Growth and Development, PDEGEN

The next speaker, Dr. Lui, was introduced by his mentor, Dr. Jeffrey Baron. Dr. Liu completed his Ph.D. training at the Chinese University of Hong Kong before joining the Baron laboratory as a postdoctoral fellow. Given the synergy between them, Dr. Baron is requesting a staff scientist appointment for Dr. Lui so they can continue to work together.

Childhood Growth: Molecular Mechanisms and Novel Treatment Approaches

Project 1: Regulation of IGF2 by E2F3 in juvenile growth and tumorigenesis
Insulin-like growth factor 2 (IGF2) is an important fetal growth factor. Its expression in both mouse and human is downregulated in multiple organs shortly after birth, and is frequently elevated in human cancers. The mechanisms that drive the postnatal downregulation of IGF2 are unclear. Here we show that transient expression of E2f3a or 3b activates a reporter construct of the mouse Igf2 promoter P2, which consists of multiple E2F-binding sites. Using chromatin immunoprecipitation, we show that E2f3 binds to the Igf2 P2 promoter in 1-wk-old mouse organs, when E2f3 expression is high; but not in 4-wk-old organs, when E2f3 expression is low. Overexpression of E2f3a or 3b induces a dramatic increase of Igf2 mRNA in primary adult hepatocytes, but not in fetal hepatocytes. In addition, we provide evidence that E2F3-overexpressing cancers have increased IGF2 expression, and levels of E2F3 and IGF2 in these cancers are positively correlated. These data suggest that E2f3 transcription factor positively regulates Igf2 expression in vivo, downregulation of E2f3 with age may drive the decline of Igf2 expression in postnatal organs, and elevated expression of IGF2 in human cancers may be due in part to overexpression of E2F3.

Project 2: Human monoclonal antibody fragments for targeting therapeutics to growth plate
Many genetic disorders impair growth plate function, resulting in short and sometimes malformed bones. Currently, recombinant growth hormone is administered systemically to treat growth plate disorders, but it has limited efficacy for severe disease, such as skeletal dysplasias, and causes adverse effects on other tissues. In addition to growth hormone, multiple endocrine and paracrine factors are capable of promoting chondrogenesis at the growth plate, which could potentially be used to increase longitudinal bone growth. We envisioned that targeting these growth factors specifically to the growth plate may augment the therapeutic skeletal effect while diminishing undesirable effects on non-target tissues, and thus provide novel treatment approaches for growth disorders. Using yeast display, we selected single-chain variable antibody fragments that bound with high affinity to human and mouse matrilin-3, an extracellular matrix protein specifically expressed in cartilage. These antibody fragments also bound with high tissue specificity to cartilage homogenates and to cartilage structures in mouse embryo sections. When injected intravenously in mice, the antibody fragments specifically homed to cartilage. Coupling these cartilage-binding antibodies to

chondrogenic endocrine and paracrine signaling molecules have the potential to open up new pharmacological approaches to treat childhood skeletal grow.

Questions and a discussion followed.

Following a short break, the meeting resumed at 12:05 p.m. The next portion of the meeting was chaired by Dr. Cathy Spong.

Dr. Spong introduced the final speaker, Dr. Ida Owens, a senior investigator in Program on Developmental Endocrinology and Genetics. Dr. Stratakis recused himself from the presentation and the discussion that followed. Dr. Owens introduced three guests she brought to the meeting, Dr. Nikhil Basu, NICHD; Dr. Masahiko Negishi, NIEHS; and Dr. Frank Gonzalez, NCI.

Dr. Ida Owens, Section on Genetic Disorders of Drug Metabolism, PDEGEN

Identification of Pro-and Anti-apoptotic Controlling Sequences in Human Prostate distributed DHT-metabolizing UGT-2B15 and UGT-2B17 Respectively

Human prostate basal cell distributed UDP-glucuronosyltransferase-2B17 (UGT-2B17) and its 97%-identical UGT-2B15 homolog metabolize both dihydrotestosterone (DHT) and its androstane- diol (Andro) metabolite. Mass spectrometry has confirmed UGT-2B17 has 4 of 5 predicted phosphorylation sites in luminal-cell-distributed UGT-2B15; dissimilarly, 2B17 contains the rare tri-phosphorylated-TYS sequence at position 98-100. Both isozymes undergo regulated phosphorylation for two different functions. Like UGT-2B15, anti-PKCalpha-immunocomplexed UGT-2B17-S172 confirmed phosphate-signaling enables a non-fixed active-site that catalyzes an unspecified number of substrates based on analysis of 6/19 human UGTs. Contrariwise, anti-PKCepsilon immnocomplexes of 2B17 generated dense smearing patterns, except for its Y99F mutant or following expression of constructs in Src-free cells. Hence, previously identified robust and critical signaling by the Src/PKCepsilon-partnership phosphorylation site, TYS, located at position 98-100 in 2B17 suggests its pivotal role. Following 2B17 expression in Src-/- versus +/- cells, glucuronidation of DHT versus its Andro metabolite indicates Src inhibits both by 50 %, which necessarily elevates anti-apoptotic DHT levels concomitantly. Exchanges of IYG in wt-UGT-2B15(IYG) and TYS in wt-UGT-2B17(TYS) at positions 98-100 followed by expression in COS-1 and PC3 cells enabled mt-UGT-2B17(IYG) to generated 4 to 10-fold greater in-cellulo caspases 8/3 activations over wt-UGT-2B15(IYG), while mt-UGT-2B15(TYS) suppressed activation of caspases 8/3 over 50% of wt-UGT-2B15 (IYG) levels. Treatment of LNCap cells that contain endogenous UGT-2B15/UGT-2B17 with Src inhibitor, curcumin, enabled a cycle of apoptosis and recovery within 16 h. Combined, evidence indicates the triple-phosphorylated TYS creates a signaling site involving Src and PKCepsilon that is anti-apoptotic, while the pro-apoptotic Src-specific binding/phosphorylation site, IYG, in UGT-2B15 at position 98-100 is blocked by Src.

SIGNIFICANCE- This study provides evidence human prostate luminal-cell distributed DHT-metabolizing UGT-2B15 uses programmed phosphorylation-based signaling to carry out luminal-cell specific apoptosis. Contrariwise, basal-cell-distributed UGT-2B17 -- contained within a Basal-cell Compartment that houses intermediate stem cells with both basal- and luminal cell-surface cytokeratin markers-- utilizes programmed anti-apoptotic signaling to protect this population of cells. For the first time, we have identified the luminal-cell specific UGT-2B15 as the pro-apoptotic agent that removes challenged luminal cells before transformation ensues. Moreover we have identified basal-cell specific UGT-2B17 as the anti-apoptotic agent that protects intermediate stem cells containing both basal- and luminal- cell surface markers that replace the departed luminal cell and gives rise to a new basal cell. These findings indicate UGT-2B15 controls luminal transformation and that UGT-2B17 controls continuity of the prostate. Endogenously expressed UGT-2B15/-2B17 in LNCap cells show a cycle of apoptosis and recovery within 16 h after treatment with Src-inhibitor, curcumin. Evidence indicates the triple-phosphorylated TYS creates an anti-apoptotic Src / PKCepsilon signaling site, while pro-apoptotic Src-specific IYG signaling site in UGT-2B15 is blocked by Src phosphorylation. Hence, UGT-2B15 and -2B17 appear to be unique clinical markers.

The BSC had no questions for Dr. Owens.

The open session concluded.