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MEETING REPORT

TT2022 meeting report on the 17th Transgenic Technology meeting in Helsinki, Finland

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The much-anticipated TT2022 conference was held with great success in mid-September of 2022 in Helsinki, Finland. After the long delay in in-person meetings, ISTT members greatly appreciated the opportunity to get together with new and old friends in person near the shore of the Baltic Sea. The Scandic Grand Marina Hotel and Conference Center was the site of the meeting from 17 to 19 September, an excellent venue that easily handled the 250 on-site delegates and the additional 250 virtual participants. Attendees could easily walk to the city center to see the magnificent white cathedral, or to the public library to see the miniscule sculpture of the literary mouse. Participants with extra time even took the ferry to Tallinn, Estonia or visited Lapland for sightings of reindeer and landscapes near the Arctic Circle.

Day 1 A warm Finnish welcome countered the autumn chill to begin the meeting on Saturday evening. Ernst-Martin Füchtbauer, the ISTT President, welcomed attendees in Finnish, eliciting a few chuckles from the Finnish members of the audience. Then the chairs of the local organizing committee, Satu Kuure and Reetta Hintala, welcomed the onsite delegates and introduced Kari Alitalo (Univ. Helsinki), who described his studies of the lymphatic system in the brain, and how various growth factors affect the lymphatic system. He has used numerous genetically modified mice to examine the pathways of this system and found unexpected connections among vascular growth factors that are used to maintain lymphangiogenesis. His research has led to a current clinical study in humans that examines the effects of VEGF-C/D in dry macular degeneration. Professor Alitalo's lecture was followed by a welcome reception, where old friends had a chance to chat and catch up on several years of new developments in their labs.

Day 2 The Sunday 18 September sessions began with talks demonstrating how animal models allow researchers to take ideas from the bench to the clinic. Emma Andersson from the Karolinska Institute showed an amazing method of delivering DNA in situ

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in the early embryo using a nano-injection device developed specifically for her studies of gene expression that contribute to the development of the nervous system. She achieved temporal and spatial specificity in her system (titled NEPTUNE) using lentiviral vectors and avoiding ubiquitous promoters (e.g., PGK). NEPTUNE is now being tested for CRISPR/Cas9 targeting and the development of a barcode lentiviral library. The second plenary talk was given by Martin Bergö (Karolinska Inst.) where he shared his analysis of the antioxidant-stimulated metastasis pathway to show that BACH1, a critical protective factor in this pathway, stimulates genes that promote metastasis in response to antioxidants.

Two short talks followed the plenary talks, both describing animal models generated to mimic human conditions. Daniel Davis (Univ. Missouri) described three mouse models of human diseases that mimic the human causal variants, including spinal muscle atrophy (SMA) models that recapitulate hotspot mutations found in humans; KCNT1 models of mutations that have been shown to generate epilepsy; and a model of cystic fibrosis (CF) where exon 8 was replaced with a mutant form of human exon 8 found in CF patients. Doug Strathdee (CRUK Beatson Inst.) followed by presenting an animal model of Barth Syndrome where the *Tafazzin* gene (a mitochondrial lipid transferase) was floxed. Analysis of mice that were knocked out for the TAFAZZIN protein showed growth delay and changes in heart function as was expected based on human disease. Subsequently, his group replaced the entire mouse gene with the human gene that contains an extra exon. They are now using this gene to add in patient-specific point mutations.

The next session included both a talk on the auxin-inducible degron (AID) system (a system adapted to degrade specific proteins) in mice, and a lively round-table discussion on quality control of CRISPR/Cas-generated animals. Philip Jordan (Johns Hopkins Univ.) described his use of the AID system to degrade proteins that maintain the structure of chromosomes, including cohesins, condensins, and SMC5/6. He is currently using AID2 technology (Yesbolatova et al. 2020) and sees tight control of the system that is rapid, specific, inducible and reversible.

Next, a highly interactive round table was chaired by Lydia Teboul (MRC Harwell) where Søren Warming (Genentech), Lauryl Nutter (The Centre for Phenogenomics) and Marie-Christine Birlling (IGBMC) presented concerns and ideas for validating animal models produced using CRISPR techniques. There are no current standards required for ensuring the CRISPR model produced is not affected by off-target mutations, indels, or even mating procedures that can fix undesired/unknown mutations in a line. Some easily adaptable ideas for rodent models include regular backcrossing to the wild-type inbred strain, obtaining both experimental and control mice from the same colony, tracking and reporting generations, and cryopreservation of early generations. After these easy validations, though, additional criteria that could or should be checked provoked a lot of discussion leading to a consensus among all present that investigators and core directors would be well-served by an ISTT-generated white-paper that can be used as a blueprint for publishing validated CRISPR animal models.

This session was followed by a short interval to view posters, have lunch, and visit with vendors. In all, more than 80 posters were presented over six short sessions during the meeting, spanning from simple culture techniques to the generation of large animal models that mimic human diseases. In addition, two lunch-and-learn presentations were given. Day 2's lunch session was given by Jean Cozzi of Charles River on methods that can be used to improve research reproducibility and animal welfare when breeding, genotyping and phenotyping animal models. He described several confounding factors commonly seen when problems occur, such as failure to properly clean instruments between taking samples (as one example).

The afternoon session began with five short, flash poster presentations. Elizabeth Bryda (Univ. of Missouri) presented the work of her group where they produced and phenotyped human ACE2 overexpression rat models generated to study SARS-CoV-2 and its impacts. Graham Duddy (Francis Crick Inst.) followed with a description of their workflow using AAV and electroporation (CRISPR-READI) to efficiently create mouse knock-in models. Next, Marie-Christine Birling (IGBMC) detailed methods to generate humanized mouse models by inserting large genomic DNAs produced by BAC engineering into mouse embryonic stem cells via CRISPR/Cas9. Roger Askew (Ozgene) then presented a new molecular screen to identify gene-targeted alleles using a fusion transcript that incorporates a polyA trap. Finally, Kathy Krentz (Univ. of Wisconsin) described

their successful recovery of the Copenhagen rat line (a tumor-resistant inbred strain) many years after the initial freeze.

The 3Rs session started with a talk from Thorsten Buch (Univ. of Zurich) describing his attempt to develop a breeding calculation program to reduce mouse usage during complex breeding. His talk was followed by an overview by Vootele Voikar (Helsinki Institute of Life) of how EU regulations and outreach, in addition to adherence to the 3Rs, can contribute to ethical use of animals in research. Finally, the 3Rs Prize recipient, Ronald Naumann (MPI-CBG), presented his work showing that, by genotyping sperm samples from chimeras and looking for those with a high percentage of embryonic stem cell contribution, one can choose animals that are more likely to transmit through the germline.

After the second short poster session, a virtual plenary talk was given by Jihan Osborne (Univ. Texas-SWMC) that described her studies of the miRNAs Lin28a and Lin28b in lung development and branching morphogenesis. Using mouse embryos and embryonic stem cells, she found that the Lin28a/b miRNAs regulate the master regulators Sox2 and Sox9. She is currently investigating how these miRNAs are regulated. The following talk by Ritva Heljasvaara (Univ. of Oulu) described her use of numerous mouse transgenic lines to assess the role of the extracellular matrix in cancer. One of the genes studied, Col18a1, has isoforms that may act as tumor promoters. Finally in this session, an enthusiastic presentation was given by Andrew Syvyk (Texas A&M Univ.) to promote the use of rat spermatogonial stem cell lines to generate animal models. He demonstrated the ease of this method by showing how an overexpressing ErbB2 stem cell line could be introduced into the rete testis of *Dazl* transgenic rats to generate progeny that were wholly modified.

The final session of the first day was the highly anticipated presentation of the ISTT Prize to Drs. Elizabeth Lacy (Professor Emeritus, Sloan Kettering Cancer Center) and Frank Costantini (Professor Emeritus, Columbia University). A video of the presentation of the sculpture took place in a park in New York City, where Wojtek Auerbach, ISTT Past-President, gave the award to Drs. Lacy and Costantini. After viewing the presentation, we heard a live lecture that incorporated both awardees' reminisces and discussions of their scientific careers which included some of the first methods described in the generation of transgenic animals. The audience was humbled to hear how these pioneers, at the very beginning of the age of molecular biology, overcame hurdles that included isolating genomic DNA for genotyping, determining how transgenes were incorporated, and figuring out how prokaryotic sequences inhibit the expression of transgenes. Their contribution to the methods now used to generate gene-modified animals has been immense and greatly appreciated within the community.

This presentation was followed by the conference banquet, held in the Scandic Marina Conference Center, where attendees were greeted with a flute of champagne and then treated to an excellent Finnish feast.

Day 3 The next full day of talks began with a session on non-mammalian models, and included a plenary lecture from Howy Jacobs (Univ. of Tampere) describing his use of *Drosophila* transgenics to study mitochondrial DNA (mtDNA). By generating mutants in nuclear DNA that function in mtDNA expression he has mimicked some human diseases. His most recent work includes the generation of cybrid flies (introducing mtDNA from one genotype into the cells from another genotype), thus establishing fruit flies as a first approach before generating the mutations in mice. In the second talk of this session, Annamarie Locasio (Stazione Zoologica Anton Dohm) described a unique animal model—*Ciona robusta*—a marine invertebrate chordate with embryonic development similar to other higher animals. Manipulation by electroporation of CRISPR/Cas9 components and comparison with similar mutations in *Danio rerio* (zebrafish) was used to identify enhancers and other means of regulating ONECUT and other HOX genes.

Short talks after the plenary lectures included one from Ralf Kühn (MDC-Mol.Med.) describing his attempts to generate the "perfect" C57BL/6 mouse by repairing known mutations and erasing endogenous retroviral sequences (ERVs), and a second talk by Mitra Cowan (McGill Univ.) describing the success she has had in generating large knock-in sequences using CRISPR/Cas9. She found that their best results were achieved using IVF to generate fertilized embryos followed by injection the following day at the 2-cell stage.

A fourth short poster session was followed by the much-anticipated "Running a Transgenic Service

Facility" Roundtable chaired by Ben Davies (Univ. Oxford, Wellcome Trust). The panelists, Ben Lowe (The Jackson Laboratory), Peter Sipila (Univ. of Turku), Natalia Moncaut (CRUK-Manchester Inst.), and Pawel Pelczar (Univ. of Basel), described methods of embryo generation, reagent delivery, delivery of large DNAs, development of new technologies and how they implemented them, and took questions and comments from the audience. A very enlightening discussion surrounded how failed projects were managed: typically, most facilities perform multiple attempts to deliver the requested service. All agreed that it was difficult to determine when to stop and that communication with the contracting investigator is critical when projects run into difficulty.

The lunchtime break included a presentation by Gurpreet Balrey from Merck that described their new Cas9Plus, along with an enticing project in development to develop a chimeric Cas9 protein that will function efficiently within closed chromatin.

Jaan-Olle Andressoo (Univ. of Helsinki) began the afternoon session with a presentation of how their conditional 3'-UTR mutants of glial-derived neurotrophic factor (GDNF) were able to recapitulate aspects of schizophrenia in humans, but that the timing of Cre-excision was critical in the process. Interestingly, they noted that an interacting adenosine receptor, *Adora2a*, is inhibited by caffeine, and determined that blocking this receptor normalized the *Gdnf* mutant mice. The subsequent lecture by Tarja Malm (Univ. of Eastern Finland) examined how non-coding RNAs regulate protein expression in complex regulatory networks. She described studies of the interactions among lncRNAs, miRNAs and circulating RNAs in Alzheimer's disease, Parkinson's disease and ischemic stroke. The plenary lectures were followed by two short talks. The first, by Lev Federov (Oregon Health & Science Univ.), described his comparison of embryo development after culture in human tubal fluid (HTF), M16, Global Media from Cooper Surgical, or KSOM, finding that more embryos developed normally in HTF than the comparison medias. The second short talk was given by Yueh-Chiang Hu (Cincinnati Children's Hospital), describing doxycycline-inducible models of the four core genotypes in mice (XX female, XX male, XY male and XY female) that were generated using CRISPR-interference and CRISPR-activation constructs targeted to the Col1a1 safe harbor site.

After a short poster break, the Orbis Pictus lecture, sponsored by the Czech Center for Phenogenomics (CCP) was given by Maria Lehtinen (Boston Children's Hospital). Dr. Lehtinen's talk was replete with beautiful images showing how the choroid plexus contributes to the cerebral spinal fluid (CSF) to control the development of the brain and subsequent neurogenesis in early mouse embryogenesis. During the response to calcium induction, unusual blebs were identified that appear to function in apocrine secretion of transthyretin (TTR), which was confirmed after generating TTR-GFP mice that secreted GFP into the CSF.

Day 4 The final day of TT2022 began with Qiangge Zhang (Massachusetts Inst. Technology) describing the process used at MIT for developing transgenic marmosets to serve as NHP models in neuroscience research. IVF-generated embryos were microinjected with transgenes to knock out SHANK3, PSEN1, MeCP2 or SETD1A. The second talk, given by Benjamin Schusser (Technical Univ. Munich), described his successful efforts to show that chickens could be protected from disease using gene editing to delete an amino acid that is critical for infection by avian leukosis virus. The short talks following these presentations included one from Stuart Newman (PetMedix LTD) where transgenic mice were generated to express canine therapeutic antibodies, and a second talk from Guillaume Pavlovic (IGBMC) where changes in the gut microbiome were assessed in response to different factors such as housing area, sex of the animal and genetic background of the animal (among others).

The final regular scientific session of the meeting focused on descriptions of emerging technologies. The plenary lecture was given by Bon-Kyong Koo (Inst. Of Molecular Biotechnology) where he presented a new artificial intron that can be used to generate conditional alleles. His short conditional intron (SCON) was used to target numerous genes, including single exon genes within specific parameters for use. The next short talk by Stephane Pelletier (Indiana Univ.) discussed his work with the "Degradation based on Cre-regulated Artificial Intron" (DECAI), where he has been able to target several genes to generate conditional alleles. The final short talk of the meeting was given by Xiaojun Xing (Yale Univ.), where he demonstrated his high-throughput method for mouse sperm

cryopreservation using microstraws and batch labeling.

In the last sessions of TT2022, the Young Investigator Award was presented to Davide Seruggia (St. Anna Children's Cancer Research Inst.) followed by his lecture, and, subsequently, an EMBO-sponsored lecture by Elizabeth Fisher (UCL Inst. Of Neurology). Dr.Seruggia described his work using CRISPR, base editing, degrons and the adeno-associated viral method to deliver components to the embryo to determine essential areas required for enhancer function and critical transcription factor sites.

Dr. Fisher then depicted the generation of mice with humanized genes used to study the genes implicated in amyotrophic lateral sclerosis (ALS), including fused in sarcoma (FUS) and transactive response DNA binding protein (TARDBP), which appear to be critical for most neurodegeneration.

The meeting ended with a joyous celebration of this successful meeting, beginning with awards, which included the Mammalian Genome-sponsored awards presented for the best oral presentations of a poster (Stephane Pelletier and Yueh-Chiang Hu), three prizes for best posters (sponsored by emeritus ISTT members) (awarded to Vanessa Chenouard from INSERM, Graham Duddy from Francis Crick Institute, and Pawel Pelczar from Univ. of Basel), and two awards from the International Mammalian Genome Society given as complimentary registration for one of their upcoming meetings (Lauri Lintott from the Hospital for Sick Children, and Tereza Nickl from the Czech Center for Phenogenomics). Jan Parker-Thornburg then issued an invitation to TT2023, to be held in Houston Texas, USA in November of 2023. Final thank-yous and closing remarks from President Füchtbauer ended this happy occasion and left delegates anticipating the next time we all could be together to present and celebrate our science.

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Declarations

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