information about potentially actionable variants will be excluded from the study to avoid a situation where the investigator has clinically important information that cannot be shared with the participant. b. Genetic testing will be performed in a CLIA-certified lab to allow investigators to share the results with the study participants. c. Results will be reported to study participants according to a standard operating procedure (SOP) that classifies the report of variants according to the relation to phenotype and the pathogenic potential. d. Participant satisfaction with the informed consent process and the return of results will be assessed by a questionnaire for obtaining participants' perceptions of their research experience, based on a standard set of validated research participation experiences measures (Kost RG et al, J Clin Transl Sci. 2018;2:31). RESULTS/ANTICIPATED RESULTS: Samples from individuals with severe null mutation hemophilia and a mild bleeding phenotype will be enriched in genetic modifier variants. After completing participation, participants will express satisfaction with the informed consent process and the results of the return of genetic information. DISCUSSION/SIGNIFICANCE OF IMPACT: Genetic risk assessment to predict bleeding risk has the potential to provide hemophilia patients with tailored therapy, allowing for very early initiation of treatment (prophylactic thrice weekly IV administration of FVIII) in patients with a high bleeding risk and deferring this costly and burdensome treatment in patients who are expected to be mild bleeders. Genetic modifier variants of hemophilia may be found to predict thrombosis in non-hemophiliac patients and profoundly impact the treatment of venous thrombosis. A structured process for obtaining consent for NGS and return of genetic results to study participants can protect them from uncertain genetic information. Moreover, this process will prevent a situation in which investigators have knowledge about clinically actionable variants but they are not allowed to report them to the participants or do not have a process for doing so. Sharing individual research results and results with clinical significance with participants of studies that involve whole exome sequencing can promote transparency and engagement of participants throughout the research enterprise.

**3579**

## The role of CypD/mPTP during osteogenic differentiation - a potential target to prevent bone loss

Rubens Sautchuk Jr.[1], Brianna H. Shares[1] and Roman A. Eliseev[1]
[1]University of Rochester Medical Center

OBJECTIVES/SPECIFIC AIMS: The study aims to further investigate how cyclophilin D (CypD), the key mPTP opening regulator, affects BMSCs fate and to determine potential regulatory mechanisms involved in CypD regulation during osteogenesis. METHODS/STUDY POPULATION: We evaluated CypD mRNA expression in mouse BMSCs and in osteogenic-like (OL) cells during the course of OB differentiation. CypD protein level was also probed. Moreover, BMSCs had their mPTP activity recorded during osteoinduction. We further analyzed the effect of CypD genetic deletion on osteogenesis in vitro and in vivo. For our in vivo model, we performed the ectopic bone formation assay to asses differences in ossicle formation when CypD KO BMSCs were transplanted compared to wild type littermate BMSCs. In our in vitro model, we transfected OL cells with either CypD gain of function or CypD loss of function vector and measured their osteogenic differentiation potential. Additionally, we treated BMSCs with CypD inhibitor and compare to non-treated BMSCs for mineralization level. To determine potential regulatory mechanisms involved in CypD regulation, we analyzed the CypD gene (Ppif) promoter for potential transcription factor (TF) binding sites and found multiple Smad-binding elements within this promoter. Smads (Smad1, 5, 8) are TFs downstream from Bone Morphogenic Protein (BMP) signaling pathway that transmit cell differentiation signaling, and exert either activating or inhibitory effects on a variety of genes. We also transfect OL cells with Smad1 vector and analyzed for CypD mRNA levels. RESULTS/ANTICIPATED RESULTS: - Our data showed that CypD mRNA levels decreased in both primary cells and OL cells at day 7 and day 14 in osteogenic media. - Osteogenic induction also decreased mPTP activity. - In vivo ectopic bone formation assay showed increased ossicle fo DISCUSSION/SIGNIFICANCE OF IMPACT: Our data suggest that downregulation of CypD increases OB differentiation due to improved OxPhos activity led by mPTP closure. Our results corroborate reports of CypD downregulation and mPTP closure during neuronal differentiation in developing rat brains as well as in cardiomyocyte differentiation in developing mouse hearts. Our studies also suggest a yet unknown mechanism linking differentiation signaling with mitochondrial function – BMP/Smad mediated downregulation of CypD transcription. As initially mentioned, in a previous study, our lab showed that CypD KO mice present higher mitochondrial function and osteogenicity in aged BMSCs and less osteoporosis burden. Taken together, these results suggest that CypD can be a potential target to prevent bone loss in aging.

**3152**

## The role of myostatin in diabetic bone disease

Evangelia Kalaitzoglou[1], Callie Knuckles and John Fowlkes
[1]University of Kentucky Center for Clinical and Translational Science

OBJECTIVES/SPECIFIC AIMS: Our primary objective is to determine the mechanism of action of myostatin on osteoblasts by measuring markers of osteoblast differentiation. With these experiments we will evaluate the effects of myostatin on an osteoblastic cell line (MC3T3 cells) and primary murine osteoblasts during baseline and hyperglycemic conditions and assess whether these effects are altered in the presence of a hyperglycemic environment. METHODS/STUDY POPULATION: Primary osteoblasts from calvaria of WT mice will be isolated and cultured per previously published protocol. MC3T3 cells (murine pre-osteoblast cell line) and primary osteoblasts will be plated in 6-well plates until they reach confluency. They will subsequently be stimulated with or without myostatin at various concentrations under control and hyperglycemic conditions. Additional experiments will assess myostatin stimulation during cell differentiation/maturation in the presence of osteogenic induction medium. Subsequently, cells will be lysed and processed for gene analysis with qPCR. Genes of interest (e.g., myostatin, RUNX2, osteocalcin etc.) will be assessed. Additionally, cells will be collected and processed for protein quantification with western blot to assess myostatin-related pathways, such as Smad2/3 and MAPK signaling. RESULTS/ANTICIPATED RESULTS: We have demonstrated that the receptor for myostatin (Activin receptor 2b, AcvR2b) is present in MC3T3 cells and we have evidence of Smad2 phosphorylation in MC3T3 cells as a result of myostatin stimulation, confirming that myostatin can exert intracellular signaling events in bone cells (Fig 1). We anticipate to observe negative effects of myostatin on differentiation of primary osteoblasts and MC3T3 cells. Specifically, we anticipate suppression of Runt-related transcription factor 2 (RUNX-2), a transcription factor known as the " master regulator" of osteogenic gene expression and programming, as a result of

https://doi.org/10.1017/cts.2019.59 Published online by Cambridge University Press

signaling downstream of Smad 2/3. Additionally we anticipate downregulation of osterix and osteocalcin, two essential genes for osteoblast differentiation and activity. We anticipate that hyperglycemia will potentiate the negative effects of myostatin on osteoblastogenesis. DISCUSSION/SIGNIFICANCE OF IMPACT: We have demonstrated that myostatin can directly act on osteoblastic cells. As myostatin is a negative regulator or bone mass, its direct effects on bone cells can be detrimental to the bone health of patients with elevated myostatin levels and/or activity. There is evidence suggesting that myostatin is elevated in Type 1 diabetes, and its effects might be potentiated in a hyperglycemic environments. Future experiments will be evaluating the role of myostatin on a diabetic animal model and in humans. Our experiments provide an additional mechanism by which muscle-bone interactions could be contributing to the development of diabetic bone disease.

**3433**

## Tissue Engineered Nigrostriatal Pathway as a Test-Bed for Evaluating Axonal Pathophysiology in Parkinson's disease

Elisia Clark[1], Laura Struzyna, Wisberty Gordián-Vélez and Kacy Cullen
[1]University of Pennsylvania School of Medicine

OBJECTIVES/SPECIFIC AIMS: Selective loss of long-projecting neural circuitry is a common feature of many neurodegenerative diseases, such as the vulnerable nigrostriatal pathway in Parkinson's disease (PD). Current in vitro approaches for studying disease development generally do not mimic complex anatomical features of the afflicted substrates such as long axonal pathways between stereotypical neural populations. Such exquisite features are not only crucial for neural systems function but may also contribute to the preferential vulnerability and pathophysiological progression of these structures in neurodegenerative disease. We have previously developed micro-tissue engineered neural networks to recapitulate the anatomy of long-projecting cortical axonal tracts encased in a tubular hydrogel.1 Recently, we have extended this work to include the first tissue-engineered nigrostriatal pathway that was anatomically-inspired to replicate the structure and function of the native pathway.2 Notably, this tissue-engineered brain pathway possesses three-dimensional (3D) structure, multicellular composition, and architecture of long axonal tracts between distinct neuronal populations. Therefore, in the current study we apply this system as a biofidelic test-bed for evaluating axonal pathway development, maturation, and pathophysiology. METHODS/STUDY POPULATION: Dopaminergic neurons from the ventral mesencephalon and medium spiny neurons (MSNs) from the striatum were separately isolated from rat embryos. Tissue-engineered nigrostriatal pathways were formed by initially seeding dopaminergic neuron aggregates at one end of hollow hydrogel micro-columns with a central extracellular matrix, collectively spanning up to several centimeters in length. Several days later, tissue-engineered MSN aggregate was seeded on the other end and was allowed to integrate. Immunocytochemistry (ICC) and confocal microscopy were used to assess health, cytoarchitecture, synaptic integration, and mitochondrial dynamics with stains that label cell nuclei (Hoechst) and mitochondria (MitoTracker Red) and antibodies that recognize axons (anti-β-tubulinIII), neurons/dendrites (anti-MAP2), dopaminergic neurons/axons (anti-tyrosine hydroxylase; TH), and MSNs (anti-DARPP-32). RESULTS/ANTICIPATED RESULTS: Seeding tubular micro-columns with dopaminergic neuronal aggregates resulted in unidirectional axonal extension, ultimately spanning >5mm by 14 days in vitro. For constructs also seeded with Tissue-engineered, ICC confirmed the presence of the appropriate neuronal sub-types in the two aggregate populations, specifically TH+ dopaminergic neurons and DARPP-32+ MSNs. Moreover, confocal microscopy revealed extensive axonal-dendritic integration and synapse formation involving the dopaminergic axons and MSN somata/dendrites. Collectively, these constructs mimicked the general cytoarchitecture of the in vivo nigrostriatal pathway: a discrete population of dopaminergic neurons with long-projecting 3D axonal tracts that were synaptically integrated with a population of MSNs. Mitochondria structure along axonal tracts was also observed using MitoTracker staining, revealing dynamic intra-axonal mitochondrial motility in this system. Ongoing studies are evaluating real-time mitochondrial dynamics and axonal physiology in this tissue-engineered nigrostriatal pathway in vitro, under both baseline conditions as well as following the addition of exogenous α-Synuclein fibrils to model synucleinopathy in PD. DISCUSSION/SIGNIFICANCE OF IMPACT: This tissue-engineered nigrostriatal pathway provides an anatomically-inspired platform with neuronal-axonal architecture that structurally and functionally emulates the nigrostriatal pathway in vivo. We are applying this paradigm as a powerful in vitro test-bed for understanding mitochondrial activity and inter-axonal energetics pathways under homeostatic as well as PD pathological conditions. Successful demonstration will serve as proof-of-concept that this technique can be used to study mitochondria pathology in personalized constructs built using cells derived from PD patients in order to evaluate pharmacological therapies targeted at improving mitochondrial resiliency and fitness so as to delay and/or prevent dopaminergic axonal/neuronal degeneration in tailored to specific PD patients.

**3174**

## Tumor suppressors p53 and ARF control oncogenic potential of triple-negative breast cancer cells by regulating RNA editing enzyme ADAR1

Che-Pei Kung[1], Emily Bross[2], Emily Bramel[2], Eric Freeman[2], Thwisha Sabloak[2], Catherine Kuzmicki[2], Mike Benjamin[2], Leonard Maggi[2] and Jason Weber[2]
[1]Washington University in St. Louis, Institute Of Clinical and Translational Sciences and [2]Washington University in St. Louis

OBJECTIVES/SPECIFIC AIMS: Triple-negative breast cancer (TNBC) accounts for one-fifth of the breast cancer patient population. The heterogeneous nature of TNBC and lack of options for targeted therapy make its treatment a constant challenge. The co-deficiency of tumor suppressors p53 and ARF is a significant genetic signature enriched in TNBC, but it is not yet clear how TNBC is regulated by this genetic alteration. METHODS/STUDY POPULATION: To answer this question, we established p53/ARF-defective murine embryonic fibroblast (MEF) to study the molecular and phenotypic consequences in vitro. Moreover, transgenic mice were generated to investigate the effect of p53/ARF deficiency on mammary tumor development in vivo. RESULTS/ANTICIPATED RESULTS: Increased transformation capability was observed in p53/ARF-defective cells, and formation of aggressive mammary tumors was also seen in p53-/-ARF-/- mice. RNA-editing enzyme ADAR1 was identified as a potential mediator for the elevated oncogenic potential. Interestingly, we found that the overexpression of ADAR1 is also prevalent in human TNBC cell lines and patient specimen.

https://doi.org/10.1017/cts.2019.59 Published online by Cambridge University Press