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.

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

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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.

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The role of myostatin in diabetic bone disease

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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

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