Diabetic Complications Consortium

Application Title: Sarm1 and diabetic complications in adolescents with type 1 diabetes

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1. Project Accomplishments:

In this 1-year pilot & feasibility study, our goal was to enroll up to 36 adolescent participants across three groups: well-controlled type 1 diabetes (T1D), poorly-controlled T1D and non-diabetic controls. Research appointments occurred in the Bone Health Clinic at the Washington University School of Medicine. Enrollment criteria included poorly controlled T1D (HbA1c ≥8% for the past 12-months) or well-controlled T1D (HbA1c <8% for 12-mo), female sex, 12- to 18-years of age, and ≥5-years since the onset of T1D.

During the past year, we have reached out to all eligible candidates with T1D within the BJC and Washington University Health Systems. We have also recruited non-diabetic control participants from the health system and surrounding community. Thanks to the efforts of our team, we have successfully completed 26 total research visits (Table 1). An additional 8 research visits are scheduled in July/August. As the pilot funding from DiaComp was not able to cover the projected research costs in full, the remaining visits this summer will be supported by funds from the Division of Bone and Mineral Diseases at Washington University. Once complete, we anticipate that these data will be used to prepare two publications and to support a follow-up R01 application to the NIH in 2022-2023.

Table 1. Summary of recruitment, demographics, and health information to date. Mean +/- standard deviation.

Demographics and Health Information	Non-Diabetic Control (8 completed, 4 scheduled)	Well Controlled T1D (9 completed, 2 scheduled)	Poorly Controlled T1D (9 completed, 2 scheduled)
Age (years)	14.87 ± 2.79	15.35 ± 1.81	15.18 ± 2.06
Height (inches)	63.75 ± 2.01	63.73 ± 2.41	65.70 ± 3.74
Weight (pounds)	111.68 ± 11.84	129.23 ± 24.16	164.56 ± 50.48
BMI	19.34 ± 2.14	22.21 ± 2.90	26.53 ± 6.35
Tanner Stage	3.8 ± 0.84	3.67 ± 1.03	3.75 ± 0.71
Activity Score (1-5)	2.04 ± 0.79	2.47 ± 0.55	2.39 ± 0.93
Duration of diabetes (years)	n/a	7.00 ± 2.68	8.06 ± 2.40
HbA1c (%)	4.80 ± 0.39	6.73 ± 0.65	9.09 ± 0.84
Fasting Glucose (mg/dL)	86.33 ± 3.39	147.00 ± 32.71	234.50 ± 80.79
Calcium (mg/dL)	9.63 ± 0.32	9.60 ± 0.32	9.53 ± 0.34
Vitamin D (ng/mL)	20.53 ± 4.06	28.90 ± 4.78	17.41 ± 5.70
Total Cholesterol (mg/dL)	153.83 ± 27.29	152.0 ± 23.49	190.38 ± 46.40

2. Specific Aims:

Specific Aim 1. Sarm1 expression and activation in adolescents with T1D. The goal of this aim is to quantify Sarm1 expression and activation in adolescents with poorly controlled T1D relative to non-diabetic participants and those with well-controlled T1D (Fig.1). We hypothesize that upregulation of Sarm1 expression and activation occurs in response to poor glycemic control, leading to Sarm1-induced tissue degeneration and damage. Our long-term goal is to define patients at high risk of Sarm1-mediated tissue damage, supporting the future clinical application of Sarm1 inhibitors.

Aim 1 Results: So far, we have collected 26 peripheral blood samples from healthy control and T1D participants. After collection, peripheral blood mononuclear cells (PBMCs) were isolated and treated with pre-optimized concentrations of Sarm1 activator, Sarm1 inhibitor, and a combination of Sarm1 activator and inhibitor for 4-hours (Fig.1A). After treatment, we processed the PBMCs for metabolite extraction and analysis by mass spectrometry for an array of NAD+ and purine pathway metabolites. The goal of this experiment was to quantify the potential for basal and stimulated Sarm1 activity in human PBMCs. This is important for two reasons. First, Sarm1 inhibition represents an emerging clinical target to treat and prevent diabetic complications such as peripheral neuropathy. However, there are no available assays to quantify the individual risk of Sarm1 activation using readily available cell types. PBMCs represent a target population for the purpose. Second, our work in mice as recently linked Sarm1 activation to diabetic skeletal complications. Thus, these results will inform the use of PBMCs as a minimally invasive means to identify patients at risk for diabetic neuroskeletal disease.

In brief, we found that Sarm1 activator 3-AP was sufficient to induce flux of both NAD+ and purine pathway metabolites in PBMCs and that this could be partially abrogated by concurrent application of the Sarm1 inhibitor DSRM-3716, in some cases in a dose-dependent manner (Fig.1B-D). Data analysis is still ongoing. However, these initial results suggest that a subset of PBMCs express a functional Sarm1 enzyme that could be used as a clinical readout or target for the management of diabetic neuroskeletal complications. We

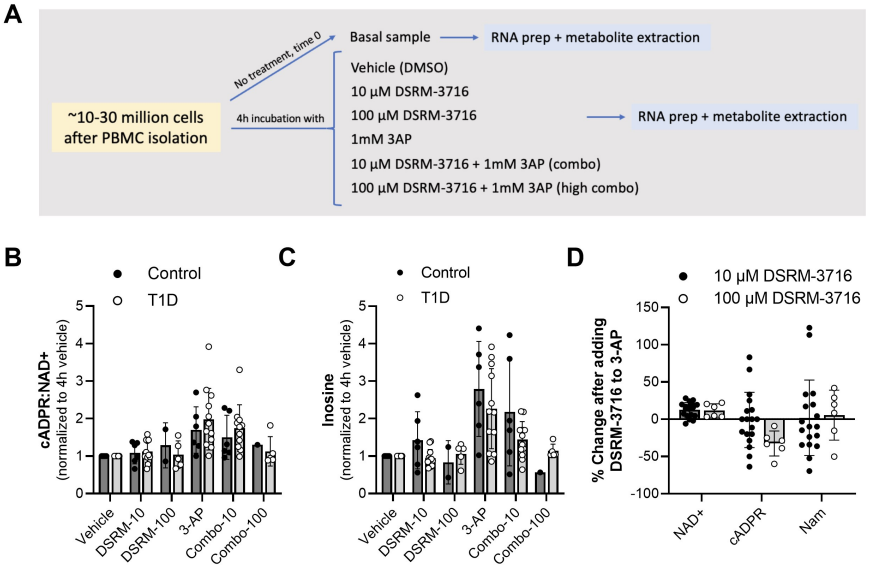

Figure 1. Novel evidence for functional Sarm1 expression in human PBMCs. (A) Workflow. **(B)** Elevation of cADPR:NAD+ is a sensitive readout of Sarm1 activation as it relates to NAD+ metabolism. **(C)** Elevation of inosine is a sensitive readout of Sarm1 activation as it relates to the purine pathway. **(D)** Dose dependent changes after application of Sarm1 inhibitors apply primarily to cADPR.

expect to complete the data collection by August of 2022 with data analysis running through the Fall to address this possibility. In addition to metabolite extraction and analysis, we purified RNA samples from both basal and stimulated PBMC fractions. The analysis of these samples is currently pending and will be completed in the coming months after completing participant recruitment in Summer of 2022.

Specific Aim 2. Sarm1 and diabetic neuroskeletal complications in adolescents with T1D. Our research team specializes in the study of neuropathy and bone disease, two major complications of T1D that can contribute to lifelong increases in morbidity and mortality. The two primary outcomes of this aim included assessment of bone microarchitecture using high-resolution micro-computed tomography and quantification of neuropathy index using the Michigan Neuropathy Screening Instrument (MNSI). The primary goal of this aim was to enhance our foundational knowledge of these understudied complications in diabetic adolescents. The secondary goal of this aim was to segment our results based on Sarm1 expression and activation as quantified in Aim 1 to begin to isolate the relationships between Sarm1 and the presence of complications in nerve and bone. We hypothesize that Sarm1 activation occurs in settings of inadequate glycemic control, putting adolescents with T1D at risk for development of complications across multiple systems. This cross-sectional pilot project will support future longitudinal studies to establish the temporal relationships between glucose control, Sarm1 activation, and tissue damage.

Aim 2 Results: Current results show that adolescents with poorly controlled T1D have increased body mass (Table 1). Consistent with previous clinical reports, this was associated with increases in cortical bone parameters as assessed by XtremeCT-II. However, we also observed a concurrent decrease in trabecular bone quality, indicating deterioration of skeletal structural integrity in patients with T1D (Fig.2). Though overt signs of neuropathy were not detected in this group, we observed a mild trending increase in neuropathy score by survey, but not exam, in the group with poorly controlled T1D. Data collection and analysis is still ongoing (includes demographics, surveys, fracture risk, activity/strength, blood labs/biomarkers, etc), however, these early results suggest a paradigm whereby structural changes in bone precede the onset of clinical neuropathy in adolescents with T1D. This questions the general assumption of neuropathy → bone that has emerged based on studies of older individuals.

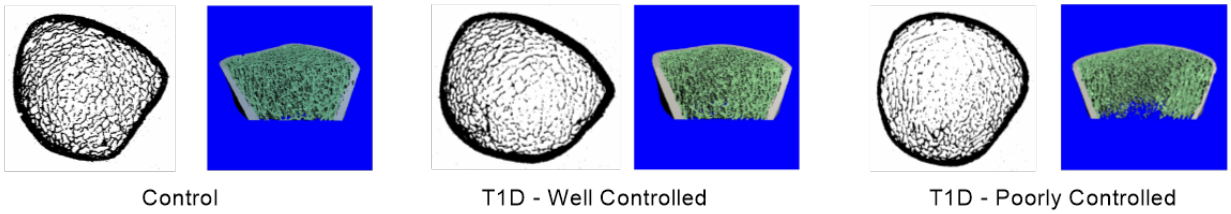

Figure 2. Representative XtremeCT-II images of the tibia in control and T1D participants.

3. Publications:

We will use the data from this project to support two primary publications. The first publication will focus on the expression of functional Sarm1 by human PBMCs and any differences in adolescents with T1D. The second publication will report the intersection of the neural and skeletal findings as it relates to the relative capacity for Sarm1 activation.