nPOD
Network for Pancreatic Organ Donors with Diabetes

Elucidating the Etiopathogenesis of Human Type 1 Diabetes

JDRF nPOD 5th Annual Scientific Meeting

February 10-13, 2013

SCIENTIFIC ABSTRACT SUBMISSION

Abstracts are due **September 17, 2012**

NOTICE OF PUBLICATION OF ABSTRACTS

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Part of nPOD's mission is to generate a comprehensive analysis of type 1 diabetes, which is enhanced through investigator collaboration. All abstracts accepted for presentation during the 2013 nPOD Annual Meeting will automatically be included in a printed program hand-out available at the meeting. By submitting your abstract, you agree that it can be printed and distributed as a program syllabus to all participants at the meeting.

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Abstract Instructions

1. Type abstract title and body only within the box below.
2. Do not repeat the authors within this section.
3. Include the following: *Purpose*, *Methods*, *Summary of Results*, and *Conclusions*.

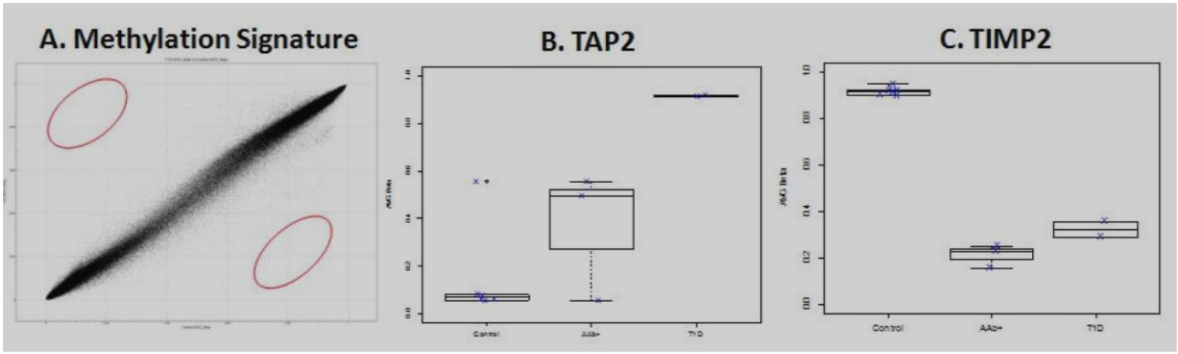
Title:
Global CpG methylation signature of pancreatic draining lymph node CD8+ T cells in type 1 diabetes

Purpose:
HLA class I overexpression within islets and the expansion of autoreactive CD8+ T cells are characteristic events during the pathogenesis of type 1 diabetes (T1D). We propose that CD8+ T cells display epigenetic markers, including CpG methylation, that are initiated during T cell activation and differentiation and persist in established disease. Further, we predict these marks foretell the transcriptional profile and effector phenotypes of these autoreactive effector populations. To address this hypothesis, we assessed the global methylation signature of CD8+ T cells isolated from tissues derived from individuals with T1D, multiple autoantibody positive at-risk subjects, and normal healthy controls.

Methods:
CD8+ T lymphocytes were isolated from peripheral blood mononuclear cells (PBMCs), pancreatic draining lymph nodes (PLN), or irrelevant draining lymph nodes by Fluorescence-Activated Cell Sorting (FACS). Total genomic DNA was extracted and bisulfite converted prior to CpG-methylation analysis on an Illumina Infinium HumanMethylation450 BeadChip comprising 485,000 CpG sites. Data were exported to R statistical program and vialized in Genome Browser.

Summary of Results:
Preliminary analyses demonstrated variable DNA methylation patterns at a number of unique loci between patients with T1D and controls (e.g., TAP2, TIMP2, IDI2, RPH3AL, FAM20C) (Fig 1, below).

Fig. 1 - *Differential CpG methylation pattern of isolated CD8+ T cells in T1D. A*) A representative plot depicting the average methylation of a subject with T1D and control subject, with differentially methylated loci identified by their non-linear position on the graph. Two representative genes of 22 highly differentially methylated at CpG residues are depicted in ***B***) for TAP2, and ***C)*** for TIMP2. Graphs shown compare methylation at each designated loci for T1D, AAb+ subjects, and controls (1=methylated CpG residue, 0=demethylated CpG)



Conclusion:
These data demonstrate our ability to isolate CD8+ T cells from the PDLN of nPOD subjects for epigenetic studies. Preliminary analyses suggest several distinct loci display differential methylation patterns in T1D, at-risk, and controls subjects. Ongoing studies are underway to validate targets differentially methylated in additional nPOD and PBMC samples, and subsequently assess their functional impact on CD8+ T cells in T1D.

Submit this document as an email attachment to npod@pathology.ufl.edu no later than **September 17, 2012**. Acknowledgement will be sent to the primary author and principal investigator within one week of receipt. Notification of accepted abstracts will be available October 2011. For any assistance, please contact Jayne Moraski at the email address above or at 352-273-9271.