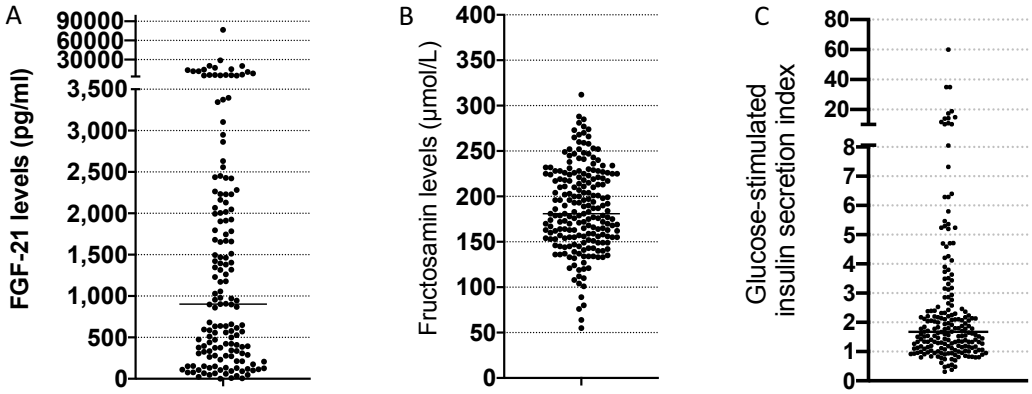
Diabetes Research Connection Grant

6-month project update

Progress report:
Milestone I - Genetic variant identification

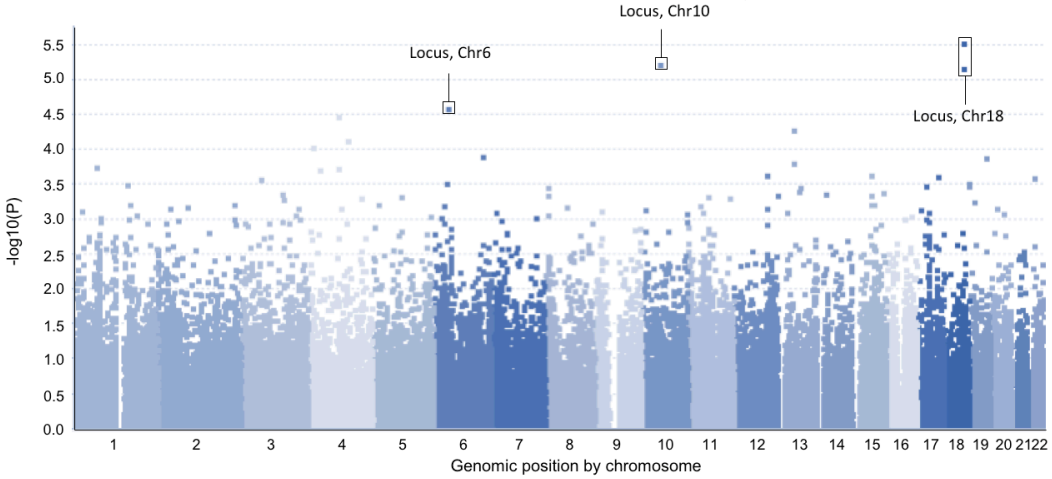
We collected complete phenotypic data on 200 pancreas donors which were all transplanted into type I diabetic patients. More specifically, we obtained donor data on FGF-21 and fructosamine levels (**Figure 1A, B**). Both are connected to "health status" of the pancreas and its ability to secrete insulin (hormone lacking in diabetic patients). For these 200 pancreas donors, we also collected data regarding islets of Langerhans, in particular, the ability of those islets to secrete insulin in response to glucose (sugar) in vitro (i.e. Glucose-Stimulated Insulin Secretion index (GSIS)). This is expressed as a ratio (see **Figure 1C** here below). A value of 10 means that islets of a given donor did secrete 10 times more insulin when exposed to glucose compared with the resting condition (low glucose).

Figure 1



We successfully isolated the DNA from 200 pancreas donors and proceeded with the genotyping (detection of Single Nucleotide Polymorphisms (SNP), namely genetic variations that may cause diseases) using the MetaboChip. We proceeded with a stringed quality control check. We used a flipping process to eliminate SNPs on the wrong DNA strand, we eliminated SNPs with a call rate inferior to 99%, we removed duplicate samples, we used HapMap to eliminate admixed elements. Following this process 123,932 SNPs were available for analysis. We then looked for association between the phenotype variables: FGF-21, fructosamine levels and GSIS (all three reflecting islet's "health status") and the genetic information (SNPs). We first look at the association between in vitro β-cell function and SNPs. We found three loci with p-value between 0.000003 and 0.00003 on chromosome 6, 10 and 18 that were associated with glucose secretion (See Manhattan plot, **Figure 2**). Genes potentially related to these loci were Cadherin-7 and Protocadherin Related 15 which were could play a key role in adhesions between cell inside the islet and thus be key molecules for an appropriate insulin secretion.

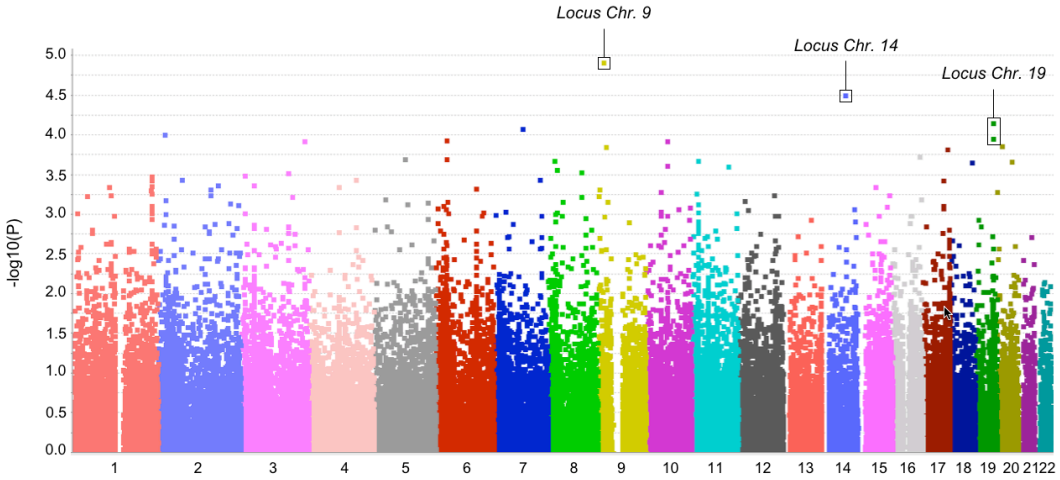
Figure 2



R. Meier - University of Maryland, Baltimore

We performed the association analysis for FGF-21 and refined the previously obtained results. We found three loci with p-value between 0.00001 and 0.0001 on chromosome 6, 10 and 18 that were associated with FGF-21 levels (See Manhattan plot, **Figure 2**). One of the locus identified was in close relation with Pleckstrin Homology MyTH4 And FERM Domain Containing H1 which is very likely to play important regulatory roles in the development of pancreas and in the differentiation of islet cells. Its manipulation is likely to modulate insulin synthesis. Another locus is associated with the gene Foxa3 which has been identified as a novel transcriptional regulator that inhibits brown fat generation during aging which in turn could alter insulin sensitivity.

Figure 3



Milestone II - *In vitro* assessment of candidate genes

We established islet cell culture, hepatocyte (HepG2) and myocyte culture (HSkMC). We started co-culture of islets and hepatocytes / myocyte using a transwell system. We assessed the response of islets using the different conditions. The next step is to extract RNA and preform a quantitative analysis of the gene expression relative to insulin secretion.

Milestone III - *In vivo* assessment of candidate genes

We first aimed to establish the model of insulin resistance in mice in our lab. We fed Black6 mice with high fat and high sucrose (HFHS) diet for 23 weeks and analyzed their insulin resistance profile (See **Figure 4**). These mice develop fatty livers, insulin resistance, and diabetes.

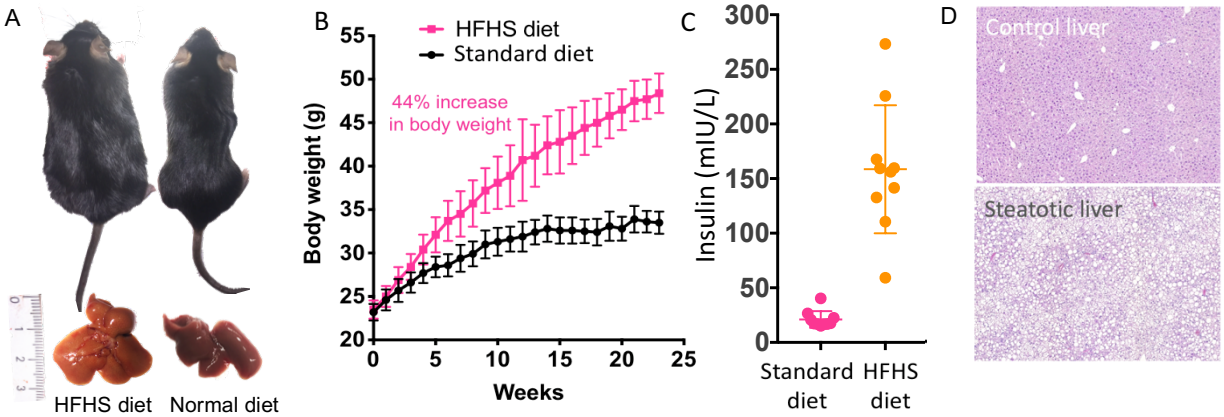


Figure 4: (A) mice fed with/without HSHF diet. (B) Mice weight. (C) Serum insulin. (D) Liver histology (H&E)

We observed 44% increase in mice weight following the introduction of the high fat high sucrose diet ("Western diet"). The mice developed much higher basal insulin levels, reflecting the development of insulin resistance and diabetes. The liver histology showed fatty livers.

We are now working on the generation mice deficient for the genes mentioned in Milestone I and confirmed in Milestone II. We will analyze the metabolic profile and insulin resistance in those mice. The aim is to connect the finding made in human pancreases (the new genes potentially implicated in diabetes) to the mechanisms causing diabetes in mice. The confirmation of these genes and pathways as key players of islet function would pave the way to the development of new molecules to target and treat diabetes.

Perspectives

Thanks to the DRC support, we already made several original findings with the identification of genes related to β-cell function and their potential key role in insulin production. Our next steps are to identify the crosstalk with the liver and the peripheral tissue, and study mechanism that connect the identified genes and pathway with insulin secretion. Our long-term goal is to detect specific metabolic disease predispositions in pre-diabetic patients and find targets for potential new drugs, as well as the promote the optimal selection of pancreatic islet donors to offer a better chance of cure for type 1 diabetic recipients.