CANDIDATE GENES IN THE GUT AND PANCREAS OF DIABETES-PRONE RATS

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ABSTRACT

BACKGROUND: Type 1 diabetes (T1D) is an autoimmune disorder, targeting the β-cells of the pancreas. T1D develops as a result of complex interplay between genetic and environmental factors. The initiating stimulus of T1D autoimmunity is unidentified, but processes occurring in the gut and pancreas are inferred to be involved. The pre-diabetic expression signature in these tissues is largely uncharacterized. **HYPOTHESIS:** Spontaneous models of T1D, the LEW.1AR1/Ztm-*iddm* rat (LEW-DP) and BioBreeding diabetes-prone rat (BBdp) exhibit a distinct transcriptional signature in the pre-diabetic phase of T1D onset. **METHODS:** Transcriptional profiling techniques were used to elucidate the transcriptional signatures of the LEW-DP gut and BBdp pancreas with qRT-PCR and IHC confirmation. **RESULTS:** The transcriptional profile of the LEW-DP gut displayed decreased expression of markers for immunoregulatory M2 macrophages compared with the control strain, LEW.1AR1 (LEW-C). The LEW-DP rats showed an upregulation of markers of the pro-inflammatory NF-κB pathway when fed a cereal diet compared with LEW-DP rats fed a protective hydrolyzed casein (HC) diet, suggesting that the cereal diet promotes transcription of genes involved in the pro-inflammatory response. Prospective pancreatectomy was used to analyze T1D development in the BBdp rat. Significant upregulation of regenerating islet-derived members, *Reg3α* and *Reg3β* and a novel transcript, *Trim26* was observed in pre-diabetic rats. These

candidates were localized to β-cells. **CONCLUSIONS:** Data from this thesis indicate that pre-diabetic rats possess divergent transcriptional signatures in the pre-diabetic period. It was also shown that the environmental influence of diet modifies the transcriptional program of diabetes-prone rats. Thus, it can be inferred that the transcriptional profile of DP rats is dynamic and is programmed early in development to promote T1D development.

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ABBREVIATIONS

BBc:	BioBreeding non-diabetes-prone control rat
BBdp:	BioBreeding diabetes-prone rat
BB DRlyp/lyp:	lymphopenic BioBreeding diabetes-resistant rat
Ccl19:	chemokine ligand C-C motif 19
CD:	celiac disease
CEL:	raw data file type extension, Affymetrix arrays
DAB:	3,3-Diaminobenzidine
DAVID:	**D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery
DP:	diabetes-prone
EIC:	extra-islet insulin^{+} cluster
ENSEMBL:	EMBL-EBI centralized bioinformatics database
FDR:	false discovery rate
FITC:	fluorescein isothiocyanate
Fn1:	fibronectin 1
GAD65:	glutamate decarboxylase 65
GALT:	gut-associated lymphoid tissue
Glycam1:	glycosylation-dependent adhesion molecule 1
GO:	gene ontology
HC:	hydrolyzed casein diet
HDAC:	histone deacetylase
HLA:	human leukocyte antigen
Hprt1:	hypoxanthine phosphoribosyltransferase 1
IAA:	anti-insulin autoantibodies
ICA:	anti-islet cell autoantibodies
IFN:	interferon
IHC:	immunohistochemistry
Il1f10:	interleukin 1 family member 10
IL-1Ra:	interleukin 1 receptor antagonist
Il1rapl2:	interleukin 1 receptor accessory protein ligand 2
Il2ra:	interleukin 2 receptor alpha
INGAP:	islet neogenesis associated protein
Ins1:	insulin 1 precursor
INS-1E:	rat insulinoma cell line 1E
Ldha:	lactate dehydrogenase A
LEW-C:	LEW.1AR1 rat non-diabetes-prone control strain
LEW-DP:	LEW.1AR1/Ztm-*iddm* diabetes-prone rat
LP:	lamina propria
M1Φ:	M1 macrophage

M2Φ:	M2 macrophage
MHC:	major histocompatibility complex
MLN:	mesenteric lymph nodes
NF-κB:	nuclear factor kappa light chain enhancer of activated B-cells
Nfkbia:	nuclear factor kappa light chain enhancer of activated B-cells inhibitor α
NOD:	non-obese diabetic mouse
Nos3:	nitric oxide synthase 3
PBMC:	peripheral blood mononuclear cells
PLN:	pancreatic lymph nodes
PP:	Peyer's patches
PPx:	partial pancreatectomy
qRT-PCR:	quantitative reverse transcription polymerase chain reaction
Reg:	regenerating islet-derived protein family member
RIN:	RNA integrity number
RINm5F:	rat insulinoma cell line m5F
RMA:	Robust Multichip Average
Rplp1:	ribosomal protein large P1
Rpl13a:	ribosomal protein L13a
SD:	standard deviation
Sell:	L-selectin
Serpine1:	serpin peptidase inhibitor clade E1
SLE:	systemic lupus erythematosus
SNP:	single nucleotide polymorphism
Stab1:	stabilin-1
STAT:	signal transducer and activator of transcription
T1D:	type 1 diabetes
TCR:	T-cell receptor
Teff:	effector T-cell
Th1:	T-helper 1 subset
Th17:	T-helper 17 subset
TJ:	tight junction
TLR:	toll-like receptor
TNFα:	tissue necrosis factor α
Tnfrsf1a:	tissue necrosis factor receptor superfamily member 1α
Tnfrsf5:	tissue necrosis factor receptor superfamily member 5
Treg:	regulatory T-cell
TRIM:	tripartite motif containing protein family
UMFIX:	**U**niversal **M**olecular **Fix**ative

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INTRODUCTION

Type 1 Diabetes and Autoimmunity

Autoimmune diseases develop as a result of the host immune system identifying self-proteins as antigens and initiating an immune attack on the host's tissue (Zhang et al., 2008). It is thought that autoimmune diseases arise from a genetic predisposition with an environmental stimulus prior to disease onset (Atkinson and Eisenbarth, 2001). Type 1 diabetes (T1D) is a chronic, inflammatory T-cell mediated autoimmune disorder that destroys the β-cells of the pancreas, impairing an individual's ability to regulate their blood glucose (Atkinson, 2012). Pancreatic islet β-cells are the primary cells that produce insulin in the body. Insulin is an endocrine hormone necessary for cellular uptake of glucose from the blood. Islets are the pancreatic endocrine units, which comprise 1 – 2% of the total pancreas. β-cells are the major cell type found in the islet core (70 – 80%) surrounded by glucagon-producing α-cells, somatostatin-producing δ-cells, pancreatic polypeptide-producing cells, and ghrelin-producing ε-cells. The pancreas is also a digestive organ that produces lytic enzymes in the exocrine tissue (acini) for the catabolism of nutrients in the duodenum. T1D is diagnosed when persistent hyperglycemia is observed; this occurs at 80 - 90% β-cell loss

that progresses in a lobular pattern in the pancreas (Gianani and Eisenbarth, 2005).

The population incidence of T1D in the United States is estimated to be 1 in 500 (Cizza et al., 2012). T1D develops spontaneously in children, adolescents, young adults, and a similar disease is observed in adults called latent autoimmune diabetes in adults (Andersen et al., 2013). Patients with T1D are insulin-dependent and manage their blood glucose concentration through diet and insulin injections. This is the most common and widely available therapy for T1D and has been used since insulin was discovered by Banting, Macleod, Best, and Collip in the 1920's (Best, 1972). Normoglycemic individuals maintain their blood glucose at ~5.5 mmol/L, but individuals with T1D have fasting blood glucose concentrations that fluctuate outside of the healthy homeostatic range (Stagner et al., 2008). Frequent high blood glucose concentrations in T1D patients cause severe complications including retinopathy, nephropathy, neuropathy, and cardiovascular disease (Kilpatrick et al., 2012).

Due to the severity of T1D and the lack of alternative therapies to insulin, there is a need to identify markers of disease for further development of diagnostic and treatment options. High-throughput genomic techniques can provide candidate markers of diabetes. The focus of this thesis will be the use of high-throughput transcriptomics in the study of the pre-diabetic period in spontaneous models of T1D to elucidate genes and processes modified in T1D development. I

hypothesize that rat spontaneous models of T1D display distinct gene expression signatures at an early age in the target tissues, gut and pancreas.

Environmental Influence in T1D Development

T1D is a complex disorder with over 40 identified genetic susceptibility loci implicated in disease onset with about half of these comprising of human leukocyte antigen (HLA) genes (Concannon et al., 2005; Steck et al., 2005). However, studies of monozygotic twins from families with first-degree relatives with T1D have shown that there is less than a 40 – 60% concordance rate between individuals, and concordance between siblings is between 5 – 16% (Larsen and Alper, 2004). Thus, there is a genetic component to T1D development, and strong evidence implicates environmental factors in T1D pathogenesis. These factors include: diet (Lefebvre et al., 2006), the gut microbiome (Neu et al., 2010), and childhood infection with enteroviruses (Coppieters et al., 2012). There is a complex interplay between heritability and environment that could be reflected by epigenetics, yet the details of these interactions remain undefined.

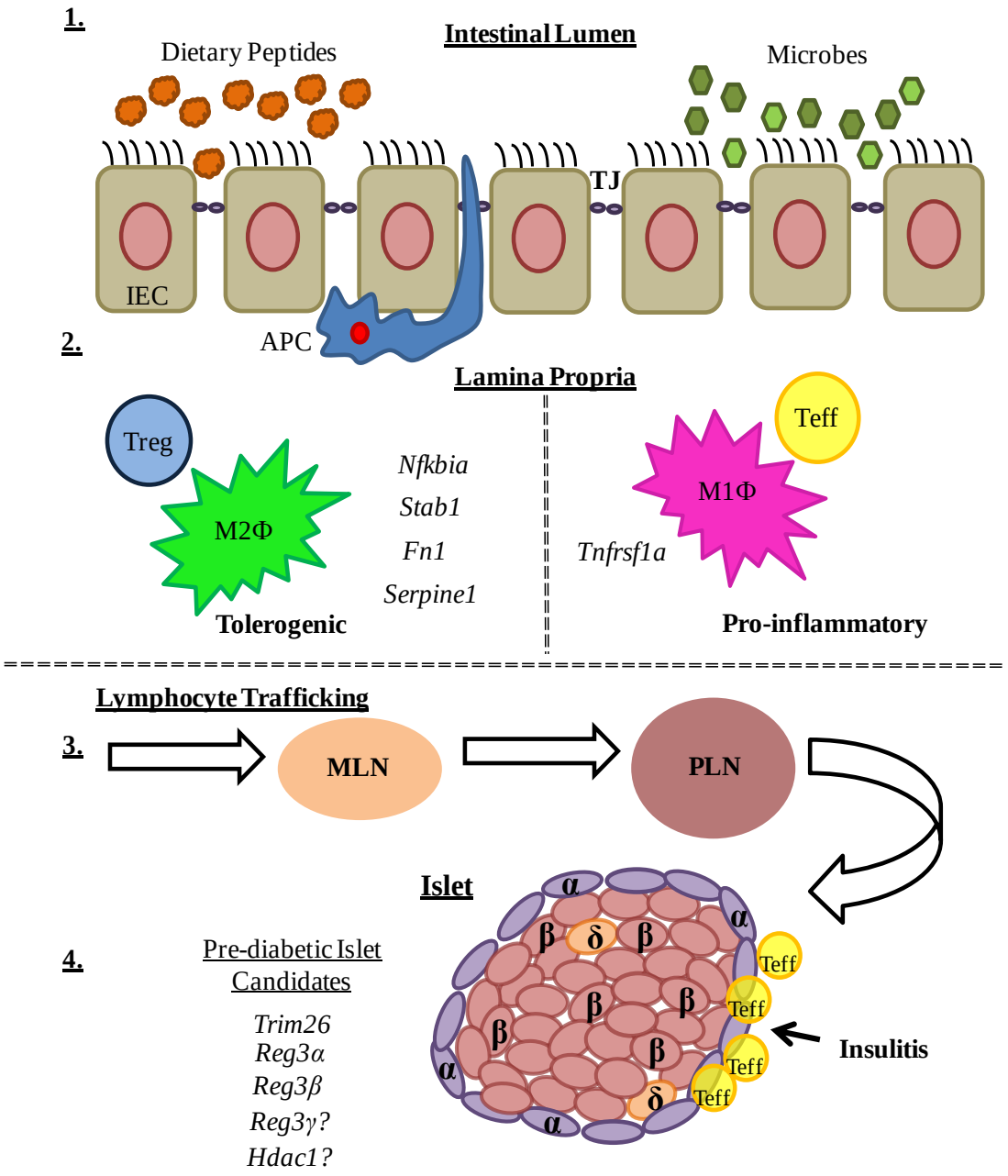
Involvement of the Gut Immune System

The gut is the body's largest lymphoid organ, and primary contact site between ingested matter from the environment and the host immune system (Weiner et al., 2011). The intestinal mucosa is separated from the

lumen by a single layer of epithelial cells that secrete mucin and anti-microbial peptides (Burger-van Paassen et al., 2012; Courtois et al., 2005). The gut-associated lymphoid tissue (GALT) consists of the lamina propria, Peyer's patches (PP), and M cells, which function to promote tolerance, antigen presentation, and lymphoid maturation (Pearson et al., 2012). Oral tolerance is established by the gut immune system to prevent an immune response against dietary and commensal antigens (Weiner et al., 2011). Differentiated lymphocytes migrate from the PP to mature in the mesenteric lymph nodes (MLN), the primary inductive site for immune response to dietary antigens (Chakir et al., 2005; Scott et al., 2000). Lymphocytes can differentiate into T-regulatory cells (Treg) that promote tolerance, or mature into T-effector (Teff) cells that are associated with pro-inflammatory conditions in the gut. A pro-inflammatory/immunoregulatory phenotype polarization is also characteristic of macrophages where M1 macrophages (M1Φ) produce pro-inflammatory factors and M2 macrophages (M2Φ) promote immune tolerance. These immune cells that mature in the MLN have tropism to the pancreatic lymph nodes (PLN), connecting the gut to the pancreas and supporting its putative role in T1D pathogenesis (Turley et al., 2005). **N.B.** "Gut" will refer to the jejunum throughout the thesis and total RNA includes RNA isolated from all cell types found in jejunum or pancreas tissue **(Figure 1)**.

Figure 1. Type 1 diabetes pathogenesis – complex interactions of gut contents, immunity, and pancreas. (1) Dietary proteins, viruses, and microbiota in the intestinal lumen are absorbed through the gut epithelia or enter the lamina propria (LP) through leaky tight junctions (TJ). **(2)** These foreign antigens interact with the gut-associated lymphoid tissue and are sampled by antigen-presenting cells (APC) of the LP. This can determine whether the gut exhibits a pro-inflammatory (M1Φ) or tolerogenic (M2Φ) status. **(3)** Activated lymphocytes (Teff and Treg) traffic to the mesenteric lymph nodes (MLN) in the gut for maturation. MLN-matured T-cells have tropism to the pancreatic lymph nodes (PLN), which influences the immune microenvironment surrounding the islets. **(4)** Infiltration of Teff cells into the islet is known as insulitis and precedes overt T1D. Pre-diabetic islets display upregulation of microarray candidates *Trim26*, *Reg3α*, *Reg3β, Reg3γ,* and *Hdac1*.

T1D Pathogenesis – complex interactions of gut contents, immunity and pancreas



It has been shown that animal models and patients with T1D possess “leaky guts”, a wider paracellular space between adjacent epithelial cells that line the intestinal villi, that allow the passage of larger molecules from the lumen into the lamina propria (Vaarala et al., 2008). Tight junctions (TJ) are formed by the interaction of claudin proteins on adjacent intestinal epithelial cells, regulating the traffic of molecules in the gut through the paracellular space (Visser et al., 2009). Zonulin is the only reported physiological modulator of intestinal TJ inducing a wider paracellular space, and it is thought to be involved in the development of oral tolerance (Fasano, 2011). The BBdp rat model of T1D exhibited 35-fold increase in zonulin levels within the intestinal lumen compared with the BioBreeding control strain (BBc) prior to autoantibody detection and T1D onset (Watts et al., 2005). BBdp rats that did not develop T1D showed a zonulin level similar to BBc rats (Watts et al., 2005). In a study of human serum zonulin levels, 42% of patients with T1D and 70% of pre-diabetic individuals had increased zonulin compared with age-matched control subjects (Sapone et al., 2006). Gene expression assays of gut biopsies from individuals with T1D showed altered expression of TJ proteins, claudin-1, claudin-2, and myoIXB with upregulation of TJ proteins that form a wider paracellular space (Sapone et al., 2006).

Diet and T1D

Several prospective studies have been initiated to investigate the effects of environmental factors in T1D development, especially the influence of diet. Studies of dietary influence on diabetes in humans include: the Trial to Reduce Diabetes in the Genetically at Risk (TRIGR) (2007), BABYDIAB (Ziegler et al., 2011), BABYDIET (Schmid et al., 2004), Diabetes Autoimmunity in the Young (DAISY) (Rewers et al., 1996), FINDIA (Vaarala et al., 2012), the environmental determinants of diabetes in the young (TEDDY) (Hagopian et al., 2006), EURODIAB (Toeller et al., 1996), and Diabetes Prediction and Prevention (DIPP) (Marjamaki et al., 2010). The first of these studies was the BABYDIAB study that was initiated in 1989 in Germany (Ziegler et al., 2011).

Diet has been demonstrated as a factor influencing T1D incidence in the population with genetic susceptibility. It is hypothesized that food ingested in childhood plays an integral role in the development of oral tolerance, the suppression of the immune response by exposure through the oral route (Mojibian et al., 2009). The introduction of wheat and cow's milk into children's diets has been widely researched as a source of T1D-related factors. It has been proposed that consumption of cow's milk outside of a developmental window promotes β-cell autoimmunity, particularly in individuals that possess the HLA-DQ8 T1D risk allele (Virtanen et al., 2012). Exposure to insulin in cow's milk could initiate the development of autoantibodies against human insulin in at risk individuals (Vaarala et al., 1998). The TRIGR trial is an investigation of

HC diet inhibition of T1D in Finnish children with a T1D HLA risk haplotype and a familial history of T1D. The investigators have found that children fed HC formula were significantly less likely to develop islet autoantibodies than children who received cow's milk (Knip et al., 2010).

Wheat peptides are also hypothesized to act as antigens that produce a diabetogenic immune response. A case study of a ~6-year old T1D patient placed on a gluten-free diet reported that this dietary regimen induced T1D remission, suggesting that a gluten-free diet prolongs the "honeymoon period" between diagnosis and insulin treatment (Sildorf et al., 2012). The gluten-free dietary regimen prevented the need of insulin therapy 20 months after initial diagnosis (Sildorf et al., 2012). Recent results from the DAISY study demonstrated that in individuals with a T1D-susceptiblity HLA haplotype, early exposure to wheat (< 6 months), and enteroviral infection have a significantly higher T1D incidence compared with children who had later introduction of wheat and/or no GI infection (Snell-Bergeon et al., 2012).

Studies to investigate the effects of diet on diabetes incidence have also been undertaken in animal models such as the non-obese diabetic mouse (NOD) and BioBreeding diabetes-prone rat (BBdp) (Flohe et al., 2003; Maurano et al., 2005; Wang et al., 2000). It has been established that controlling the protein source of the diet fed to diabetes-prone animals modifies the T1D incidence. Previous studies by several groups researching the BBdp have shown that feeding rats a hydrolyzed casein (HC) diet prevents T1D and delays disease onset compared with

rats fed an antigenic cereal diet (Scott et al., 2002). Peptides produced from the metabolism of wheat and milk have been hypothesized as antigens that trigger T1D (Flohe et al., 2003). Wheat peptides were identified as an inducer of pro-inflammatory cytokines in the gut of animal T1D models and patients with T1D (Funda et al., 2008). The production of a pro-inflammatory environment in the gut by the interaction of dietary peptides with the immune system could contribute to the induction of reactive T-cells with tropism to the pancreas. A recent publication studying germ-free and specific pathogen free BBdp rats has reported that diet exerts more influence over T1D development than the gut microbiome (Patrick et al., 2013). Thus, diet serves an important role in the etiology of T1D, but further investigation to determine the mechanisms involved is required.

Animal Models of T1D

The pancreas is situated behind the liver, deep in the abdominal cavity, making it difficult to biopsy for research purposes. Rodent models of T1D are commonly used instead for the study of target tissues in disease development. T1D is modeled with one of three methods: chemical induction of T1D, viral induction, or using rodents that spontaneously develop disease through mutations. Still, these models are imperfect. In fact, there are over 190 different methods to cure T1D in the non-obese diabetic (NOD) mouse, but none of these methods has had translational benefit for patients with T1D (Atkinson, 2005). However,

these models have provided many insights into T1D pathogenesis and factors contributing to T1D development. Models of spontaneous T1D include the NOD mouse, BioBreeding diabetes-prone rat (BBdp rat), LEW.1AR.1/Ztm-*iddm* rat (LEW-DP rat), and Komeda/Long-Evans Tokushima Lean diabetes-prone rat (KDP/LETL rat) (Mordes et al., 2004), each of which arose from mutations in a congenic control strain. The basis of this thesis will be transcriptional profiling of the LEW-DP and BBdp models.

The LEW-DP Model of Spontaneous T1D

The LEW-DP is a relatively new rat model of type 1 diabetes that exhibits many similarities to the human disease (Weiss et al., 2005). The LEW-DP is congenic with the diabetes-resistant LEW.1AR1 (LEW-C) strain (Lenzen et al., 2001). Autoimmunity arises in the LEW-DP from mutations in the rat MHC class II haplotype (*Iddm1*), which confers susceptibility to diabetes development (Arndt et al., 2009). The diabetes incidence of the German colony of LEW-DP is 60% (Wedekind et al., 2005). Hyperglycemia is not apparent until 60 – 70% of β-cells have been destroyed (Jorns et al., 2005), and it is hypothesized that β-cell loss occurs through apoptosis (Lenzen et al., 2001). The window for T1D development in the LEW-DP model is narrow, occurring around 60 d for both males and females and does not exhibit a sex-bias in T1D incidence as observed in the NOD mouse (Lenzen et al., 2001). The disease onset is extremely rapid, destroying the population of β-cells within one week

(Lenzen et al., 2001). The immune infiltrate begins with innate immune cells such as macrophages and progresses to insulitis with T-cells in the islets (Jorns et al., 2005).

Gene expression analysis of the LEW-DP lymphocytes in the PLN has shown that pro-inflammatory cytokines were upregulated and anti-inflammatory cytokine expression was decreased (Jorns et al., 2010). *Il-1β* and *Tnfα* were significantly upregulated in activated PLN $CD8^+$ T-cell populations (Jorns et al., 2010). *In-situ* RT-PCR analysis of monocyte chemoattractant protein-1, cytokines, and CD8 demonstrated that genes for pro-inflammatory cytokines and markers of immune activation were upregulated (Jorns et al., 2010).

The BBdp Model of Spontaneous T1D

The inbred BBdp strain is the most studied rat model of T1D. It was derived from an outbred Wistar rat colony with mutations that occurred in several genes including GTPase IMAP family member, *Gimap5 (Iddm2)*, and the MHC (RT1) class II u region (*Iddm1*), that resulted in severe T-cell lymphopenia (Dalberg et al., 2007; Mordes et al., 2004; Wallis et al., 2009). At least fourteen other gene loci are thought to be implicated in T1D development in the BBdp (Wallis et al., 2009). Disease onset occurs equally in both male and female rats between 50 – 90 d. Immune infiltrate into the pancreas consists of Th1 cells and the rat displays a deficiency in regulatory T-cells (Treg). Adoptive transfer of concanavalin A-activated $\alpha\beta TCR^+$ (T-cell receptor) splenocytes from

diabetes-prone or diabetic BBdp rats induced T1D in 30 d BBdp rats, suggesting that $CD4^+$ T-cells were responsible for β-cell apoptosis (Metroz-Dayer et al., 1990). Dietary intervention with an isonitrogenous HC diet or thymectomy have been shown to prevent disease (Mordes et al., 2004).

Preliminary transcriptional profiling of the peripheral blood mononuclear cells (PBMCs) isolated from the lymphopenic BioBreeding diabetes-resistant (BB DRlyp/lyp) rat has been published (Kaldunski et al., 2010). Studies of the immune cell profile of the BB DRlyp/lyp rat have shown a similar upregulation of processes observed in human patients with T1D demonstrating the value of the BioBreeding rat as a model in the study of the T1D transcriptome (Kaldunski et al., 2010).

Transcriptomics and Markers of T1D Development in the Pre-diabetic Period

Genomics has been employed in T1D research since the late 1970's and has expanded during the 1990's and onward with the development of sophisticated genomic technology. T1D research has focused in the past on identifying patients at risk for T1D by autoantibody markers against islet-antigens and markers detectable in patient serum for diagnostic use including anti-zinc transporter 8 autoantibodies (ZnT8) (Ingemansson et al., 2013), anti-glutamate decarboxylase 65 (GAD65) (Stayoussef et al., 2011), anti-insulin autoantibodies (IAA) (Adler et al., 2011), and anti-islet cell autoantibodies (ICA) (Bottazzo and Doniach,

1978). Alternatively, genome-wide association studies and characterization of single-nucleotide polymorphisms (SNP) have identified several genes that confer T1D risk (Barrett et al., 2009), but are not definitively linked to T1D development.

The expression profile of the T1D fingerprint remains poorly understood as T1D integrates defects of the immune, endocrine, and gastrointestinal systems. High-throughput gene expression technology has become widely available enabling the interrogation of the transcriptional signatures of target tissues. These methods could be used to elucidate changes in the pre-diabetic phase of T1D for screening of those at risk for developing T1D. A number of studies have been undertaken to characterize the transcriptome of T1D using peripheral blood, islets, and lymphoid tissue in order to identify novel candidate genes participating in T1D development (Eckenrode et al., 2004; Jia et al., 2011; Planas et al., 2010a; Planas et al., 2010b). Although the proteome and transcriptome have only moderate concordance, investigation of transcription can provide valuable insight into potential biomarkers and biological processes implicated in T1D development (Griffin et al., 2002; Mootha et al., 2003).

Transcriptional Signature of the Immune System in T1D

There has been a consistent bias in the literature toward the investigation of immune system gene expression instead of global expression changes in target tissues. The bias toward the immune system

has occurred for several reasons including the convenience of sample collection, and the interest in the mechanism of T1D autoimmunity. Another reason is that a majority of the T1D candidate genes (~60%) are hypothesized to have immune function (Concannon et al., 2005). The majority of research into the T1D transcriptional signature has been performed using patient samples of peripheral blood to study T1D indirectly. This approach allows for the investigation of immune pathways, but lacks the tissue specificity of directly investigating target tissues. These designs also make the assumption that circulating peripheral immune cells are reflective of those found in the pancreas.

Pro-inflammatory Status and Adaptive Immunity Defects of Peripheral Tissues in T1D

Peripheral blood samples from individuals with T1D are often collected due to ethical constraints. Advantages of collecting this sample are the ability to enroll a large number of study participants, collect sample at different time points, and obtain a heterogeneous cell population. Analyses of PBMCs from patients with autoimmune disorders including T1D, multiple sclerosis, rheumatoid arthritis, ulcerative colitis, systemic lupus erythmatosus (SLE) and Crohn's disease have found that the expression signature of individuals with T1D diverged from other autoimmune disorders (Tuller et al., 2012). T1D patients had significant clustered interactions with eukaryotic initiation factor members and upregulation of prostaglandin endoperoxidase synthase-2 and

cyclooxygenase. In an earlier study of the T1D and pre-diabetic whole blood transcriptome, Reynier *et al*. revealed that individuals with T1D expressed an interferon signature that diverged from other autoimmune disorders such as SLE (Reynier et al., 2010). The type I IFN transcriptional signature is preferentially expressed in pre-diabetic individuals compared with longstanding cases of T1D, and they concluded that the type I IFN pathway is central to early T1D pathogenesis (Reynier et al., 2010).

Additionally, PBMC analyses have facilitated twin and familial studies to improve insight into the role of epigenetics and environment in T1D. Stechova *et al*. published a case report of PBMCs isolated from monozygotic quadruplets where all quadruplets were homozygous for the risk allele HLA-DQ8 and two were diagnosed with T1D. They found that the antiviral IFN signaling pathway was the most upregulated, and the pro-inflammatory cytokine signaling pathway was the second most affected pathway (Stechova et al., 2012). However, while all quadruplets possessed genetic susceptibility and previous enteroviral infection, only half of the quadruplets had T1D. This group has also proposed that first degree relatives of T1D patients have a different basal gene expression signature in immune cells compared with controls including upregulated innate immunity genes and cytokine signaling pathways (Stechova et al., 2011). The greatest expression profile differences between groups were observed in relatives of individuals with T1D and control subjects. Interestingly, they discovered that first-degree relatives that became

diabetic had a divergent expression profile until autoantibody presence at which point differences in gene expression between relatives and patients with T1D were not significant.

Profiling the PBMCs of recent-onset and autoantibody positive patients permitted Elo *et al.* to discover a highly significant network of genes downregulated in T1D patients through network analysis (Elo et al., 2010). Individuals with T1D displayed decreased expression of TCR and MHC genes. Consistent with previous research published, they found that the T-cells of T1D patients were anergic with dysfunctional TCR signaling that likely caused $CD8^+$ cytotoxic T-cells to play a major role in the pre-diabetic phase.

Investigation of animal model peripheral immune cells has produced results consistent with those obtained from patient studies since more invasive studies using lymphoid tissue or pancreas samples can be performed using animal models. Gene ontology interrogation using **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (DAVID) database determined that the NF-κB, antigen presentation, and the immune response functions were significantly upregulated in the BB DRlyp/lyp rats compared with the BB DR+/+ strain (Kaldunski et al., 2010). Profiling PBMCs from recent-onset 60 d BB DRlyp/lyp rats showed a significant involvement of IL-1 in T1D development (Kaldunski et al., 2010). When BB DRlyp/lyp rats were administered IL-1 receptor antagonist (IL-1Ra), T1D was prevented. Furthermore, the

expression signature of cultured PBMCs isolated from IL1-Ra treated BB DRlyp/lyp rats showed a decrease in pro-inflammatory immune genes.

Alternatives to using PBMCs as a source of immune cells are the use of spleen or thymus tissues. Spleen leukocytes of NOD mice compared with a control strain C57BL/6 mice demonstrated strong influence of interaction networks involving pro-inflammatory cytokines, TNFα and IFNγ (Wu et al., 2012). Eckenrode *et al.* profiled spleen lymphocytes in 5 week old pre-diabetic NOD mice and discovered several novel candidate genes of interest: *Hspa8, Irf4,* and *Ubl1* (Eckenrode et al., 2004). Fornari *et al.* dissected thymi from 1 month old NOD mice and separated the T-cells ($CD3^+$) for microarray analysis of T-cell transcriptomes in pre-diabetic and diabetic mice (Fornari et al., 2011). Diabetic NOD T-cells showed increased expression in $CD4^+/CD8^+$ differentiation and downregulated expression of Treg and tolerance-promoting gene, *Foxp3* (Fornari et al., 2011).

In summary, it is evident that animal models and patients with T1D display a distinct immunological gene expression signature at various time points even in peripheral blood. The trend observed for the diabetic groups was a bias towards pro-inflammatory signaling including NF-κB, IFN, and Th1 pathways. Innate immunity is not the focus of many published transcriptional analyses and there is an over-representation of profiling for T-cells and adaptive immune system markers. The paucity of data regarding the innate immune system in T1D transcriptomics is likely due to its potential early role in disease

pathogenesis that often goes undetected in patients in the pre-diabetic period. Overall, the use of peripheral blood samples has shown that the immune expression signature is polarized toward T1D development in pre-diabetic individuals prior to disease presentation.

Understanding the Pancreas Transcriptome in the Context of T1D

Transcriptional analyses of primary target tissues of T1D have been employed with *in vitro* and *ex vivo* approaches using β-cell lines and donor islets. Collection of pancreas biopsies from recent onset or long term T1D patients is highly invasive and samples are difficult to obtain. Cadaveric islet samples can be obtained through The Network for Pancreatic Organ Donors with Diabetes (nPOD) (Campbell-Thompson et al., 2012). Another alternative to *in situ* study is the use of rodent insulinoma cell lines. Some researchers have attempted to expand human β-cells in culture to study them; however, it remains a challenge to establish a successful culture since only 1 – 2% of pancreas cells are β-cells. Only recently was a human β-cell line established from fetal pancreatic buds (Weir and Bonner-Weir, 2011). As Weir and Bonner-Weir noted, *in vitro* β-cells are not biologically representative of those found *in vivo*, which are heterogeneous and surrounded by a complex microenvironment (Weir and Bonner-Weir, 2011). There are currently no publications detailing the *in situ* molecular signature from pancreas biopsies isolated from individuals with T1D.

Several groups have attempted to study T1D development in the NOD mouse using PLN and islets sampled from 2 – 20 weeks (Aspord et al., 2004). By comparing the NOD mouse with immunodeficient NOD-SCID and a control strain (BALB/c), Aspord *et al*. attempted to document the early events occurring in the pancreas that initiate β-cell death. This study examined the immune/endocrine similarities in the pancreas of pre-diabetic animals by sampling the PLN and islets of NOD mice during the pre-diabetic period and during insulitis. They found that there was a 0.76 – 0.86 correlation among the genes upregulated in the endocrine and immune tissues sampled, suggesting that the same set of genes is upregulated in both the islets and PLN of pre-diabetic NOD mice. Additionally, they found that there is a Th1 bias in the NOD mice, which was previously suspected. IL-11 was downregulated in the NOD in the pre-diabetic period (4 weeks). IL-11 is an immunoregulatory cytokine that can inhibit IFNγ and NF-κB function. Pro-apoptotic genes *Bax* and *Casp3* were also upregulated at 4 weeks of age in the NOD islets. This was a notable finding that suggested β-cells alter their transcriptional program before destruction.

In another investigation of the PLN, NOD mice were screened for the development of insulin autoantibodies (IAA) to indicate the impending onset of T1D (Regnault et al., 2009). PLN RNA was isolated from IAA positive and negative mice. Gene ontology (GO) analysis showed the pro-inflammatory Th1 response and tissue remodeling processes were the enriched functional annotations in PLN samples from

autoantibody positive NOD mice. It is well known that Th1 phenotype T-cells are the lymphocytes involved in insulitis. In addition, they found that clusters of genes involved in inflammation and tissue regeneration were upregulated in 3 – 5 week old autoantibody positive mice. These clusters include the regenerating islet-derived proteins (Reg) and elastase proteins, yet the function of these groups during the pre-diabetic period in the NOD mouse has not been confirmed.

Insulitis, the infiltration of immune cells into an islet, is associated with upregulated inflammatory gene expression in both humans and animal models of T1D. Non-diabetic human islets exposed to serum from diabetic patients had gene expression signatures that demonstrated increased angiogenesis and decreased protection (Jackson et al., 2012). During inflammation, the expression of the survival pathway, JunB, is upregulated in pancreatic β-cells of the NOD mouse in response to TNFα and IFNγ treatment (Gurzov et al., 2011). The investigation of intracellular β-cell stress-induced signaling is regularly overlooked in the study of T1D in favor of examining interactions occurring at the cell membrane that induce an apoptotic cascade.

The work of Eizirik's group has been instrumental in establishing a database of information on the β-cell transcriptome. They are responsible for organizing and curating the β-cell gene atlas accessed through T1DBase and EuroDia. These are repositories for gene expression data collected from primary β-cells and insulinoma cell lines (Hulbert et al., 2007; Liechti et al., 2010). They have identified numerous

candidate genes using high-throughput genomic analysis including microarrays and the first RNA-Seq study of islets (Eizirik et al., 2012) including *Ptpn2, Usp18, Mda5, Il1β, Tnfα, Stat1, Ifnγ, Bcl6*, and *Mcp1* (Barthson et al., 2011; Colli et al., 2010; Igoillo-Esteve et al., 2011; Moore et al., 2009; Moore et al., 2011; Ortis et al., 2012; Santin et al., 2012). Through both *in silico* and *in vitro* methods, the group and collaborators have identified putative pathways involved in T1D pathogenesis including: NF-κB, endoplasmic reticulum (ER) stress response, STAT1 signaling, interleukin-17 pathway, interferon regulatory network, and pro-inflammatory cytokine signaling (Arif et al., 2011; Callewaert et al., 2007; Eldor et al., 2006; Gysemans et al., 2005; Miani et al., 2012; Moore et al.; Ortis et al., 2012; Pirot et al., 2007). Many researchers choose high-throughput gene expression methods as an unbiased technique to elucidate changes in a target tissue as a discovery tool. This approach often results in the publication of an article with a focus on *in silico* identification, functional enrichment of candidates, and a preliminary investigation into the biological function of select candidates. Eizirik's group has gone beyond the chip, publishing multiple studies of in depth genomic and biological analyses for specific processes of β-cell biology relating to apoptosis from data collected in high-throughput studies.

Planas *et al.* have published the most comprehensive gene expression analysis of islets isolated from longstanding and newly-diagnosed T1D cadaveric donors (Planas et al., 2010a). Their study was

the first data set of human pancreata with biological validation for significant candidates. Pancreas samples were dissected to isolate islet samples for microarray processing and qRT-PCR validation. Microarray analyses were performed with total pancreas and dissected islet RNA. Their results showed that antigen presentation pathways were upregulated in all four diabetic samples. Significant differentially expressed genes included those encoding autoantibodies GAD65 and IAA as well as chemokine genes in T1D patients. The gene expression signature of the recent onset and long-term T1D patients was significantly different indicating a shift in biological processes during disease progression. They concluded that T1D is a chronic inflammatory disease with a central role for innate immunity, which contrasts the conclusions made from the PBMC studies. They hypothesized that the upregulation of β-cell transcripts such as *REG4* measured in patients with T1D is potentially the result of compensation for β-cell loss. It can be inferred from their work that novel candidates with differential expression between recent and long-term onset have the potential to act as diagnostic markers to identify pre-diabetic individuals.

As T1D has an undefined development period that often goes undetected, many researchers have attempted to understand the molecular changes occurring during the pre-diabetic period. Benoist and Mathis used a time-course study of cyclophosphamide-induced T1D in the NOD mouse to study T1D progression in purified islets and resultant changes in gene expression (Matos et al., 2004). They found that T-cell expression

was unperturbed in pre-diabetic mice, but observed a downregulation in B-cell transcripts. Upregulated genes were predominantly interferon-regulated. Of the transcripts identified, only one family of genes expressed in the pancreas was affected, the regenerating islet-derived 3 genes. Subsequently, Benoist and Mathis have published several reviews evaluating the power of the microarray as a tool in immunology research (Hyatt et al., 2006). Transcriptional analysis is one element of systems immunology that requires consideration and integration of genetics and proteomics to extend beyond bioinformatics algorithms for practical and biological value (Benoist et al., 2006).

In conclusion, integrative genomics and bioinformatics methods have provided insight into complex disorders such as T1D; however, many of the published studies employ study designs that do not reflect the complex interaction between the immune system and target tissues involved in T1D pathogenesis. Data obtained from a sorted cell type or peripheral sample do not provide insight into communication occurring between cell types in the pancreas. Reductionism in study design does not necessarily model processes occurring in T1D development in the intact organism. These studies have emphasized the identification of candidates through the statistical analysis of arrays and functional annotation validation through additional *in silico* gene ontology interrogation. Thus, genomic technologies yield vast quantities of data, but downstream biological confirmation is essential to deriving meaningful results from high-throughput genomic analyses. Ultimately, systems approaches

present the possibility of providing insight into T1D disease mechanisms when considered in the context of the disease.

OVERALL HYPOTHESIS AND OBJECTIVES

Despite multiple risk loci and putative environmental factors identified, the initiating event(s) of T1D autoimmunity remain undetermined. Molecular changes that occur throughout the pre-diabetic period in T1D target tissues are mainly uncharacterized. The central hypothesis of this thesis concentrates on the divergent gene expression of diabetes-prone rats during the pre-diabetic phase of T1D development. I hypothesize that the gene expression signature of the LEW-DP gut and BBdp pancreas will display distinct transcriptional profiles early in development. The major objectives of this thesis were to (1) identify strain and diet induced gene expression changes in the 45 days of age (d) LEW-DP rat jejunum and (2) to retrospectively study the pre-diabetic period in pancreas biopsy samples from partially pancreatectomized 30 d BBdp rats using high-throughput genomic methods.

EXPRESSION PROFILING OF THE LEW-DP GUT

Currently, there is very limited literature on the LEW-DP spontaneous model and whether the small intestine contributes to disease development in the LEW-DP is not known. Since the gut is the primary contact site with environmental agents, it is a target tissue implicated in type 1 diabetes development. Preliminary studies from Lenzen *et al.* suggested that there was no involvement of the small intestine in the LEW-DP rats and that only the pancreas is affected (Lenzen et al., 2001). However, the BBdp rat displays overt enteropathy prior to T1D onset suggesting that gut inflammation could be present in the LEW-DP as well. In a follow-up paper from the same group, the small intestine was examined by immunohistochemistry for immune infiltrate, but was not analyzed for molecular signs of inflammation or enteropathy (Jorns et al., 2004). Few transcriptional analyses were pursued in any of the T1D target tissues of the LEW-DP and much remains to be determined about the transcriptome in this model.

OBJECTIVE: To determine transcriptional differences in the gut of 45 d diabetes-prone rats versus the control strain (LEW-C) and evaluate the effect of the protective HC diet on gene expression in the LEW-DP gut using high-throughput genomics.

RESEARCH QUESTIONS

1. What pro-inflammatory genes are upregulated in the gut of the cereal-fed LEW-DP rat compared with LEW-DP rats fed a hypoallergenic HC diet (diet comparison)?
2. What genes and biological processes are upregulated in the diabetes-prone LEW-DP rat compared with the LEW-C background strain (strain comparison)?

MATERIALS AND METHODS

Animal Husbandry: LEW-C and LEW-DP rats were obtained from Drs. Dirk Wedekind and Sigurd Lenzen (Hannover, Germany) and a colony was established at the Ottawa Hospital Research Institute (OHRI, Ottawa, ON, Canada) animal care facility. Rats were fed *ad libitum* a standard rodent cereal diet (Teklad Global 18% Protein Rodent Diet, Harlan Laboratories, Montreal, QC, Canada) or an AIN-93G HC diet (Scott et al., 2002). In the HC diet, hydrolyzed casein is the only amino acid source (Scott et al., 2002). Animals were monitored for glucosuria and diabetes status was confirmed by a fasting blood glucose level above 11.1 mmol/L; diabetic animals were euthanized within 48 h by exsanguination under 3% isoflurane (Abbott Laboratories, Montreal, QC, Canada). Diabetes incidence was documented and 45 d and 100 d animals were euthanized to harvest pancreas and jejunum. Tissues were snap frozen in liquid N_2 for RNA studies or fixed in Bouin's fixative for

immunohistochemistry. Animals were maintained and euthanized according to the Canadian Council on Animal Care guidelines.

RNA Isolation: Samples of whole LEW jejunum were homogenized using a Polytron PT-2100 homogenizer (Kinematica, Bohemia, NY, USA). Total RNA was isolated from samples of 45 d LEW-C cereal fed (n=5), LEW-DP cereal-fed (n=6), and LEW-DP HC-fed (n=7) jejunum using the RNA II kit according to the manufacturer's instructions (Macherey-Nagel, Düren, Germany). RNA integrity was assessed and concentration was measured using Agilent RNA 6000 Nano chips in an Agilent 2100 Bioanalyzer at the institute core facility, Stemcore (Ottawa, ON, Canada). All samples displayed RNA integrity numbers (RIN) above 9.0.

Microarray Processing: RNA from each animal was equally pooled in 5 μL aliquots with a final RNA concentration of 100 μg/μL. Individual Affymetrix Rat Gene 1.0 ST arrays (n=1/group) were used for analysis at Stemcore. Affymetrix Rat Gene 1.0 ST arrays cover over 27,000 protein coding transcripts and more than 24,000 Entrez gene IDs (GeneChip Rat Gene ST Arrays, www.affymetrix.com). Resultant CEL[1] files were processed using Affymetrix Powertools plug-in on AltAnalyze

[1]CEL: Is a file extension for the raw data chip files. These are generated from intensity calculations of the pixels from Affymetrix DNA microarray image software.

bioinformatics platform and normalized using RMA[2] following the default instructions of AltAnalyze programmers (Gladstone Institute, San Francisco, CA, USA) (Emig et al., 2010; Irizarry et al., 2003). Normalization of the raw data allowed for experimental error and correction of nonspecific binding. Normalization of the array data was verified using FlexArray (McGill University, Montreal QC, Canada and Genome Quebec, Montreal QC, Canada). Upregulated and downregulated gene lists for each comparison consisted of genes with arbitrary cut off of 1.5 fold change.

Gene Ontology Analysis: (1) *DAVID* (Huang da et al., 2007): ENSEMBL gene IDs corresponding to the list ± 1.5 fold change were uploaded into the functional annotation tool to obtain enrichments with highest stringency setting. Enrichment scores above 2.0 with lowest *p*-value and false discovery rate (FDR) values were of interest (http://david.abcc.ncifcrf.gov/). (2) *GOrilla* (Eden et al., 2009): The organism was set to *Rattus Norvegicus* for correct ENSEMBL ID recognition. Two unranked gene lists were used. The "target set" was the ENSEMBL identifiers ± 1.5 fold change. The "background set" was the remaining ENSEMBL identifiers. The process ontology was selected, and the ontologies with the lowest *p*-value, lowest FDR q-value, and enrichment score ~10 were considered to be of interest (http://cbl-gorilla.cs.technion.ac.il/). FDR q-value is the correction of the FDR

[2]RMA: Robust Multichip Average (RMA) is an algorithm used for the normalization of Affymetrix probe values to remove background noise.

versus the number of gene IDs tested. Enrichment of biological process is calculated using the following formula:

$$\text{Enrichment} = (b/n) / (B/N)$$

Where b is the number of genes overlapping the target and background set, n is the number of genes in the target set, B is all of the genes associated with one GO category, and N is the total number of IDs input.

Candidate Selection: AltAnalyze gene lists were sorted with Microsoft Excel and studied for upregulation or downregulation of putative transcripts involved in inflammation or processes related to T1D development. Candidate genes of interest were selected for validation based on the following criteria using semi-quantitative qRT-PCR:

i) Fold change (± 1.5 fold change)
ii) GO functional enrichments (DAVID and GOrilla)
iii) Literature search for publications relating to T1D, inflammation and autoimmunity

qRT-PCR: 2 μg of whole jejunum RNA was reverse transcribed using Moloney Murine Leukemia Virus (MMLV) Reverse Transcriptase according to manufacturer's directions with modifications (Life Technologies/Invitrogen, Carlsbad Springs, CA, USA). The reaction was incubated at 42 °C for 90 minutes and inactivated for 5 minutes at 90 °C in an Eppendorf Mastercycler PCR machine. Semi-quantitative qRT-PCR was performed in an ABI Prism 7000 Sequence Detection System with

TaqMan Gene Expression Assays (Applied Biosystems). Primers for *Ccl19, Il-20, Glycam1,* and *Ins1* were used (Applied Biosystems). Data from qRT-PCR experiments were analyzed by ΔΔCt method in Microsoft Excel and exported to Statistica (StatSoft, Tulsa, OK, USA) for analysis using ANOVA with post-hoc Sheffé test.

PCR Array: RT^2Profiler Innate and Adaptive Immune Response focused arrays (PARN-052A, SABiosciences, Mississauga, ON, Canada) were used for each group to identify pathways involved in gut inflammation. RNA from LEW-C cereal-fed (n = 4), LEW-DP cereal-fed (n = 4) and LEW-DP HC-fed (n = 4) animals was reversed transcribed (RT) to cDNA using SABiosciences RT^2 First Strand kit using 1 µg of RNA according to manufacturer's instructions in an Eppendorf Mastercycler PCR machine. cDNA from the RT reaction was used for RT^2Profiler Innate and Adaptive Immune Response PCR arrays and reactions were run in an ABI Prism 7000 Sequence Detection System qRT-PCR machine following manufacturer's guidelines. Each PCR plate contained 84 primers, loading controls, and 5 internal controls (*Actb, Ldha, Hprt1, Rplp1, Rpl13a*). Data were normalized through the web-based data analysis service (SABiosciences) using the manual selection method to run normalization against all control primers. PCR array data were analyzed and figures were generated using SABiosciences web-based software (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php).

Immunohistochemistry (IHC): Pancreas and jejunum from LEW-DP HC-fed, LEW-DP cereal-fed and LEW-C animals were Bouin's fixed and sent for processing at the University of Ottawa Pathology Department. Slides from asymptomatic 45 d and 100 d animals were de-waxed and rehydrated using serial Citrisolv (Fisher Scientific, Nepean, ON, Canada) and ethanol washes. Antigen retrieval was performed using citrate buffer (0.05 M, pH 6) for 4 or 10 minutes at 125 ˚C. Endogenous peroxidases were inhibited using a 70% methanol, 1% hydrogen peroxide wash. Sections were blocked with 5% bovine serum albumin (Fisher Scientific) in phosphate buffered saline (pH 7.5) for a minimum of 30 minutes. Sections of LEW-DP and LEW-C pancreas were stained with rabbit anti-guinea pig insulin antibody (1:150, Dako, A0564, Burlington, ON, Canada) with a donkey anti-guinea pig secondary antibody (1:300, 706-065-148, Jackson ImmunoResearch, West Grove, PA, USA) and avidin-biotin conjugation (Vectastain, Vector Labs, Burlingame, CA, USA) and 3-3'-diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO, USA) development for 5 minutes followed by a hematoxylin counterstain (Electron Microscopy Sciences, Hattfield, PA, USA).

Morphometry: Sections of insulin stained pancreas from 45 d and asymptomatic 100 d were scanned with a Zeiss Axioplan 2 (Zeiss, Toronto, ON, Canada) microscope to generate superimages at 5x to study total pancreas section area. $Insulin^+$ area was quantified using Northern

Eclipse software (Empix Imaging Inc., Mississauga, ON, Canada) and normalized against the total pancreas section area:

(% normalized insulin$^+$ area = insulin$^+$ area/total section area x 100)

One-way ANOVA with post-hoc Sheffé test was performed using Statistica (StatSoft) to determine statistical significance.

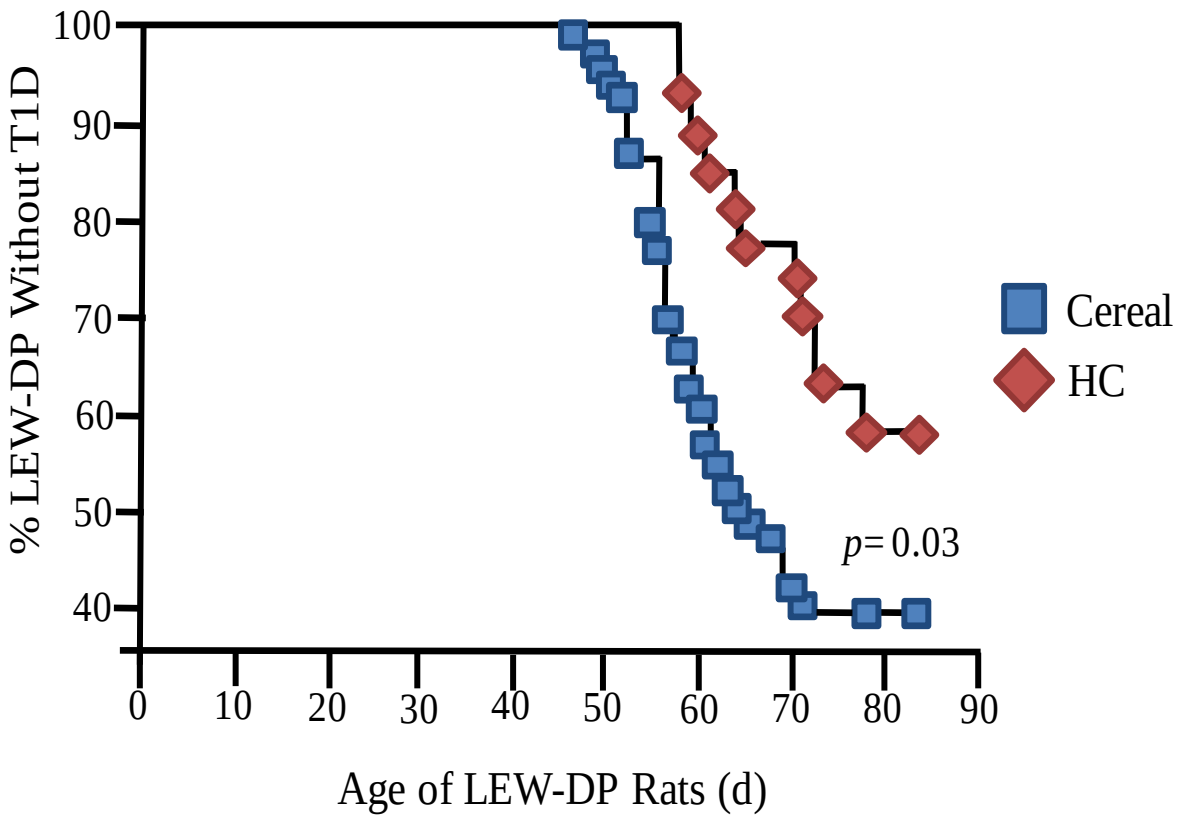
RESULTS

The HC Diet Inhibits T1D Development in the LEW-DP Model

LEW-DP and LEW-C rats were fed a standard cereal diet with protein derived from plant-based peptides after weaning (~23 days). A separate group of LEW-DP rats was placed on the HC diet. HC is a mixture of trypsin-digested milk protein, casein, which contains short peptides 2 – 3 amino acids in length. The HC diet is considered to be hypoallergenic because the peptides are too small to interact with MHC class II molecules to elicit an immune response.

The LEW-DP rats fed an HC diet were protected from T1D development and showed a diabetes incidence of 38.8% and delayed disease onset. Cereal-fed LEW-DP rats had a 61.5% T1D-incidence and developed T1D ~2 weeks earlier than the LEW-DP HC-fed group. The HC diet had a significantly protective effect in the LEW-DP model (**Figure 2**, $p = 0.03$). **Note:** The LEW-C strain is diabetes-resistant.

Figure 2. A low antigen HC diet protects LEW-DP rats from T1D development. Percentage of LEW-DP rats that did not develop T1D is shown. Diabetes incidence in the cereal-fed strain was 61.5% and HC-fed LEW-DP rats had a 38.8% T1D incidence ($p = 0.03$, log rank test).


100
90
80
70
60
50
40
% LEW-DP Without T1D
0
10
20
30
40
50
60
70
80
90
Age of LEW-DP Rats (d)
p= 0.03
Cereal
HC

Microarray LEW-DP Diet Comparison (Cereal vs. HC)

The microarray analysis of LEW-DP cereal-fed vs. LEW-DP HC-fed concentrated on transcripts that were upregulated in the rats fed a diabetes-promoting diet. There were 443 genes upregulated over 1.50 fold change, and 486 genes with downregulated expression below -1.50 fold change. Beyond insulin and *Il-20* gene transcription, the lists were not validated further **(Tables 1 – 4).**

Table 1. Candidate genes of interest – LEW-DP diet (cereal vs. HC)

Gene Symbol	Definition	Fold Change	Validated
Il-20	Interleukin-20	1.43	Yes
Ins1	Insulin-1 precursor	-3.17	Yes

Table 2. Summary of LEW diet candidate ENSEMBL IDs and normalized probe values

ENSEMBL ID	Gene	DP Cereal	DP HC
ENSRNOG00000004633	*Il-20*	4.79	4.28
ENSRNOG00000012052	*Ins1*	4.65	6.31

LEW-DP Diet Justification of Candidates

Insulin was one of the top downregulated candidates. A controversial report was published by Bendayan and Park that indicated extra-pancreatic islets localized to the duodenum between the duodenal connective tissue and submucosa; however, these findings have not been confirmed (Bendayan and Park, 1991).

Table 3. Microarray analysis of LEW-DP cereal-fed vs. LEW-DP HC-fed whole gut - upregulated genes (diet comparison)*

Gene Symbol	Description	Fold change (Cereal vs HC)
Igl-V1	Productively rearranged V-lambda-1 precursor	15.29
GC1_RAT	Ig gamma-1 chain C region	7.37
Q4KM66_RAT	LOC500183 protein	7.09
Tsga2	testis specific gene A2	5.95
Vsnl1	Visinin-like protein 1 (VILIP) (Neural visinin-like protein 1)	4.91
Ecel1	Endothelin-converting enzyme-like 1	3.59
Cyp1a1	Cytochrome P450 1A1	3.43
Gprc5a	G protein-coupled receptor, family C, group 5, member A	3.31
2010109I03Rik	intectin	3.04
A330021E22Rik	Uncharacterized protein C7orf63 homolog	2.96
Tpo	Thyroid peroxidase precursor	2.91
Maf	Transcription factor Maf	2.91
C030011O14Rik	Protein FAM73A	2.71
Slc27a2	Very long-chain acyl-CoA synthetase	2.63
LAC2_RAT	Ig lambda-2 chain C region	2.62
Odz2	Teneurin-2 (Ten-2)	2.60
Dlx2	Homeobox protein DLX-2	2.60
Ces5	Carboxylesterase precursor	2.60
Slc39a4_predicted	Zinc transporter ZIP4 precursor	2.58
LAC2_RAT	Ig lambda-2 chain C region	2.57
Atf3	Cyclic AMP-dependent transcription factor ATF-3	2.52
Tgm4	Protein-glutamine gamma-glutamyltransferase 4	2.50
Dhrs9	Dehydrogenase/reductase SDR family member 9 precursor	2.39
Lamc2	Laminin chain (Fragment)	2.38
Gsg1	germ cell associated 1	2.38
Srd5a2	3-oxo-5-alpha-steroid 4-dehydrogenase 2	2.37
Fmo1	Dimethylaniline monooxygenase	2.35
Camta1	Calmodulin-binding transcription activator 1	2.33
RGD1561089_predicted	late cornified envelope 1F	2.31
PTRF_RAT	Polymerase I and transcript release factor	2.29
Hbegf	Proheparin-binding EGF-like growth factor precursor	2.27
KLK-L3	kallikrein 9	2.21
Vom2r38	vomeronasal 2 receptor 38	2.20
Etv4	ets variant gene 4	2.16
Kif6	Kinesin-related protein 3B	2.13
Cilp	cartilage intermediate layer protein	2.13
Il12rb2	Interleukin 12 receptor beta2 subunit	2.09
Defb24	beta-defensin 24	2.09

*Probes on gene list without symbol removed.

Table 4. Microarray analysis of LEW-DP cereal-fed vs. LEW-DP HC-fed whole gut - downregulated genes (diet comparison)*

Gene Symbol	Description	Fold change (Cereal vs HC)
RGD1561307	similar to preferentially expressed antigen in melanoma-like 3	-13.45
Bai3	brain-specific angiogenesis inhibitor 3	-7.52
Cyp2b21	cytochrome P450, family 2, subfamily b, polypeptide 21	-4.88
LOC689415	Metallothionein-2 (MT-2) (Metallothionein-II) (MT-II)	-4.30
Cga	Glycoprotein hormones alpha chain precursor	-3.48
Ins1	Insulin-1 precursor [Contains: Insulin-1 B chain; Insulin-1 A cha	-3.17
Ca3	Carbonic anhydrase 3	-3.06
tGap1	GTPase activating protein testicular GAP1	-3.03
Kpl2	Sperm flagellar protein 2 (Protein KPL2)	-3.02
Scd1	Acyl-CoA desaturase 1	-2.92
Q7M082_RAT	Metallothionein 1	-2.81
SNORA36	Small nucleolar RNA SNORA36	-2.66
Mt1a	Metallothionein-1 (MT-1)	-2.61
Acsbg1	Long-chain-fatty-acid--CoA ligase ACSBG1	-2.55
Gm1960	Macrophage inflammatory protein 2-beta precursor (MIP2-beta)	-2.48
Actr3b	Actin-related protein 3B (Actin-like protein 3B)(ARP3-beta)	-2.39
Nrxn2	Neurexin-2-alpha precursor (Neurexin II-alpha)	-2.36
Prg4	Proteoglycan 4 Precursor (Lubricin)	-2.36
Dcx	Neuronal migration protein doublecortin	-2.33
Lrrc46	Leucine-rich repeat-containing protein 46	-2.33
Cnksr2	Connector enhancer of kinase suppressor of ras 2	-2.32
Fcrls	macrophage scavenger receptor 2	-2.31
F13a1	Coagulation factor XIII A chain precursor	-2.30
Gprk2l	G protein-coupled receptor kinase 4	-2.26
Cr2	complement receptor 2	-2.26
LOC501621	similar to nuclear RNA export factor 7	-2.26
Ar	Androgen receptor (Dihydrotestosterone receptor)	-2.23
Cd72	CD72 antigen	-2.20
C130038G02Rik	Uncharacterized protein KIAA0774	-2.20
Cdh18	Putative uncharacterized protein Fragment	-2.19
Ttn	Titin protein homolog (Fragment)	-2.15
LOC499330	Nicotinamide riboside kinase 1	-2.12
Cyp4x1	Cytochrome P450 4X1	-2.11
Sv2b	Synaptic vesicle glycoprotein 2B	-2.10
Ranbp17	Ran binding protein 17	-2.09
Tmem198	Transmembrane protein 198	-2.08
SHC3_RAT	SHC-transforming protein 3	-2.08
Itih4	inter-alpha-inhibitor H4 heavy chain	-2.08
Kcnj11	ATP-sensitive inward rectifier potassium channel 11	-2.06
NP_001100065.1	SET and MYND domain containing 1	-2.06
Q91ZP8_RAT	Metallothionein 1	-2.06
Adam3	a disintegrin and metalloprotease domain 3 (cyritestin)	-2.05
NP_001101672.1	EGF-like-domain, multiple 9	-2.04
Aqp9	Aquaporin-9 (AQP-9)	-2.04
Kcnd1	potassium voltage-gated channel, Shal-related family, member	-2.01
P2rx5	P2X purinoceptor 5 (ATP receptor) (P2X5) (Purinergic receptor	-2.01
Kcne4	potassium voltage-gated channel, Isk-related subfamily, gene 4	-2.00

*Validated genes highlighted in red. Probes on gene list without gene symbol removed.

LEW Diet Gene Ontology Functional Enrichment

The upregulated candidates from the LEW cereal-fed versus HC-fed diet comparison showed increased enrichment of genes involved with triacyl glycerol and cholesterol metabolism using both DAVID and GOrilla programs **(Tables 5, 7)**. The downregulated genes displayed enriched functions related to transport across the cell membrane and cholesterol biosynthesis **(Tables 6, 8).**

Table 5. DAVID functional annotation enrichment of upregulated cereal vs. HC-fed genes

Enrichment Score: 4.82	**Count**	***p*-Value**	**FDR**
Peptidase S1/S6, chymotrypsin/Hap, active site	11	9.83E-06	0.01
Tryp_SPc	11	1.78E-05	0.02
Peptidase S1 and S6, chymotrypsin/Hap	11	1.99E-05	0.03
Enrichment Score: 3.28	**Count**	***p*-Value**	**FDR**
CCP	6	5.05E-04	0.55
Sushi/SCR/CCP	6	5.10E-04	0.72
Complement control module	6	5.78E-04	0.81
Enrichment Score: 2.07	**Count**	***p*-Value**	**FDR**
glycosaminoglycan binding	7	5.66E-03	7.73
pattern binding	7	1.04E-02	13.78
polysaccharide binding	7	1.04E-02	13.78
Enrichment Score: 2.07	**Count**	***p*-Value**	**FDR**
vitamin A biosynthetic process	3	2.16E-03	3.56
9-cis-retinoic acid metabolic process	3	2.16E-03	3.56
9-cis-retinoic acid biosynthetic process	3	2.16E-03	3.56
fat-soluble vitamin biosynthetic process	3	3.21E-03	5.25
retinoic acid metabolic process	3	2.33E-02	32.61
isoprenoid biosynthetic process	3	6.53E-02	67.74
vitamin biosynthetic process	3	6.95E-02	70.07
Enrichment Score: 2.02	**Count**	***p*-Value**	**FDR**
triglyceride metabolic process	5	6.54E-03	10.41
acylglycerol metabolic process	5	9.74E-03	15.11
neutral lipid metabolic process	5	1.10E-02	16.89
glycerol ether metabolic process	5	1.17E-02	17.82

Table 6. DAVID functional annotation enrichment of downregulated cereal vs. HC-fed genes

Enrichment Score: 5.63	**Count**	***p* -Value**	**FDR**
substrate specific channel activity	22	1.59E-06	2.25E-03
channel activity	22	2.86E-06	4.05E-03
passive transmembrane transporter activity	22	2.86E-06	4.05E-03
Enrichment Score: 5.25	**Count**	***p* -Value**	**FDR**
voltage-gated cation channel activity	14	6.93E-07	9.83E-04
voltage-gated channel activity	14	1.60E-05	2.27E-02
voltage-gated ion channel activity	14	1.60E-05	2.27E-02
Enrichment Score: 3.04	**Count**	***p* -Value**	**FDR**
Cholesterol biosynthesis	5	2.73E-04	0.36
sterol biosynthesis	5	6.80E-04	0.89
cholesterol biosynthetic process	5	1.34E-03	2.22
sterol biosynthetic process	5	2.68E-03	4.41
Enrichment Score: 3.02	**Count**	***p* -Value**	**FDR**
potassium transport	9	7.36E-04	0.96
potassium ion binding	9	8.91E-04	1.26
potassium	9	1.34E-03	1.74
Enrichment Score: 2.69	**Count**	***p* -Value**	**FDR**
Cyclic nucleotide-binding	5	1.80E-03	2.54
Cyclic nucleotide-binding, conserved site	5	1.80E-03	2.54
RmlC-like jelly roll fold	5	2.08E-03	2.92
cNMP	5	2.45E-03	2.69
Enrichment Score: 2.66	**Count**	***p* -Value**	**FDR**
cellular ion homeostasis	18	1.05E-03	1.76
cellular chemical homeostasis	18	1.21E-03	2.01
cellular homeostasis	18	8.25E-03	13.00
Enrichment Score: 2.28	**Count**	***p* -Value**	**FDR**
oxidoreductase activity	5	5.03E-03	6.90
dioxygenase	5	5.18E-03	6.58
oxidoreductase activity	5	5.53E-03	7.56
Enrichment Score: 2.00	**Count**	***p* -Value**	**FDR**
chemokine activity	5	2.67E-03	3.72
Small chemokine, interleukin-8-like	5	2.73E-03	3.82
chemokine receptor binding	5	3.00E-03	4.17
SCY	5	3.70E-03	4.03
cytokine	5	9.45E-02	72.78
cytokine activity	5	1.45E-01	89.06

Table 7. GOrilla biological processes upregulated in LEW-DP cereal vs. HC

Description	*p*-value	FDR q-value	Enrichment
9-cis-retinoic acid biosynthetic process	4.55E-05	0.17	35.75
regulation of keratinocyte migration	4.55E-05	0.13	35.75
benzene-containing compound metabolic process	1.55E-04	0.29	14.02
secretion by lung epithelial cell involved in lung growth	2.80E-04	0.39	59.59
retinoic acid biosynthetic process	3.64E-04	0.45	19.86
diterpenoid biosynthetic process	3.64E-04	0.40	19.86
drug transmembrane transport	5.13E-04	0.44	17.88
terpenoid biosynthetic process	6.97E-04	0.48	16.25
lipoprotein transport	6.97E-04	0.45	16.25
zymogen activation	6.97E-04	0.43	16.25
positive regulation of keratinocyte proliferation	8.32E-04	0.44	39.73
drug export	8.32E-04	0.42	39.73

Table 8. GOrilla biological processes downregulated in LEW-DP cereal vs. HC

Description	*p* -value	FDR q-value	Enrichment
cholesterol biosynthetic process	8.90E-05	0.98	10.51

Microarray LEW Strain Comparison (LEW-DP vs. LEW-C)

The LEW strain microarray analysis concentrated on the gene expression differences in the LEW-DP that could result in strain differences that are associated with T1D susceptibility. There were 571 genes upregulated above 1.50 fold increase in the LEW-DP and 346 genes downregulated below -1.50 fold change **(Tables 9 – 12).**

Table 9. LEW-DP vs. LEW-C strain comparison candidates of interest

Symbol	Definition	Fold Change
Glycam1	Glycosylation-dependent adhesion molecule 1	3.75
Ccl19	Chemokine ligand (C-C motif) 19	2.36
Sell	L-selectin	2.13
Il20	Interleukin 20	1.60
Ins1	Insulin 1 precursor	-4.54
Il2ra	Interleukin 2 receptor alpha	1.94

Table 10. Summary of LEW strain candidate ENSEMBL IDs and normalized probe values

		Normalized Probes	
ENSEMBL ID	**Gene**	**LEW-DP**	**LEW-C**
ENSRNOG00000036826	*Glycam1*	7.15	5.24
ENSRNOG00000015668	*Ccl19*	6.22	4.98
ENSRNOG00000002776	*Sell*	6.55	5.46
ENSRNOG00000004633	*Il-20*	4.80	4.11
ENSRNOG00000012052	*Ins1*	4.65	6.83
ENSRNOG00000031623	*Il2ra*	6.39	7.35

LEW-DP vs. LEW-C Justification of Candidates

Candidates selected from the LEW strain microarray analysis have immune functions. Glycosylation-dependent adhesion molecule 1 (*Glycam1*) is the ligand to L-selectin (*Sell*) both of which were upregulated in the LEW-DP above the 1.50 fold cut off (Girard and Amalric, 1998). L-selectin participates in T-cell activation (Girard and Amalric, 1998). Interleukin-2 receptor alpha/CD25 (*Il2ra*) is constitutively expressed on regulatory T-cells and an upregulation of this transcript indirectly indicates a decreased number of Tregs in the gut of the diabetes-prone strain (Dendrou and Wicker, 2008). *IL2RA* is also a risk gene for T1D (Dendrou and Wicker, 2008). Chemokine ligand (C-C motif) 19 (*Ccl19*) is involved in trafficking of lymphocytes from the thymus (Shannon et al., 2012). An early downregulation of insulin transcription in the gut was an interesting result that requires further inquiry with IHC to localize the cell population with positive insulin staining. Extra-pancreatic islets were identified with immunogold staining in the duodenum of Sprague Dawley rats suggesting the intestine could also produce insulin. However, these reports were highly controversial (Bendayan and Park, 1991; Bendayan and Park, 1997).

Table 11. Microarray analysis of LEW-DP vs. LEW-C whole gut - upregulated genes (strain comparison)*

Gene Symbol	Description	Fold change (LEW-DP vs LEW-C)
Ebp42	erythrocyte protein band 4.2	4.45
Kif27	Kinesin-like protein KIF27	4.04
U6	U6 spliceosomal RNA	3.81
Glycam1	Sulfated 50 kDa glycoprotein precursor	3.75
Col28a1	Collagen alpha-1(XXVIII) chain precursor	3.49
Olig2_predicted	Oligodendrocyte transcription factor 2	3.13
Gk11	glandular kallikrein 11	2.99
Cr2	complement receptor 2	2.78
Siglec10	sialic acid binding Ig-like lectin 10	2.75
Ka17	Keratin, type I cytoskeletal 17 (Cytokeratin-17)	2.71
Gpc5	glypican 5	2.67
Cpne5	copine V	2.63
Luzp2	Leucine zipper protein 2 precursor	2.62
Epha8	Ephrin type-A receptor 8	2.60
Igfl3	Insulin growth factor-like family member precursor	2.50
RT1-CE4	RT1 class I, CE4	2.50
5S_rRNA	5S ribosomal RNA	2.49
Tsga2	testis specific gene A2	2.45
Olr964_predicted	olfactory receptor Olr964	2.41
RT1-CE2	RT1 class I, CE2	2.39
Myct1	myc target 1	2.39
Adam7	Disintegrin and metalloproteinase domain-containing protein 7	2.38
Ccl19	chemokine (C-C motif) ligand 19	2.36
5S_rRNA	5S ribosomal RNA	2.33
SNORD57	Small nucleolar RNA SNORD57	2.32
A8WCG0_RAT	Truncated serine incorporator 4	2.32
Lrrc38	leucine rich repeat containing 38	2.30
Hrg	histidine-rich glycoprotein	2.25
Slco6c1	solute carrier organic anion transporter family, member 6c1	2.24
Scd2	Acyl-CoA desaturase 2	2.23
Trpm1	transient receptor potential cation channel	2.21
Cdig1l	cadmium-inducible gene 1L	2.20
P4ha3	Procollagen-proline, 2-oxoglutarate 4-dioxygenase	1.61
Mgl1	Macrophage asialoglycoprotein-binding protein	1.61
GRZ1_RAT	Granzyme-like protein 1 precursor	1.61
Stx1b2	Syntaxin-1B (Syntaxin-1B2) (P35B)	1.61
Il20	Interleukin-20 Precursor (IL-20)	1.61
Pgr	Progesterone receptor (PR)	1.60
Hal	Histidine ammonia-lyase	1.60
Apba2	Amyloid beta A4 precursor protein-binding family A member 2	1.60

*Candidates highlighted in red. Probes on gene list without gene symbol removed.

Table 12. Microarray analysis of LEW-DP vs. LEW-C whole gut - downregulated genes (strain comparison)*

Gene Symbol	Description	Fold change (LEW-DP vs LEW-C)
RGD1561307	similar to preferentially expressed antigen in melanoma-like 3	-12.02
Ins1	Insulin-1 precursor [Contains: Insulin-1 B chain; Insulin-1 A chain]	-4.54
Spata18	Spermatogenesis-associated protein 18	-4.03
Kif20b	M-phase phosphoprotein 1 (MPP1)	-3.49
Gm1960	Macrophage inflammatory protein 2-beta precursor (MIP2-beta)	-2.87
Gabrb2	Gamma-aminobutyric acid receptor subunit beta-2 precursor	-2.85
Tnfrsf17	tumor necrosis factor receptor superfamily, member 17	-2.72
Pcdhgb7	protocadherin gamma subfamily B, 7	-2.63
Bai3	brain-specific angiogenesis inhibitor 3	-2.60
Lrrc46	Leucine-rich repeat-containing protein 46	-2.57
SNORA36	Small nucleolar RNA SNORA36 family	-2.31
Smp2a	Alcohol sulfotransferase A	-2.26
Armcx6	Protein ARMCX6	-2.25
Sele	E-selectin precursor	-2.22
Bcan	Brevican core protein precursor	-2.19
Phyh2	phytanoyl-CoA 2-hydroxylase 2	-2.18
Cyp2b21	cytochrome P450, family 2, subfamily b, polypeptide 21	-2.18
Ca3	Carbonic anhydrase 3	-2.15
LAC2_RAT	Ig lambda-2 chain C region	-2.11
Kcnk2	potassium channel, subfamily K, member 2 isoform 2	-2.10
Spata9	spermatogenesis associated 9	-2.08
Dync2li1	Cytoplasmic dynein 2 light intermediate chain 1	-2.05
Arntl	Aryl hydrocarbon receptor nuclear translocator-like protein 1	-2.04
Prg4	Proteoglycan 4 Precursor	-2.01
Olr1233_predicted	olfactory receptor Olr1233	-2.01
Kpl2	Sperm flagellar protein 2 (Protein KPL2)	-2.00
tGap1	GTPase activating protein testicular GAP1	-1.98
PO6F1_RAT	POU domain, class 6, transcription factor 1	-1.97
Cstl1	cystatin-like 1	-1.97
Edil3	EGF-like repeat and discoidin I-like domain-containing protein 3 Precursor	-1.96
GCB_RAT	Ig gamma-2B chain C region (Immunoglobulin heavy chain 1a)	-1.95
Il2ra	Interleukin-2 receptor alpha chain Precursor	-1.94
Kctd1	BTB/POZ domain-containing protein KCTD1	-1.94
LOC291823	similar to C-terminal binding protein 2 CtBP2	-1.94
Igsf4a	immunoglobulin superfamily, member 4A	-1.93
Q5M837_RAT	Ly49s6 protein	-1.92
Nkx2-2_predicted	NK-2 homeobox 2	-1.89
Adad2	Adenosine deaminase domain-containing protein 2	-1.89
Olr990_predicted	olfactory receptor Olr990	-1.89
Sfrp4	Secreted frizzled-related protein 4 precursor (sFRP-4)	-1.87
Ar	Androgen receptor (Dihydrotestosterone receptor)	-1.87
Olr1082	Olfactory receptor 1082	-1.87
Chrnd	Acetylcholine receptor subunit delta precursor	-1.86
Smyd1	SET and MYND domain containing 1	-1.86
Ikzf2	zinc finger protein, subfamily 1A, 2 (Helios)	-1.86
Disp2	Protein dispatched homolog 2	-1.85
Actr3b	Actin-related protein 3B	-1.85
Armetl1_predicted	ARMET-like protein 1 precursor	-1.85
Cadps	Calcium-dependent secretion activator 1	-1.84
U6	U6 spliceosomal RNA	-1.84
Q5M858_RAT	Tcrb protein	-1.83
A2NB68_RAT	Productively rearranged V-lambda-1 precursor (Fragment)	-1.83
P2rx5	P2X purinoceptor 5 (ATP receptor) (P2X5) (Purinergic receptor)	-1.83
LOC682474	similar to EEA1 (Early Endosome Antigen, Rab effector) homolog family member	-1.82
Spata6	sperm associated antigen 6	-1.81
Mcpt10	Granzyme-like protein 2 precursor	-1.81
RGD1310043_predict	similar to solute carrier family 16, member 5	-1.80
Hapln1	Hyaluronan and proteoglycan link protein 1 precursor	-1.80
RGD1311975	aldehyde oxidase 4	-1.80
Acox2	Acyl-coenzyme A oxidase 2, peroxisomal	-1.80
Ano5	Anoctamin-5	-1.80
Usp18	ubiquitin specific peptidase 18	-1.80
Acsbg1	Long-chain-fatty-acid--CoA ligase	-1.80

*Candidates highlighted in red. Probes on gene list without gene symbols removed.

LEW Strain Gene Ontology Functional Annotation Enrichment

DAVID GO analysis showed enrichment in peptidases and intermediate filament proteins in the upregulated genes and no functions were enriched for the downregulated list **(Table 13)**. GO analyses using GOrilla showed processes that were upregulated and downregulated in the LEW-DP rat involved neuronal functions **(Tables 14-15).**

Table 13. DAVID functional annotation enrichment of LEW-DP vs. LEW-C upregulated genes

Enrichment Score: 3.23	**Count**	***p*-Value**	**FDR**
keratin	7	2.20E-04	0.29
Intermediate filament protein, conserved site	7	6.96E-04	0.96
Intermediate filament protein	7	7.60E-04	1.05
Intermediate filament	7	7.62E-04	0.99
Filament	7	8.27E-04	1.15
Enrichment Score: 2.70	**Count**	***p*-Value**	**FDR**
Peptidase S1/S6, chymotrypsin/Hap, active site	8	1.62E-03	2.23
Tryp_SPc	8	1.98E-03	2.12
Peptidase S1 and S6, chymotrypsin/Hap	8	2.55E-03	3.49
Enrichment Score: 2.41	**Count**	***p*-Value**	**FDR**
CCP	5	3.44E-03	3.65
Sushi/SCR/CCP	5	3.96E-03	5.37
Complement control module	5	4.36E-03	5.90

Table 14. GOrilla biological processed upregulated in LEW-DP vs. LEW-C genes

Description	***p*-value**	**FDR q-value**	**Enrichment**
spinal cord oligodendrocyte cell fate specification	9.09E-04	1.00	38.01
spinal cord oligodendrocyte cell differentiation	9.09E-04	1.00	38.01

Table 15. GOrilla biological processes downregulated in LEW-DP vs. LEW-C genes

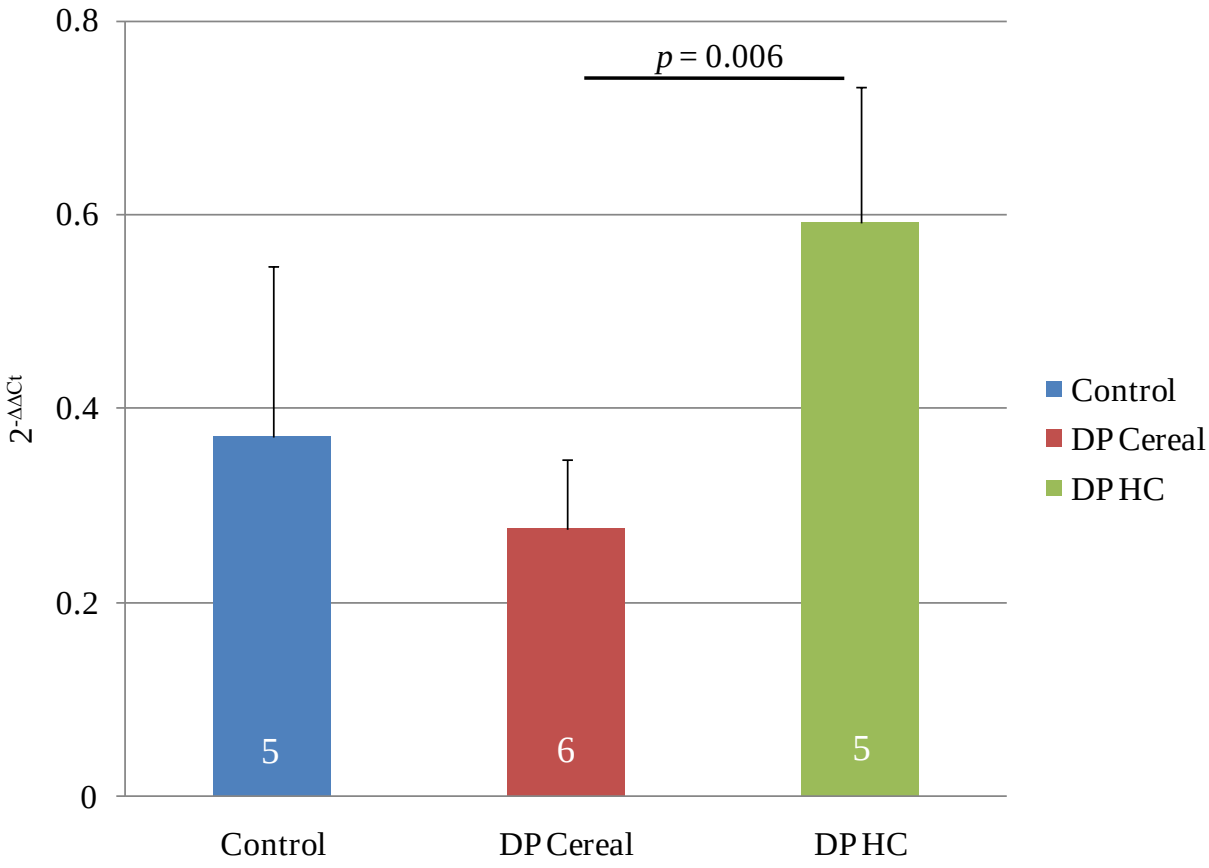
Description	*p* -value	FDR q-value	Enrichment
detection of mechanical stimulus involved in sensory perception	5.19E-04	1.00	10.48

Validation of Pooled Microarray Candidate Genes by qRT-PCR

Confirmation of array candidates by qRT-PCR for both the diet and strain comparisons did not yield significant results for *Glycam1, Sell, Ins1, Il2ra, or Ccl19* (data not shown).

Il-20 (1.61 fold change, diet comparison, **Figure 3**) – IL-20 is a member of the IL-10 family of cytokines (Sabat, 2010). IL-20 is secreted by monocytes and non-immune cells during inflammation (Sabat, 2010). IL-20 likely acts through STAT3 signaling and STAT3 promotes T-cell memory and is activated by pro-inflammatory cytokines in the gut (Siegel et al., 2011). It has been hypothesized that IL-20 plays a role in modulating cross talk between epithelial tissue and immune cells in inflammatory environments (Wegenka, 2010). Meta-analysis and genome-wide association study have identified both IL-10 and IL-20 as a candidate genes in T1D (Barrett et al., 2009). *Il-20* expression was confirmed by qRT-PCR to be significantly upregulated in LEW-DP HC-fed rats (p=0.006, data not shown). IL-20 is a Th2 cytokine and it has been shown that HC diet promotes a Th2 T-effector cell phenotype (Scott et al., 1997).

Figure 3. *Il-20* is significantly upregulated in LEW-DP HC-fed rats. qRT-PCR results for interleukin-20 were significant between the LEW-DP cereal and HC-fed rats ($p = 0.006$). Biological replicates and technical triplicates of each sample were tested. Number of biological replicates is shown on graph. Mean ± the standard deviation (SD) is shown. One-way ANOVA with post-hoc Sheffé test was performed using Statistica.


0.8
0.6
0.4
0.2
0
2-ΔΔCt
p = 0.006
5
6
5
Control
DP Cereal
DP HC
Control
DP Cereal
DP HC

PCR Array Analysis of LEW-DP Gut Inflammation

Innate and Adaptive Immune Response focused PCR arrays (PARN-052, SABiosciences) with n=4/group were used to study pro-inflammatory pathway gene expression in total gut RNA from the same rats used for microarray analysis. Significance boundaries were set at $p < 0.05$ and fold change ± 1.5 to filter genes of interest from the dataset.

LEW-DP Cereal-fed vs. LEW-DP HC-fed PCR Array

Table 16. Diet (cereal vs. HC) PCR array candidates

Symbol	Definition	Fold Change	*p*-value
Tnfrsf1a	TNF receptor superfamily 1α	1.46	0.001
Il1f10	Interleukin 1 family member 10	-2.28	0.04
Nfkbia	Nuclear factor kappa B inhibitor α	-1.63	0.03

Diet (Cereal vs. HC-fed LEW-DP) Significantly Upregulated Genes

Tnfrsf1a – $p = 0.001$, 1.46 fold change **(Figure 4)**. Tissue necrosis factor receptor superfamily member 1α is upregulated in apoptotic and cytokine interaction pathways. It is the primary receptor for the pro-inflammatory cytokine TNFα and is implicated in T1D pathogenesis (Moore et al., 2012). TNFRSF1A also interacts with LTA (lymphotoxin alpha) which was found to contain a risk SNP for T1D as part of the HLA haplotype (Perez et al., 2010). In a comprehensive study of HLA susceptibility interactomes in T1D, Brorsson *et al*. found that TNFRSF1A was among the most significant interactors with the HLA-DR3 locus (Brorsson et al., 2010). This makes TNFRSF1A a valuable candidate because HLA

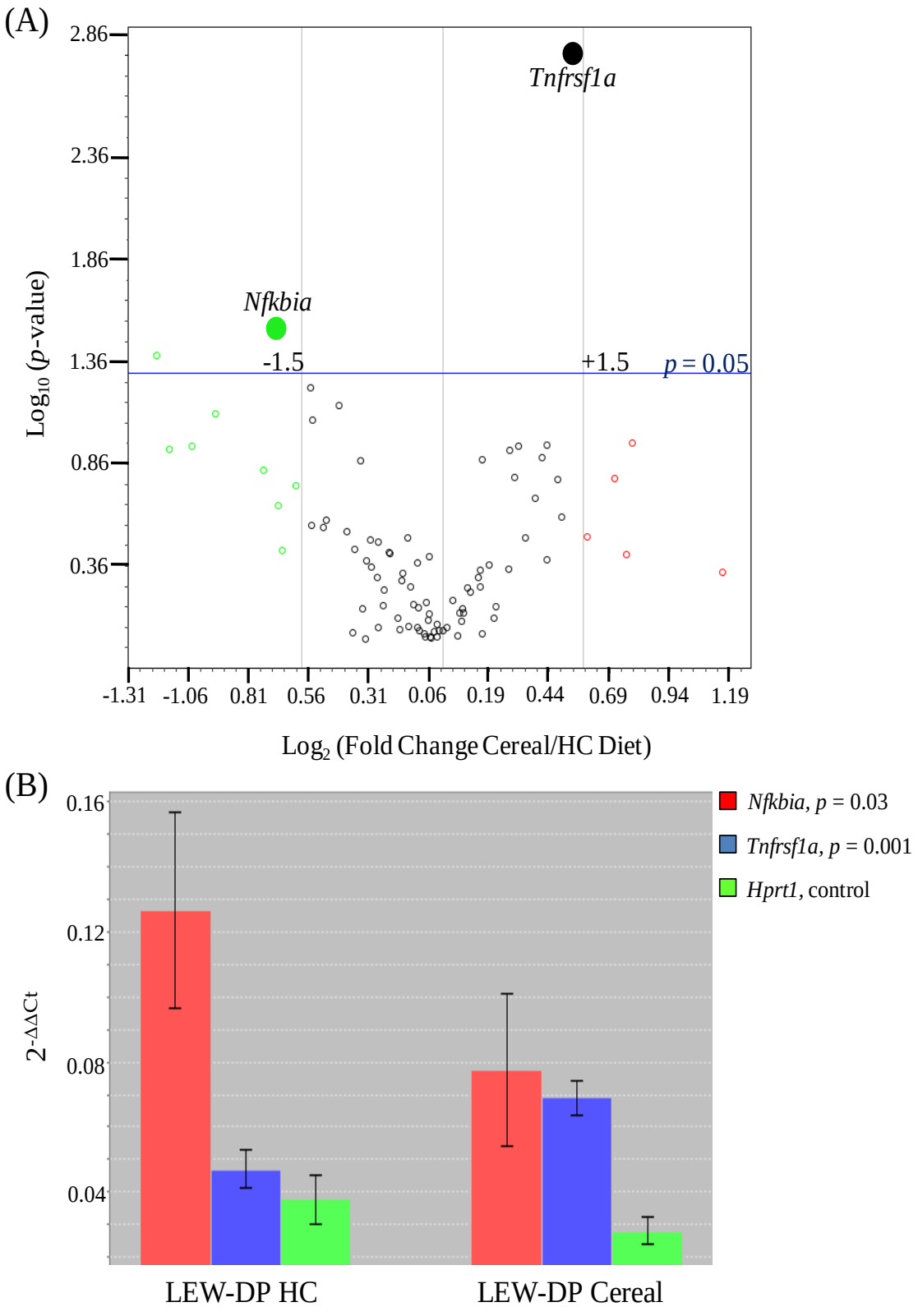
susceptibility regions including HLA-DR3 constitute 40 - 50% of familial inheritance of T1D (Noble and Valdes, 2011).

Diet (Cereal vs. HC-fed LEW-DP) Significantly Downregulated Genes

Il1f10 – p = 0.04, -2.28 fold change. *Il1f10* is a family member of the interleukin 1 cytokines that was identified as a novel cytokine in 2001 (Bensen et al., 2001). In a large patient study, *Il1f10* was confirmed to be a novel binding partner to the chronic inflammation marker, c-reactive protein (Dehghan et al., 2011). *Il1f10* is not shown in **Figure 4** due to minimal expression level.

Nfkbia – p = 0.03, -1.63 fold change **(Figure 4).** *Nfkbia* (nuclear factor kappa-b inhibitor-α, IkBα) acts as an inhibitor of the subunit of the pro-inflammatory gene transcription factor NF-κB by preventing NF-κB entry into the nucleus (Klein et al., 2004). NFKBIA protein binds to NF-κB to prevent transcriptional activation of genes that influence a pro-inflammatory immune response. The NF-κB pathway was identified to be affected in NOD mice at 4 weeks of age around the time insulitis begins in this model (Wu et al., 2012). The *Nfkbia* gene was previously profiled in a study by Katarina *et al.* that found an association with *Nfkbia* polymorphism and latent autoimmune diabetes in adults (LADA) (Katarina et al., 2007).

Figure 4. NF-κB signaling could be implicated in the development of pro-inflammatory conditions of the cereal-fed LEW-DP gut. Innate and Adaptive Immune Response PCR arrays for the diet comparison (DP cereal-fed vs. DP HC-fed) showed an upregulation of genes involved in the pro-inflammatory NF-κB pathway. n=4 arrays/group were performed with technical triplicates. **(A)** A volcano plot shows the distribution of fold changes with $p < 0.05$ and ± 1.5 fold change boundaries (student's *t*-test, ΔΔCt method). Upregulated genes are red and downregulated genes are green. **(B)** Fold change of significant PCR array candidates in cereal-fed rats compared with HC diet (baseline). *Hprt1* is included as a control. Mean ±SD is shown. Figures produced using SABiosciences PCR array web analysis software.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2.36
1.86
1.36
0.86
0.36
Log10 (p-value)
Tnfrsf1a
Nfkbia
-1.5
+1.5
p = 0.05
-1.31
-1.06
0.81
0.56
0.31
0.06
0.19
0.44
0.69
0.94
1.19
Log2 (Fold Change Cereal/HC Diet)
(B)
0.16
0.12
0.08
0.04
2-ΔΔCt
Nfkbia, p = 0.03
Tnfrsf1a, p = 0.001
Hprt1, control
LEW-DP HC
LEW-DP Cereal

LEW-DP vs. LEW-C Jejunum PCR Array

Table 17. Strain (DP vs. control) PCR array candidates

Symbol	Fold Change	*p*-value
Il1rapl2	1.56	0.024
Fn1	-1.70	0.012
Serpine1	-1.64	0.051
*Stab1**	-1.65	0.007

**Stab1* is referred to as rat protein identifier, LOC100363145, by SABiosciences

Strain (LEW-DP vs. LEW-C) Significantly Upregulated Candidates

Il1rapl2 – p = 0.02, 1.56 fold change **(Table 17, Figure 5)**. *Il1rapl2* (interleukin 1 receptor accessory protein like-2) is a member of the interleukin 1 cytokine family. It is an X-linked gene in both rat and human that has been implicated in X-linked mental retardation disorders (Ferrante et al., 2001). It has been identified as a surface antigen on PBMCs from a study of methylation profiles of monozygotic twins with scleroderma, an autoimmune disorder of the skin (Selmi et al., 2012).

Strain (LEW-DP vs. LEW-C) Significantly Downregulated Candidates

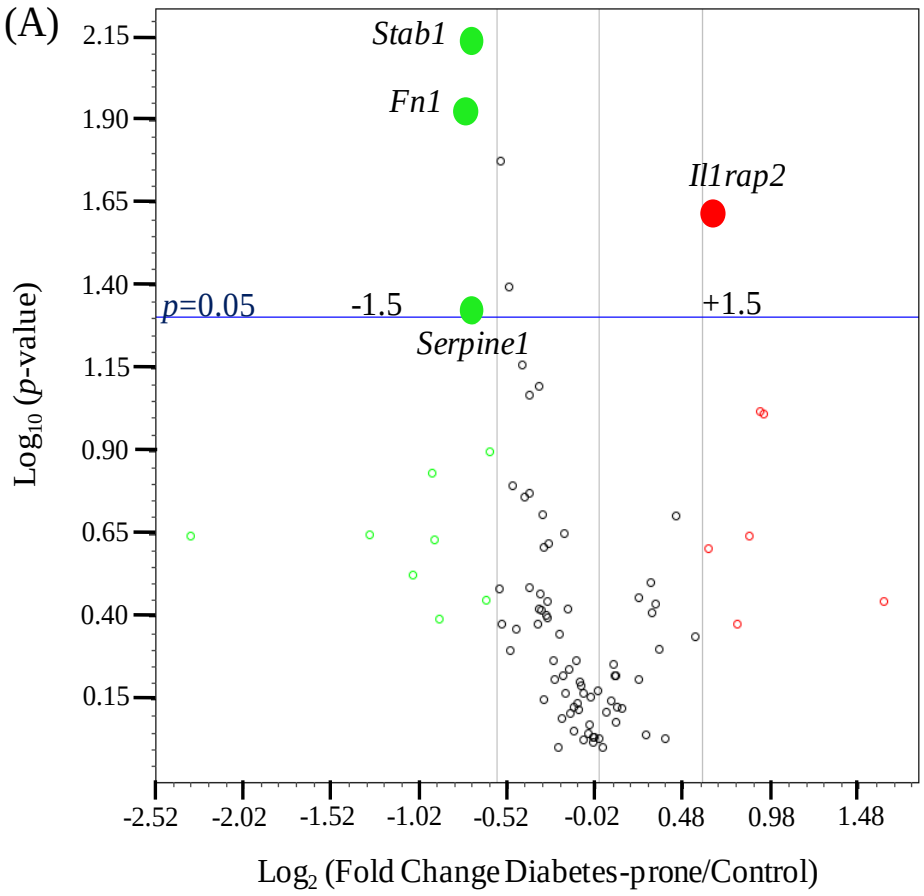
Fn1 – p = 0.012, -1.70 fold change **(Table 17, Figure 5).** Fibronectin-1 is an extracellular matrix component and basement membrane adhesion protein in the gut epithelium with greatest expression in the crypts of the villi (Goke et al., 1996). Fibronectin-1 participates in α5β1 integrin signaling and is also secreted by M2 macrophages in the wound healing

process (Vial and McKeown-Longo, 2008). In the gut, fibronectin downregulation is characteristic of colitis-related inflammation (Assi et al., 2011). As an α-integrin, fibronectin regulates inflammatory cytokines and is also capable of regulating Treg differentiation in the gut (Assi et al., 2011).

Stab1 – p = 0.007, -1.65 fold change **(Table 17, Figure 5)**. Stabilin-1 (Clever-1) is a surface marker of a subset of immunoregulatory M2 macrophages and is a scavenger receptor similar to CD163 (Palani et al., 2011). STAB1 also has trafficking properties and is responsible for endothelial transmigration of immune cells into inflammatory lesions (Shetty et al., 2011). Leukocyte recruitment is increased and T-cell migration is inhibited by STAB1 under inflammatory conditions (Shetty et al., 2011). STAB1 mediates the clearing of unwanted antigens through phagocytosis and has been suggested as a therapeutic target for chronic inflammatory disease (Kzhyshkowska, 2010). STAB1 promotes leukocyte and Treg cell migration into inflamed tissues to mitigate inflammation (Karikoski et al., 2009). Suppression of STAB1 function by siRNA altered the cytokine signature secreted by placental macrophages suggesting STAB1 plays an important role in maintaining regulatory M2 macrophage character (Palani et al., 2011).

Serpine1 – $p = 0.05$, -1.65 fold change **(Table 17, Figure 5)**. SERPINE1 or plasminogen-activator inhibitor-1 (PAI-1) is a basement membrane adhesion protein in the gut and is involved with fibrinolytic pathways and the induction of fibronectin-1 in the α5β1 integrin cascade (Vial and McKeown-Longo, 2008). Also secreted by M2 macrophages, SERPINE1 enhances wound healing and dampens inflammation in the gut epithelium. SERPINE1 stimulates macrophage recruitment to sites of inflammation and its expression is increased by the same activators of macrophages (Thapa et al., 2012). Circulating levels of SERPINE1 increase during severe inflammatory disorders as a protective agent against neutrophil apoptosis (Zmijewski et al., 2011).

Figure 5. LEW-DP rats show a decrease in immunosuppressive/regulatory M2 macrophage markers. Analysis of the Innate and Adaptive Immune Response PCR arrays for the strain comparison (LEW-DP vs. LEW-C both cereal-fed) showed downregulated markers of alternatively activated M2 macrophages. n=4 arrays/group. **(A)** Volcano plot of fold changes using $p < 0.05$ and ± 1.5 fold change boundaries (student's *t*-test, ΔΔCt method). Upregulated genes are red and downregulated genes are green. **(B)** PCR array candidates with highest fold changes in the LEW-DP samples with significant *p*-values. *Hprt1* primer shown as control. Mean ±SD is shown. Figures produced using SABiosciences PCR array web analysis software.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Fn1
Il1rap2
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+1.5
Serpine1
2.15
1.90
1.65
1.40
1.15
0.90
0.65
0.40
0.15
Log10 (p-value)
-2.52
-2.02
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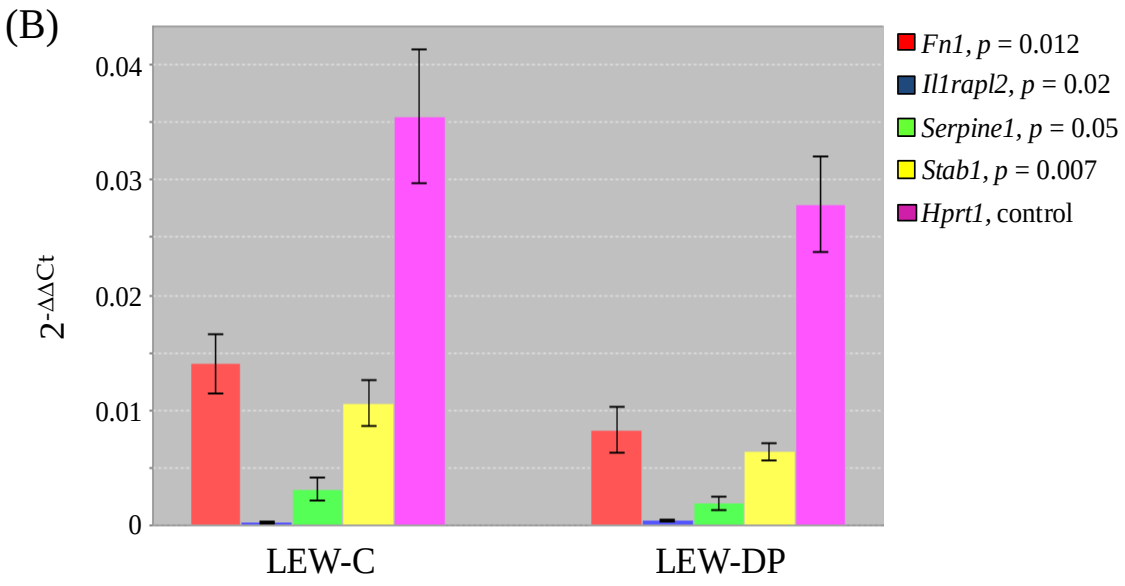
-1.02
-0.52
-0.02
0.48
0.98
1.48
Log2 (Fold Change Diabetes-prone/Control)


(B)
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0.03
0.02
0.01
0
2-ΔΔCt
LEW-C
LEW-DP
Fn1, p = 0.012
Il1rapl2, p = 0.02
Serpine1, p = 0.05
Stab1, p = 0.007
Hprt1, control

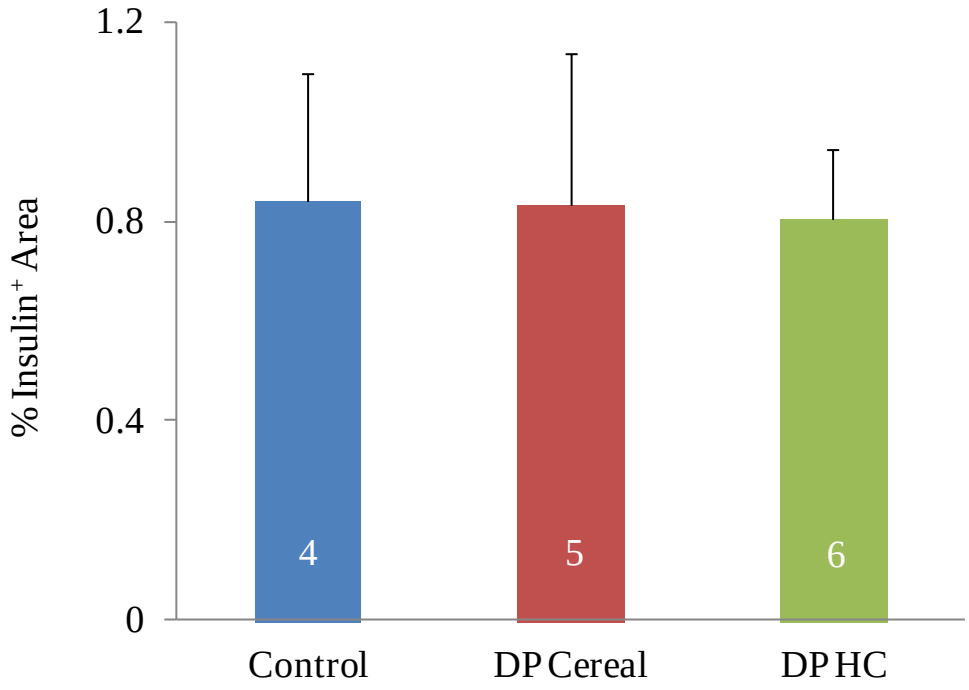
Morphometry of LEW Pancreata

Pancreas sections from 45 d and 100 d LEW-DP cereal-fed, LEW-DP HC-fed, and LEW-C cereal-fed rats (n = 4 - 5/group) were stained with anti-insulin antibody and analyzed for insulin$^+$ area **(Figure 6).** All animals selected for the study were asymptomatic for T1D and showed no signs of insulitis. The 45 d analysis of the islets and extra-islet insulin$^+$ (EIC) of the LEW rats showed no difference between the LEW-DP HC-fed group and the cereal-fed LEW-DP group (0.80% vs. 0.83%, $p = 0.83$). The 45 d LEW-C (0.84%) and LEW-DP cereal-fed groups were not different ($p = 0.98$).

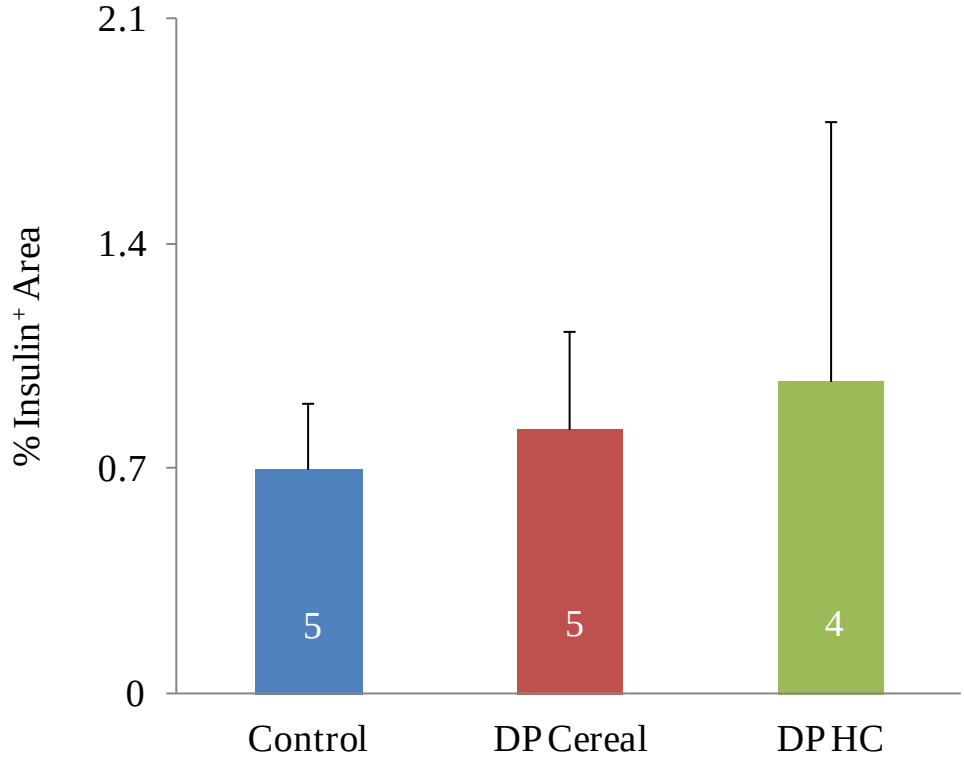
The 100 d normalized insulin area morphometry showed a greater insulin$^+$ area in the asymptomatic HC-fed rats (0.97%), but overall the area of insulin staining decreased in the 100 d rats. The LEW-C (0.70%) and LEW-DP cereal-fed (0.82%) rats showed no difference in percent insulin$^+$ area when compared to the 45 d LEW rats. Neither the strain ($p = 0.46$) nor diet ($p = 0.72$) comparison had a significant difference in percent insulin$^+$ area between groups by one-way ANOVA with post-hoc Sheffé analysis**.**

Figure 6. There are no diet or strain differences observed for percent insulin$^+$ area in the LEW model. Pancreas sections of 45 d **(A)** and 100 d **(B)** were stained for insulin to determine the percent area of insulin-producing β-cells. Pancreas sections from LEW-C (n = 4 – 5), LEW-DP cereal-fed (DP Cereal, n = 5), and LEW-DP HC-fed (DP HC, n = 4 - 6) were investigated by morphometric analysis. Percent of total insulin$^+$ area was measured and normalized by total area of the pancreas section. Mean ±SD is shown.

(A) 45 d LEW



(B) 100 d LEW



PRE-DIABETIC SIGNATURE OF THE PANCREAS IN THE BBDP RAT

To better understand the molecular mechanisms of T1D, high-throughput transcriptional and proteomic profiling has been done using islets and peripheral blood mononuclear cells (PBMCs) to identify candidate biomarkers in patients or animals with established diabetes versus healthy controls or recent onset cases. However, the impact of transcriptional changes occurring early in T1D development continues to be poorly understood.

The BioBreeding diabetes-prone rat (BBdp) is a spontaneous model of T1D that is well-characterized, yet its pre-diabetic signature is currently incomplete. A recent study from Hessner's group using high-throughput genomic techniques revealed a distinct immunological signature in the sera of diabetic BB DRlyp/lyp rats comparable to changes observed in diabetic patients (Kaldunski et al., 2010). Similar to the NOD and human data, the BB rat profiling is primarily immune cell marker-focused and lacks data about global gene expression. Thus, the body of previous profiling data has mainly relied on research derived from expression changes in genes with immunomodulatory function with less emphasis on differentially expressed genes with other ontologies.

In this study, young BBdp rats were partially pancreatectomized (PPx) at 30 d, at a point before diabetes-onset or T-cell infiltration into the pancreas (insulitis) to determine early changes in the pancreas and to gain insight into disease mechanism. This study design has not been

previously performed to identify candidate biomarkers of T1D, and supplies novel insight into the molecular changes occurring prior to autoimmunity. It is of interest to define the pre-diabetic signature of animal models and patients for the development of therapeutic and diagnostic tools for T1D.

HYPOTHESIS: Pre-diabetic BBdp rats display a distinct gene expression signature early in T1D development predictive of disease development.

OBJECTIVE: To study gene expression changes retrospectively in the BBdp pre-diabetic and T1D-resistant pancreata and determine candidate genes potentially involved in T1D development.

RESEARCH QUESTIONS

1. How does the pre-diabetic gene expression signature differ in regards to candidates and pathways upregulated?
2. What are the roles of the upregulated and downregulated pre-diabetic candidate genes in the development of T1D?

MATERIALS AND METHODS

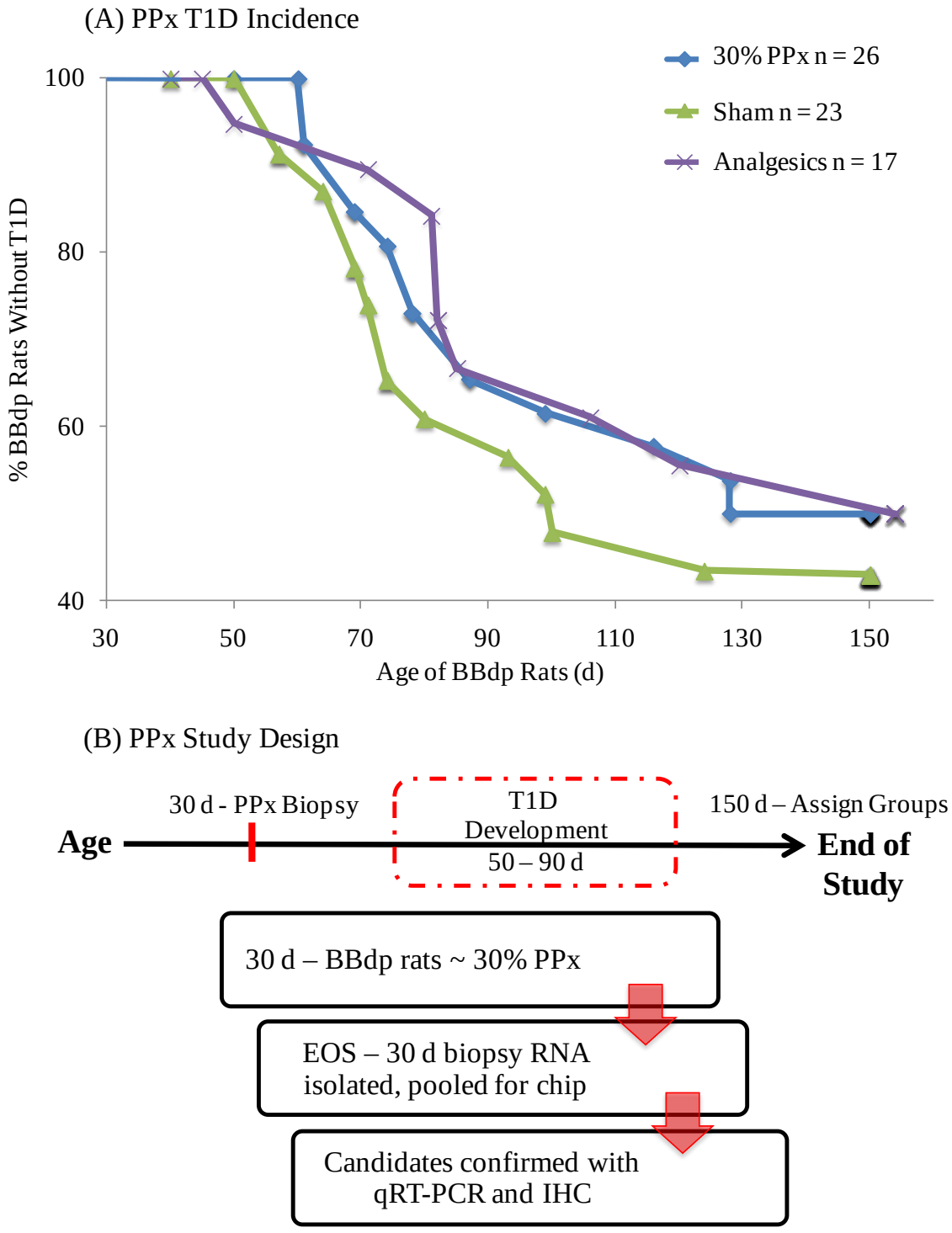
Partial Pancreatectomy Surgery: Control and experimental rats were fed a standard cereal diet and given access to water *ad libitum* at the OHRI animal care facility. BBdp rats underwent 30% partial pancreatectomy (PPx) surgery at 30 d (n = 26). Age and diet matched BBdp rats underwent sham surgery (n = 23). Prior to surgery, rats were injected with ketamine (5 mg/kg). The tail of the pancreas adjacent to the spleen was biopsied. Each surgical group received post-operative analgesics with subcutaneous injection of temgesic (0.03 mg/kg) and meloxicam (0.2 mg/kg) for three days. Control rats with no surgical treatment (n = 20) and rats administered analgesics without surgery (n = 17) were used. All rats were monitored for diabetes development until 150 d. Animals were euthanized by exsanguination under 3% isoflurane (Abbott Laboratories). All animal procedures were approved by local ethics committees and animals were maintained according to the Canadian Council on Animal Care guidelines. Compared with the sham-operated rats, the PPx did not alter the diabetes incidence **(Figure 7A)**. Samples of pancreata were collected at time of PPx and at end of study when the samples were classified as pre-diabetic, diabetic, or T1D-resistant.

RNA Isolation: Total pancreas RNA from 30 d pancreatectomized rats was isolated on ice immediately following dissection to prevent RNA degradation. Tissue samples were mechanically homogenized on ice

using a Polytron PT-2100 homogenizer (Kinematica) in buffer RA1 (Macherey-Nagel) with β-mercaptoethanol. Buffer RA1 contains a chaotropic salt, guanidinium thiocyanate, which stabilizes the RNA from degradation by endogenous RNAses. RNA was isolated using the Nucleospin RNA L kit (Macherey-Nagel) following manufacturer's protocol. Purified pancreatic RNA samples were stored at -80 °C until use. RNA integrity was assessed and concentration was measured using Agilent RNA 6000 Nano chips in an Agilent 2100 Bioanalyzer at the institute core facility, Stemcore. All RNA produced RNA integrity numbers (RIN) above 7.0, indicating a high quality sample.

Microarray analysis (Affymetrix Rat Gene 1.0 ST, n = 1/group), GO analysis, and qRT-PCR protocols for the PPx study were the same as outlined for the LEW-DP study (p. 28 – 30, **Figure 7B**).

Figure 7. Prospective partial pancreatectomy - gene expression analysis of 30 d pancreas biopsies from BBdp rats (A) Compared with sham-operated rats, 30% pancreatectomy (PPx) surgery did not affect diabetes incidence. Animals were monitored for T1D development and euthanized upon diabetes onset or at end of study at 150 d if T1D-resistant. Diabetes incidence for experimental (PPx), and controls (analgesics, sham surgery, and no treatment) are shown. **(B)** Workflow for PPx time points, RNA isolation, and microarray analysis.


(A) PPx T1D Incidence
30% PPx n = 26
Sham n = 23
Analgesics n = 17
% BBdp Rats Without T1D
100
80
60
40
30
50
70
90
110
130
150
Age of BBdp Rats (d)
(B) PPx Study Design
30 d - PPx Biopsy
T1D Development
50 – 90 d
150 d – Assign Groups
Age
End of Study
30 d – BBdp rats ~ 30% PPx
EOS – 30 d biopsy RNA isolated, pooled for chip
Candidates confirmed with qRT-PCR and IHC

Immunohistochemistry: Pancreata from 30 d and 150 d pre-diabetic and T1D-resistant PPx rats were fixed in Universal Molecular Fixative (UMFIX, Sakura Tissue-Tek, Torrance, CA) upon dissection (n=6/group). INS-1E and RINm5F cells were trypsinized (0.5%, Invitrogen) and spun down at 400 g for 10 minutes. Pellets were washed with PBS and flash frozen for fixation in Bouin's fixative. Samples were sent to the University of Ottawa Pathology Department to be paraffin-embedded and cut into 5 μm sections. Slides were de-waxed in Citrisolv (Fisher Scientific) and ethanol, and rehydrated. Antigen retrieval was carried out in citrate for 10 minutes at 125 °C or enzymatic retrieval (pronase E or proteinase K) for 15 – 30 minutes. Sections were incubated with 5% BSA blocking buffer for 30 minutes. Primary antibodies for TRIM26, REG3β, REG3γ, HDAC1, insulin, and glucagon were applied for 2 h at room temperature or overnight at 4 °C. Sections were stained with avidin-biotin complex (ABC, Vectastain) and hematoxylin counterstain (Electron Microscopy Sciences). Secondary antibodies (IgG) for goat anti-rabbit, donkey anti guinea-pig and rabbit anti-mouse were applied for 30 minutes (1:300, Dako). For fluorescence staining, slides were incubated with secondary antibodies conjugated to Alexa 488 (1:400, A21206 (rabbit) and A21202 (mouse), Invitrogen), fluorescein isothiocyanate (FITC) (1:400, 706-075-151, Jackson ImmunoResearch), Texas Red (1:400, 706-075-151, Jackson ImmunoResearch), Rhodamine Red (1:400, 706-295-148, Jackson ImmunoResearch), or Cy3 (1:400, 711-165-152, Jackson ImmunoResearch) fluorophores. Hoechst (1:100,

Sigma-Aldrich) was used as a nuclear counterstain. Co-localization was analyzed with a LSM 700 laser scanning confocal microscope (Zeiss). LSM images were processed using ZEN LE 2009 (Zeiss) and Northern Eclipse software (Empix).

Table 18. IHC and co-localization antibodies (Ab)

Ab	Dilution	Species	Supplier	Cat. No.	Secondary Ab
REG3β	1:200	Rabbit	Abgent	AP9210a	anti-rabbit
REG3γ	1:100	Rabbit	Abgent	AP5606c	anti-rabbit
HDAC1	1:3500	Rabbit	Abcam	ab19845	anti-rabbit
TRIM26	1:50	Mouse	Abcam	ab89290	anti-mouse
Insulin	1:150	Guinea pig	Dako	A0564	anti-guinea pig
Glucagon	1:200	Rabbit	Dako	A0565	anti-rabbit

Morphometric Analysis: 30 d islets were analyzed using an Axioplan 2 microscope (Zeiss). Positively stained cells were quantified using Northern Eclipse software (Empix) and normalized against islet area. Alternatively, stained sections were analyzed using a ScanScope (Aperio) and ImageScope software to convert image format to TIF files (Aperio). TIF files of pancreas sections were analyzed for positive stained area using Northern Eclipse (Empix). Excel and Statistica (Statsoft) were used to analyze results using student's *t*-test.

RESULTS

Microarray Analysis of BBdp PPx Biopsies

The aim of this study was to identify novel genes and transcripts implicated in T1D development. Criteria used to determine candidates of interest were the same as outlined in the LEW-DP study (p. 30). The validation of array candidates was concentrated on the pre-diabetic gene list. Candidates of interest with biological relevance to T1D were identified in the downregulated gene list, but have not yet been confirmed.

Table 19. Summary of candidates from PPx pre-diabetic vs. T1D-resistant BBdp rats

Symbol	Definition	Fold Change
Trim26	Tripartite motif containing protein 26	9.76
Ngfb	Nerve growth factor beta	5.03
Hdac1	Histone deacetylase 1	3.91
Reg3α	Regenerating islet-derived 3α	3.76
Reg3β	Regenerating islet-derived 3β	3.14
Reg3γ	Regenerating islet-derived 3γ	1.86
Nos3	Endothelial nitric oxide synthase	-5.58
Tnfrsf5	Tissue necrosis factor receptor superfamily 5	-5.13
Ins1	Insulin 1 precursor	-1.54

Table 20. Summary of PPx candidates and normalized probe values

	Normalized Probes	
Gene	**Pre-diabetic**	**T1D-resistant**
Trim26	7.17	3.88
Ngfb	6.63	4.30
Hdac1	3.99	2.02
Reg3α	10.17	8.26
Reg3β/Pap	9.04	7.39
Reg3γ	7.57	6.66
Nos3	3.72	6.20
Tnfrsf5/Cd40	4.57	6.93
Ins1	10.00	10.62

Table 21. Microarray analysis of total pancreas from pre-diabetic (pre-T1D) vs. resistant BBdp rats - upregulated genes*

Symbol	Description	Fold change (Pre-T1D vs Resistant)
Trim26	Tripartite motif-containing protein 26 (Zinc finger protein 173)	9.76
Ngfb	Beta-nerve growth factor precursor (Beta-NGF)	5.03
Hdac1_predicted	Histone deacetylase 1 (HD1)	3.91
Robo4	Roundabout homolog 4 precursor	3.87
NP_001099949.1	chloride channel 6	3.76
Reg3a	Regenerating islet-derived protein 3 alpha precursor	3.76
Serpinb6b	serine (or cysteine) proteinase inhibitor, clade B, member 6b	3.49
Cgm3	carcinoembryonic antigen gene family (CGM3)	3.47
Slc6a13	Sodium- and chloride-dependent GABA transporter 2	3.34
Adcy4	Adenylate cyclase type 4 (EC 4.6.1.1) (Adenylate cyclase type IV)	3.26
NP_001100993.1	chromodomain helicase DNA binding protein 2	3.14
Pap	Regenerating islet-derived protein 3 beta precursor (Reg III-beta)	3.14
RGD1561089_pre	late cornified envelope 1F	3.00
NP_001099720.1	sialic acid binding Ig-like lectin 10	2.98
Slc26a5	Prestin (Solute carrier family 26 member 5)	2.98
MGC94199	Uncharacterized protein C8orf37 homolog	2.84
Zfp707	zinc finger protein 707	2.78
Cbwd1	COBW domain-containing protein 1	2.78
Egr1	Early growth response protein 1 (EGR-1)	2.77
NP_001101769.1	ets variant gene 4 (E1A enhancer binding protein, E1AF)	2.76
Gp49b	glycoprotein 49b	2.73
NP_001103001.1	similar to expressed sequence C79407 (LOC689296)	2.70
ISK7_RAT	Serine protease inhibitor Kazal-type 7 precursor	2.70
Mlph	melanophilin	2.69
Tesb	hypothetical protein LOC407788	2.69
LOC503419	similar to Expressed sequence AW146242	2.68
Ccdc32	Protein CCDC32	2.63
Ly49i3	immunoreceptor Ly49i3	2.62
Sh2d2a	SH2 domain protein 2A	2.58
Dgkg	Diacylglycerol kinase gamma	2.55
U6	U6 spliceosomal RNA	2.54
Opn4	Melanopsin (Opsin-4)	2.52
Cdk2	Cell division protein kinase 2	2.52
Kcnab2	Voltage-gated potassium channel subunit beta-2	2.51
Palb2	Partner and localizer of BRCA2	2.50
Tg	Thyroglobulin precursor.	2.50
Arhgap20	Rho GTPase-activating protein 20	2.50
NP_001100600.1	F-box only protein 24	2.41
Ndnl2	Ndnl2 protein (Fragment)	2.40
PTRF_RAT	Polymerase I and transcript release factor (Calvin) (cav-p60)	2.38
Olr1654_predicte	olfactory receptor Olr1654	2.37
Lgi1	Leucine-rich glioma-inactivated protein 1 precursor	2.34
Cntnap2	Contactin-associated protein-like 2 Precursor	2.33

*Candidates highlighted in red. Probes on gene list without gene symbol removed.

Table 22. Microarray analysis of total pancreas from pre-diabetic (pre-T1D) vs. resistant BBdp rats - downregulated genes*

Gene Symbol	Description	Fold Change (PRE-T1D Vs. Resistant)
NP_001099806.1	membrane-spanning 4-domains, subfamily A, member 10	-11.96
5S_rRNA	5S ribosomal RNA	-9.52
Robo3	roundabout homolog 3	-7.95
Nos3	Nitric oxide synthase, endothelial	-5.58
LOC502310	Cytochrome P450 2B15	-5.35
rno-mir-142	rno-mir-142	-5.32
Tnfrsf5	tumor necrosis factor receptor superfamily, member 5	-5.13
Vcsa1	SMR1 protein precursor (VCS-alpha 1)	-5.03
Bex1	Protein BEX1	-4.83
Dsg4	Desmoglein-4 precursor.	-4.18
Il1a	Interleukin-1 alpha precursor (IL-1 alpha).	-4.15
LOC305166	1-acyl-sn-glycerol-3-phosphate acyltransferase theta	-4.13
Gnb3	Guanine nucleotide-binding protein	-4.00
Cntn4	Contactin-4 precursor	-3.73
rno-mir-349	rno-mir-349	-3.60
LOC499823	LRRG00114 (LOC499823)	-3.50
LOC301165	Putative uncharacterized protein LOC301165	-3.44
Abcg2	ATP-binding cassette sub-family G member 2	-3.42
5S_rRNA	5S ribosomal RNA	-3.40
Q63274_RAT	Kallikrein (Fragment).	-3.36
NP_001013945.1	similar to Ndr3 protein	-3.36
SNORD34	Small nucleolar RNA SNORD34	-3.33
Psmb9	Proteasome subunit beta type-9 precursor	-3.32
U6	U6 spliceosomal RNA	-3.29
Pkd1l2	Polycystic kidney disease protein 1-like 2 Precursor	-3.29
ACPL2_RAT	Acid phosphatase-like protein 2 precursor	-3.29
Ugt2b3	UDP-glucuronosyltransferase 2B3 precursor	-3.17
Tekt2	Tektin-2 (Testicular tektin) (Tektin-t)	-3.15
Tmc2	Transmembrane channel-like protein 2	-3.10
Vgcnl1	Sodium leak channel non-selective protein	-3.07
Pnma1	Paraneoplastic antigen Ma1 homolog.	-3.06
Dmpk	Myotonin-protein kinase	-3.06
4930562C15Rik	Uncharacterized protein ENSP00000371449 homolog	-3.03
Cyp2b12	Cytochrome P450 2B1	-2.98
SNORD25	Small nucleolar RNA SNORD25	-2.98
Itgad	Integrin alpha-D precursor (CD11d antigen)	-2.98
Lonrf3	LON peptidase N-terminal domain and RING finger protein 3	-2.95
Cte1	Acyl-coenzyme A thioesterase 1	-2.92
U6	U6 spliceosomal RNA	-2.88
Ddx58	Probable ATP-dependent RNA helicase DDX58	-2.81

*Candidates highlighted in red. Probes on gene list without gene symbol removed.

There were 874 genes upregulated above 1.5 fold change, and 1282 genes downregulated below -1.5 fold. Candidates of interest upregulated in pre-diabetic rats included regenerative proteins and transcriptional regulators:

Trim26 (9.76 fold change, **Tables 19 – 21**): The tripartite motif containing family of proteins is a group of E3 ubiquitin ligases involved in antiviral, autoimmune, and inflammatory functions (Hatakeyama, 2011; Jefferies et al., 2011). A review published on the role of the TRIM family reported that TRIM proteins act as autoantigens in autoimmune disorders such as SLE and Sjogren's syndrome (Jefferies et al., 2011). This could be explained by TRIM localization in the MHC class I region where many known autoantigens are found (Meyer et al., 2003). The TRIM family also mediates inflammasome pathways resulting in the activation of pro-apoptotic caspase-1 (Jefferies et al., 2011). *Trim* gene expression is upregulated in response to interferon I and interferon II proteins (Rajsbaum et al., 2008). IFNγ, an IFN class II member is a well-recognized pro-inflammatory cytokine implicated in T1D development. TRIM26 involvement in T1D has not been investigated. Validation by qRT-PCR confirmed that *Trim26* expression is significantly upregulated in the diabetes-prone cohort (p = 0.05), and protein studies showed positive staining isolated to the islet core**.**

Hdac1 (4.52 fold change, **Tables 19 – 21**): Histone deacetylase 1 is a part of the 18 member HDAC family of transcriptional regulators that removes acetyl groups from lysine residues to influence gene

transcription (Lundh et al., 2010). Histone deacetylases are implicated in the regulation of innate immunity, adaptive immunity and control of inflammation (Shakespear et al., 2011; Sweet et al., 2012). HDAC induction affects T-cells, B-cells, and macrophages to promote cytotoxicity and pro-inflammatory signaling pathways (Sweet et al., 2012). HDAC1 has been recently identified as acting in M1/M2 macrophage polarization and is differentially regulated by cytokines (Wu et al., 2012). Due to HDAC1 role in both inflammation and differentiation, it is a candidate of interest in β-cell biology.

Regenerating islet-derived family of proteins (*Reg3α* 3.76, *Reg3β* 3.14, *Reg3γ* 1.86 fold change, **Tables 19 – 21**): The Reg family of proteins is a group of secreted proteins with the greatest expression in the gut (Paneth cells) and pancreas (Wang et al., 2011). Regs have previously been reported in pancreas regeneration post-pancreatectomy (Terazono et al., 1988). The Reg family of proteins possesses a broad range of functions from proliferation (REG3α) to anti-microbial properties (REG3γ and REG3β) (Cui et al., 2009; Lai et al., 2012; Lee et al., 2012).

The role of the Reg family proteins in T1D is controversial. They are hypothesized to promote pro-islet gene transcription to prevent T1D (REG3β), and have been reported as putative autoantigens in the NOD mouse (Gurr et al., 2002; Xiong et al., 2011). It has been proposed that members of the Reg family (REG1 and REG2) are T1D autoantigens present prior to the honeymoon phase immediately after diagnosis (Gurr

et al., 2002). In the NOD mouse, Reg genes have been upregulated in response to chemically-induced diabetes (Lu et al., 2006). The Reg family is also reported to interact with IL-22, a pro-inflammatory cytokine in the NOD mouse (Singh et al., 2011). REG3β in the pancreas regulates inflammatory responses in macrophages and is hypothesized to modulate M1/M2 shift (Viterbo et al., 2008a; Viterbo et al., 2008b).

Despite multiple putative functions, the only identified receptor of a Reg protein is exostosin-like 3 (EXTL3) (Gurr, 2011). The Reg family is a group of C-type lectins involved in the innate immune response with gram-positive and gram-negative bactericidal properties although it is not known how Reg proteins identify target carbohydrate epitopes (Gurr, 2011; Vaishnava et al., 2011; van Ampting et al., 2012). REG3α and REG3β are discrete isoforms in rodent species, but both are orthologous to the human, REG3α protein (Gurr, 2011). Rat REG3γ is orthologous to human REG3γ.

Downregulated candidates from the pre-diabetic vs. T1D-resistant gene list included:

Nos3 (-5.58 fold change, **Tables 19, 20, 22**): Endothelial nitric oxide synthase (NOS3) produces nitric oxide, an inducer of pancreatic β-cell necrosis signaling (Tanioka et al., 2011). Nitric oxide production is initiated due to the presence of pro-inflammatory cytokines IL-1β, TNFα, and IFNγ in the pancreas (Chan et al., 2011).

Tnfrsf5 (-5.13 fold change, **Tables 19, 20, 22**): Tissue necrosis factor receptor superfamily member 5, more commonly known as CD40, is a pro-inflammatory factor linked with decreased tolerance and activation of B-cells (Ozcan et al., 2011). The T-cell subset of $CD4^{+}CD40^{+}$ is necessary and sufficient for developing autoimmunity in T1D (Carter et al., 2012). If this was result was further analyzed by qRT-PCR with biological replicates and technical triplicates, I would hypothesize that *Cd40* would be upregulated rather than downregulated in the pre-diabetic group.

Gene Ontology Functional Enrichment of PPx Microarray Genes

DAVID bioinformatics software was used to identify functional clusters of genes from the PPx microarray. A decrease in cyclase activity was observed for the upregulated dataset **(Table 23)** and female sexual differentiation was enriched for the downregulated genes **(Table 24).** GOrilla analysis indicated that cell division processes in pre-diabetic rats were decreased compared with the remaining dataset (spindle checkpoint and negative regulation of metaphase/anaphase) **(Figure 8)**. No processes were enriched for the downregulated PPx gene list using GOrilla. Further analyses are required to clarify pathways involved.

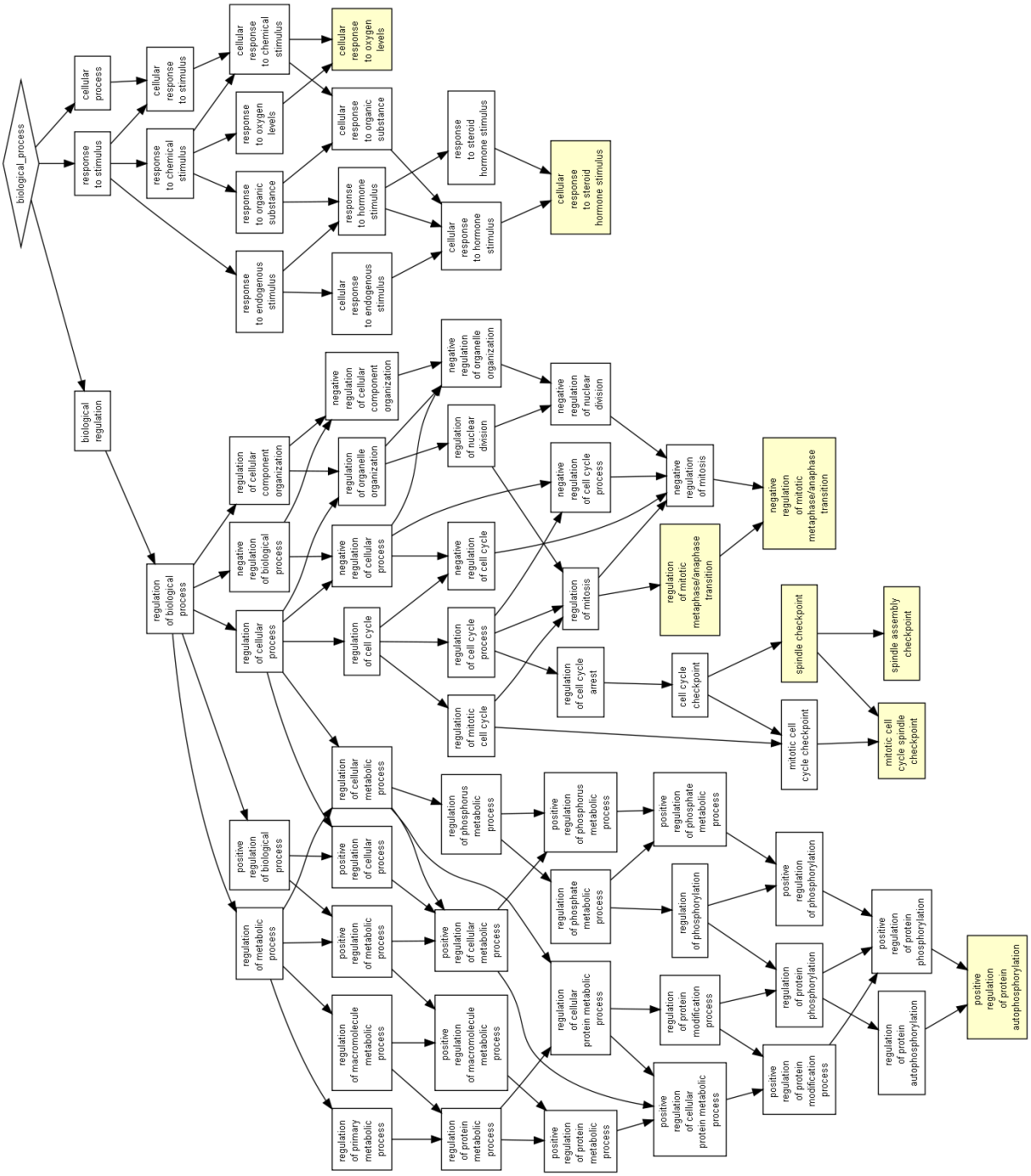
Table 23. DAVID functional annotation enrichment of upregulated pre-diabetic vs. T1D-resistant genes

Enrichment Score: 2.88	Count	*p* -Value	FDR
negative regulation of adenylate cyclase activity	7	1.31E-03	2.26
negative regulation of lyase activity	7	1.31E-03	2.26
negative regulation of cyclase activity	7	1.31E-03	2.26

Table 24. DAVID functional annotation enrichment of downregulated pre-diabetic vs. T1D-resistant genes

Enrichment Score: 2.15	Count	*p* -Value	FDR
female gonad development	9	4.62E-03	7.77
development of primary female sexual characteristics	9	6.93E-03	11.45
female sex differentiation	9	1.08E-02	17.24

Figure 8. Cell cycle functions are enriched in pre-diabetic 30 d rats. Gene ontology (GO) directed acyclic graph of upregulated pre-diabetic vs. T1D-resistant gene biological processes. Functions are organized hierarchically from least specific to most specific. Yellow boxes show functions with *p*-value between 10^{-3} – 10^{-5}. Pre-diabetic gene list target set cut-off was 1.50. Processes with enrichment scores above ~10 are shown. Data generated using GOrilla bioinformatics software.



Description	*p*-value	FDR q-value	Enrichment
spindle checkpoint	8.79E-05	0.94	9.98
spindle assembly checkpoint	4.37E-04	1.00	10.17
mitotic cell cycle spindle checkpoint	4.37E-04	1.00	10.17
negative regulation of mitotic metaphase/anaphase transition	4.37E-04	0.93	10.17

qRT-PCR Validation of Pre-diabetic vs. T1D-resistant Candidates

qRT-PCR for candidates *Trim26, Reg3α, Reg3β, Reg3γ, Hdac1,* and *Ngfb* was evaluated (n = 6 - 7/group) in technical triplicates. Candidates that were upregulated in pre-diabetic pancreas, *Trim26, Reg3α,* and *Reg3β,* had a *p*-value < 0.05 by the student's *t*-test or Mann-Whitney *U*-test **(Table 25, Figure 9)**. One outlier was excluded from the *Reg3β* pre-diabetic group. The significant candidates showed higher expression in the pre-diabetic group by qRT-PCR in accordance with the microarray data.

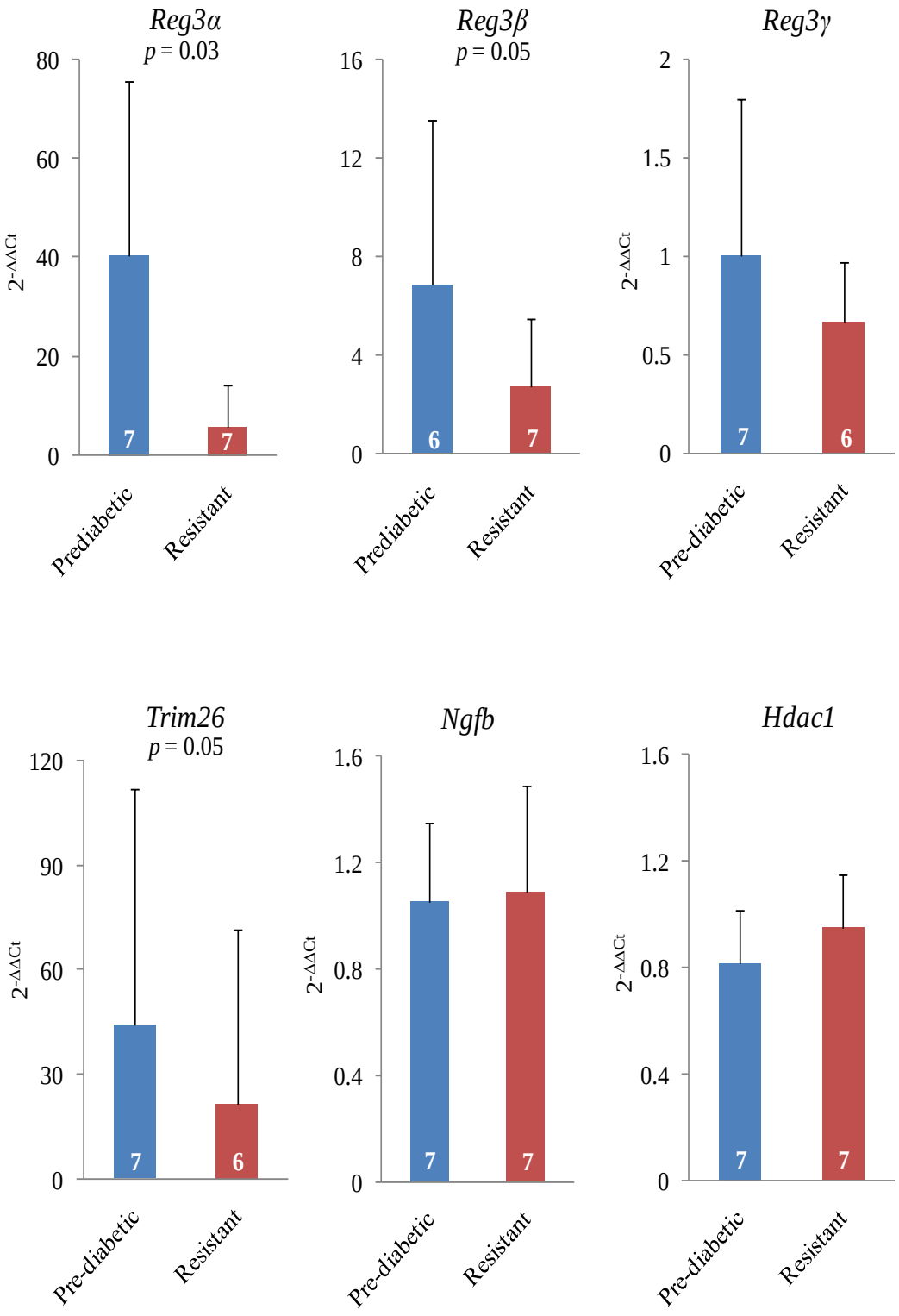
Table 25. Summary of pre-diabetic PPx candidate validation by qRT-PCR

Primer	*p*-value	Pre-T1D±SD (n)	T1D-resistant ±SD (n)
Trim26	**0.05**	**44.4±67.39 (7)**	**21.6±50.11 (6)**
Reg3α	**0.03**	**40.6±34.99 (7)**	**5.88±8.29 (7)**
Reg3β	**0.05**	**6.90±6.69 (6)**	**2.74±2.74 (7)**
Hdac1	0.24	0.82±0.20 (7)	0.95±0.20 (7)
Ngfb	0.85	1.05±0.29 (7)	1.09±0.40 (7)
Reg3γ	0.35	1.00±0.79 (7)	0.66±0.30 (6)

Protein Expression of Candidates Measured by Morphometry

Measurement of positively stained area or positive cell counting of PPx array candidates REG3β, REG3γ, and HDAC1 was studied. There were no differences in candidate expression observed between the pre-diabetic or T1D-resistant groups (data not shown). The protein expression studied by morphometry showed trends similar to the qRT-PCR results with greater expression of PPx array candidates in the pre-diabetic cohort. Further proteomic confirmation with immunoblotting is needed.

Figure 9. Pre-diabetic rats displayed increased expression of regenerative and anti-microbial markers at 30 d. The pre-diabetic vs. T1D-resistant microarray candidates were validated using qRT-PCR with biological replicates (n = 6 - 7 per group) and technical triplicates of each sample. Student's *t*-test statistical analysis was performed for *Reg3α, Reg3β, Reg3γ,* and *Ngfb* and Mann-Whitney *U*-test for *Trim26,* and *Hdac1* using Statistica. One outlier was removed from the pre-diabetic group for *Reg3β*. Mean ±SD and n numbers are shown. *p*-values are shown for significant candidates, $p < 0.05$.


Reg3α
p = 0.03
2-ΔΔCt
80
60
40
20
0
7
7
Prediabetic
Resistant
Reg3β
p = 0.05
16
12
8
4
0
6
7
Prediabetic
Resistant
Reg3γ
2
1.5
1
0.5
0
7
6
Pre-diabetic
Resistant
Trim26
p = 0.05
120
90
60
30
0
7
6
Pre-diabetic
Resistant
Ngfb
1.6
1.2
0.8
0.4
0
7
7
Pre-diabetic
Resistant
Hdac1
1.6
1.2
0.8
0.4
0
7
7
Pre-diabetic
Resistant

Pre-diabetic Upregulated Candidates Are Islet Markers

β-cells are exclusively REG3β^{+}

Preliminary REG3β IHC using the DAB method revealed positive staining exclusively in islets **(Figure 10)**. REG3β showed positive staining in many of the cells in the islet core and no staining was observed in the islet periphery, which indicated that it was β-cell specific. This was confirmed by confocal microscopy of REG3β with insulin. REG3β co-localized with insulin in 30 - 33 d BBdp rats fed a standard cereal diet **(Figure 11)**. Co-localization was also observed in extra-islet insulin^{+} clusters (EIC) in the pancreas, suggesting REG3β expression may have a role in islet neogenesis. There was no co-localization of islet α-cell marker, glucagon, and REG3β, supporting the exclusive expression of REG3β in β-cells. REG3α was not validated by IHC because the primary antibody used was anti-human REG3α, which displays the same published localization as rat REG3β (Gurr, 2011). Bouin's fixed pellets of RINm5F cells were stained for REG3β **(Figure 12).** REG3β was expressed in almost all RINm5F cells with a perinuclear expression pattern.

TRIM26 is localized to pancreatic islets

TRIM26 staining by DAB immunohistochemistry displayed positive staining throughout the islets, with no positive cells in the acini **(Figure 10).** EICs were also positive for TRIM26 **(Figure 10).** There are no reports identifying TRIM26 as an islet marker and therefore this is the

first report of TRIM26 expression in β-cells. Cell compartment studies of the TRIM family have been preliminary, and the only published localization of TRIM26 is in human spermatozoa (Linschooten et al., 2009). Co-localization was studied in Bouin's fixed pancreas sections of 30 – 33 d cereal-fed BBdp rats. TRIM26 displayed positive staining in the cytoplasm of cells in the islet core **(Figure 11).** TRIM26 staining showed co-localization with insulin in a majority of the insulin^{+} area **(Figure 11).** RINm5F and INS-1E pellets stained for TRIM26 displayed foci of TRIM26 positive staining throughout the cell pellets that were not observed in the control. Positive staining in the cell pellet was noted in the cytoplasm and nuclei **(Figure 13)**. These foci could represent localized areas of the cell pellet that were undergoing proteasomal degradation or cellular differentiation both of which are new functions of TRIM26 reported by Zhao *et al.* (2013); however, the staining could be an artifact and requires protein validation be immunoblotting.

HDAC1 and REG3γ are expressed throughout the pancreas

HDAC1 was observed in the nuclei of most islet cells as well as many of the acinar cells **(Figure 10)**. HDAC1 is well documented to be one of the major HDACs involved with transcriptional regulation in the pancreas (Papizan et al., 2011). REG3γ acts as an anti-microbial peptide in the gut that preserves the barrier between the epithelium and commensal microbiota (Vaishnava et al., 2011). REG3γ expression in the pancreas is less studied than their function in the gut. A known role of Reg proteins in the pancreas is their anti-inflammatory properties during pancreatitis, which involves the inflammation of the exocrine tissue (acini) (Fu et al., 2012). The REG3γ staining observed in BBdp pancreata was also largely found within the acini and to a lesser extent in the cytoplasm of islets **(Figure 10)**.

Figure 10. Pre-diabetic upregulated candidate proteins are localized to islets. (A) REG3β showed punctate staining in the cytoplasm of islets, but not in acinar tissue. **(B)** The islet core was TRIM26^{+} and TRIM26 staining appeared β-cell specific. Extra-islet insulin^{+} clusters (EIC) were TRIM26^{+} **(C)** REG3γ^{+} staining was observed in the islet and acinar tissue of 30 d BBdp PPx rats. **(D)** Islet and acinar nuclei were HDAC1^{+}. A, C, D pancreas sections were from 30 d PPx rats fixed with UMFIX fixative. TRIM26 was stained on a 47 d asymptomatic BBdp rat pancreas fixed in Bouin's fixative. Islets are outlined. Insets show detail of islet staining. Bar = 100 μm.

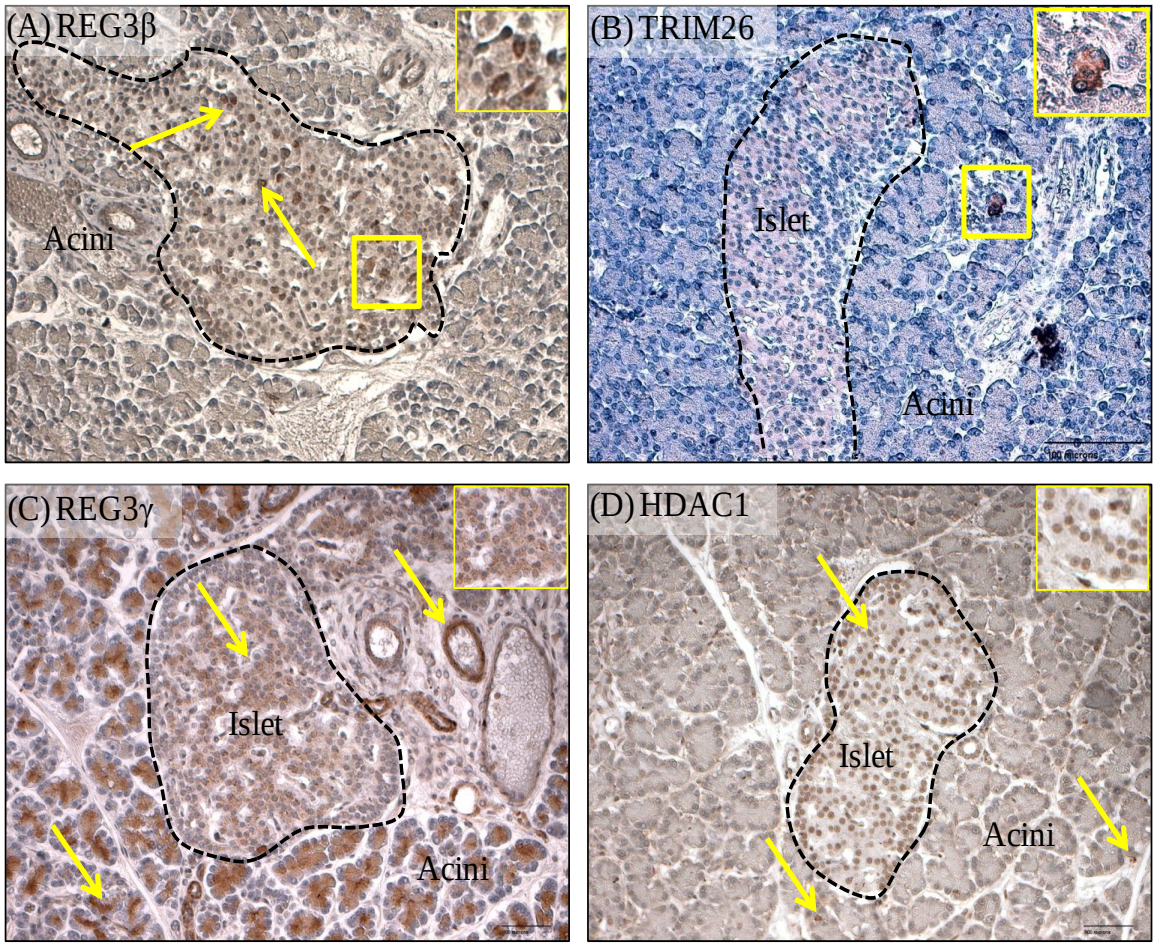
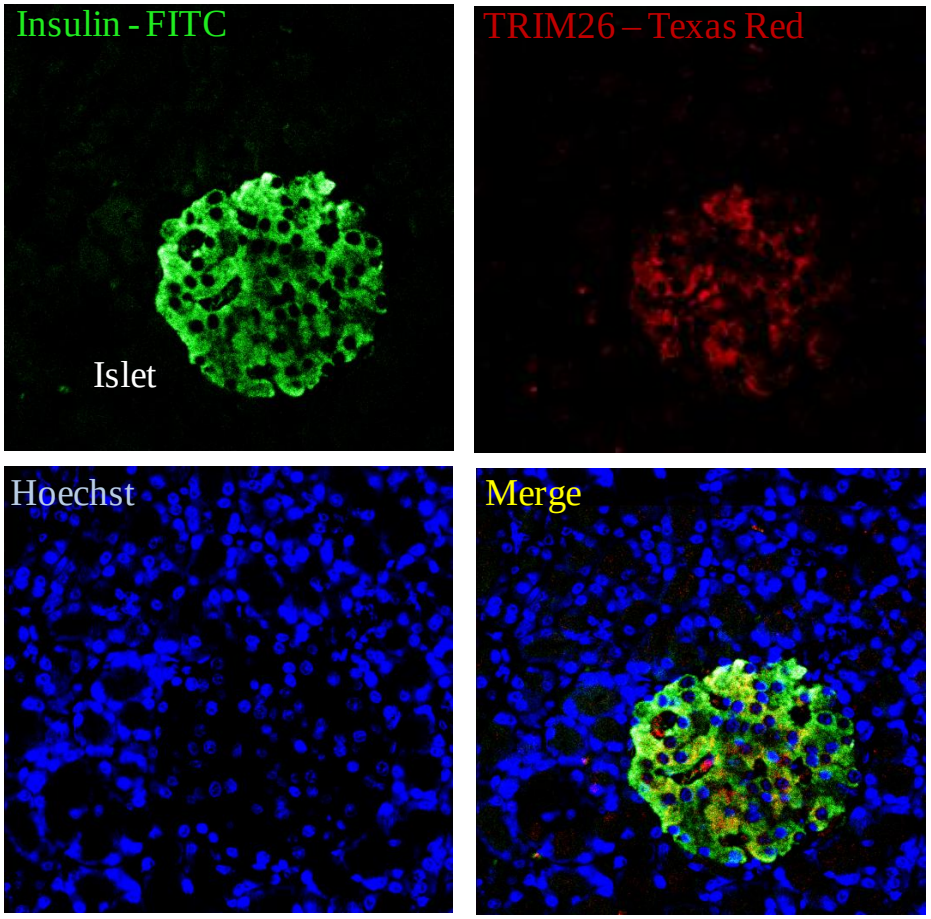

(A) REG3β
Acini
(B) TRIM26
Islet
Acini
(C) REG3γ
Islet
Acini
(D) HDAC1
Islet
Acini

Figure 11. Pre-diabetic candidate proteins are β-cell specific shown by confocal analysis. TRIM26 **(A)** and REG3β **(B),** top array candidates, are exclusively localized in β-cells. Fluorescent co-localization staining of the array candidates was tested with β-cell marker, insulin. TRIM26 was observed in islets and REG3β co-localized with insulin throughout the islet and EIC. Images were taken at 40x magnification.

(A)


Insulin - FITC
Islet
TRIM26 – Texas Red
Hoechst
Merge

(B)

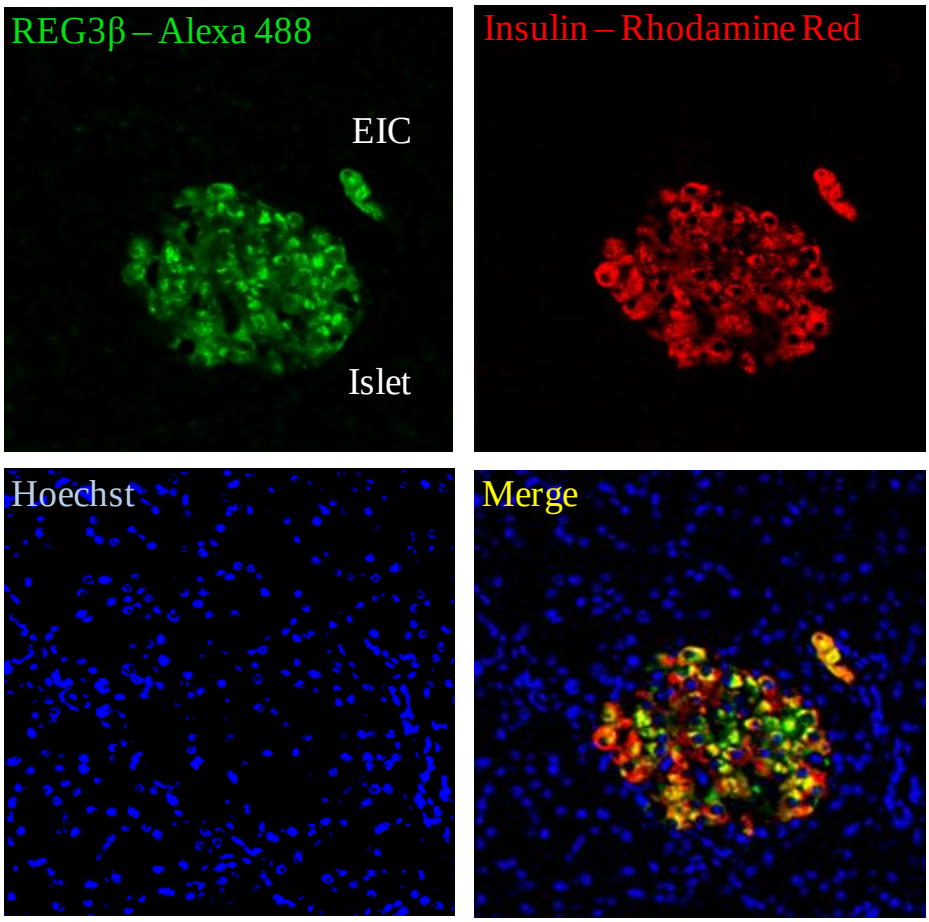
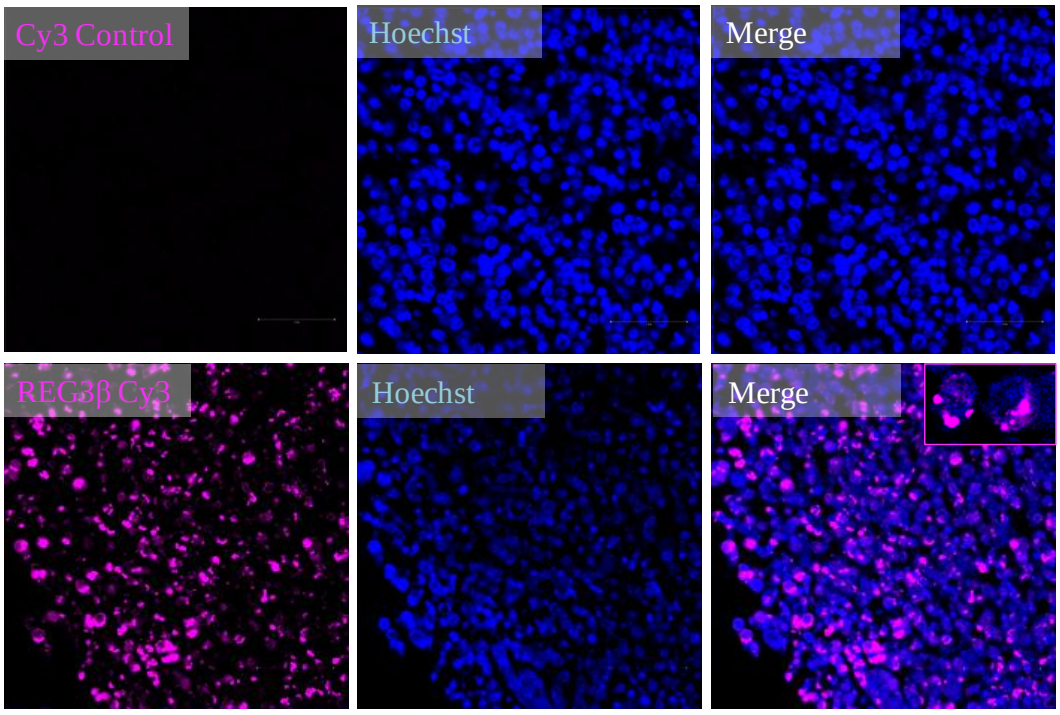

REG3β – Alexa 488
EIC
Islet
Insulin – Rhodamine Red
Hoechst
Merge

Figure 12. REG3β is endogenously expressed in rat insulinoma cells, RINm5F. REG3β is expressed throughout the cells observed by fluorescent confocal microscopy (40x). REG3β displayed a perinuclear staining pattern (63x inset). Fluorescent staining was confirmed with avidin-biotinylated immunohistochemistry. Bar = 100 μm.


Cy3 Control
Hoechst
Merge
REG3β Cy3
Hoechst
Merge

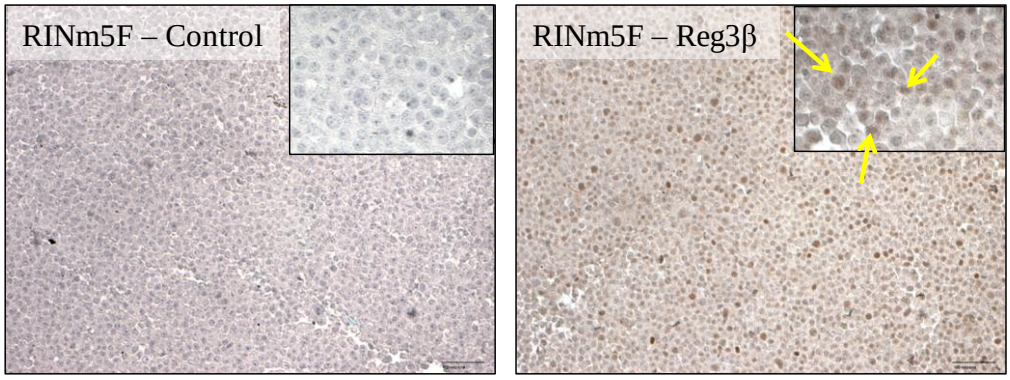
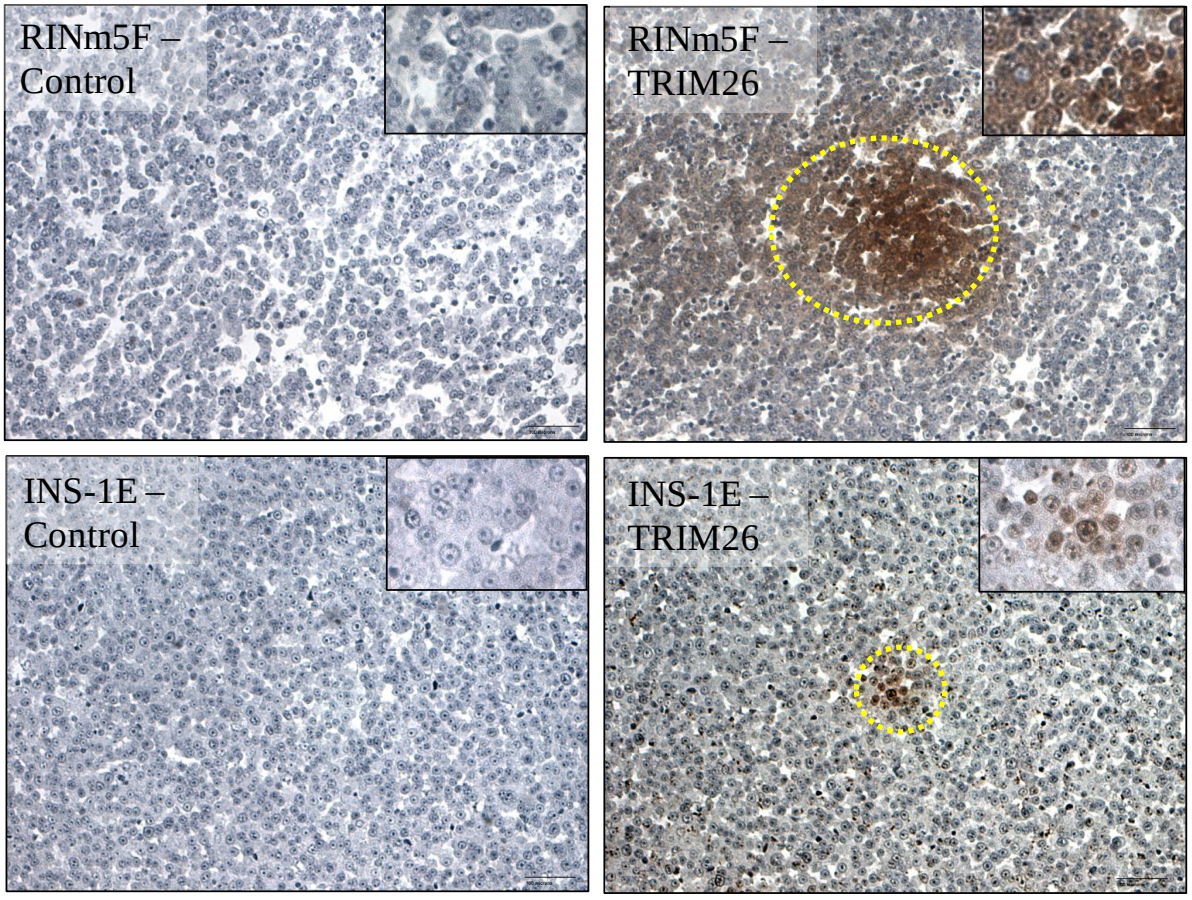

RINm5F – Control
RINm5F – Reg3β

Figure 13. TRIM26 displayed concentrated areas of positive staining in rat insulinoma cell pellets. Pellets displayed positive staining of TRIM26 in focal patterns in both RINm5F and INS-1E cells. Cell pellets were fixed in Bouin's fixative. Staining was mostly cytoplasmic in RINm5F cells and nuclear staining in INS-1E pellets. Foci are outlined. Insets were taken at 40x magnification. Bar = 100 μm.


RINm5F –
Control
RINm5F –
TRIM26
INS-1E –
Control
INS-1E –
TRIM26

DISCUSSION

Ongoing Characterization of the T1D Transcriptome

Type 1 diabetes is an autoimmune disorder with genetic susceptibility that is influenced by poorly defined environmental factors. The HLA haplotype plays a predominant role in conferring genetic predisposition (Larsen and Alper, 2004). ~28% of the Caucasian population possesses risk alleles, however, the overall T1D incidence is between 0.3 – 0.5%. This indicates that the environment plays an integral role in initiating development of T1D, yet involvement of environmental factors has only been inferred. Interaction of ingested antigens with the gut immune system could result in the activation of a pro-inflammatory response, migration to the PLN, immune cell infiltration into the pancreas, and eventual β-cell apoptosis (Lefebvre et al., 2006; Turley et al., 2005) **(Figure 1)**. The initiating event that causes an autoimmune response has not yet been elucidated as a result of the variable timeframe for disease development and multiple factors contributing to disease susceptibility.

Systems methods integrate and define complex biological interactions. The consideration of the immune, gastrointestinal, and endocrine systems as an interconnected network is necessary to elucidate the etiology of T1D. For example, an analysis of the β-cell transcriptome showed 30% homology in global gene expression of β-cells, gut mucosa, and immune cells (Martens et al., 2011). In the past, a reductionist approach to studying a complex disease was preferred due to lack of

technology, or to simplify a research question. Microarray technology permits researchers to obtain vast amounts of biological information with the potential of determining many pathways of interest with a small sample of RNA. Genomics is appropriate for both discovery-based and hypothesis-driven approaches with the availability of whole genome arrays or pathway-focused chips. Genomics permits researchers to interrogate the molecular events of the pre-diabetic phase, which can easily proceed undetected and predicting T1D risk and understanding pathogenesis are potential outcomes from the use of this technology.

Despite the advantages that high-throughput genomics provides, data analysis is subjective and is left to the interpretation of the bioinformatician and programmed biases of bioinformatics software. Fold change cut off is just one aspect of data analysis over which the analyst has license; therefore, analyses of the same cell population in the same animal model may not result in the same candidates. Furthermore, many of the figures produced by bioinformatics software require literacy of clustergrams, ball and stick networks, and heatmaps. These figures have been criticized as "hair balls" for their quantity of information and lack of direction (Lander, 2010). Many of the available bioinformatics software programs use programming code, which is not common outside of computer sciences and engineering. High-throughput genomic studies produce a large amount of *in silico* data that stems from mathematical modeling. Thorough downstream analysis with *in vivo* and *in vitro* methods is needed to establish the biological validity of the array

candidates beyond the statistical analysis of normalized probe values and these candidates must be subjected to proof of principle studies in animal models to evaluate their role in the pathogenesis of a disease.

Gene Expression is Dynamic in Response to Dietary Influence – Lessons from the LEW-DP

Gene expression profiling has been widely introduced in the last 10 years as a method of exploring nutrient influence on disease in a field called nutrigenomics, which has provided evidence that dietary factors can modify transcription. In this study, diet has been shown to confer a protective effect against T1D in animal models. This possibly occurs through the mechanism of transcriptional reprogramming in response to dietary macromolecules. The LEW-DP rat is a newer model in which methods of T1D prevention have not been extensively investigated. HC-fed LEW-DP rats had a 23% inhibition in T1D incidence in comparison with the LEW-DP cohort placed on a cereal diet, suggesting dietary antigens have a role in T1D development in this model. The LEW-DP rats fed the HC diet developed diabetes ~2 weeks later than the rats administered a cereal diet **(Figure 2).**

This project was the first to examine the transcriptional profile of the LEW-DP gut and the first use of microarrays to investigate dietary protection in the LEW-DP spontaneous model of T1D. Interestingly, the results of the LEW-DP diet microarray analysis provided evidence of the environmental modulation of transcription in an animal model of T1D.

Since environmental factors are hypothesized to have an important role in T1D development, the results of the diet comparison support that there is a dietary influence for transcriptional modification that occurred early in development of the LEW-DP. GO analysis of the LEW-DP showed enriched processes involving fatty acid and cholesterol metabolism, indicating that diet altered biological processes related to a nutrient response.

The LEW-DP gut has never been studied for markers of inflammation at the molecular level. Total gut RNA was used to analyze total gut expression profiles with the aim of discerning pathways of interest in the pre-diabetic gut. The results of the diet comparison PCR arrays suggest that LEW-DP rats fed a cereal diet have a pro-inflammatory bias with induction of the NF-κB signaling pathway **(Figure 4)**. Additionally, it provides evidence that gut inflammation is a factor in T1D development in the LEW-DP as the HC diet downregulated markers of the NF-κB pathway. The NF-κB pathway has been identified in network analysis of the NOD mouse spleen leukocytes in pre-diabetes, and is a pro-apoptotic pathway in β-cells (Eldor et al., 2006; Wu et al., 2012). Upregulation of the NF-κB pathway has been reported in celiac disease, which demonstrates similarity to gut defects and wheat peptide intolerance exhibited in T1D. There is a ~10% coincidence between celiac disease (CD) and T1D, and T1D diagnosis usually precedes CD (Schuppan and Hahn, 2001). The wheat peptide gliadin increased NF-κB gene transcription in celiac patients (Cinova et al., 2007). Both T1D and

CD patients present with enteropathy and intolerance to wheat peptides, so studies of inflammatory pathways in the gut of individuals with CD could coincide with pro-inflammatory pathways upregulated in T1D patients. These observations provide strong rationale for the use of the gut as a target tissue used in transcriptional analysis of the pre-diabetic period.

In a prior study of HC diet intervention in the BBdp rat, the HC diet was found to significantly increase mucin production and decrease gut permeability (Courtois et al., 2005). A potential explanation for the antigenic effect of a cereal diet is intestinal inflammation resulting from pro-inflammatory polarization of the cytokine profile (Scott et al., 2002). The HC diet also confers an indirect protective effect on insulin production with increased number of EIC in HC-fed rat pancreata (Wang et al., 2000). Similar publications in the NOD mouse corroborate the efficacy of hypoallergenic diets in reducing T1D incidence (Shehadeh et al., 2004). This research translates to dietary intervention in individuals with T1D as infants weaned to an HC diet were less likely to develop islet autoimmunity (Knip et al., 2010). Recent evidence suggests a gluten-free diet can prevent T1D progression, prolonging insulin independence (Sildorf et al., 2012). Furthermore, an update from the DAISY trial reported that children who were exposed to wheat and were subsequently infected with an enterovirus had greater susceptibility to developing islet autoimmunity (Snell-Bergeon et al., 2012).

The LEW-DP Rat Has Decreased Expression of Immunoregulatory M2Φ Transcripts

The data obtained from the strain comparison of the LEW-DP and LEW-C demonstrate that the diabetes-prone rats could be deficient in tolerogenic M2Φ markers **(Figure 5)**. Morphometric analyses from our group of the M2Φ marker, CD163, in LEW-DP versus LEW-C rats showed a significant decrease in M2Φ population in the LEW-DP gut (Ariel Hendin, unpublished data). Previous studies of macrophage polarization have reported that M2Φ confer protection from T1D development and animals/individuals with T1D show a decrease in M2Φ. M2Φ are immunosuppressive, tissue resident macrophages responsible for abrogating inflammation (Parsa et al., 2012). Adoptive transfer of M2Φ into the NOD mouse prevented T1D for over 3 months, and the cells had tropism to the pancreas to prevent insulitis (Parsa et al., 2012). Calderon *et al.* reported that the macrophage population in NOD-SCID mice with T1D was polarized to the M1Φ phenotype and T1D-protected mice displayed an increased population of M2Φ (Calderon et al., 2008). BBdp rats have a decreased number of M2Φ ($CD163^+$) compared with BBc rats and BBdp rats placed on the HC diet showed increased $CD163^+$ staining in the gut (Patrick et al., 2013). It was also shown that newly diagnosed T1D patients had fewer M2Φ in the jejunum than control subjects (Patrick et al., 2013). The LEW-DP model showed significant differences in the expression of alternatively-activated M2Φ markers, *Fn1*, *Stab1,* and *Serpine1*. PCR array data indirectly suggest there are

decreased numbers of M2Φ, reduced production of basement membrane adhesion proteins in diabetes-prone animals, and increased expression of pro-inflammatory cytokine receptors in response to a cereal diet.

Therefore, the LEW-DP data support the gut as a target tissue of T1D. Transcriptional profiling of the gut to investigate T1D is a novel approach to studying the pre-diabetic period of spontaneous models of T1D. The environment is a strong influence in T1D development, and the gut is the primary contact site of the host immune system and environmental antigens. Diet can confer a protective effect against T1D, suggesting that diet is part of the environmental influence of T1D development. LEW-DP rats fed an antigenic cereal diet had an increased incidence of T1D and displayed a pro-inflammatory bias in the gut with upregulation of the NF-κB pathway. The diabetes-prone LEW strain had decreased expression of tolerogenic M2Φ markers compared with LEW-C rats. The LEW-DP expression analysis demonstrated that the LEW model of T1D displays a distinct expression signature in the gut before T1D onset, which was modifiable by diet.

PPx – A Novel Approach to Prospectively Profiling T1D

The influence of the pancreatic transcriptome in diabetogenesis of young rats and individuals is still unknown. In this study, a novel design was used with biopsies sampled from young BBdp rats that progressed to diabetes or remained asymptomatic at end of study. High-throughput genomic techniques were used to characterize the transcriptional profiles of total pancreas RNA isolated from pre-diabetic and T1D-resistant

groups. We have shown that the transcriptional profile of pre-diabetic BBdp rats diverges from those rats that do not develop T1D.

Total pancreatic RNA was used for the PPx microarray analysis. Total RNA is often used for microarrays with pancreatic samples because digestive enzymes produced by the acini cause rapid degradation of RNA upon dissection, producing a poor RIN value for further genomic analysis. Successful pancreatic RNA isolation requires rapid homogenization and fixation or freezing. Selection of a single cell population is a challenge because of the lack of time before RNA degradation. Laser capture microdissection on frozen pancreas sections or collagenase digest with freshly dissected pancreas can be used to allow for more specific selection of the cells sampled. Despite using total pancreas RNA, some of the upregulated pre-diabetic candidate genes were shown to be localized to islets. Regardless, the use of pooled RNA with a single chip/group presents a caveat that must be considered in interpreting the results.

Partial pancreatectomy studies have been used to study pancreatic morphology, regeneration, transplantation, and cancer. A PPx design was previously used in T1D research as a prospective approach to investigating the development of T1D (Logothetopoulos et al., 1984). PPx sections were used for immunohistochemical study of immune infiltrate into BBdp islets (Logothetopoulos et al., 1984). However, the use of a prospective partial pancreatectomy design followed by genomic analysis has not been done. PPx surgery did not change diabetes incidence

significantly compared with sham-operated BBdp rats, and the sample taken resulted in a gene list enriched with markers of β-cells and not only acini-specific genes. The use of microarrays with pre-diabetic and asymptomatic BBdp biopsies was an unbiased method to determine which biological processes are affected before insulitis or overt T1D.

Prospective Pre-diabetic Profiling Identified β-cell Specific T1D Candidates

The PPx microarray analysis identified several candidate genes and processes upregulated in the pre-diabetic rats. *Reg3β* and *Trim26* are gene candidates implicated in the pathogenesis of T1D. *Reg3β* has previously been found to be upregulated at diabetes-onset while *Trim26* has not been reported as participating in T1D development prior to this study. Both of these candidates were localized to pancreatic islets. Currently, the receptors and modes of action of both of these candidates are undefined. *Ins1* gene transcription was downregulated in the pre-diabetic PPx group at 30 d, but no differences were observed between pre-diabetic and T1D-resistant groups by qRT-PCR (data not shown, Christopher Patrick). Contrary to previous profiling of the BB rat serum by Kaldunski *et al.*, an upregulation of pro-inflammatory immune genes and enrichment of immune response processes was not observed in the 30 d pre-diabetic or T1D-resistant BBdp pancreata. According to the PPx microarray data, the transcriptome of the pancreas does not display an observable difference in immune pathways at this point in T1D development **(Figure 8)**.

Tripartite Motif Containing Protein – A Novel T1D Transcript?

Trim26 is a member of the TRIM family, several of which are putative autoantigens and antiviral proteins (McNab et al., 2011); however, the function of TRIM26 is not fully characterized. The TRIM family is reportedly a group of E3 ubiquitin ligases, but this action has not been observed for TRIM26. The antiviral activity of TRIM26 has been explored at a preliminary level and it is known that type I IFN upregulates *Trim26* expression (Rajsbaum et al., 2008). IFN interactions have been implicated in T1D development, and TRIM26 could have a role in this process. IFN signaling networks have been shown to be affected in the pre-diabetic period (Reynier et al., 2010; Stechova et al., 2011). The restriction of *Trim26* expression to BBdp islets, its significant increase in pre-T1D rats, and hypothesized role in autoimmune disorders provide a rationale to further investigate TRIM26 as a novel T1D autoantigen **(Figures 10, 11, 13).** TRIM26 is a novel β-cell marker and further investigation into its role in β-cell biology and T1D development is required.

A Janus Faced Candidate – The Regenerating Islet-derived 3 Family

Members of the regenerating islet-derived family are expressed throughout the pancreas. The β-cell specificity of several Reg proteins and their abundant transcription make *Reg3α* and *Reg3β* attractive candidates for further inquiry. It has been shown in models of acute pancreatitis that REG3β/PAP1 is a significant anti-inflammatory protein that mitigates acinar inflammation (Fu et al., 2012). A shortened peptide

derived from PAP, PAPep, was shown to have potent anti-inflammatory action outside of its native tissue (Yang et al., 2011). The mechanism of Reg participation in inflammation or immune infiltration in T1D has not been determined. REG3α was identified as a paracrine factor promoting regeneration in a PPx rat model (Choi et al., 2010). Hamster Reg3δ peptide (INGAP) was developed as a therapy to induce β-cell regeneration for T1D and is currently in clinical trials (Fleming and Rosenberg, 2007; Lipsett et al., 2007). The human ortholog to INGAP is REG3α (rat REG3α/REG3β), which intriguingly was significantly upregulated in the prospective PPx pre-diabetic cohort.

The Reg proteins are pleiotropic in function. They are documented as anti-inflammatory peptides, and have been reported to be autoantigens in T1D. I identified two Reg3 family members, *Reg3β* and *Reg3α,* which were significantly upregulated in the pre-diabetic phase of T1D in the BBdp model **(Figure 9)**. Interestingly, the human *REG3α* gene is found in the *IDDM14* gene region, a T1D risk locus in a non-MHC region that has been shown to confer susceptibility in animal models (Mordes et al., 2009). Adoptive transfer of T-cells isolated from NOD PLN expressing the *Reg3β* gene caused T1D in NOD-SCID mice (Gurr et al., 2002). REG3α was also identified as an autoantigen from a recent onset T1D cadaveric islet expression profile (Gurr et al., 2002).

Reg genes are known to be upregulated in response to inflammation. For example, the Reg gene promoter possesses the pro-inflammatory cytokine, IL-6 response element (Gurr et al., 2002). It has

been reported that inflammation associated with partial pancreatectomy surgery can induce Reg genes. However, many publications profiling T1D show Reg gene upregulation without the use of surgical intervention. It has been hypothesized that *Reg3* expression in β-cells is activated by the pro-inflammatory Th17 cytokine, IL-22 (Singh et al., 2011). Therefore, the function of Reg proteins in T1D development could simultaneously act as both promoter and antagonist of T1D onset.

The Reg family has been identified as candidates in T1D development in the pancreas and islet transcriptome in both animal models and human samples (Planas et al., 2010a; Regnault et al., 2009). In microarray studies of cyclophosphamide-induced T1D, *Reg3α*, *Reg3β*, and *Reg3γ* were significantly upregulated (Matos et al., 2004). Benoist and Mathis stated that cyclophosphamide T1D elicited a response mostly in immune infiltrate, and that the Reg3 family was the only group with increased expression in the target tissue (Matos et al., 2004). Interestingly, in a study of the human β-cell transcriptome by Massively Parallel Signature Sequencing (MPSS), *REG3α* was the second most enriched transcript in β-cells after insulin (Kutlu et al., 2009). *Reg1* and *Reg2* expression was observed by Planas *et al.* in islets of NOD mice associated with pro-inflammatory cytokine IFNβ activation (Planas et al., 2006). The induction of Reg genes likely occurs in response to pancreatic stress, be it chemical, surgical or immunological. However, it has not been defined whether Reg induction occurs in response to a specific stressor or is a broad spectrum response. It has been speculated that Reg

proteins have regenerative properties to compensate for β-cell loss, and this function could promote autoimmunity in what Planas *et al.* referred to as a "relapsing-remitting process" (Planas et al., 2010a). Thus, the Reg3 family should be explored further as diagnostic markers for pre-diabetes.

Additionally, REG3β could have chemoattractant properties as a secreted protein targeting the β-cells for immune intervention. A study of immune infiltration into the pancreas at 30 d in the PPx BBdp rats showed significant vasculitis and infiltration of $CD68^+$ macrophages into the pancreatic vasculature in pre-diabetic rats ($p = 0.04$, data not shown, Christopher Patrick). This finding is supported by a previous report that is consistent with the primary role of macrophages early in T1D pathogenesis (Kolb-Bachofen and Kolb, 1989). No difference was observed in degree of insulitis, islet inflammation, between the pre-diabetic and T1D-resistant groups. Previous research has demonstrated a role for Reg proteins in macrophage polarization to a pro-inflammatory phenotype (Viterbo et al., 2008a; Viterbo et al., 2008b). Therefore, the secreted Regs could influence the immune phenotype of infiltrating macrophages. While the role of Reg proteins as anti-microbials in the gut has been well-documented, the function of Regs as anti-microbial agents in the pancreas is currently unexplored, and for this reason, investigation of Reg in innate immunity of the pancreas may provide knowledge of early mechanisms of disease.

The data obtained from analysis of PPx BBdp pancreata show that rats that develop T1D possess a divergent transcriptome from the T1D-

resistant PPx rats at 30 d. T1D development in the BBdp occurs between 50 – 90 d, and these data suggest that pre-diabetic rats have a distinct transcriptional program prior to this window that predisposes them to develop T1D. RNA isolated from pre-diabetic pancreata produced a gene list enriched in islet markers despite the low proportion of islets in total pancreas RNA. Confirmed pre-diabetic candidates displayed β-cell specificity, co-localizing with TRIM26 and REG3β **(Figure 11)**. Thus, PPx profiling produced novel candidate transcripts with biological relevance to T1D.

Consequently, gene expression profiling in spontaneous models of T1D provides valuable insight of the pre-diabetic period. I have shown that diabetes-prone rats exhibit distinct transcriptional signatures early in disease development in both the gut and pancreas. The HC diet inhibition of T1D supports the importance of environmental influence in T1D development. Furthermore, it has been shown that an environmental factor can alter gene expression profile as observed with the LEW-DP HC-fed versus LEW-DP cereal-fed results. It has also been shown in the PPx study that early in T1D development there are dynamic gene expression changes occurring in islets. Analyses of total RNA from the gut and pancreas of potentially pre-diabetic animals have translational value. Gene expression analyses from both studies provided candidates that are biologically relevant to T1D in individuals. Still, data obtained from *in vivo* and *in vitro* investigation are necessary. Therefore, the

confirmation of systems data through molecular biology holds potential for progress in understanding T1D pathogenesis.

FUTURE DIRECTIONS

Despite many high-throughput genomic studies in various T1D models and patient samples, much remains to be elucidated about T1D etiology. In this study, novel candidate genes associated with T1D have been identified in both the LEW-DP and BBdp model through the use of high-throughput gene expression analyses. Further functional studies and identification of candidate interactions are required to better understand how known candidates influence T1D incidence. For both studies, it would be optimal to repeat the microarray experiments with biological replicates; this would allow for statistical significance and detailed pathway and network analyses. It would also improve the identification of GOs that were biologically relevant to the tissue and disease studied. A complete genomic analysis was not possible with the pooled single array approach, however, pooled arrays are known to produce results comparable to individual arrays when done with replicates (Do et al., 2010). In this project, the pooled array acted as a discovery tool to identify genes differentially expressed in the pre-diabetic period prior to T1D onset.

The development of a holistic multi-system method to study T1D pathogenesis *in situ* is crucial to the understanding of T1D development. Researchers currently lack the technology to investigate T1D progression

in the pancreas *in vivo* due to technical challenges associated with RNA quality and the invasive nature of the biopsy. It is also critical that transcriptional analysis be complemented by proteomics since an organism functions at the post-translational level and not merely at the transcriptional level.

Currently, early detection of autoantibodies is the only diagnostic method in practice for identifying individuals who are pre-diabetic. Otherwise, individuals with T1D are diagnosed by hyperglycemia and glucose intolerance when insulin treatment is already required. Microarray profiling is readily performed with blood samples and common pre-diabetic gene candidates obtained from research with animal models could be used as biomarkers in patient samples in the future.

A protective diet has been shown to be an effective method of preventing T1D in the LEW-DP model and possibly in patients. Results of the TRIGR trial have reported that early dietary intervention with HC formula significantly decreased autoantibodies in children that possess a risk haplotype (Knip et al., 2010). Profiling the gut of diabetes-prone rats demonstrated a pro-inflammatory bias in the transcriptional signature. Using the gut as a target tissue for transcriptional investigation of pre-diabetes has not been previously documented. If minimally invasive means of gut biopsy could be developed, this would provide new markers of pre-diabetes.

The prospective pancreatectomy surgery method used to study diabetes development is a novel approach to studying the gene expression

of pre-diabetic rats. Several significant candidates of interest were identified. Continuing *in vitro* and *in vivo* proteomic investigation into the function of REG3α, REG3β, and especially TRIM26 would provide insight into the potential role of these candidates in T1D pathogenesis. Proteomic analyses of these candidates would further identify receptors and targets of action as well as clarify their roles in T1D.

CONCLUSIONS

In conclusion, T1D is a complex disorder with genetic and environmental influences that results from defects in the immune, endocrine, and gastrointestinal systems. The pre-diabetic phase of T1D often progresses undetected to overt diabetes, bypassing the potential therapeutic window to treat defects in both immune system and target tissue. Molecular changes in the target tissues of T1D require further characterization. Genomic profiling of the gut, pancreas, and immune cells demonstrates a distinct transcriptional signature early in the disease program. Transcriptional analysis of the LEW-DP gut and BBdp pancreas showed that both systems are target tissues with significant differences in gene expression in diabetes-prone rats.

In the LEW-DP rat, it was demonstrated that diet induced reprogramming of the gene expression profile in diabetes-prone rats fed a T1D-protective versus T1D-promoting diet. The antigenic diet induced a pro-inflammatory transcriptional signature in rats with genetic susceptibility to T1D. It was also determined that transcription of genes

involved in M2Φ function was downregulated in the diabetes-prone rats compared with the diabetes-resistant control strain, suggesting that there is an important role for innate immunity in T1D development.

The prospective partial pancreatectomy/retrospective genomic analysis study design has not been previously published as a method of investigating the pre-diabetic period in animal models or individuals with T1D. Resultant gene lists from 30 d pancreas biopsies exhibited a pre-diabetic fingerprint enriched with putative T1D candidate genes specific to β-cells despite the use of total pancreatic RNA for the microarrays. *Reg3α, Reg3β*, and the previously unreported *Trim26* were significantly upregulated in pre-diabetic rats.

Therefore, these results demonstrate that the pre-diabetic period presents a divergent transcriptome in the LEW-DP and BBdp rat models. Furthermore, the data support the ability of the environment to affect gene expression in T1D target tissues thereby modifying disease onset. Thus, pre-diabetic rats express a distinct transcriptional fingerprint preceding disease, which could predispose them to T1D development, and be used to identify individuals at risk prior to overt disease onset.

REFERENCES

1. (2007). Study design of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR). Pediatr Diabetes *8*, 117-137.

2. Adler, K., Mueller, D. B., Achenbach, P., Krause, S., Heninger, A. K., Ziegler, A. G., and Bonifacio, E. (2011). Insulin autoantibodies with high affinity to the bovine milk protein alpha casein. Clin Exp Immunol *164*, 42-49.

3. Andersen, M. K., Harkonen, T., Forsblom, C., Groop, P. H., Knip, M., and Tuomi, T. (2013). Zinc transporter type 8 autoantibodies (ZnT8A): prevalence and phenotypic associations in latent autoimmune diabetes in adults and type 1 diabetes diagnosed >35 years. Autoimmunity.

4. Arif, S., Moore, F., Marks, K., Bouckenooghe, T., Dayan, C. M., Planas, R., Vives-Pi, M., Powrie, J., Tree, T., Marchetti, P., *et al.* (2011). Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated beta-cell death. Diabetes *60*, 2112-2119.

5. Arndt, T., Wedekind, D., Weiss, H., Tiedge, M., Lenzen, S., Hedrich, H. J., and Jorns, A. (2009). Prevention of spontaneous immune-mediated diabetes development in the LEW.1AR1-iddm rat by selective CD8+ T cell transfer is associated with a cytokine shift in the pancreas-draining lymph nodes. Diabetologia *52*, 1381-1390.

6. Aspord, C., Rome, S., and Thivolet, C. (2004). Early events in islets and pancreatic lymph nodes in autoimmune diabetes. J Autoimmun *23*, 27-35.

7. Assi, K., Patterson, S., Dedhar, S., Owen, D., Levings, M., and Salh, B. (2011). Role of epithelial integrin-linked kinase in promoting intestinal inflammation: effects on CCL2, fibronectin and the T cell repertoire. BMC Immunol *12*, 42.

8. Atkinson, M. A. (2005). ADA Outstanding Scientific Achievement Lecture 2004. Thirty years of investigating the autoimmune basis for type 1 diabetes: why can't we prevent or reverse this disease? Diabetes *54*, 1253-1263.

9. Atkinson, M. A. (2012). The pathogenesis and natural history of type 1 diabetes. Cold Spring Harb Perspect Med *2*, 1-18.

10. Atkinson, M. A., and Eisenbarth, G. S. (2001). Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet *358*, 221-229.

11. Barrett, J. C., Clayton, D. G., Concannon, P., Akolkar, B., Cooper, J. D., Erlich, H. A., Julier, C., Morahan, G., Nerup, J., Nierras, C., *et al.* (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet *41*, 703-707.

12. Barthson, J., Germano, C. M., Moore, F., Maida, A., Drucker, D. J., Marchetti, P., Gysemans, C., Mathieu, C., Nunez, G., Jurisicova, A., *et al.* (2011). Cytokines tumor necrosis factor-alpha and interferon-gamma induce pancreatic beta-cell apoptosis through STAT1-mediated Bim protein activation. J Biol Chem *286*, 39632-39643.

13. Bendayan, M., and Park, I. S. (1991). Presence of extrapancreatic islets of Langerhans in the duodenal wall of the rat. Diabetologia *34*, 604-606.

14. Bendayan, M., and Park, I. S. (1997). Extrapancreatic islets of Langerhans: ontogenesis and alterations in diabetic condition. J Endocrinol *153*, 73-80.

15. Benoist, C., Germain, R. N., and Mathis, D. (2006). A plaidoyer for 'systems immunology'. Immunol Rev *210*, 229-234.

16. Bensen, J. T., Dawson, P. A., Mychaleckyj, J. C., and Bowden, D. W. (2001). Identification of a novel human cytokine gene in the interleukin gene cluster on chromosome 2q12-14. J Interferon Cytokine Res *21*, 899-904.

17. Best, C. H. (1972). Nineteen hundred twenty-one in Toronto. Diabetes *21*, 385-395.

18. Bottazzo, G. F., and Doniach, D. (1978). Islet-cell antibodies (ICA) in diabetes mellitus (evidence of an autoantigen common to all cells in the islet of Langerhans). Ric Clin Lab *8*, 29-38.

19. Brorsson, C., Tue Hansen, N., Bergholdt, R., Brunak, S., and Pociot, F. (2010). The type 1 diabetes - HLA susceptibility interactome--identification of HLA genotype-specific disease genes for type 1 diabetes. PLoS One *5*, e9576.

20. Burger-van Paassen, N., Loonen, L. M., Witte-Bouma, J., Korteland-van Male, A. M., de Bruijn, A. C., van der Sluis, M., Lu, P., Van Goudoever, J. B., Wells, J. M., Dekker, J., *et al.* (2012). Mucin Muc2 deficiency and weaning influences the expression of the innate defense genes Reg3beta, Reg3gamma and angiogenin-4. PLoS One *7*, e38798.

21. Calderon, B., Suri, A., Pan, X. O., Mills, J. C., and Unanue, E. R. (2008). IFN-gamma-dependent regulatory circuits in immune inflammation highlighted in diabetes. J Immunol *181*, 6964-6974.

22. Callewaert, H. I., Gysemans, C. A., Ladriere, L., D'Hertog, W., Hagenbrock, J., Overbergh, L., Eizirik, D. L., and Mathieu, C. (2007). Deletion of STAT-1 pancreatic islets protects against streptozotocin-induced diabetes and early graft failure but not against late rejection. Diabetes *56*, 2169-2173.

23. Campbell-Thompson, M., Wasserfall, C., Kaddis, J., Albanese-O'Neill, A., Staeva, T., Nierras, C., Moraski, J., Rowe, P., Gianani, R., Eisenbarth, G., *et al.* (2012). Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. Diabetes Metab Res Rev *28*, 608-617.

24. Carter, J., Vaitaitis, G. M., Waid, D. M., and Wagner, D. H., Jr. (2012). CD40 engagement of CD4+ CD40+ T cells in a neo-self antigen disease model ablates CTLA-4 expression and indirectly impacts tolerance. Eur J Immunol *42*, 424-435.

25. Chakir, H., Lefebvre, D. E., Wang, H., Caraher, E., and Scott, F. W. (2005). Wheat protein-induced proinflammatory T helper 1 bias in mesenteric lymph nodes of young diabetes-prone rats. Diabetologia *48*, 1576-1584.

26. Chan, J. Y., Cooney, G. J., Biden, T. J., and Laybutt, D. R. (2011). Differential regulation of adaptive and apoptotic unfolded protein response signalling by cytokine-induced nitric oxide production in mouse pancreatic beta cells. Diabetologia *54*, 1766-1776.

27. Choi, J. H., Lee, M. Y., Kim, Y., Shim, J. Y., Han, S. M., Lee, K. A., Choi, Y. K., Jeon, H. M., and Baek, K. H. (2010). Isolation of genes involved in pancreas regeneration by subtractive hybridization. Biol Chem *391*, 1019-1029.

28. Cinova, J., Palova-Jelinkova, L., Smythies, L. E., Cerna, M., Pecharova, B., Dvorak, M., Fruhauf, P., Tlaskalova-Hogenova, H., Smith, P. D., and Tuckova, L. (2007). Gliadin peptides activate blood monocytes from patients with celiac disease. J Clin Immunol *27*, 201-209.

29. Cizza, G., Brown, R. J., and Rother, K. I. (2012). Rising incidence and challenges of childhood diabetes. A mini review. J Endocrinol Invest *35*, 541-546.

30. Colli, M. L., Moore, F., Gurzov, E. N., Ortis, F., and Eizirik, D. L. (2010). MDA5 and PTPN2, two candidate genes for type 1 diabetes, modify pancreatic beta-cell responses to the viral by-product double-stranded RNA. Hum Mol Genet *19*, 135-146.

31. Concannon, P., Erlich, H. A., Julier, C., Morahan, G., Nerup, J., Pociot, F., Todd, J. A., and Rich, S. S. (2005). Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. Diabetes *54*, 2995-3001.

32. Coppieters, K. T., Wiberg, A., Tracy, S. M., and von Herrath, M. G. (2012). Immunology in the clinic review series: focus on type 1 diabetes and viruses: the role of viruses in type 1 diabetes: a difficult dilemma. Clin Exp Immunol *168*, 5-11.

33. Courtois, P., Nsimba, G., Jijakli, H., Sener, A., Scott, F. W., and Malaisse, W. J. (2005). Gut permeability and intestinal mucins, invertase, and peroxidase in control and diabetes-prone BB rats fed either a protective or a diabetogenic diet. Dig Dis Sci *50*, 266-275.

34. Cui, W., De Jesus, K., Zhao, H., Takasawa, S., Shi, B., Srikant, C. B., and Liu, J. L. (2009). Overexpression of Reg3alpha increases cell growth and the levels of cyclin D1 and CDK4 in insulinoma cells. Growth Factors *27*, 195-202.

35. Dalberg, U., Markholst, H., and Hornum, L. (2007). Both Gimap5 and the diabetogenic BBDP allele of Gimap5 induce apoptosis in T cells. Int Immunol *19*, 447-453.

36. Dehghan, A., Dupuis, J., Barbalic, M., Bis, J. C., Eiriksdottir, G., Lu, C., Pellikka, N., Wallaschofski, H., Kettunen, J., Henneman, P., *et al.* (2011). Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels. Circulation *123*, 731-738.

37. Dendrou, C. A., and Wicker, L. S. (2008). The IL-2/CD25 pathway determines susceptibility to T1D in humans and NOD mice. J Clin Immunol *28*, 685-696.

38. Do, G. M., Kwon, E. Y., Kim, E., Kim, H. S., and Choi, M. S. (2010). Hepatic transcription response to high-fat treatment in mice: microarray comparison of individual vs. pooled RNA samples. Biotechnol J *5*, 970-973.

39. Eckenrode, S. E., Ruan, Q., Yang, P., Zheng, W., McIndoe, R. A., and She, J. X. (2004). Gene expression profiles define a key checkpoint for type 1 diabetes in NOD mice. Diabetes *53*, 366-375.

40. Eden, E., Navon, R., Steinfeld, I., Lipson, D., and Yakhini, Z. (2009). GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics *10*, 48.

41. Eizirik, D. L., Sammeth, M., Bouckenooghe, T., Bottu, G., Sisino, G., Igoillo-Esteve, M., Ortis, F., Santin, I., Colli, M. L., Barthson, J., *et al.* (2012). The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. PLoS Genet *8*, e1002552.

42. Eldor, R., Yeffet, A., Baum, K., Doviner, V., Amar, D., Ben-Neriah, Y., Christofori, G., Peled, A., Carel, J. C., Boitard, C., *et al.* (2006). Conditional and specific NF-kappaB blockade protects pancreatic beta cells from diabetogenic agents. Proc Natl Acad Sci U S A *103*, 5072-5077.

43. Elo, L. L., Mykkanen, J., Nikula, T., Jarvenpaa, H., Simell, S., Aittokallio, T., Hyoty, H., Ilonen, J., Veijola, R., Simell, T., *et al.* (2010). Early suppression of immune response pathways characterizes children with prediabetes in genome-wide gene expression profiling. J Autoimmun *35*, 70-76.

44. Emig, D., Salomonis, N., Baumbach, J., Lengauer, T., Conklin, B. R., and Albrecht, M. (2010). AltAnalyze and DomainGraph: analyzing and visualizing exon expression data. Nucleic Acids Res *38*, W755-762.

45. Fasano, A. (2011). Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev *91*, 151-175.

46. Ferrante, M. I., Ghiani, M., Bulfone, A., and Franco, B. (2001). IL1RAPL2 maps to Xq22 and is specifically expressed in the central nervous system. Gene *275*, 217-221.

47. Fleming, A., and Rosenberg, L. (2007). Prospects and challenges for islet regeneration as a treatment for diabetes: a review of islet neogenesis associated protein. J Diabetes Sci Technol *1*, 231-244.

48. Flohe, S. B., Wasmuth, H. E., Kerad, J. B., Beales, P. E., Pozzilli, P., Elliott, R. B., Hill, J. P., Scott, F. W., and Kolb, H. (2003). A wheat-based, diabetes-promoting diet induces a Th1-type cytokine bias in the gut of NOD mice. Cytokine *21*, 149-154.

49. Fornari, T. A., Donate, P. B., Macedo, C., Sakamoto-Hojo, E. T., Donadi, E. A., and Passos, G. A. (2011). Development of type 1 diabetes mellitus in nonobese diabetic mice follows changes in thymocyte and peripheral T lymphocyte transcriptional activity. Clin Dev Immunol *2011*, 158735.

50. Fu, S., Stanek, A., Mueller, C. M., Brown, N. A., Huan, C., Bluth, M. H., and Zenilman, M. E. (2012). Acute pancreatitis in aging animals: loss of pancreatitis-associated protein protection? World J Gastroenterol *18*, 3379-3388.

51. Funda, D. P., Kaas, A., Tlaskalova-Hogenova, H., and Buschard, K. (2008). Gluten-free but also gluten-enriched (gluten+) diet prevent diabetes in NOD mice; the gluten enigma in type 1 diabetes. Diabetes Metab Res Rev *24*, 59-63.

52. Gianani, R., and Eisenbarth, G. S. (2005). The stages of type 1A diabetes: 2005. Immunol Rev *204*, 232-249.

53. Girard, J. P., and Amalric, F. (1998). Biosynthesis of sulfated L-selectin ligands in human high endothelial venules (HEV). Adv Exp Med Biol *435*, 55-62.

54. Goke, M., Zuk, A., and Podolsky, D. K. (1996). Regulation and function of extracellular matrix intestinal epithelial restitution in vitro. Am J Physiol *271*, G729-740.

55. Griffin, T. J., Gygi, S. P., Ideker, T., Rist, B., Eng, J., Hood, L., and Aebersold, R. (2002). Complementary profiling of gene expression at the transcriptome and proteome levels in Saccharomyces cerevisiae. Mol Cell Proteomics *1*, 323-333.

56. Gurr, W. (2011). The Role of Reg Proteins, a Family of Secreted C-Type Lectins, in Islet Regeneration and as Autoantigens in Type 1 Diabetes, In Type 1 Diabetes - Pathogenesis, Genetics and Immunotherapy, D. H. Wagner, ed. (New Haven: InTech), pp. 1 - 23.

57. Gurr, W., Yavari, R., Wen, L., Shaw, M., Mora, C., Christa, L., and Sherwin, R. S. (2002). A Reg family protein is overexpressed in islets from a patient with new-onset type 1 diabetes and acts as T-cell autoantigen in NOD mice. Diabetes *51*, 339-346.

58. Gurzov, E. N., Barthson, J., Marhfour, I., Ortis, F., Naamane, N., Igoillo-Esteve, M., Gysemans, C., Mathieu, C., Kitajima, S., Marchetti, P., *et al.* (2011). Pancreatic beta-cells activate a JunB/ATF3-dependent survival pathway during inflammation. Oncogene *31*, 1723-1732.

59. Gysemans, C. A., Ladriere, L., Callewaert, H., Rasschaert, J., Flamez, D., Levy, D. E., Matthys, P., Eizirik, D. L., and Mathieu, C. (2005). Disruption of the gamma-interferon signaling pathway at the level of signal transducer and activator of transcription-1 prevents immune destruction of beta-cells. Diabetes *54*, 2396-2403.

60. Hagopian, W. A., Lernmark, A., Rewers, M. J., Simell, O. G., She, J. X., Ziegler, A. G., Krischer, J. P., and Akolkar, B. (2006). TEDDY--The Environmental Determinants of Diabetes in the Young: an observational clinical trial. Ann N Y Acad Sci *1079*, 320-326.

61. Hatakeyama, S. (2011). TRIM proteins and cancer. Nat Rev Cancer *11*, 792-804.

62. Huang da, W., Sherman, B. T., Tan, Q., Collins, J. R., Alvord, W. G., Roayaei, J., Stephens, R., Baseler, M. W., Lane, H. C., and Lempicki, R. A. (2007). The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol *8*, R183.

63. Hulbert, E. M., Smink, L. J., Adlem, E. C., Allen, J. E., Burdick, D. B., Burren, O. S., Cassen, V. M., Cavnor, C. C., Dolman, G. E., Flamez, D., *et al.* (2007). T1DBase: integration and presentation of complex data for type 1 diabetes research. Nucleic Acids Res *35*, D742-746.

64. Hyatt, G., Melamed, R., Park, R., Seguritan, R., Laplace, C., Poirot, L., Zucchelli, S., Obst, R., Matos, M., Venanzi, E., *et al.* (2006). Gene expression microarrays: glimpses of the immunological genome. Nat Immunol *7*, 686-691.

65. Igoillo-Esteve, M., Gurzov, E. N., Eizirik, D. L., and Cnop, M. (2011). The transcription factor B-cell lymphoma (BCL)-6 modulates pancreatic {beta}-cell inflammatory responses. Endocrinology *152*, 447-456.

66. Ingemansson, S., Vaziri-Sani, F., Lindblad, U., Gudbjornsdottir, S., and Torn, C. (2013). Long-term sustained autoimmune response to beta cell specific zinc transporter (ZnT8, W, R, Q) in young adult patients with preserved beta cell function at diagnosis of diabetes. Autoimmunity *46*, 50-61.

67. Irizarry, R. A., Bolstad, B. M., Collin, F., Cope, L. M., Hobbs, B., and Speed, T. P. (2003). Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res *31*, e15.

68. Jackson, A. M., Kanak, M. A., Grishman, E. K., Chaussabel, D., Levy, M. F., and Naziruddin, B. (2012). Gene expression changes in human islets exposed to type 1 diabetic serum. Islets *4*, 312-319.

69. Jefferies, C., Wynne, C., and Higgs, R. (2011). Antiviral TRIMs: friend or foe in autoimmune and autoinflammatory disease? Nat Rev Immunol *11*, 617-625.

70. Jia, S., Kaldunski, M., Jailwala, P., Geoffrey, R., Kramer, J., Wang, X., and Hessner, M. J. (2011). Use of transcriptional signatures induced in lymphoid and myeloid cell lines as an inflammatory biomarker in Type 1 diabetes. Physiol Genomics *43*, 697-709.

71. Jorns, A., Gunther, A., Hedrich, H. J., Wedekind, D., Tiedge, M., and Lenzen, S. (2005). Immune cell infiltration, cytokine expression, and beta-cell apoptosis during the development of type 1 diabetes in the spontaneously diabetic LEW.1AR1/Ztm-iddm rat. Diabetes *54*, 2041-2052.

72. Jorns, A., Kubat, B., Tiedge, M., Wedekind, D., Hedrich, H. J., Kloppel, G., and Lenzen, S. (2004). Pathology of the pancreas and other organs in the diabetic LEW.1AR1/Ztm- iddm rat, a new model of spontaneous insulin-dependent diabetes mellitus. Virchows Arch *444*, 183-189.

73. Jorns, A., Rath, K. J., Terbish, T., Arndt, T., Meyer Zu Vilsendorf, A., Wedekind, D., Hedrich, H. J., and Lenzen, S. (2010). Diabetes prevention by immunomodulatory FTY720 treatment in the LEW.1AR1-iddm rat despite immune cell activation. Endocrinology *151*, 3555-3565.

74. Kaldunski, M., Jia, S., Geoffrey, R., Basken, J., Prosser, S., Kansra, S., Mordes, J. P., Lernmark, A., Wang, X., and Hessner, M. J. (2010). Identification of a serum-induced transcriptional signature associated with type 1 diabetes in the BioBreeding rat. Diabetes *59*, 2375-2385.

75. Karikoski, M., Irjala, H., Maksimow, M., Miiluniemi, M., Granfors, K., Hernesniemi, S., Elima, K., Moldenhauer, G., Schledzewski, K., Kzhyshkowska, J., *et al.* (2009). Clever-1/Stabilin-1 regulates lymphocyte migration within lymphatics and leukocyte entrance to sites of inflammation. Eur J Immunol *39*, 3477-3487.

76. Katarina, K., Daniela, P., Peter, N., Marianna, R., Pavlina, C., Stepanka, P., Jan, L., Ludmila, T., Michal, A., and Marie, C. (2007). HLA, NFKB1 and NFKBIA gene polymorphism profile in autoimmune diabetes mellitus patients. Exp Clin Endocrinol Diabetes *115*, 124-129.

77. Kilpatrick, E. S., Rigby, A. S., Atkin, S. L., and Frier, B. M. (2012). Does severe hypoglycaemia influence microvascular complications in Type 1 diabetes? An analysis of the Diabetes Control and Complications Trial database. Diabet Med *29*, 1195-1198.

78. Klein, W., Tromm, A., Folwaczny, C., Hagedorn, M., Duerig, N., Epplen, J. T., Schmiegel, W. H., and Griga, T. (2004). A polymorphism of the NFKBIA gene is associated with Crohn's disease patients lacking a predisposing allele of the CARD15 gene. Int J Colorectal Dis *19*, 153-156.

79. Knip, M., Virtanen, S. M., Seppa, K., Ilonen, J., Savilahti, E., Vaarala, O., Reunanen, A., Teramo, K., Hamalainen, A. M., Paronen, J., *et al.* (2010). Dietary intervention in infancy and later signs of beta-cell autoimmunity. N Engl J Med *363*, 1900-1908.

80. Kolb-Bachofen, V., and Kolb, H. (1989). A role for macrophages in the pathogenesis of type 1 diabetes. Autoimmunity *3*, 145-154.

81. Kutlu, B., Burdick, D., Baxter, D., Rasschaert, J., Flamez, D., Eizirik, D. L., Welsh, N., Goodman, N., and Hood, L. (2009). Detailed transcriptome atlas of the pancreatic beta cell. BMC Med Genomics *2*, 3.

82. Kzhyshkowska, J. (2010). Multifunctional receptor stabilin-1 in homeostasis and disease. ScientificWorldJournal *10*, 2039-2053.

83. Lai, Y., Li, D., Li, C., Muehleisen, B., Radek, K. A., Park, H. J., Jiang, Z., Li, Z., Lei, H., Quan, Y., *et al.* (2012). The antimicrobial protein REG3A regulates keratinocyte proliferation and differentiation after skin injury. Immunity *37*, 74-84.

84. Lander, A. D. (2010). The edges of understanding. BMC Biol *8*, 40.

85. Larsen, C. E., and Alper, C. A. (2004). The genetics of HLA-associated disease. Curr Opin Immunol *16*, 660-667.

86. Lee, K. S., Kalantzis, A., Jackson, C. B., O'Connor, L., Murata-Kamiya, N., Hatakeyama, M., Judd, L. M., Giraud, A. S., and Menheniott, T. R. (2012). Helicobacter pylori CagA triggers expression of the bactericidal lectin REG3gamma via gastric STAT3 activation. PLoS One *7*, e30786.

87. Lefebvre, D. E., Powell, K. L., Strom, A., and Scott, F. W. (2006). Dietary proteins as environmental modifiers of type 1 diabetes mellitus. Annu Rev Nutr *26*, 175-202.

88. Lenzen, S., Tiedge, M., Elsner, M., Lortz, S., Weiss, H., Jorns, A., Kloppel, G., Wedekind, D., Prokop, C. M., and Hedrich, H. J. (2001). The LEW.1AR1/Ztm-iddm rat: a new model of spontaneous insulin-dependent diabetes mellitus. Diabetologia *44*, 1189-1196.

89. Liechti, R., Csardi, G., Bergmann, S., Schutz, F., Sengstag, T., Boj, S. F., Servitja, J. M., Ferrer, J., Van Lommel, L., Schuit, F., *et al.* (2010). EuroDia: a beta-cell gene expression resource. Database (Oxford) *2010*, baq024.

90. Linschooten, J. O., Van Schooten, F. J., Baumgartner, A., Cemeli, E., Van Delft, J., Anderson, D., and Godschalk, R. W. (2009). Use of spermatozoal mRNA profiles to study gene-environment interactions in human germ cells. Mutat Res *667*, 70-76.

91. Lipsett, M., Hanley, S., Castellarin, M., Austin, E., Suarez-Pinzon, W. L., Rabinovitch, A., and Rosenberg, L. (2007). The role of islet neogenesis-associated protein (INGAP) in islet neogenesis. Cell Biochem Biophys *48*, 127-137.

92. Logothetopoulos, J., Valiquette, N., Madura, E., and Cvet, D. (1984). The onset and progression of pancreatic insulitis in the overt, spontaneously diabetic, young adult BB rat studied by pancreatic biopsy. Diabetes *33*, 33-36.

93. Lu, Y., Ponton, A., Okamoto, H., Takasawa, S., Herrera, P. L., and Liu, J. L. (2006). Activation of the Reg family genes by pancreatic-specific IGF-I gene deficiency and after streptozotocin-induced diabetes in mouse pancreas. Am J Physiol Endocrinol Metab *291*, E50-58.

94. Lundh, M., Christensen, D. P., Rasmussen, D. N., Mascagni, P., Dinarello, C. A., Billestrup, N., Grunnet, L. G., and Mandrup-Poulsen, T. (2010). Lysine deacetylases are produced in pancreatic beta cells and are differentially regulated by proinflammatory cytokines. Diabetologia *53*, 2569-2578.

95. Marjamaki, L., Niinisto, S., Kenward, M. G., Uusitalo, L., Uusitalo, U., Ovaskainen, M. L., Kronberg-Kippila, C., Simell, O., Veijola, R., Ilonen, J., *et al.* (2010). Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring. Diabetologia *53*, 1599-1607.

96. Martens, G. A., Jiang, L., Hellemans, K. H., Stange, G., Heimberg, H., Nielsen, F. C., Sand, O., Van Helden, J., Van Lommel, L., Schuit, F., *et al.* (2011). Clusters of conserved beta cell marker genes for assessment of beta cell phenotype. PLoS One *6*, e24134.

97. Matos, M., Park, R., Mathis, D., and Benoist, C. (2004). Progression to islet destruction in a cyclophosphamide-induced transgenic model: a microarray overview. Diabetes *53*, 2310-2321.

98. Maurano, F., Mazzarella, G., Luongo, D., Stefanile, R., D'Arienzo, R., Rossi, M., Auricchio, S., and Troncone, R. (2005). Small intestinal enteropathy in non-obese diabetic mice fed a diet containing wheat. Diabetologia *48*, 931-937.

99. McNab, F. W., Rajsbaum, R., Stoye, J. P., and O'Garra, A. (2011). Tripartite-motif proteins and innate immune regulation. Curr Opin Immunol *23*, 46-56.

100. Metroz-Dayer, M. D., Mouland, A., Brideau, C., Duhamel, D., and Poussier, P. (1990). Adoptive transfer of diabetes in BB rats induced by CD4 T lymphocytes. Diabetes *39*, 928-932.

101. Meyer, M., Gaudieri, S., Rhodes, D. A., and Trowsdale, J. (2003). Cluster of TRIM genes in the human MHC class I region sharing the B30.2 domain. Tissue Antigens *61*, 63-71.

102. Miani, M., Colli, M. L., Ladriere, L., Cnop, M., and Eizirik, D. L. (2012). Mild endoplasmic reticulum stress augments the proinflammatory effect of IL-1beta in pancreatic rat beta-cells via the IRE1alpha/XBP1s pathway. Endocrinology *153*, 3017-3028.

103. Mojibian, M., Chakir, H., Lefebvre, D. E., Crookshank, J. A., Sonier, B., Keely, E., and Scott, F. W. (2009). Diabetes-specific HLA-DR-restricted proinflammatory T-cell response to wheat polypeptides in tissue transglutaminase antibody-negative patients with type 1 diabetes. Diabetes *58*, 1789-1796.

104. Moore, F., Colli, M. L., Cnop, M., Esteve, M. I., Cardozo, A. K., Cunha, D. A., Bugliani, M., Marchetti, P., and Eizirik, D. L. (2009). PTPN2, a candidate gene for type 1 diabetes, modulates interferon-gamma-induced pancreatic beta-cell apoptosis. Diabetes *58*, 1283-1291.

105. Moore, F., Cunha, D. A., Mulder, H., and Eizirik, D. L. (2012). Use of RNA interference to investigate cytokine signal transduction in pancreatic beta cells. Methods Mol Biol *820*, 179-194.

106. Moore, F., Naamane, N., Colli, M. L., Bouckenooghe, T., Ortis, F., Gurzov, E. N., Igoillo-Esteve, M., Mathieu, C., Bontempi, G., Thykjaer, T., *et al.* STAT1 is a master regulator of pancreatic {beta}-cell apoptosis and islet inflammation. J Biol Chem *286*, 929-941.

107. Moore, F., Naamane, N., Colli, M. L., Bouckenooghe, T., Ortis, F., Gurzov, E. N., Igoillo-Esteve, M., Mathieu, C., Bontempi, G., Thykjaer, T., *et al.* (2011). STAT1 is a master regulator of pancreatic {beta}-cell apoptosis and islet inflammation. J Biol Chem *286*, 929-941.

108. Mootha, V. K., Bunkenborg, J., Olsen, J. V., Hjerrild, M., Wisniewski, J. R., Stahl, E., Bolouri, M. S., Ray, H. N., Sihag, S., Kamal, M., *et al.* (2003). Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. Cell *115*, 629-640.

109. Mordes, J. P., Bortell, R., Blankenhorn, E. P., Rossini, A. A., and Greiner, D. L. (2004). Rat models of type 1 diabetes: genetics, environment, and autoimmunity. Ilar J *45*, 278-291.

110. Mordes, J. P., Cort, L., Norowski, E., Leif, J., Fuller, J. M., Lernmark, A., Greiner, D. L., and Blankenhorn, E. P. (2009). Analysis of the rat Iddm14 diabetes susceptibility locus in multiple rat strains: identification of a susceptibility haplotype in the Tcrb-V locus. Mamm Genome *20*, 162-169.

111. Neu, J., Lorca, G., Kingma, S. D., and Triplett, E. W. (2010). The intestinal microbiome: relationship to type 1 diabetes. Endocrinol Metab Clin North Am *39*, 563-571.

112. Noble, J. A., and Valdes, A. M. (2011). Genetics of the HLA region in the prediction of type 1 diabetes. Curr Diab Rep *11*, 533-542.

113. Ortis, F., Miani, M., Colli, M. L., Cunha, D. A., Gurzov, E. N., Allagnat, F., Chariot, A., and Eizirik, D. L. (2012). Differential usage of NF-kappaB activating signals by IL-1beta and TNF-alpha in pancreatic beta cells. FEBS Lett *586*, 984-989.

114. Ozcan, E., Rauter, I., Garibyan, L., Dillon, S. R., and Geha, R. S. (2011). Toll-like receptor 9, transmembrane activator and calcium-modulating cyclophilin ligand interactor, and CD40 synergize in causing B-cell activation. J Allergy Clin Immunol *128*, 601-609 e601-604.

115. Palani, S., Maksimow, M., Miiluniemi, M., Auvinen, K., Jalkanen, S., and Salmi, M. (2011). Stabilin-1/CLEVER-1, a type 2 macrophage marker, is an adhesion and scavenging molecule on human placental macrophages. Eur J Immunol *41*, 2052-2063.

116. Papizan, J. B., Singer, R. A., Tschen, S. I., Dhawan, S., Friel, J. M., Hipkens, S. B., Magnuson, M. A., Bhushan, A., and Sussel, L. (2011). Nkx2.2 repressor complex regulates islet beta-cell specification and prevents beta-to-alpha-cell reprogramming. Genes Dev *25*, 2291-2305.

117. Parsa, R., Andresen, P., Gillett, A., Mia, S., Zhang, X. M., Mayans, S., Holmberg, D., and Harris, R. A. (2012). Adoptive transfer of immunomodulatory M2 macrophages prevents type 1 diabetes in NOD mice. Diabetes *61*, 2881-2892.

118. Patrick, C., Wang, G. S., Lefebvre, D. E., Crookshank, J. A., Sonier, B., Eberhard, C., Mojibian, M., Kennedy, C. R., Brooks, S. J., Kalmokoff, M. L., *et al.* (2013). Promotion of autoimmune diabetes by cereal diet in the presence or absence of microbes associated with gut immune activation, regulatory imbalance, and altered cathelicidin antimicrobial peptide. Diabetes, In Press.

119. Pearson, C., Uhlig, H. H., and Powrie, F. (2012). Lymphoid microenvironments and innate lymphoid cells in the gut. Trends Immunol *33*, 289-296.

120. Perez, A. B., Sierra, B., Garcia, G., Aguirre, E., Babel, N., Alvarez, M., Sanchez, L., Valdes, L., Volk, H. D., and Guzman, M. G. (2010). Tumor necrosis factor-alpha, transforming growth factor-beta1, and interleukin-10 gene polymorphisms: implication in protection or susceptibility to dengue hemorrhagic fever. Hum Immunol *71*, 1135-1140.

121. Pirot, P., Naamane, N., Libert, F., Magnusson, N. E., Orntoft, T. F., Cardozo, A. K., and Eizirik, D. L. (2007). Global profiling of genes modified by endoplasmic reticulum stress in pancreatic beta cells reveals the early degradation of insulin mRNAs. Diabetologia *50*, 1006-1014.

122. Planas, R., Alba, A., Carrillo, J., Puertas, M. C., Ampudia, R., Pastor, X., Okamoto, H., Takasawa, S., Gurr, W., Pujol-Borrell, R., *et al.* (2006). Reg (regenerating) gene overexpression in islets from non-obese diabetic mice with accelerated diabetes: role of IFNbeta. Diabetologia *49*, 2379-2387.

123. Planas, R., Carrillo, J., Sanchez, A., de Villa, M. C., Nunez, F., Verdaguer, J., James, R. F., Pujol-Borrell, R., and Vives-Pi, M. (2010a). Gene expression profiles for the human pancreas and purified islets in type 1 diabetes: new findings at clinical onset and in long-standing diabetes. Clin Exp Immunol *159*, 23-44.

124. Planas, R., Pujol-Borrell, R., and Vives-Pi, M. (2010b). Global gene expression changes in type 1 diabetes: insights into autoimmune response in the target organ and in the periphery. Immunol Lett *133*, 55-61.

125. Rajsbaum, R., Stoye, J. P., and O'Garra, A. (2008). Type I interferon-dependent and -independent expression of tripartite motif proteins in immune cells. Eur J Immunol *38*, 619-630.

126. Regnault, B., Osorio, Y. F. J., Miao, D., Eisenbarth, G., and Melanitou, E. (2009). Early over expression of messenger RNA for multiple genes, including insulin, in the Pancreatic Lymph Nodes of NOD mice is associated with Islet Autoimmunity. BMC Med Genomics *2*, 63.

127. Rewers, M., Norris, J. M., Eisenbarth, G. S., Erlich, H. A., Beaty, B., Klingensmith, G., Hoffman, M., Yu, L., Bugawan, T. L., Blair, A., *et al.* (1996). Beta-cell autoantibodies in infants and toddlers without IDDM relatives: diabetes autoimmunity study in the young (DAISY). J Autoimmun *9*, 405-410.

128. Reynier, F., Pachot, A., Paye, M., Xu, Q., Turrel-Davin, F., Petit, F., Hot, A., Auffray, C., Bendelac, N., Nicolino, M., *et al.* (2010). Specific gene expression signature associated with development of autoimmune type-I diabetes using whole-blood microarray analysis. Genes Immun *11*, 269-278.

129. Sabat, R. (2010). IL-10 family of cytokines. Cytokine Growth Factor Rev *21*, 315-324.

130. Santin, I., Moore, F., Grieco, F. A., Marchetti, P., Brancolini, C., and Eizirik, D. L. (2012). USP18 is a key regulator of the interferon-driven gene network modulating pancreatic beta cell inflammation and apoptosis. Cell Death Dis *3*, e419.

131. Sapone, A., de Magistris, L., Pietzak, M., Clemente, M. G., Tripathi, A., Cucca, F., Lampis, R., Kryszak, D., Carteni, M., Generoso, M., *et al.* (2006). Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes *55*, 1443-1449.

132. Schmid, S., Buuck, D., Knopff, A., Bonifacio, E., and Ziegler, A. G. (2004). BABYDIET, a feasibility study to prevent the appearance of islet autoantibodies in relatives of patients with Type 1 diabetes by delaying exposure to gluten. Diabetologia *47*, 1130-1131.

133. Schuppan, D., and Hahn, E. G. (2001). Celiac disease and its link to type 1 diabetes mellitus. J Pediatr Endocrinol Metab *14 Suppl 1*, 597-605.

134. Scott, F. W., Cloutier, H. E., Kleemann, R., Woerz-Pagenstert, U., Rowsell, P., Modler, H. W., and Kolb, H. (1997). Potential mechanisms by which certain foods promote or inhibit the development of spontaneous diabetes in BB rats: dose, timing, early effect on islet area, and switch in infiltrate from Th1 to Th2 cells. Diabetes *46*, 589-598.

135. Scott, F. W., Olivares, E., Sener, A., and Malaisse, W. J. (2000). Dietary effects on insulin and nutrient metabolism in mesenteric lymph node cells, splenocytes, and pancreatic islets of BB rats. Metabolism *49*, 1111-1117.

136. Scott, F. W., Rowsell, P., Wang, G. S., Burghardt, K., Kolb, H., and Flohe, S. (2002). Oral exposure to diabetes-promoting food or immunomodulators in neonates alters gut cytokines and diabetes. Diabetes *51*, 73-78.

137. Selmi, C., Feghali-Bostwick, C. A., Lleo, A., Lombardi, S. A., De Santis, M., Cavaciocchi, F., Zammataro, L., Mitchell, M. M., Lasalle, J. M., Medsger, T., Jr., and Gershwin, M. E. (2012). X chromosome gene methylation in peripheral lymphocytes from monozygotic twins discordant for scleroderma. Clin Exp Immunol *169*, 253-262.

138. Shakespear, M. R., Halili, M. A., Irvine, K. M., Fairlie, D. P., and Sweet, M. J. (2011). Histone deacetylases as regulators of inflammation and immunity. Trends Immunol *32*, 335-343.

139. Shannon, L. A., McBurney, T. M., Wells, M. A., Roth, M. E., Calloway, P. A., Bill, C. A., Islam, S., and Vines, C. M. (2012). CCR7/CCL19 controls expression of EDG-1 in T cells. J Biol Chem *287*, 11656-11664.

140. Shehadeh, N., Weis, R., Teninboum, G., Benderly, A., Etzioni, A., and Shamir, R. (2004). The influence of oral insulin on the development of autoimmune diabetes in NOD mice fed a hypoallergenic diet. Diabetes Nutr Metab *17*, 1-5.

141. Shetty, S., Weston, C. J., Oo, Y. H., Westerlund, N., Stamataki, Z., Youster, J., Hubscher, S. G., Salmi, M., Jalkanen, S., Lalor, P. F., and Adams, D. H. (2011). Common lymphatic endothelial and vascular endothelial receptor-1 mediates the transmigration of regulatory T cells across human hepatic sinusoidal endothelium. J Immunol *186*, 4147-4155.

142. Siegel, A. M., Heimall, J., Freeman, A. F., Hsu, A. P., Brittain, E., Brenchley, J. M., Douek, D. C., Fahle, G. H., Cohen, J. I., Holland, S. M., and Milner, J. D. (2011). A critical role for STAT3 transcription factor signaling in the development and maintenance of human T cell memory. Immunity *35*, 806-818.

143. Sildorf, S. M., Fredheim, S., Svensson, J., and Buschard, K. (2012). Remission without insulin therapy on gluten-free diet in a 6-year old boy with type 1 diabetes mellitus. BMJ Case Rep *2012*, [Epub ahead of print].

144. Singh, B., Nikoopour, E., Huszarik, K., Elliott, J. F., and Jevnikar, A. M. (2011). Immunomodulation and regeneration of islet Beta cells by cytokines in autoimmune type 1 diabetes. J Interferon Cytokine Res *31*, 711-719.

145. Snell-Bergeon, J. K., Smith, J., Dong, F., Baron, A. E., Barriga, K., Norris, J. M., and Rewers, M. (2012). Early Childhood Infections and the Risk of Islet Autoimmunity: The Diabetes Autoimmunity Study in the Young (DAISY). Diabetes Care *35*, 2553-2558.

146. Stagner, J., Ahren, B., Sundler, F., and White, K. (2008). Reconstructing the pancreas: restoration of normoglycemia, exocrine function, and islet innervation by islet transplantation to the pancreas. Transplant Proc *40*, 452-454.

147. Stayoussef, M., Benmansour, J., Al-Jenaidi, F. A., Said, H. B., Rayana, C. B., Mahjoub, T., and Almawi, W. Y. (2011). Glutamic acid decarboxylase 65 and islet cell antigen 512/IA-2 autoantibodies in relation to human leukocyte antigen class II DR and DQ alleles and haplotypes in type 1 diabetes mellitus. Clin Vaccine Immunol *18*, 990-993.

148. Stechova, K., Halbhuber, Z., Hubackova, M., Kayserova, J., Petruzelkova, L., Vcelakova, J., Kolouskova, S., Ulmannova, T., Faresjo, M., Neuwirth, A., *et al.* (2011). Case report: type 1 diabetes in monozygotic quadruplets. Eur J Hum Genet.

149. Stechova, K., Halbhuber, Z., Hubackova, M., Kayserova, J., Petruzelkova, L., Vcelakova, J., Kolouskova, S., Ulmannova, T., Faresjo, M., Neuwirth, A., *et al.* (2012). Case report: type 1 diabetes in monozygotic quadruplets. Eur J Hum Genet *20*, 457-462.

150. Stechova, K., Kolar, M., Blatny, R., Halbhuber, Z., Vcelakova, J., Hubackova, M., Petruzelkova, L., Sumnik, Z., Obermannova, B., Pithova, P., *et al.* (2011). Healthy first degree relatives of patients with type 1 diabetes exhibit significant differences in basal gene expression pattern of immunocompetent cells compared to controls: expression pattern as predeterminant of autoimmune diabetes. Scand J Immunol *75*, 210-219.

151. Steck, A. K., Bugawan, T. L., Valdes, A. M., Emery, L. M., Blair, A., Norris, J. M., Redondo, M. J., Babu, S. R., Erlich, H. A., Eisenbarth, G. S., and Rewers, M. J. (2005). Association of non-HLA genes with type 1 diabetes autoimmunity. Diabetes *54*, 2482-2486.

152. Sweet, M. J., Shakespear, M. R., Kamal, N. A., and Fairlie, D. P. (2012). HDAC inhibitors: modulating leukocyte differentiation, survival, proliferation and inflammation. Immunol Cell Biol *90*, 14-20.

153. Tanioka, T., Tamura, Y., Fukaya, M., Shinozaki, S., Mao, J., Kim, M., Shimizu, N., Kitamura, T., and Kaneki, M. (2011). Inducible nitric-oxide synthase and nitric oxide donor decrease insulin receptor substrate-2 protein expression by promoting proteasome-dependent degradation in pancreatic beta-cells: involvement of glycogen synthase kinase-3beta. J Biol Chem *286*, 29388-29396.

154. Terazono, K., Yamamoto, H., Takasawa, S., Shiga, K., Yonemura, Y., Tochino, Y., and Okamoto, H. (1988). A novel gene activated in regenerating islets. J Biol Chem *263*, 2111-2114.

155. Thapa, B., Kim, Y. H., Kwon, H. J., and Kim, D. S. (2012). Novel regulatory mechanism and functional implication of plasminogen activator inhibitor-1 (PAI-1) expression in CpG-ODN-stimulated macrophages. Mol Immunol *49*, 572-581.

156. Toeller, M., Klischan, A., Heitkamp, G., Schumacher, W., Milne, R., Buyken, A., Karamanos, B., and Gries, F. A. (1996). Nutritional intake of 2868 IDDM patients from 30 centres in Europe. EURODIAB IDDM Complications Study Group. Diabetologia *39*, 929-939.

157. Tuller, T., Atar, S., Ruppin, E., Gurevich, M., and Achiron, A. (2012). Common and specific signatures of gene expression and protein-protein interactions in autoimmune diseases. Genes Immun, 1-16.

158. Turley, S. J., Lee, J. W., Dutton-Swain, N., Mathis, D., and Benoist, C. (2005). Endocrine self and gut non-self intersect in the pancreatic lymph nodes. Proc Natl Acad Sci U S A *102*, 17729-17733.

159. Vaarala, O., Atkinson, M. A., and Neu, J. (2008). The "perfect storm" for type 1 diabetes: the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. Diabetes *57*, 2555-2562.

160. Vaarala, O., Ilonen, J., Ruohtula, T., Pesola, J., Virtanen, S. M., Harkonen, T., Koski, M., Kallioinen, H., Tossavainen, O., Poussa, T., *et al.* (2012). Removal of Bovine Insulin From Cow's Milk Formula and Early Initiation of Beta-Cell Autoimmunity in the FINDIA Pilot Study. Arch Pediatr Adolesc Med *166*, 608-614.

161. Vaarala, O., Paronen, J., Otonkoski, T., and Akerblom, H. K. (1998). Cow milk feeding induces antibodies to insulin in children--a link between cow milk and insulin-dependent diabetes mellitus? Scand J Immunol *47*, 131-135.

162. Vaishnava, S., Yamamoto, M., Severson, K. M., Ruhn, K. A., Yu, X., Koren, O., Ley, R., Wakeland, E. K., and Hooper, L. V. (2011). The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science *334*, 255-258.

163. van Ampting, M. T., Loonen, L. M., Schonewille, A. J., Konings, I., Vink, C., Iovanna, J., Chamaillard, M., Dekker, J., van der Meer, R., Wells, J. M., and Bovee-Oudenhoven, I. M. (2012). The intestinal secreted C-type lectin Reg3b attenuates Salmonellosis but not Listeriosis in mice. Infect Immun *80*, 1115-1120.

164. Vial, D., and McKeown-Longo, P. J. (2008). PAI1 stimulates assembly of the fibronectin matrix in osteosarcoma cells through crosstalk between the alphavbeta5 and alpha5beta1 integrins. J Cell Sci *121*, 1661-1670.

165. Virtanen, S. M., Nevalainen, J., Kronberg-Kippila, C., Ahonen, S., Tapanainen, H., Uusitalo, L., Takkinen, H. M., Niinisto, S., Ovaskainen, M. L., Kenward, M. G., *et al.* (2012). Food consumption and advanced beta cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes: a nested case-control design. Am J Clin Nutr *95*, 471-478.

166. Visser, J., Rozing, J., Sapone, A., Lammers, K., and Fasano, A. (2009). Tight junctions, intestinal permeability, and autoimmunity: celiac disease and type 1 diabetes paradigms. Ann N Y Acad Sci *1165*, 195-205.

167. Viterbo, D., Bluth, M. H., Lin, Y. Y., Mueller, C. M., Wadgaonkar, R., and Zenilman, M. E. (2008a). Pancreatitis-associated protein 2 modulates inflammatory responses in macrophages. J Immunol *181*, 1948-1958.

168. Viterbo, D., Bluth, M. H., Mueller, C. M., and Zenilman, M. E. (2008b). Mutational characterization of pancreatitis-associated protein 2 domains involved in mediating cytokine secretion in macrophages and the NF-kappaB pathway. J Immunol *181*, 1959-1968.

169. Wallis, R. H., Wang, K., Marandi, L., Hsieh, E., Ning, T., Chao, G. Y., Sarmiento, J., Paterson, A. D., and Poussier, P. (2009). Type 1 diabetes in the BB rat: a polygenic disease. Diabetes *58*, 1007-1017.

170. Wang, G. S., Gruber, H., Smyth, P., Pulido, O., Rosenberg, L., Duguid, W., and Scott, F. W. (2000). Hydrolysed casein diet protects BB rats from developing diabetes by promoting islet neogenesis. J Autoimmun *15*, 407-416.

171. Wang, Y., Jacovetti, C., Li, B., Siddique, T., Xiong, X., Yin, H., Wang, M., Zhao, H., and Liu, J. L. (2011). Coordinated age-dependent and pancreatic-specific expression of mouse Reg2Reg3alpha, and Reg3beta genes. Growth Factors *29*, 72-81.

172. Watts, T., Berti, I., Sapone, A., Gerarduzzi, T., Not, T., Zielke, R., and Fasano, A. (2005). Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats. Proc Natl Acad Sci U S A *102*, 2916-2921.

173. Wedekind, D., Weiss, H., Jorns, A., Lenzen, S., Tiedge, M., and Hedrich, H. J. (2005). Effects of polyinosinic-polycytidylic acid and adoptive transfer of immune cells in the Lew.1AR1-iddm rat and in its coisogenic LEW.1AR1 background strain. Autoimmunity *38*, 265-275.

174. Wegenka, U. M. (2010). IL-20: biological functions mediated through two types of receptor complexes. Cytokine Growth Factor Rev *21*, 353-363.

175. Weiner, H. L., da Cunha, A. P., Quintana, F., and Wu, H. (2011). Oral tolerance. Immunol Rev *241*, 241-259.

176. Weir, G. C., and Bonner-Weir, S. (2011). Finally! A human pancreatic beta cell line. J Clin Invest *121*, 3395-3397.

177. Weiss, H., Bleich, A., Hedrich, H. J., Kolsch, B., Elsner, M., Jorns, A., Lenzen, S., Tiedge, M., and Wedekind, D. (2005). Genetic analysis of the LEW.1AR1-iddm rat: an animal model for spontaneous diabetes mellitus. Mamm Genome *16*, 432-441.

178. Wu, C., Li, A., Leng, Y., Li, Y., and Kang, J. (2012). Histone Deacetylase Inhibition by Sodium Valproate Regulates Polarization of Macrophage Subsets. DNA Cell Biol *31*, 592-599.

179. Wu, J., Kakoola, D. N., Lenchik, N. I., Desiderio, D. M., Marshall, D. R., and Gerling, I. C. (2012). Molecular phenotyping of immune cells from young NOD mice reveals abnormal metabolic pathways in the early induction phase of autoimmune diabetes. PLoS One *7*, e46941.

180. Xiong, X., Wang, X., Li, B., Chowdhury, S., Lu, Y., Srikant, C. B., Ning, G., and Liu, J. L. (2011). Pancreatic islet-specific overexpression of Reg3beta protein induced the expression of pro-islet genes and protected the mice against streptozotocin-induced diabetes mellitus. Am J Physiol Endocrinol Metab *300*, E669-680.

181. Yang, X., Jin, H., Liu, K., Gu, Q., and Xu, X. (2011). A Novel Peptide Derived from Human Pancreatitis-Associated Protein Inhibits Inflammation In Vivo and In Vitro and Blocks NF-Kappa B Signaling Pathway. PLoS One *6*, e29155.

182. Zhang, L., Gianani, R., Nakayama, M., Liu, E., Kobayashi, M., Baschal, E., Yu, L., Babu, S., Dawson, A., Johnson, K., *et al.* (2008). Type 1 diabetes: chronic progressive autoimmune disease. Novartis Found Symp *292*, 85-94; discussion 94-88, 122-129, 202-123.

183. Ziegler, A. G., Pflueger, M., Winkler, C., Achenbach, P., Akolkar, B., Krischer, J. P., and Bonifacio, E. (2011). Accelerated progression from islet autoimmunity to diabetes is causing the escalating incidence of type 1 diabetes in young children. J Autoimmun *37*, 3-7.

184. Zmijewski, J. W., Bae, H. B., Deshane, J. S., Peterson, C. B., Chaplin, D. D., and Abraham, E. (2011). Inhibition of neutrophil apoptosis by PAI-1. Am J Physiol Lung Cell Mol Physiol *301*, L247-254.

STATEMENT OF CONTRIBUTION FROM COLLABORATORS

Dr. Gen-Sheng Wang, M.D., Ph.D., Research Associate, designed the partial pancreatectomy study and performed the PPx surgeries. Dr. Wang generated the PPx diabetes incidence curve.

Christopher Patrick, Ph.D. Candidate, performed the sham PPx surgeries, designed the PPx study, and isolated the pancreatic RNA used for microarray analysis and qRT-PCR in the PPx study. Christopher analyzed vasculitis and insulitis on 30 d BBdp PPx rat pancreas sections.

Jennifer A. Crookshank, M.Sc., Senior Research Technician, designed the LEW-DP study, generated the LEW-DP diabetes incidence curve, and collected the LEW-DP and LEW-C samples.

Ariel Hendin, B.Sc., Summer Student, analyzed LEW-DP and LEW-C gut sections for $CD163^{+}$ staining.

Cory Batenchuk, Ph.D. Candidate, Bell Lab, recommended bioinformatics resources and provided instruction of AltAnalyze and GOrilla workflow.

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2006 – 2010: Honours B.Sc. Biomedical Science, minor in Music
Faculty of Science, University of Ottawa

SCHOLARSHIPS

2011 – 2012: Ontario Graduate Scholarship (OGS)

2010 – 2011: National Science and Engineering Research Council Alexander Graham Bell Canadian Graduate Scholarship (CGS-M)

2010 – 2012: University of Ottawa Excellence Scholarship
Faculty of Graduate and Postdoctoral Studies
University of Ottawa

2010 – 2012: University of Ottawa Graduate Admission Scholarship
Faculty of Graduate and Postdoctoral Studies,
University of Ottawa

2009 – 2010: Institutes of Musculoskeletal Health and Arthritis Summer Undergraduate Research Award
Canadian Institutes of Health Research

2006 – 2010: Queen Elizabeth II Aiming for the Top Award
University of Ottawa

2006 – 2010: Undergraduate Admission Scholarship
Faculty of Science, University of Ottawa

TRAVEL BURSARIES

June 2012: Biochemistry, Microbiology, and Immunology
Conference Travel Award
Faculty of Medicine, University of Ottawa
12th International Conference on the Immunology of Diabetes
Victoria, BC

June, 2012: Faculty of Graduate and Postdoctoral Studies
Conference Travel Grant
University of Ottawa
12th International Conference on the Immunology of Diabetes
Victoria, BC

PAPERS IN PREPARATION

Crookshank JA, Patrick C, **Noel A**, Hendin A, Wang GS, and Scott FW. Diabetes development is associated with decreased $FoxP3^+$ and $CD163^+$ M2 macrophage populations and loss of oral tolerance in the gut immune system of the Lew.1AR1-*iddm* rat.

PUBLISHED ABSTRACTS

Patrick C, Crookshank JA, Wang GS, **Noel A,** Hendin A, Scott FW. Decreased intestinal immune regulatory cells in autoimmune diabetes-prone LEW.1AR1-*iddm* rats-modification by diet. *Accepted for poster presentation at the 2nd International Conference on Immune Tolerance in Amsterdam, the Netherlands (October 2011).*

Noel A, Wang GS, Patrick C, Abujamel T, Stintzi A, Scott FW. Pre-diabetic Signature in Prospective Pancreas Biopsies and Microbiome of the Diabetes-prone Rat. *Accepted for poster presentation at the 12th International Conference on the Immunology of Diabetes in Victoria, British Columbia (June 2012).*

CONFERENCES ATTENDED

The 12th International Conference on the Immunology of Diabetes, Victoria BC, June 15 – 19, 2012.

TEACHING ASSISTANTSHIPS

Course Teaching Assistant – General Intermediary Metabolism (BCH3120), Faculty of Medicine, January 2011 – April 2013.

Exam Proctor – Faculty of Science, January 2011 – April 2013.