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# BETA CELLS CIS-REGULATORY NETWORKS AND TYPE 1 DIABETES

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## 1. Summary of the project

Type 1 Diabetes (T1D) is a chronic autoimmune disease that develops as a consequence of a combination of genetic predisposition and environmental factors. Combined these events trigger an aggressive autoimmune assault against pancreatic β cells provoking local inflammation of pancreatic islets (insulitis) and progressive loss of β cells due to apoptosis. Nevertheless our knowledge about the molecular mechanisms that trigger T1D remains limited.

During early insulitis, inflammation contributes to both the primary induction and secondary amplification of the immune assault with inflammatory mediators contributing to the functional suppression and apoptosis of β cells. In this context a "dialogue" between the invading immune cells and the target β cells, mediated by cytokines/chemokines released by both cell populations, is established and subsequently maintained by putative immunogenic signals delivered by dying or "altered" β cells. Although several advances allowed a partial understanding of the disease physiopathogenesis, the precise mechanisms by which autoimmunity is triggered and aggravated in T1D remain to be clarified.

In the current project we demonstrated that immune stress has a profound impact on the pancreatic β-cell chromatin landscape. We found that the β cell response to cytokines is mediated by the induction of new regulatory regions as well as the activation of primed regulatory elements prebound by islet-specific transcription factors.
Strikingly, we observed that T1D associated variants are enriched in human islets cytokine-responsive regulatory elements and identify T1D-associated variants disrupting cytokine-responsive enhancer activity in human β cells.

Overall our study illustrates how β cells respond to a proinflammatory environment and imply a role for stimulus-response islet enhancers in T1D.

## 2. Results

By exposing human pancreatic islets to proinflammatory cytokines we uncovered profound chromatin remodeling and pervasive activation of distal regulatory elements in cytokine-treated human islet cells. Data from ATACseq showed than ~12,500 chromatin sites became accessible as defined by a $log_2$fold change >1 and an adjusted p value <0.05 (shown in green in Fig.1a). Such changes in chromatin accessibility were coupled with the acquisition of activating H3K27ac chromatin marks, unmasking ~3,800 previously uncharacterized human islet cytokine-IREs (Fig, 1b), which correlated with expression and protein changes of nearby genes (Fig.1c-e).

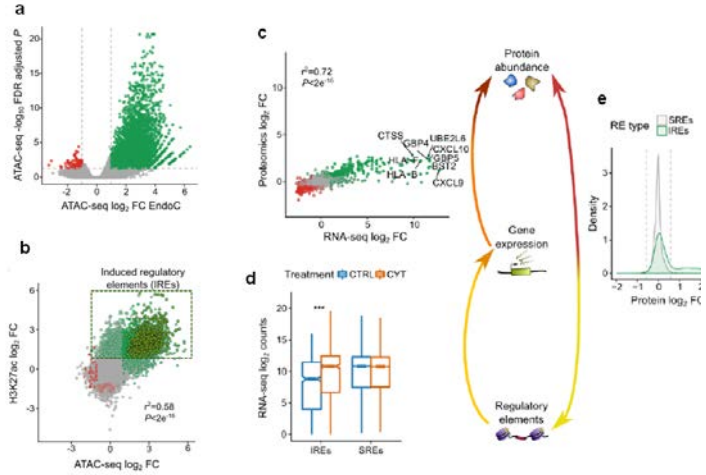


**Figure 1.** a. Volcano plot of ATAC-seq changes obtained after exposure of pancreatic islets to IFN-γ + IL-1β; green and red dots correspond to sites with $|log_2$ FC$|>1$ and FDR adjusted $p<0.05$. **b**. Correlation between chromatin accessibility and H3K27ac deposition. Each dot corresponds to a chromatin site, point fill refers to the ATAC-seq and the border to the H3K27ac classification (gained=green; lost=red; not significant=grey). The dotted box depicts the regulatory elements referred as induced regulatory elements (IREs). **c**. Correlation between changes in RNA expression and the corresponding protein abundance. The x-axis shows the $log_2$ FC for the RNA expression and the y-axis the $log_2$ FC for the protein abundance (both in the EndoC-βH1 human β cell line). **d**. Genes located <20 kb from IREs show cytokine-induced expression in β cells exposed or not to proinflammatory treatment. **e**. Abundance of proteins encoded by IRE-associated genes (genes located <20 kb from IREs or stable regulatory elements (SREs)) is induced by cytokine exposure in EndoC-βH1 cells.

Analysis of islet ChIP-seq data and sequence composition of the activated regulatory sites showed that >1/3 of the islet cytokine-responsive regulatory elements were pre-bound by islet-specific transcription factors (HNF1A/B, NEUROD1, PDX1, MAFB, NKX6.1, among others) and were further bound by inflammatory response-activated transcription factors such as IRFs, STATs and NF-kB following cytokine treatment. These observations, together with results obtained from other non-islet

studies, lead us to hypothesize that enhancers prebound by tissue-specific transcription factors may facilitate cell-type-specific responses to proinflammatory signals under disease conditions.

Strikingly, we found that cytokine-IREs are enriched in T1D associated loci, opening an avenue to the identification of gene targets in the β cell with a potential role in T1D pathogenesis.

These findings suggest a new mechanism for T1D pathogenesis that links T1D genetic susceptibility with β-cell responses to external stimuli. In other words, T1D variants may act at the β cell level, but this may only become manifest in response to extrinsic inflammatory cues. Hence, T1D islet functional variants do not map to islet regulatory elements in an unperturbed state, but can be captured by stimulus-specific cis regulatory maps. These findings also suggest that a subset of T1D functional variants may interfere with regulatory responses to external stress stimuli.

## 3. Relevance

The limited success of drugs blocking autoimmunity in T1D is due mainly to two factors. First, the poor understanding of the molecular mechanism triggering and driving the β cells' autoimmune destruction, limits the development of etiological target drugs. Second, the limited success of drugs blocking autoimmunity at clinical diagnosis suggests that therapies should be introduced early, prior to the irreversible damage to the insulin-producing cells. These observations point to the need for early genetic or molecular disease biomarkers.

The results obtained in the current project allowed the identification of functional genetic variants that could be used to enable early diagnosis, prior to the β cells autoimmune destruction. Integration of our data to the present knowledge of T1D genetic architecture will allow refining polygenetic risk score to detect individuals at risk of developing the disease, thus influencing clinical management.

Moreover the identification of target genes of such variants opens the possibility of testing drug targets that could allow preventing β cell disruption during T1D pathogenesis as a follow-up of the current study.

## 4. Publications


The impact of proinflammatory cytokines on the β-cell regulatory landscape provides insights into the genetics of type 1 diabetes.
Ramos-Rodríguez M, Raurell-Vila H, Colli ML, Alvelos MI, Subirana-Granés M, Juan-Mateu J, Norris R, Turatsinze JV, Nakayasu ES, Webb-Robertson BM, Inshaw JRJ, Marchetti P, Piemonti L, Esteller M, Todd JA4, Metz TO, Eizirik DL, Pasquali L.
Nature Genetics 51(11):1588-1595. 2019

Pancreatic β-cells in human type 1 and type 2 diabetes mellitus: different pathways to failure
Decio L. Eizirik Lorenzo Pasquali and Miriam Cnop
Nature Endocrine Reviews 16 (7), 349-362 2020

An integrated multi-omics approach identifies the landscape of interferon-α-mediated responses of human pancreatic beta cells.
Maikel Colli, Mireia Ramos-Rodriguez, Ernesto Nakayasu, Maria Alvelos, Miguel Lopes, Jessica Hill, Jean-Valery Turatsinze, Alexandra Coomans de Brachène, Mark Russell, Helena Raurell-Vila, Angela Castela, Jonas Juan-Mateu, Bobbie-Jo Webb-Robertson, Lars Krogvold, Knut Dahl-Jorgensen, Lorella Marselli, Sarah Richardson, Noel Morgan, Thomas Metz, Piero Marchetti, Lorenzo Pasquali, Décio Eizirik
Nature communications 11 (1), 1-17 25 2020

Assay for Transposase Accessible Chromatin (ATAC-Seq) to Chart the Open Chromatin Landscape of Human Pancreatic Islets.
Raurell-Vila H, Ramos-Rodríguez M, Pasquali L.
Methods Mol Biol. 1766:197-208. 2018