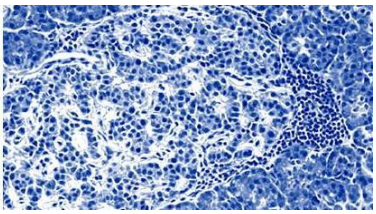
nPOD
Network for Pancreatic Organ Donors with Diabetes



Unmethylated CHTOP and INS Provide Evidence for Islet Cell Death in Youth with Obesity and Diabetes

AUTHORS

Farooq Syed*, Sarah A. Tersey*, Jean-Valery Turatsinze, Jamie L. Felton, Nicole Jiyun Kang, Jennifer B. Nelson, Emily K. Sims, Mathieu Defrance, Francois Fuks, Miriam Cnop, Marco Bugliani, Piero Marchetti, Anette-Gabriele Ziegler, Ezio Bonifacio, Bobbie-Jo Webb-Robertson, Carmella Evans-Molina, Decio L. Eizirik, Kieren J. Mather, Silva Arslanian, and Raghavendra G Mirmira

Indiana University School of Medicine, Indianapolis, Indiana, USA
Université Libre de Bruxelles, Brussels, Belgium
University of Pisa, Pisa, Italy
Technische Universität Dresden, Dresden, Germany
Technische Universität München, Munich, Germany
Pacific Northwest National Laboratories, Richland, Washington, USA
Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, PA
****Authors contributed equally***

PURPOSE

Circulating unmethylated DNA fragments arising from the human *INS* gene have been proposed as biomarkers of β-cell death for the presymptomatic detection of diabetes. However, given the variability of CpG methylation in the *INS* gene in different cell types, this gene alone may not yield sufficiently specific information to unambiguously report β-cell death. In an effort to address the current limitations of differentially-methylated INS as a biomarker for β-cell damage, we hypothesized that other differentially-methylated genes would show either greater specificity for β-cells or could be used as complementary biomarkers to increase β-cell specificity.

METHODS

Human non-pancreatic tissue samples were obtained from National Disease Research Interchange (NDRI). Human islet and pancreatic acinar tissues were obtained from cadaveric donors from the University of Pisa, University of Louisville, or the Integrated Islet Distribution Program (IIDP). We performed a methylation specific Infinium HumanMethylation450 array (Illumina) of 64 human islet preparations and compared them with 27 human tissues/cell lines (control data retrieved from ENCODE DNA methylation database using GEO repository GSE40699). DNA from tissue and cell samples was isolated using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich). DNA from serum was isolated from 50 ul of serum samples spiked in with 5 µg carrier DNA (poly-A) using QIAamp DNA Blood Mini Kit (Qiagen). DNA recovery from serum samples (of the poly-A carrier) was quantified using a nanophotometer (Implen). All samples showed ≥85% recovery of DNA following isolation. DNA bisulfite conversion was carried out using EZ DNA Methylation-Lightning kit (Zymo

Research), and conversion was verified using a pre- and post-conversion sample in the droplet dPCR.

SUMMARY OF RESULTS

We employed an unbiased array-based approach to identify the *CHTOP* gene as a candidate biomarker whose CpGs show a greater frequency of unmethylation in human islets. When tested across an array of non-islet human tissues by digital PCR, both *INS* and *CHTOP* contained unmethylated CpG sites in several of these tissues, but in a non-overlapping pattern. Both *INS* and *CHTOP* genes were unmethylated in β-cells and α-cells (with *CHTOP* showing only slightly greater specificity for β-cells compared to *INS*), implying that either of these genes alone cannot definitively report on β-cell death, as previously assumed. To evaluate if the utilization of both assays together improves the ability to predict the tissue type, we utilized a Naïve Bayes classifier to predict tissue type specificity using single features models with *INS* and *CHTOP* alone, as well as a two feature model with *INS* and *CHTOP*. Five-fold cross-validation (CV) was preformed and each sample was classified based on the posterior probability at a threshold of 0.5. When the two assays were combined, distinct tissue type specificities could be predicted: (1) β-cell specificity was 88.8% when using both assays, (2) islet cell specificity (which include α- and β-cells) was 100% when using both assays, (3) pancreas specificity (which included exocrine and endocrine cells) was 94.8% using both assays. Next, to assess unmethylated *CHTOP* and *INS* as biomarkers for islet damage in human samples, we measured circulating DNA in human populations by digital PCR. Compared to healthy controls, differentially methylated *CHTOP* and *INS* levels were higher in youth with new onset type 1 diabetes and, unexpectedly, in healthy, autoantibody-negative youth who have first-degree relatives with T1D. When tested in youth across a spectrum of metabolic disease, increased levels of unmethylated *INS* and *CHTOP* were observed in obese individuals compared to lean controls.

CONCLUSIONS

In conclusion, our data suggest that using two distinct DNA assays, *INS* and *CHTOP*, outperforms each one separately in determining islet cell specificity compared. Additionally, these data suggest that islet death may be a feature in youth at risk for both T1D and T2D. Our data support the use of multiple parameters to increase the confidence of detecting islet damage in youth with diabetes.