Diabetic Complications Consortium

Application Title: Identification of Novel Targets in the Development of Complications of Diabetes

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1. Project Accomplishments:

This DiaComp Pilot and Feasibility Program project had one specific aim covering two hypotheses:

Specific Aim 1. Pathway analysis of differentially expressed gene transcripts identified by deep sequencing in diabetic gastroparesis will reveal further genes and molecules associated with diabetic gastroparesis.

Hypothesis 1a.

The abundance of certain gene related transcripts in RNA extracted from gastric tissue is different between samples from diabetic patients with and without gastroparesis.

Hypothesis 1b.

The abundance of the affected transcripts is due to changes in the phenotype of key cells responsible for protecting against or causing diabetic gastroparesis.

We have accomplished the following:

i) We have collected all of the 12 diabetic control and 12 diabetic gastroparetic tissue samples necessary to complete the study as outlined in our proposal. We have completed sequencing and pathway analysis on 7 samples from diabetic control patients and 6 samples from diabetic gastroparetic patients and from a total of 60 gene transcripts that were differentially expressed, we have identified 6 transcripts for further investigation. The proteins coded by these transcripts have been detected by immunohistochemistry.

ii) The resulting data contributed to abstract presentations at a Keystone meeting on "Complications of Diabetes" (ref1) and at Digestive Diseases Week 2104 (the annual meeting of the American Gastroenterological Association) (ref2). The data also contributed to an application for the renewal of our project, "Pathobiology of Diabetic Gastroenteropathy" (PI G. Farrugia) on the Program Project Grant: "Pathobiology of the Enteric System", (PI J.H. Szurszewski) submitted in September 2014.

iii) For the tissues that have not yet been sequenced we have extracted the RNA and submitted them for sequencing, these will be added to our analysis when they are complete.

iv) A manuscript is in preparation to report the first part of these data and when that manuscript is accepted for publication, we will make the data available as required. Further data will be released as we complete additional manuscripts.

2. Specific Aim:

i) As outlined in the proposal, our preliminary studies identified 7 transcripts differentially expressed between diabetic gastroparetic and diabetic control tissues. Examination of those data found significant heterogeneities in gene expression within the groups that confounded the detection of differentially expressed genes and which were determined to be due to sample collection problems. These issues were resolved and analysis of RNA expression in identified gastric smooth muscle from well-defined, homogeneous patient groups resulted in identification of 60 differentially expressed genes from only half of our planned cohort size. This number was sufficient to proceed with initial pathway analysis. However, we have collected and sent for analysis the additional samples to complete the cohort, which will fully power our study.

ii) Analysis of RNA-Seq data was done by using a computational pipeline for secondary analysis and differentially expressed genes were determined using a DESeqR package. At least 60 million reads from each sample were mapped to 21,667 identified gene transcripts. Principal component analysis using the Partek Genomics Suite software (Partek,St Louis MO) indicated that the abundance and diversity of the identified gene transcripts were different between the diabetic control and diabetic gastroparetic samples. 60 gene transcripts were significantly expressed, which is more than the 20-40 that we predicted in our proposal. We are currently confirming the differential expression of this large cohort of transcripts by quantitative real-time PCR.

iii) 17 of the differentially expressed genes had known associations with the regulation of macrophage phenotype and others are known to alter immune function. Ingenuity Pathway Analysis predicted 5 canonical pathways to be significantly affected by the differences in RNA expression including two associated with TNF signaling. This is consistent with our previous data indicating that gastroparesis is associated with an immune infiltrate in the gastric muscle wall and generated hypotheses to be tested in our recently submitted grant proposal on the role of macrophages in diabetic gastroparesis.

iv) In 30 of the gene transcripts that were either differentially expressed or previously identified as important for normal gastric motility, 73 single nucleotide variants were identified. 23 of these variants have not been previously annotated.

In conclusion, we have completed the majority of the work proposed in this application and, as intended, we have generated sufficient data for one manuscript in preparation and significant amounts of preliminary data to support a PPG application with new hypotheses regarding the mechanisms behind development of diabetic complications in general and more specifically diabetic gastroparesis.

3. Publications:

1. S.J. Gibbons, C.E. Bernard, A.S. Zubair, K.L. Boone, M.L. Kendrick, K.R. Shen, M.G. Sarr, K.M. Reid Lombardo, Gastroparesis Clinical Research Consortium, P.J. Pasricha and G. Farrugia. Next Generation Sequencing of Gastric Smooth Muscle RNA Identifies Gene Markers for Inflammation in Patients with Diabetic Gastroparesis. Keystone Symposium on Complications of Diabetes. Organizers: M.A. Brownlee, M.D. Breyer, S. Quaggin. Abstract X7-1013.
2. Simon J. Gibbons, Cheryl E. Bernard, Adeel S. Zubair, Kiley L. Boone, Michael L. Kendrick, K. Robert Shen, Michael G. Sarr, Kaye M. Reid Lombardo, GpCRC Consortium, Henry P. Parkman, Pankaj J. Pasricha, Gianrico Farrugia. Next Generation Sequencing of Gastric Smooth Muscle RNA Identifies Gene Markers for Altered Immune Function and Reduced Cellular Proliferation and Differentiation in Patients with Gastroparesis. Gastroenterology, Vol. 146, Issue 5, S-606.