Diabetic Complications Consortium

Application Title: Epigenetic changes and methylglyoxal adducts in patients with type 1 diabetes that develop diabetic kidney disease

Principal Investigator: Sarah Shuck

1. Project Accomplishments:

We discovered that methylglyoxal adducts were significantly associated with the development of diabetic kidney disease (DKD) in individuals with type 1 diabetes at various time points throughout their disease. This is the first time these adducts have been shown to be associated with DKD diagnosis. We have also built a bioinformatics and statistical frameworks for extending our proposed analyses to include additional clinical variables and address how this association changes over time prior to the diagnosis of DKD. Our collaborator, Dr. Natarjan, has also published data describing sites of DNA-methylation (DNA-Me) that are associated with the development of DKD. (*Nature Metabolism*, 2020 Aug;2(8):744-762. doi: 10.1038/s42255-020-0231-8).

2. Specific Aims:

Specific Aim 1. Determine the combined predictive capacity of MG-adducts and DNA-Me for DKD. The association DKD with both MG-adducts and DNA-Me prior to its onset suggests that they may be predictive biomarkers. Because of the link between pathways regulated by DNA-Me and MG production, we ***hypothesize*** that a combination of these analytes will be more predictive than either analyte alone.

Results: From our analyses, we have determined that MG-adducts, specifically the MG-RNA adduct CEG, are associated with DKD up to fifteen years pre-diagnosis (Figure 1). Our collaborator, Dr. Natarajan, also published results identifying specific sites of DNA-Me associated with DKD from these same individuals (*Nature Metabolism*, 2020 Aug;2(8):744-762. doi: 10.1038/s42255-020-0231-8). Now that we have both MG-adducts and DNA-Me patterns for this patient cohort along with bioinformatics and statistics frameworks established for these analyses, we are prepared to combine our datasets and determine the combined predictive capacity of MG-adducts and DNA-Me for DKD. Using this framework, we determined that CEG was significantly ($p<0.001$) associated with the development of DKD when controlling for additional variables including age, BMI, HbA1c, triglycerides and glomerular filtration rate (Table 1). Significance was determined using multivariate logistic regression analysis. We also analyzed the association of CEG with clinical variables at DCCT closeout and found a significant association with cholesterol, triglycerides, albumin excretion rate, HbA1c and retinopathy (Table 2). This suggests there is a correlation of CEG with markers of metabolic dysfunction as well as other diabetic complications.

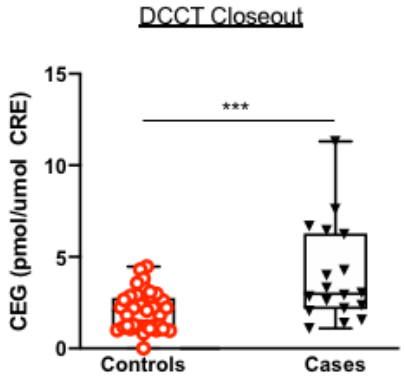

Figure 1. CEG (MG-RNA adduct) is significantly elevated in cases compared to controls. Cases were defined as individuals who received conventional treatment during DCCT and developed DKD. Controls received intensive treatment during DCCT and did not develop DKD. Significance was determined using t-test. ***p=0.003

Characteristic	OR[1]	95% CI[1]	p-value
AGE	0.89	0.83, 0.96	0.002
BMI	1.01	0.88, 1.15	0.90
HBA1C	0.95	0.65, 1.38	0.77
TRIG	1.00	0.99, 1.00	0.48
GFR	0.92	0.89, 0.96	<0.001
log2(CEG)	5.34	2.78, 11.5	<0.001

[1] OR = Odds Ratio, CI = Confidence Interval

Table 1. The association of multiple variables found to be independently associated with DKD was determined. Multi-variate logistic regression was used to determine the influence of each variable on the association of each other with DKD. This analysis was performed for samples collected at DCCT closeout.

	CEG	
Clinical Variables	Eta squared	P-value
AGE	0.001	0.717
BMI	0.002	0.630
CHOL	0.037	**0.043**
TRIG	0.041	**0.032**
GFR	0.007	0.397
DUR	0.006	0.411
BCVALS	0.001	0.743
INSULN	0.009	0.322
AER	0.094	**0.001**
CLR	0.001	0.763
MBP	0.015	0.204
FSCORE36	0.001	0.745
HBA1C	0.084	**0.002**
ETDRSPAT.cat	0.049	**0.020**
ANYCCN	0.005	0.440
case.status	0.142	**0.000**

Table 2. The correlation between CEG and clinical variables at DCCT closeout was determined.

Specific Aim 2. Associate MG-adducts, DNA-Me, and H3K9Ac with prior periods of poor glycemic control (metabolic memory). DCCT/EDIC is an excellent cohort in which to study metabolic memory because it demonstrated that poor glycemic control increases the risk of DKD, despite subsequent strong glycemic control. In our preliminary data, we showed that MG-adducts, DNA-Me, and H3K9Ac are associated with metabolic memory. Because many pathways impacted by DNA-Me and H3K9Ac produce MG, we ***hypothesize*** that a combination of these measurements will be more significantly associated with metabolic memory than any alone.

Results: We extended our preliminary data analysis to include the entire DCCT/EDIC cohort to determine the association of MG-adducts with metabolic memory. We determined that at EDIC year 15, CEG (MG-RNA adduct) was significantly (p=0.0034) higher in cases compared to controls (Figure 2). Analysis of the odds ratio of CEG with DKD at EDIC year 15 found it was significantly (p=0.02), independently associated (Table 3). Analysis of the correlation of CEG with clinical variables at EDIC year 15 revealed significant correlation with C-peptide (BCVAL5), HbA1c, and DKD status (Table 4). This data shows that CEG remains associated with DKD, despite future glycemic control, suggesting that this adduct may contribute to metabolic memory.

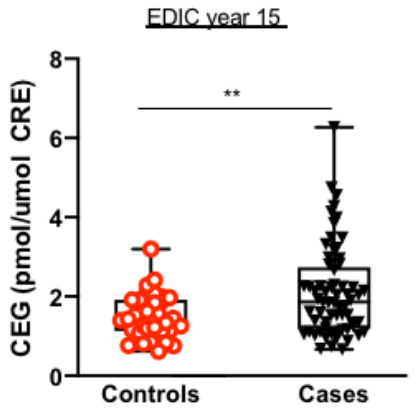

Figure 2. CEG (MG-RNA adduct) is significantly elevated in cases compared to controls at EDIC year 15. Cases were defined as individuals who received conventional treatment during DCCT and developed DKD. Controls received intensive treatment during DCCT and did not develop DKD. Significance was determined using t-test. **p=0.0034

Characteristic	OR[1]	95% CI[1]	p-value
AGE	0.90	0.83, 0.96	0.003
BMI	1.09	0.99, 1.21	0.10
HBA1C	1.80	1.30, 2.60	<0.001
TRIG	1.00	0.99, 1.01	0.88
GFR	0.93	0.89, 0.96	<0.001
log2(CEG)	1.90	1.12, 3.36	0.020

[1] OR = Odds Ratio, CI = Confidence Interval

Table 3. CEG is independently associated with DKD at EDIC year 15. The association of multiple variables with DKD was determined. Multi-variate logistic regression was used to determine the influence of each variable on the association of each other with DKD. This analysis was performed for samples collected at EDIC year 15.

	CEG	
Clinical Variables	Eta squared	P-value
AGE	0.009	0.261
BMI	0.007	0.320
CHOL	0.006	0.347
TRIG	0.020	0.090
GFR	0.002	0.645
DUR	0.016	0.137
BCVAL5	0.030	**0.037**
INSULN	0.003	0.513
AER	0.010	0.238
CLR	0.019	0.100
MBP	0.000	0.946
FSCORE36	0.009	0.395
HBA1C	0.073	**0.001**
ETDRSPAT.cat	0.007	0.322
ANYCCN	0.015	0.146
case.status	0.029	**0.040**

Table 4. The correlation between CEG and clinical variables at EDIC year 15 was determined. BCVAL5=C-peptide

Specific Aim 3. Identify potential therapeutic targets associated with DKD and metabolic memory. Our preliminary data showed that DKD-related DNA-Me and H3K9Ac modifications occur on genes that regulate metabolism and MG production. We **propose** to identify the specific proteins interrelated between epigenetics and MG that are associated with DKD and metabolic memory. To expand our analysis, we will also identify SNPs from patients in our cohort that are associated with metabolic memory and DKD. This is a hypothesis-generating approach to identify potential therapeutic targets that will be validated experimentally in future studies.

Results: Dr. Natarajan's analysis revealed that the loss of TXNIP through increased DNA-Me was associated with the risk of diabetic complications (Chen et al. *Nature Metabolism*, 2020 Aug;2(8):744-762). TXNIP was recently shown to be regulated by glyoxalase 1 (GLO1), the primary enzyme cells use to detoxify methylglyoxal (Jandova and Wondrak, Redox Biol, 2021 Feb;39:101838. doi: 10.1016/j.redox.2020.101838). This suggests an explanation for why both MG-adducts and DNA-Me are associated with the risk of DKD. We are currently expanding this finding with the help of Dr. Ching Ouyang at City of Hope, who is a bioinformatics expert who is helping us to access genetic information from dbGaP that is from the individuals in our cohort.

3. Publications:

- Chen et al. *Nature Metabolism*, 2020 Aug;2(8):744-762. PMID: 32694834; PMCID: PMC7590966