Diabetic Complications Consortium

Application Title: Kidney Iron by Haptoglobin Genotype (KIRHA study)

Principal Investigator: Tina Costacou.

1. Project Accomplishments:

Provide broad overview of the accomplishments of this project

In the past decade, the Haptoglobin (Hp) 2-2 genotype has risen as a major contributor to type 1 and type 2 diabetes (T1D/T2D) complication risk. Hp's main role is to bind free hemoglobin (Hb), reducing its oxidative potential. The Hp-Hb complex formed is cleared by monocyte/macrophage receptor CD163. In diabetes, impaired Hp 2-2 - Hb CD163 clearance and abnormal glomerular permeability allow the large Hp 2-2 – Hb complex to cross the barrier, where it is thought that its redox active iron leads to cellular toxicity; this does not happen with the more stable Hp 1-1 – Hb complex. Hp genotype 2-2 was has been shown to predict renal function decline in two large T1D cohorts. We sought to obtain pilot data on kidney iron deposition in humans with T1D.

The protocol and methods of this pilot study were approved by the University of Pittsburgh Institutional Review Board (IRB).

Given the financial and time constraints of this funding mechanism, we sought to recruit individuals with T1D and known Hp genotype among participants of other ongoing research projects (the EDC (PI: Trevor Orchard) and HapE (PI: Tina Costacou) studies). During the past year, a letter was mailed to 76 individuals, followed by a phone call to assess their willingness and eligibility to participate. Of these:

- No contact could be established for 18
- 8 were ineligible to participate. Reasons for ineligibility included iron supplementation/treatment (n=2); anemia (n=1); alcohol intake of >2 drinks daily (n=1); claustrophobia (n=2); and MRI scan ineligibility (n=2)
- 11 were not interested
- 38 expressed interest in participating and were initially deemed eligible

However, among these 38 willing and initially eligible:

- We were unable to schedule an appointment for a clinic visit for 4 within the study time frame
- 2 were deemed ineligible upon further assessment (claustrophobia (n=1); shoulders too wide to fit in the MRI scanner (n=1))
- 2 cancelled (1 for health reasons and 1 due to jury duty); and
- 30 completed a clinic visit.

Two weeks prior to their visit, participants were mailed containers (and detailed instructions) for the collection of a 24-h urine sample, which was kept refrigerated or frozen until the visit. On arrival at the clinic in the fasted state, participants provided informed consent, eligibility for an MRI scan was confirmed and a blood sample was obtained for biomarker measurement. After taking insulin and receiving breakfast, participants were taken to the MR Research Center; any metal objects (e.g. watch) were removed; the participant changed into light clothing and the one hour, non-contrast, kidney MRI scan was performed. The radiologist conducting the MRI scans and the assessment of kidney iron was blinded to the participants' Hp genotype.

Biomarkers to be assessed as part of this effort comprised:

HbA1c

Fasting plasma glucose and lipids

Serum albumin and creatinine
Serum iron
Ferritin
Urinary KIM-1
Urinary hemoglobin
Urinary iron
Urinary isoprostanes

Thus far, we have received results on all participants for all blood biomarkers. No data have yet been received on the proposed urinary biomarkers, though the lab director assured us that we could expect those results by the end of February 2017.

We have also obtained preliminary data on kidney iron. A second "reading" will be performed within the first two months of 2017 and any discrepancies between the two readings will be resolved before we can proceed with the final analyses and publication of study results.

With the exception of kidney cysts and a horseshoe kidney, no other structural abnormalities of the participants' kidneys were observed. No adverse events have been reported.

2. Specific Aims:

For each specific aim provide the data (includes figures and tables) and progress in each aim.

Specific Aim 1. **Assess, using magnetic resonance imaging (MRI), kidney iron deposition and structural abnormalities (i.e. lesions, hypertrophy) by Hp genotype in T1D**. We hypothesize that kidney iron will be increased and structural abnormalities will be most apparent in Hp 2-2 vs. Hp 1-1 carriers.

Results: A kidney MRI was performed among 30 individuals with type 1 diabetes and known Hp genotype (15 with Hp 1-1 and 15 with Hp 2-2 of similar age (53 yrs), duration (45 yrs) and gender distribution (50% men)). Renal failure, anemia, blood transfusion/donation within 3 months of the study, iron supplement use and alcohol intake >20 g/d were exclusion criteria. The standard renal Blood Oxygen Level Dependent (BOLD), a multi-echo gradient echo approach, was used to measure the T2* value in the renal cortex and renal medulla. Total kidney iron was estimated as the sum of the cortex and medulla iron. Albuminuria was defined as urinary albumin to creatinine ratio >30 mg/g in 2 of 3 samples.

No differences were observed in demographic or clinical characteristics by Hp genotype (Table 1).

Table 1. Characteristics of participants of the KIRHA study by Haptoglobin genotype 1-1 vs. 2-2

	Hp 1-1 (n=15)	Hp 2-2 (n=15)	p-value
Age (years)	53.6 (9.2)	51.8 (6.7)	0.53
Diabetes duration (years)	45.9 (7.8)	44.6 (4.2)	0.58
Females (%, n)	53.3 (8)	46.7 (7)	0.71
BMI (kg/m^2)	28.3 (5.1)	25.9 (4.9)	0.19
$HbA1_c$ (%)	8.0 (1.4)	8.0 (1.5)	0.93
Systolic blood pressure (mmHg)	129.5 (18.5)	134.4 (18.7)	0.47
Diastolic blood pressure (mmHg)	67.9 (10.0)	72.6 (8.3)	0.17
Pre-clinic glucose (mg/dL)	154.5 (55.6)	163.1 (96.5)	0.77
Final glucose (mg/dL)	139.3 (51.9)	141.3 (61.1)	0.92

	Hp 1-1 (n=15)	Hp 2-2 (n=15)	p-value
Total cholesterol (mg/dL)	179.8 (39.8)	185.7 (52.1)	0.73
HDL cholesterol (mg/dL)	79.0 (43.0, 87.0)	54.0 (52.0, 68.0)	0.37
Non-HDL cholesterol (mg/dL)	111.8 (33.7)	126.2 (51.3)	0.37
Albumin to creatinine ratio (24 hr, mg/g)	12.3 (7.3, 85.0)	9.8 (6.3, 35.9)	0.43
Albumin to creatinine ratio (overnight, mg/g)	9.0 (6.2, 34.7)	10.9 (7.4, 23.9)	1.00
Albumin to creatinine ratio (pre-clinic, mg/g)	16.2 (8.2, 67.3)	13.4 (9.7, 29.7)	0.41
Albuminuria (%, n)	33.3 (5)	26.7 (4)	1.00*
Serum albumin (g/dL)	4.2 (4.1, 4.5)	4.3 (4.1, 4.4)	0.83
Serum creatinine (mg/dL)	0.94 (0.78, 1.2)	0.93 (0.75, 1.1)	0.59
eGFR by CKD-Epi	77.4 (19.8)	81.2 (25.3)	0.65

* Fisher's exact test p-value

Preliminary results for kidney iron deposition.

The MRI results were corrupted for one participant and have not been used in the results below. Total kidney iron deposition did not differ by sex (not shown) or Hp (Table 2) but was higher in those with albuminuria (p=0.05). This association appeared confined to Hp 2-2 carriers as no difference in kidney iron deposition was seen by albuminuria with Hp 1-1 (Table 3).

Table 2. Kidney iron deposition (sec^{-1}) of participants of the KIRHA study by Haptoglobin genotype 1-1 vs. 2-2

	Hp 1-1 (n=15)	Hp 2-2 (n=15)	p-value	Age/sex adjusted p-value
Total Kidney iron (sec^{-1})	82.5 (8.2)	82.9 (8.8)	0.90	0.77
Cortex iron	48.7 (4.8)	48.7 (4.7)	0.98	0.74
Medullar iron	33.8 (4.2)	34.2 (4.9)	0.83	0.86

Table 3. Kidney iron deposition (sec^{-1}) by albuminuria status and Hp genotype

	Normoalbuminuria	Albuminuria	p-value
Hp 1-1 carriers	*n*=9	*n*=5	
Total kidney iron	81.4 (9.7)	84.5 (5.0)	0.51
Cortex	48.4 (5.6)	49.2 (3.5)	0.78
Medullar	33.0 (4.6)	35.4 (3.1)	0.33
Hp 2-2 carriers	*n*=11	*n*=4	
Total kidney iron	80.1 (8.2)	90.6 (5.9)	0.04
Cortex	47.3 (4.5)	52.6 (3.1)	0.05
Medullar	32.8 (4.8)	38.0 (3.5)	0.07

Data presented are mean (standard deviation)

No structural abnormalities of the kidneys were observed with the exception of small cysts. In addition, a horseshoe kidney was observed in an Hp 1-1 carrier who exhibited normal renal function.

Thus, our preliminary pilot results support the hypothesis of increased kidney iron deposition in carriers of the Hp 2-2 genotype (compared to Hp 1-1 carriers) among individuals with T1D and albuminuria. These results would need to be confirmed (upon a second assessment of kidney iron) in this study and larger cohorts.

Specific Aim 2. **Obtain preliminary data on blood/urine biomarkers of proximal tubular injury, oxidation and iron by Hp in T1D**. We hypothesize that biomarker levels will be increased in Hp 2-2 vs. Hp 1-1 carriers.

Results: Unfortunately, we have not yet received data on urinary biomarkers of renal tubular damage. However, data on blood biomarkers of iron suggested no differences by Hp genotype, with the exception of serum ferritin concentrations being non-significantly (due to the small sample size) higher among Hp 2-2, compared to Hp 1-1, carriers.

Table 4. Serum iron biomarkers by Haptoglobin genotype 1-1 vs. 2-2 in the KIRHA study

	Hp 1-1 (n=15)	Hp 2-2 (n=15)	p-value	Age/sex adjusted p-value
Serum iron (mcg/dL)	90.0 (67.0, 102.0)	97.0 (74.0, 118.0)	0.37	0.82
Total iron binding capacity (mcg/dL)	315 (284, 329)	294 (275, 336)	0.39	0.23
Amount of saturated transferrin (%)	31.4 (18.0)	33.9 (13.4)	0.67	0.69
Serum ferritin (ng/mL)	34.0 (28.0, 81.0)	84.0 (35.0, 139.0)	0.15	0.22

3. Publications:

As we currently only have preliminary data, there are no publications at present resulting from this project. However, an abstract has been submitted to the 77th Scientific Sessions of the American Diabetes Association:

Tina Costacou, Trevor J. Orchard, Chan-Hong Moon, Kyotang Bae, Linda Fried, Rhobert W. Evans. Magnetic Resonance Detection of Kidney Iron Deposition by Haptoglobin (Hp) Genotype in Type 1 Diabetes (T1D): Results from the KIRHA Study. Submitted to the 77th Scientific Sessions of the American Diabetes Association, June 9-13, 2017, San Diego.