Children's Hospital of Philadelphia

Annual Progress Report: 2010 Formula Grant

Reporting Period

July 1, 2014 – December 31, 2014

Formula Grant Overview

The Children's Hospital of Philadelphia received $3,548,977 for grant award period January 1, 2011 to December 31, 2014. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Magnetic Resonance Imaging and Neurocognitive Assessment in Chronic Kidney Disease - Utilizing state of the art neuroimaging technology, including standard magnetic resonance imaging (MRI), arterial spin labeling (ASL), resting state functional MRI (fMRI), and a concurrent matched control group in a cross-sectional study of 180 individuals, aged 8-25 years, we will test the hypothesis that subclinical vascular disease affects cognitive function in chronic kidney disease (CKD). Using novel pattern recognition methods, we will integrate these measures to develop a multi-parametric imaging phenotype in CKD that can identify high-risk groups. We hypothesize that level of kidney function, hypertension (HTN) and anemia affect resting cerebral blood flow (CBF) and functional performance. Achievement of our aims will enable targeted interventions to prevent and treat the cognitive deficits associated with CKD.

Duration of Project

1/1/2011 - 12/31/2014

Project Overview

To identify the causal mechanisms underlying the cognitive decline seen in CKD, we propose to use state of the art multimodal MRI and cognitive testing in a cross-sectional study of 90 subjects with Stage III, IV and V CKD (estimated GFR <90 ml/min/1.73m2) , aged 8 – 25 years, and 90 controls matched on age and socioeconomic status, using insurance status as a proxy. Our specific aims are:

1. To demonstrate differences in brain imaging between the group of individuals with CKD and age-matched controls, manifested by a higher prevalence of subcortical hyperintensities on T2-weighted (FLAIR) MRI and reduction in ASL signal in subjects with CKD. We hypothesize that HTN and anemia in CKD cause alterations in CBF measured using ASL perfusion MRI, as well

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as hypoxic-ischemic injury in the brain, which can be measured via multi-parametric MRI (combination of FLAIR, T2, Proton density and T1-weighted images).

2. To explore the impact of CKD on structural brain integrity as measured by anatomic MRI with quantitative volumetric measurements with a particular focus on prefrontal cortex and frontotemporal regions. We hypothesize that evidence of frontotemporal brain injury will be correlated with deficits in executive function in subjects with kidney disease.

3. To characterize the impact of longer duration and increased severity of CKD, HTN and anemia on neurocognitive abilities, and correlate these with fMRI findings. We hypothesize that cognitive deficits will be correlated with changes in regional CBF and/or connectivity within specific brain networks supporting these neurocognitive functions.

4. To develop individual-patient biomarkers by combining structural, ASL and fMRI data in multi-parametric classification. Our goal is to ultimately find imaging patterns that in future studies can predict high risk of neurocognitive decline and thereby identify subjects for targeted interventions.

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Expected Research Outcomes and Benefits

We will identify differences in brain structure and functional performance between CKD and control group children and young adults. Structural MRI (sMRI) will assess the regional distribution of grey matter, white matter and cerebrospinal fluid (CSF), which will determine whether there are correlations between CKD related clinical variables, brain atrophy and cognitive performance, and whether or not these relationships are spatially heterogeneous. Structural MRI will provide measurements of the regional distribution of cerebrovascular disease (CVD), as HTN is exceedingly common in CKD and is one of the highest risk factors for CVD.

Additionally, ASL MRI will provide measurements of regional blood perfusion, which will provide mechanistic insights into the pathogenesis of observed structural changes based on correlations between CKD-related clinical variables, brain atrophy and cognitive performance, and whether or not these relationships are spatially heterogeneous. Using both ASL MRI and resting state fMRI we will examine brain activity in the absence of external stimuli and this will allow us to evaluate functional changes in these patients that correlate with clinical and cognitive variables. State of the art pattern recognition methods will allow us to integrate all these types of measurements and develop a multi-parametric imaging phenotype in CKD.

This phenotype can be used to predict subsequent cognitive decline in future longitudinal studies so that interventions can be implemented to improve functional performance in this population. We are studying children and young adults with CKD, as this will allow us to identify neurocognitive and neuroanatomic changes due to kidney disease in the absence of underlying CVD, which is ubiquitous in adults with CKD. The long term benefit of this project will be improvement in the health status of the 1 in 9 Americans with CKD, as cognitive impairment may affect adherence to the complex medical regimens which these patients are routinely prescribed, and may contribute substantially to decreasing the adverse outcomes and substantial cost associated with end stage renal disease (>$36 billion in 2007).

Summary of Research Completed

Methods and Results: Enrollment is complete, with 92 Chronic Kidney Disease (CKD**)** subjects (3% on dialysis, 26% with transplant) and 70 controls have been enrolled. Twenty additional study visits (repeated measures) were obtained, yielding a total of 182 person visits. Data presented in this report shows data analyzed as of 12.31.14. We present effect sizes (Cohen's d = difference of the means/average standard deviation) rather than statistical significance as data analysis is in progress. CKD participants are 65% Male, 65% African American (AA), with median age: 15.7yrs; compared to controls: 55% Male, 59% AA, median age: 15.6 years. The most significant laboratory differences between CKD and controls (effect size ≥0.8) are seen in estimated glomerular filtration rate (eGFR) (CKD 47.85 ± 24.4 vs. 97.9 ± 19.8 ml/min/1.73m^2), blood urea nitrogen (28.9 ± 17.4 vs. 12.7 ± 16.2 mg/dL) hemoglobin (CKD 12.6 ± 1.8 vs. 14.0 ± 1.4 g/dL), hematocrit (CKD 37.7 ± 5.0 vs. 41.5 ± 3.9 %), and triglycerides (CKD 133.2 ± 69.2 vs. 84.1 ± 41.4 mg/dL). 80 CKD and 57 Controls had valid ambulatory blood pressure monitor (ABPM) studies, with greatest differences shown in sleeping and total diastolic loads, sleeping and total diastolic indices, and diastolic dipping, with effect sizes ≥0.5 SD. (Table 1). A longitudinal arm was also added to look for within person changes related to significant change in kidney function (received a transplant, started dialysis, or had ≥30% improvement or decrease in kidney function since baseline). 10 CKD participants with significant change (6 improved due to transplant, 4 worsened) and 10 CKD Controls (≤30% change in eGFR since baseline) repeated all study measures ≥6 months after baseline. Analysis of this longitudinal data is preliminary and is not presented in this report.

Traditional and Computerized Neurocognitive (NC) Assessment (Table 2): 91 CKD and 70 Controls had complete data from the traditional NC assessment. Relevant tests were grouped into

domains of Language, Visual Spatial, Verbal Memory, Visual Memory, Verbal Working Memory, Visual Working Memory, Attention, Set Shift, Inhibitory Control, Problem Solving, and Depression. Age adjusted t-scores were converted to z-scores. For domains defined by multiple tests, the median z-score of tests in that domain was used. CKD patients performed worse than controls (lower z scores) with an effect size of ≥ 0.5 in Visual Spatial, Verbal Memory, and Attention. 92 CKD and 70 Controls completed the Computerized NC Battery. CKD participants scored worse in accuracy (lower z scores) with an effect size of ≥ 0.5 in Language Reasoning, Nonverbal Reasoning, and Spatial Processing. Domains of significance are presented in Table 2.

The Behavior Rating Inventory of Executive Function (BRIEF completed by parents for ≤ 18 years, BRIEF-Adult self-report for >18 years) was used to assess facets of executive function using parent and self-ratings. There are 90 individuals with CKD who have completed BRIEFs and 69 Controls. Efforts this year were devoted to examining whether the data from the two versions (BRIEF and BRIEF-A) could be combined or whether the version of the BRIEF needed to be controlled for in the analyses. These initial efforts showed group x BRIEF version interactions in Behavior Regulation ($p < .07$), Metacognition ($p < .01$), and the Global Executive Composite ($p < .01$). In general, the parents of children with CKD tended to rate their children as lower on various executive functions than the young adults with CKD rated themselves. This was particularly evident on the executive functions scales of Initiate ($p < .01$), Working Memory ($p < .0001$), Plan/Organize ($p < .0001$), Monitoring ($p < .003$), and Organization ($p < .002$). When examining these differences, the effect sizes fell within the medium to large range, but only the parents' ratings of Metacognition fell within the clinically significant range (Mean = 60.26, SD = 12.03). Significant results for the BRIEF are presented in Table 3. No BRIEF-A scores were statistically significant when examining group differences.

Neuroimaging Data: *Arterial Spin Labeling (ASL) magnetic resonance imaging (MRI) and cardiac-induced modulation of BOLD signal.* This study systematically investigated the pathophysiological effects on the changes of brain function in pediatric patients with CKD using both quantitative cerebral blood flow (CBF) measured using arterial spin labeling (ASL) and cardiac-induced modulation of blood-oxygen-level dependent (BOLD) signal, correlating with findings of physiological measurements for those patients. ASL MRI analysis of CBF was completed for 73 CKD and 57 Controls. Marked changes were found in CBF in the CKD group, including increased CBF in global brain areas affecting both gray and white matter (Fig.1 and Table 4). The expected effects of age, gender and other physiological factors on CBF were seen in controls, but abnormalities were found in CKD cohort, which deviated from the expected effects (Table 5). Hematocrit (HCT) is a known modulator of CBF, and HCT-correlated CBF changes were responsible most of the group differences in CBF, with the CKD group having lower HCT levels and hence higher CBF. However, additional changes in both CBF and cardiac-modulated BOLD signal were observed that likely reflect disturbed CBF autoregulation. Patients with CKD showed both increased blood pressure and increased CBF, suggesting that the autoregulatory system may compensate for decreased oxygen delivery due to lower HCT levels in the CKD group by increasing CBF. BOLD fMRI data with concurrent pulse oximetric monitoring were analyzed in 45 CKD patients and 42 controls using Retrospective Image

Correction software to derive cardiac phase regressors in the BOLD signal. Patients with CKD showed marked cerebral abnormalities of physiology-related signal variance on BOLD signals, with a focal increase of cardiac modulation of resting BOLD signal in the ventromedial prefrontal cortex (vmPFC), hippocampus, amygdala and posterior cingulate cortex (PCC), that colocalized with the brain areas showing the most significant increased CBF (Fig.1 and 2), and could not be explained by HCT differences between groups. As these brain regions are key structures in the autonomic nervous system (ANS) that control respiration, heart rate and blood pressure, the observed changes in cardiac-correlated BOLD in CKD may be attributed to a focal disturbance of cerebrovascular autoregulation as reflected in the increased CBF and blood pressure, or may also represent the neural correlates of altered cardiovascular function. Regional changes of brain function observed in vmPFC, hippocampus, amygdale and PCC as shown in CBF and BOLD modulation signals may underlie some of the cognitive dysfunction observed in CKD.

Functional MRI: 68 CKD and 58 Controls have successfully completed resting state fMRI scanning (i.e., artifact- and motion-free data). Multiple regression-based connectivity analysis, whereby time series data from a seed region is correlated separately with every intracerebral voxel, was implemented for each individual. Multiple regression incorporates a 36-parameter nuisance regression model recently shown to be comparable to "scrubbing" (removal of motion volumes) in reducing spurious motion-driven connectivity effects. The resulting statistical maps are then used as the dependent variable in analyses of group effects. The most noteworthy fMRI connectivity results to date come from a constellation of brain structures called the Default Mode Network (DMN), encompassing posterior cingulate, lateral prefrontal, medial prefrontal, and parietal cortices. The intact functioning of DMN has been associated with the ability to meet ongoing attentional and information processing demands. Conversely, diminished DMN is associated with attentional deficits in multiple psychiatric populations, including Attention Deficit/Hyperactivity Disorder and Alzheimer's disease. A standard approach to isolating DMN connectivity on a per-subject basis is to carry out the aforementioned voxel-wise multiple regression procedure using a seed region within posterior cingulate cortex. In this dataset, this procedure elicited robust DMN connectivity from both CKD and control groups; however, the CKD group shows significantly decreased connectivity relative to controls in medial and lateral prefrontal cortex (specifically, anterior cingulate and dorsolateral prefrontal cortex). Furthermore, within the CKD group, disease duration is significantly correlated with decreased PCC/lateral prefrontal cortex connectivity, and eGFR is significantly positively correlated with PCC/medial prefrontal connectivity. Overall, these data indicate that attentional deficits found in CKD are associated with abnormal connectivity within a major attentional network (DMN). A publication manuscript on these results is currently in preparation.

Structural MRI: An automated pipeline for processing structural images was applied on the complete NICK dataset. The pipeline included: multi-atlas brain extraction, inhomogeneity correction and tissue segmentation, calculation of tissue density maps (Regional Analysis of Volumes Examined in Normalized Space [RAVENS] maps) in template space, white matter lesion segmentation, and segmentation into anatomic regions of interest (ROIs) using a multi-atlas label fusion method. Volumetric measurements for normal and abnormal (lesion) tissues

were calculated in each ROI. A voxel based morphometry type analysis was performed to detect spatial patterns of group differences between CKD group and controls, as well as patterns associated with main clinical variables such as eGFR and hematocrit. Statistical parametric maps of group differences on GM and WM RAVENS maps (i.e. tissue density at each voxel) were calculated both using the more traditional VBM approach, and optimally-discriminative voxel-based analysis. A multi-variate regression model was applied to investigate the relationship between volumetric measurements in specific anatomical regions and clinical variables. The control group showed better preservation of brain tissue, while CKD group showed cortical atrophy and larger ventricles.

Milestones: During this period, 3 of 3 milestones were met:

1) *Final analysis of data obtained:* All data has now been collected and analysis is either complete or ongoing.
2) *Write manuscripts and submit for publication:* Manuscripts from all imaging and neurocognitive groups have been written and submitted.
3) *Disseminate research findings:* In addition to publications, research findings have been disseminated via conference presentations and published abstracts.

Publications and Abstracts:

- Hartung E, Kim J, Laney N, et al: A Computerized Neurocognitive Battery Reveals Deficits in Executive Control and Complex Cognition in Children and Young Adults with Chronic Kidney Disease [Abstract]. J Am Soc Nephrol 25, 2014: Page 251A.
- Laney N, Hooper S, Radcliffe J, et al: Elevated Blood Pressure Index and Decreased Nocturnal Dip are Risk Factors for Cognitive Dysfunction in Children and Young Adults [Abstract]. J Am Soc Nephrol 25, 2014: Page 24A.
- Ruebner R, Laney N, Kim J: Children and Young Adults with Chronic Kidney Disease Have Neurocognitive Deficits Compared to Healthy Controls [Abstract]. J Am Soc Nephrol 25, 2014: Page 51A.

Table 1: Ambulatory Blood Pressure Monitor Data **n = 80 CKD 57 Controls**

		Load %			**Index**			
		Waking	Sleeping	Total	Waking	Sleeping	Total	**Dipping %**
Systolic	**CKD**	23 ± 2	25 ± 3	24 ± 2	0.9 ± 0.08	0.9 ± 0.09	0.9 ± 0.08	11 ± 6
	Control	16 ± 2	15 ± 2	16 ± 2	0.9 ± 0.09	0.9 ± 0.08	0.9 ± 0.08	13 ± 5
	Effect Size	d=0.3	d=0.4	d=0.4	d=0.3	d=0.4	d=0.3	d=0.2
Diastolic	**CKD**	17 ± 17	23 ± 24	19 ± 18	0.9 ± 0.09	0.9 ± 0.12	0.9 ± 0.09	18 ± 7
	Control	11 ± 13	10 ± 14	11 ± 11	0.8 ± 0.07	0.8 ± 0.08	0.8 ± 0.07	21 ± 6
	Effect Size	d=0.4	**d=0.7**	**d=0.6**	d=0.4	**d=0.6**	**d=0.5**	**d=0.5**

Load: Percentage of readings ≥ 95th %ile, Normal <25%; Intermediate 25 – 50%; Abnormal >50%
Index: Mean ambulatory BP, an index > 1.0 is considered hypertensive **Reference values for Dipping:** Normal ≥ 10%

Table 2: Traditional and Computerized Neurocognitive (NC) Assessment **n = 91 CKD 70 Controls**

Traditional NC Assessment Domains	**CKD (z-scores ± SD)**	**Control (z-scores ± SD)**	**Effect Size**
Attention	-0.18 ± 0.6	0.2 ± 0.6	d = 0.5
Verbal Memory	0.3 ± 1.6	1.1 ± 1.9	d = 0.5
Visual Spatial	-0.3 ± 0.9	0.1 ± 0.8	d = 0.5
Computerized NC Assessment (Accuracy)			
Language Reasoning	-0.7 ± 1.1	-0.2 ± 0.9	d = 0.5
Nonverbal Reasoning	-0.4 ± 0.9	-0.01 ± 1.0	d = 0.5
Spatial Processing	-0.4 ± 1.0	0.1 ± 1.1	d = 0.5

Table 3: BRIEF scores (t-scores ± SD)

	BRIEF Parent Form		
	CKD (n = 65) (t-scores ± SD)	**Control (n = 50)** (t-scores ± SD)	**P value**
Initiate	57.15 ± 11.07	51.72 ± 11.36	0.011
Working Memory	62.02 ± 13.66	52.52 ± 11.10	0.0001
Plan/Organize	59.88 ± 13.26	50.80 ± 10.58	0.0001
Organization of Materials	56.06 ± 10.49	49.84 ± 10.08	0.002
Monitor	57.29 ± 11.58	50.74 ± 10.96	0.003
Metacognition Index	60.26 ± 12.03	51.46 ± 11.17	0.0001
Global Executive Composite	58.71 ± 11.54	51.50 ± 11.96	0.002

Table 4: Group comparison of CBF between CKD and control subjects. Data are presented as mean±std and P values. *P<0.05.

CBF (ml/100g/min)	**GM**	**WM**	**Global**
CKD	75.76 ± 13.40	34.31 ± 5.89	64.14 ± 10.88
Control	67.79 ± 11.69	31.38 ± 5.54	57.55 ± 9.55
P-value	**0.0004***	**0.004***	**0.0003***

Table 5: Correlations for univariate associations between CBF and other clinical variables in CKD and subjects. Data are presented as correlation coefficients and P values. *P<0.05.

Rho	CKD			Control		
p-value	GM-CBF	WM-CBF	Global-CBF	GM-CBF	WM-CBF	Global-CBF
Age	-0.506 **<0.0005***	-0.301 **0.01***	-0.535 **<0.0005***	-0.460 **<0.0005***	-0.469 **<0.0005***	-0.527 **<0.0005***
Gender	0.058 0.629	-0.052 0.662	0.041 0.73	0.354 **0.007***	0.301 **0.023***	0.382 **0.003***
Hct	-0.626 **<0.0005***	-0.618 **<0.0005***	-0.643 **<0.0005***	-0.681 **<0.0005***	-0.689 **<0.0005***	-0.715 **<0.0005***
eGFR	-0.233 **0.047***	-0.149 0.207	-0.224 0.057	0.584 **<0.0005***	0.356 **0.007***	0.555 **<0.0005***
Calcium × phos	0.315 **0.008***	0.243 **0.043***	0.339 **0.004***	0.180 0.185	0.146 0.283	0.229 0.089

Figure 1. Voxel-wise group comparison in CBF. The figure shows the contrast CKD > control subjects. Color bar indicates t scores. FWE cluster corrected P<0.0005.

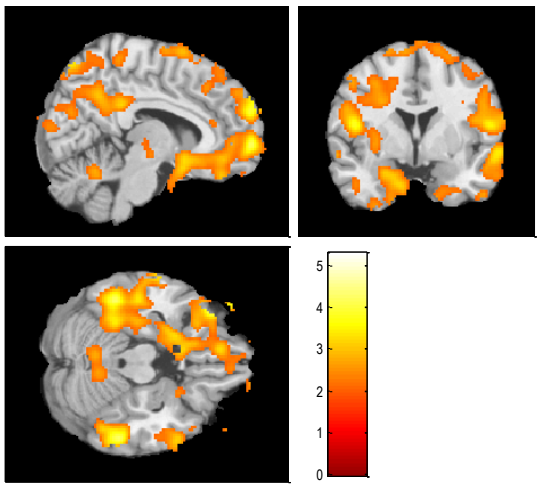

Fig. 2. Group difference of cardiac-induced modulation of BOLD signal. The figure shows the contrast CKD > control subjects. Color bar indicates t scores. FWE Cluster corrected P<0.001.