Children's Hospital of Philadelphia

Annual Progress Report: 2010 Formula Grant

Reporting Period

July 1, 2013 – June 30, 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.

Anticipated 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 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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Stephen Hooper, PhD –employed by University of North Carolina

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: To date, 92 Chronic Kidney Disease (CKD**)** subjects (4% on dialysis, 26% with transplant, 70% estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73 m^2), and 62 controls have been enrolled. Data presented in this report shows data analyzed as of 6.20.14. Some enrolled participants have not completed visits; data from some visits has not yet been analyzed, so sample sizes vary. We present effect sizes (mean CKD- Mean control)/standard deviation (SD) (Mean CKD – Mean Control) rather than statistical significance as data collection is not yet complete. CKD participants are 65% Male, 25% African American (AA), with median age: 15yrs; compared to controls: 53% Male, 43% AA, median age: 15 years. Recruitment of controls lags to enable matching on age, gender, and SES. The most significant laboratory differences between CKD and controls are seen in kidney function, acidosis and anemia: estimated GFR (CKD 48.69 ± 23.6 vs. 100.84 ± 20.4 ml/min/1.73m^2), serum bicarbonate (CKD 25.19 ± 3.4 vs. 26.75 ± 1.9 meq/L), hemoglobin (CKD 12.62 ± 1.8 vs. 13.91 ± 1.4 mg/dl). Subjects with CKD also show higher blood pressure, with greatest differences shown in ambulatory blood pressure monitoring (ABPM) readings in total diastolic index, sleeping diastolic index, total diastolic load, waking diastolic load, sleeping diastolic load, and diastolic dipping, with effect sizes ≥0.5 SD. (Table 1)

Traditional and Computerized Neurocognitive (NC) Assessment (Table 2): 76 CKD and 55 Controls completed 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 test results were converted to z-scores. For domains defined by multiple tests, the median z-score of tests in that domain was used as a summary measure. CKD patients performed worse than controls (indicated by lower z scores) with medium effect sizes ranging from 0.3 to 0.5 SD in Attention, Verbal Memory, Verbal Working Memory, Visual Spatial, and Inhibitory Control. 74 CKD and 48 Controls completed the 1 hour Computerized NC Battery. Domains where CKD participants performed worse in accuracy (with lower z scores), with an effect size ≥ 0.5 SD were Attention, Language Reasoning, and Nonverbal Reasoning. Domains of significance are presented in Table 2. The Behavior Rating Inventory of Executive Function (BRIEF for ≤ 18 years, BRIEF-Adult for >18 years) was used to assess facets of executive

function using parent and self-ratings. CKD participants showed relative weaknesses (indicated by higher z-scores) in the Working Memory, Plan/Organize, Organization of Materials, and Monitor subtests of the Metacognition Index, as well as the General Executive Composite overall score, with effect sizes $\geq$ 0.5 SD. Significant results are presented in Table 3. For ages older than 18, self-ratings on the BRIEF-A revealed little in the way of significant differences between groups, but that could be due to the small sample size in this age group.

Neuroimaging Data: *Arterial Spin Labeling (ASL) magnetic resonance imaging (MRI)* analysis of cerebral blood flow (CBF) was completed for 50 CKD and 39 Controls. The CKD group had increased CBF in global brain areas affecting both gray matter (GM) and white matter (WM) (Table 4). Age, gender, and other physiological factors had significant effects on CBF, and abnormalities in CBF were found in the CKD group, which likely reflect disturbed autoregulation and developmental changes in regional brain function (Table 5). Hematocrit, a risk factor for CBF changes, seems to be driving most of the group differences in CBF, with the CKD group having lower hematocrit levels and hence higher CBF. While the cause is not yet known, it could be due to the autoregulatory system compensating for decreased oxygen delivery due to lower hematocrit levels in the CKD group by increasing CBF. Blood level oxygen-dependent (BOLD) functional MRI (fMRI) time series data include a variety of physiological "noise" sources, including the effects of gross motion and cardiac and respiratory activity. These effects are often discarded to enhance sensitivity to task or resting state effects, however the same procedures used to discard these effects can conversely be used to explore differences in BOLD signal modulation that may reflect pathophysiological differences between CKD and controls. BOLD fMRI data with concurrent pulse oximetric monitoring was analyzed in 38 CKD patients and 29 controls using Retrospective Image Correction (RETROICOR) software to derive cardiac phase regressors in the BOLD signal. Group effects were assessed while controlling for group differences in hematocrit, a major modulator of CBF. Subjects with CKD demonstrated focal increases in cardiac modulation of BOLD signal in the orbitofrontal cortex, hippocampus, and amygdala. These effects cannot be explained based purely on group differences in CBF, blood pressure, pulse, or hematocrit. The physiological basis for these group differences are not yet known, but might reflect focal alterations in cerebrovascular autoregulation or conceivably the neural correlates of altered cardiovascular function.

Functional MRI: The sample of participants who have successfully completed resting state fMRI scanning (i.e., artifact- and motion-free data) has now reached 56 for the CKD group and 32 for the control group. This past year more robust nuisance regression procedures were implemented - in particular, the use of a 36-parameter nuisance regression model that was recently shown to be comparable to "scrubbing" (removal of motion volumes) in reducing spurious motion-driven connectivity effects. The general approach has been to implement multiple regression-based connectivity analysis whereby time series data from seed regions are correlated separately with every intracerebral voxel, for each individual. The resulting statistical maps are then used as the dependent variable in analyses of group effects (i.e., Student's t-tests [t-tests]).

Analyses have focused on brain networks involved in language and executive function - two domains in which the CKD sample shows significant deficits. The overall pattern in the resting state fMRI data involves significant over- as well as underconnectivity in the CKD sample relative to controls. Interestingly, the CKD group showed increased connectivity between two

key language regions: Wernicke's and Broca's areas. The CKD group also showed increased connectivity between left medial temporal areas (involved in language processing) and medial frontal areas. In order to examine connectivity among executive function areas, seeds from Shirer et al.'s executive control network were used. Results from these analyses included increased connectivity among PFC structures in CKD (in particular, right middle frontal gyrus and large portions of right prefrontal cortex), but decreased connectivity between the frontal poles and contralateral portions of insula. The overall pattern indicates diminished efficiency within brain areas implementing executive function - overconnectivity among PFC regions but diminished communication with more distant cognitive control structures. Furthermore, the pattern of contralateral deficit in frontal pole/insula connectivity suggests deficits in intrahemispheric communication. Next steps in this line of analyses include graph theoretical analyses to examine global patterns of connectivity differences among individuals with CKD.

Structural MRI: An automated pipeline was applied for processing structural images, including: multi-atlas brain extraction, inhomogeneity correction and tissue segmentation, calculation of tissue density maps (Regional Analysis of Volumes Examined in Normalized Space [RAVENS maps]), WM 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. All images were verified for quality using both visual inspection and automated tools. The distribution of tissue volumes in single ROIs as well as in larger anatomical regions are verified in comparison to reference values reported in previous studies (Figure 1). A series of pattern analysis methods were applied on RAVENS maps and volumetric measurements to detect regional patterns of structural brain changes related to clinical variables. a) Pattern analysis of group differences between CKD group and controls: Statistical parametric maps of group differences on GM and WM RAVENS (i.e., tissue density at each voxel) are calculated both using the more traditional voxel-based morphometry approach, and optimally-discriminative voxel-based analysis (ODVBA). b) Classification of CKD vs. controls: A supervised method using machine learning techniques is applied to learn a classifier that discriminates the two groups of subjects. The method is independently applied on imaging data from RAVENS maps and the lower dimensional volumetric data. The highest classification accuracy (ACC) was ACC=0.66, and was obtained using the combination of GM and WM RAVENS maps. Four ROIs were found to have small but significant volume differences: the third ventricle, the right lateral ventricle, and the left basal forebrain were larger in CKD, and the left post-central gyrus larger in controls. c) Calculation of Brain development index (BDI): A predictive model of age is trained using ROI volumes of typically developing subjects from the Philadelphia Neurodevelopmental Cohort (PNC) study (n=621). The model is applied on all CKD subjects to predict the BDI. A prediction accuracy (using Pearson Correlation Coefficient [r]) of r=0.67 was obtained. The actual ages and predicted BDIs are shown in Figure 2 for CKD and control subjects. No significant group differences were detected for the deviation of the BDI from the actual age.

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

1) *Ongoing recruitment of subjects, assess progress on meeting recruitment goals*: 92 CKD subjects and 62 Controls are currently enrolled with scheduled study visits.
2) *Submit abstracts of data obtained to national meetings*: In total, 8 abstracts have been submitted to national conferences: 3 are pending acceptance and 4 were previously accepted

for presentation. One review manuscript has already been published.

3) *Prepare proposals for additional grant funding based on data*: The investigators are currently working on proposals for additional grant funding and plan to apply before the end of this grant.

Publications and Abstracts:

- Moodalbail DG, Reiser KA, Detre JA, Schultz RT, Herrington JD, Davatzikos C, Doshi JJ, Erus G, Liu HS, Radcliffe J, Furth SL, Hooper SR. Systematic Review of structural and functional neuroimaging findings in children and adults with CKD. Clin J Am Soc Nephrol. 2013 Aug;8(8):1429-48. doi: 10.2215/CJN.11601112.
- Moodalbail D, Laney N, Gur R, et al. Cognitive Performance on a Computerized Neurocognitive Battery in Children and Young Adults with CKD. Poster presented at: American Society of Nephrology Kidney Week Conference; November 5-10, 2013; Atlanta, Georgia. Abstract publication: *J Am Soc Nephrol* 2013, Vol. 24, Supp. 1, pp 663A.
- Liu H, Jawad A, Laney N, et al. Alterations in Cardiac-Correlated Brain BOLD Signal in Children with Chronic Kidney Disease. E-Poster presented at: International Society for Magnetic Resonance in Medicine Conference; May 10-16, 2014; Milan, Italy.

Table 1: Ambulatory Blood Pressure Monitor Data

	CKD (n = 65)				**Controls (n = 39)**			
	Systolic		**Diastolic**		**Systolic**		**Diastolic**	
	% Load	**Index**	**% Load**	**Index**	**% Load**	**Index**	**% Load**	**Index**
Waking	23.40 ± 22.77	0.92 ± 0.08	16.89 ± 17.06	0.87 ± 0.09	15.00 ± 19.00	0.90 ± 0.08	9.77 ± 8.43	0.84 ± 0.07
Sleeping	25.04 ± 28.94	0.93 ± 0.09	24.68 ± 24.92	0.91 ± 0.12	14.71 ± 16.77	0.90 ± 0.06	10.52 ± 12.22	0.84 ± 0.08
Total	24.03 ± 23.62	0.92 ± 0.08	20.03 ± 18.86	0.89 ± 0.09	15.01 ± 17.25	0.90 ± 0.07	10.02 ± 8.63	0.84 ± 0.07
Dipping	11.48% ± 6.12%		17.38% ± 7.34%		12.10% ± 5.03%		20.57% ± 6.49%	

Load: Percentage of readings $\geq$ 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 $\geq$ 10%

Table 2: Traditional and Computerized Neurocognitive (NC) Assessment

Traditional NC Assessment Domains	**CKD (n = 76) (z-scores ± SD)**	**Control (n = 55) (z-scores ± SD)**
Attention	-0.22 ± 0.70	0.06 ± 0.60
Verbal Memory	0.21 ± 1.59	0.82 ± 1.75
Verbal Working Memory	0.10 ± 1.25	0.54 ± 1.43
Visual Spatial	-0.26 ± 0.81	0.03 ± 0.87
Inhibitory Control	-0.12 ± 0.57	0.09 ± 0.53
Computerized NC Assessment (Accuracy Component)		
Language Reasoning	-0.72 ± 1.15	-0.21 ± 0.89
Nonverbal Reasoning	-0.43 ± 0.89	0.03 ± 0.97
Attention	-0.31 ± 1.06	0.19 ± 0.66

Table 3: BRIEF scores (t-scores ± SD)

	BRIEF Parent Form	
	CKD (n = 54) (t-scores ± SD)	**Control (n = 35)** (t-scores ± SD)
Working Memory	1.23 ± 1.38	0.42 ± 1.10
Plan/Organize	0.98 ± 1.36	0.08 ± 1.06
Organization of Materials	0.66 ± 1.04	-0.08 ± 1.09
Global Executive Composite	0.91 ± 1.15	0.21 ± 1.23

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	78.45 ± 12.08	34.25 ± 5.5	66.13 ± 10.04
Control	69.96 ± 11.11	31.73 ± 5.67	59.20 ± 9.17
P-value	**0.0009***	**0.038***	**0.001***

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	-0.542 **<0.0005***	-0.328 **0.02***	-0.569 **<0.0005***	-0.469 **0.003***	-0.451 **0.004***	-0.517 **0.001***
Gender	-0.054 0.709	0.104 0.473	-0.019 0.896	0.416 **0.008***	0.439 **0.005***	0.448 **0.004***
Age of onset	-0.574 **0.001***	-0.346 0.056	-0.563 **0.001***			
Hct	-0.653 **<0.0005***	-0.704 **<0.0005***	-0.670 **<0.0005***	-0.691 **<0.0005***	-0.744 **<0.0005***	-0.723 **<0.0005***
Hb	-0.614 **<0.0005***	-0.647 **<0.0005***	-0.622 **<0.0005***	-0.668 **<0.0005***	-0.712 **<0.0005***	-0.698 **<0.0005***
eGFR	-0.168 0.245	-0.017 0.908	-0.121 0.401	0.592 **<0.0005***	0.374 **0.019***	0.519 **0.001***
Calcium × phos	0.345 **0.016***	0.261 0.073	0.361 **0.012***	0.112 0.496	0.115 0.485	0.150 0.362

Figure 1: Gray matter (GM, left) and white matter (WM, right) volumes of CKD subjects plotted against age. Globally, the WM volume increases with age while the GM volume decreases, as a result of both progressive myelination and regressive pruning processes, as previously reported.

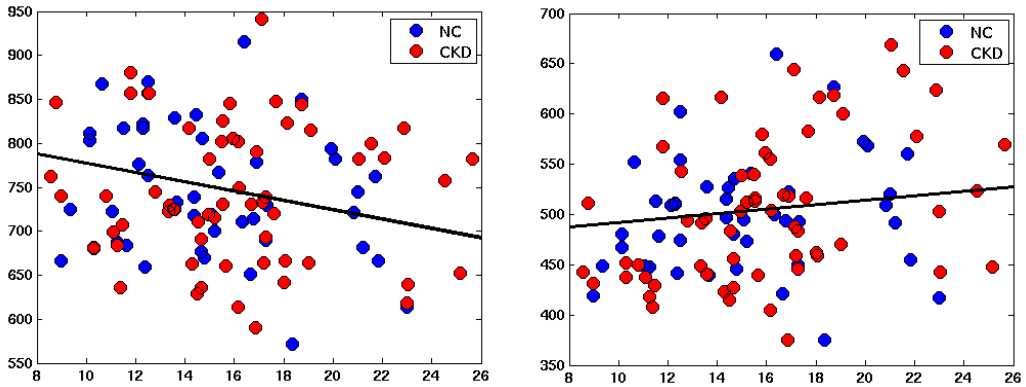

Figure 2: Structural MRI Age Prediction