The Role of the Renin-Angiotension System in Pediatric Essential Hypertension
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Background
Pediatric essential hypertension is increasingly common, occurring in 5-10% of normal-weight children and up to 25% of children with obesity. It is a risk factor for adult cardiovascular and renal disease. But even during childhood, hypertension is associated with significant morbidity, including cognitive impairment and organ damage. In the heart and kidneys, this organ damage is characterized by thickened heart muscle (left ventricular hypertrophy) and spillage of protein in the urine (albuminuria). Obese children are also at risk for fatty liver disease. However, the cause of pediatric essential hypertension, the role of obesity, and the mechanisms behind heart and kidney injury are poorly understood. Due to these limitations, there are no first-line medications, and treatment is often inadequate. An altered renin-angiotensin system may cause pediatric essential hypertension and organ damage. Evidence suggests uric acid, FGF23, and obesity play a role in renin-angiotensin system-mediated injury. An improved comprehension of the pathophysiology of pediatric essential hypertension could enhance clinical care by targeting treatment to the cause of disease and informing novel measures of organ damage.

Specific Aims
The **goal** of this proposal is to begin to elucidate the origins of pediatric essential hypertension and determine how it causes cardiac and renal disease. Our **primary hypothesis** is that an altered renin-angiotensin system leads to the development of pediatric essential hypertension and organ damage in the heart and kidney, specifically left ventricular hypertrophy and albuminuria. We postulate that a relative increase in angiotensin (Ang) II tone compared to Ang-(1-7) tone in the circulation and the kidney (measured in the plasma and urine, respectively) leads to disease. Our **secondary hypotheses** are that abnormalities in renin-angiotensin system tone are related to higher uric acid and FGF23 and, with concurrent obesity, contribute to nonalcoholic fatty liver disease. We propose to test our hypotheses by recruiting 100 subjects aged 5-17 years who are referred for a new diagnosis of pediatric essential hypertension to the Pediatric Nephrology clinic at Brenner Children's Hospital. Our **Specific Aims** are to:

Aim 1: Determine the associations among the Ang II:Ang-(1-7) ratio, blood pressure, left ventricular hypertrophy, and albuminuria at baseline and after 12 months of treatment in incident cases of pediatric essential hypertension.
Hypothesis 1: *An increased ratio of Ang II to Ang-(1-7) is associated with higher blood pressure, left ventricular hypertrophy, and albuminuria at baseline and at 12 months.*

Aim 2: Investigate the relationships between the Ang II:Ang-(1-7) ratio and additional markers of cardiovascular and renal disease including uric acid and FGF23 to further elucidate the mechanisms behind the renin-angiotensin system's role in organ damage.
Hypothesis 2: *An increased ratio of Ang II to Ang-(1-7) is correlated with uric acid and FGF23.*

Aim 3: Assess the association between the Ang II:Ang-(1-7) ratio and nonalcoholic fatty liver disease, defined by elevated liver enzymes and hepatic fat infiltration on ultrasound, to investigate the role of the renin-angiotensin system in obesity-associated organ damage.
Hypothesis 3: *An increased ratio of Ang II to Ang-(1-7) is associated with elevated aspartate aminotransferase and alanine aminotransferase and hepatic fat infiltration on ultrasound.*

Study Design
Primary Study Subjects - Subjects between the ages of 5 and 17 years who are referred for a new diagnosis of hypertension will be recruited over one year from the tertiary care Pediatric

Nephrology clinic at Brenner Children's Hospital. We will obtain parental consent and subject assent. Inclusion criteria include a diagnosis confirmed with three separate blood pressure measurements ≥95%ile for age, sex, and height. Exclusion criteria include secondary causes such as renovascular hypertension and renal disease. The clinic sees approximately 20 new hypertension patients a month, and we anticipate enrolling 100 patients within the one-year study period. The target population is diverse (approximately 40% black, 40% white, and 20% Hispanic), predominantly male (60%), and 50% obese. Each subject will undergo a thorough evaluation per the standard of care. After baseline study assessment, subjects will be started on an antihypertensive medication per the standard of care by an independent clinician not involved in the study. Subjects will be seen in the clinic every three months per standard of care to obtain labs, monitor their blood pressure, and adjust their medication (see **Figure 1** below). Subjects will be evaluated at 12 months with repeat measurements of the outcomes.

Control Arm 1 – Ten healthy normotensive subjects between the ages of 5 and 17 years will be recruited from a general pediatrics clinic (Cornerstone Pediatrics, part of the Wake Forest Baptist Health system) to obtain samples for measurements of the following outcomes for comparison with the hypertensive participants over the same age range: left ventricular hypertrophy on echocardiograms, urine albumin, uric acid in the blood, FGF23 in the blood, and klotho in the blood and urine, as well as the predictors angiotensin II and angiotensin-(1-7) measured in the blood and urine.

Control Arm 2 – Fifty normotensive subjects with obesity between the ages of 5 and 17 years will be recruited from the Brenner FIT program or the Gastroenterology clinic under the direction of my collaborator Dr. Joseph Skelton, who is an associate professor of Pediatric Gastroenterology and the Director of Brenner FIT. Eligible patients will be approached about the study by their physician. Control Arm 2 subjects will undergo measurement of the following outcomes: fatty liver disease on ultrasound, liver function testing (AST, ALT), and uric acid in the blood, all of which are obtained as standard of care. They will also undergo collection of angiotensin ll and angiotensin –(1-7) in the blood and urine at the same time as clinical samples are obtained.

We will obtain parental consent and subject assent for all control subjects. Exclusion criteria include any significant medical problems or current treatment with an antihypertensive medication. Control subjects in both arms will be evaluated at baseline and at one year with only blood pressure measurements and blood and urine for collection of angiotensin ll, angiotensin-(1-7), FGF23, and klotho.

Data Collection
Clinical data will be collected from the electronic medical record, including height, weight, age, sex, parent-reported race, and past medical and family histories. We will calculate body mass index and define overweight/obesity as a body mass index ≥85%ile for age and sex. We will note antihypertensive medication type and dosage. We will calculate the estimated glomerular filtration rate to measure renal function standardized to age, sex, and height. All data will be de-identified and stored securely in an encrypted system.

Laboratory Assessment
Study Subjects - Blood (less than 5 mL) and urine samples will be collected at baseline and every three months up to 12 months at the same time as routine clinical labs at the Brenner Children's Hospital Clinic laboratory or the Downtown Health Plaza laboratory, so as to avoid additional study-specific blood draws. These samples will be sent to the Clinical Research Unit for processing. The predictors Ang II and Ang-(1-7) will be measured in the plasma and urine

using radioimmunoassays in a CLIA-certified laboratory within the Biomarker Analytical Core. We will calculate the ratio of the two peptides and, in the urine, standardize their values to urine creatinine. Uric acid, aspartate aminotransferase, alanine aminotransferase, lipid profile, creatinine, urine albumin, and urine creatinine will be collected per standard of care and analyzed in the clinical laboratory. FGF23 will be measured by ELISA (Immutopics, San Clemente, CA) in the Core. We will bank de-identified and securely-stored blood and urine samples for future analysis.

Control Subjects – Blood (less than 5 mL) and urine samples will be collected from all control subjects at the Brenner Children's Hospital Clinic laboratory. The predictors Ang ll and Ang-(1-7) will be measured in the plasma and urine using radioimmunoassays in a CLIA-certified laboratory with the Biomarker Analytical Core. We will calculate the ratio of the two peptices and, in the urine, standardize their values to urine creatinine. Uric acid, aspartate aminotransferase, alanine aminotransferase, lipid profile, creatinine, urine albumin, and urine creatinine will be collected per standard of care and analyzed in the clinical laboratory. FGF23 will be measured by ELISA (Immunotopic, San Clemente, CA) in the Core. Klotho will be measured by ELISA (IBL, MN) in the Core. We will bank de-identified and securely-stored blood and urine samples for future analysis.

Imaging
Study Subjects - All patients seen in our clinic for pediatric essential hypertension receive baseline and follow-up echocardiograms to evaluate for left ventricular hypertrophy. Patients with obesity receive baseline and follow-up ultrasounds of the liver to evaluate for hepatic fat infiltration. Imaging studies are interpreted by pediatric cardiologists and radiologists at Brenner Children's Hospital.

Control Arm 1 – Subjects (10 subjects) will have baseline echocardiograms to evaluate for left ventricular hypertrophy. Imaging studies are interpreted by pediatric cardiologists and radiologists at Brenner Children's Hospital.

Control Arm 2 – Subjects (50 subjects will have baseline ultrasounds of the liver to evaluate for hepatic fat infiltration as part of the standard of care. Imaging studies are interpreted by pediatric cardiologists and radiologists at Brenner Children's Hospital.

Blood Pressure Measurements
Blood pressure will be measured at clinic visits per standard guidelines. Because age, sex, and height define normative pediatric values, we will standardize blood pressure with z scores. Continuous blood pressure (measured in clinic for 10 minutes) will be collected at the baseline and 12-month visits. Ambulatory blood pressure monitoring will be employed at baseline and at 12 months per clinical practice and has been validated in patients as young as five years old. Subjects wear a monitor (Spacelabs 90207, Redmond, WA) at home for 24 hours; the monitor records a minimum of one valid blood pressure an hour. Parameters include i) mean blood pressure and ii) blood pressure load (percent of readings ≥95th percentile) for the wake, sleep, and 24-hour periods; and iii) percent nocturnal dipping (percent drop in sleep mean blood pressure from wake mean BP). Ambulatory blood pressure index will be calculated as the mean divided by the 95th percentile. All parameters will be referenced to normal values.
Clinic blood pressure will be measured at the study visit per standard guidelines for all control subjects at baseline and at one year. In Control Arm1, continuous blood pressure (measured noninvasively in clinic for 10 minutes) and ambulatory blood pressure monitoring will be assessed in control subjects at baseline.

Outcomes

The primary outcomes are blood pressure, left ventricular hypertrophy, and albuminuria at baseline and after 12 months of treatment. Blood pressure will be measured in clinic, by continuous blood pressure, and by ambulatory blood pressure monitoring. A left ventricular mass index ≥95%ile for age and sex on echocardiography defines left ventricular hypertrophy, and a urinary albumin-to-creatinine ratio >30 mg/g creatinine defines albuminuria. The secondary outcomes are serum uric acid, plasma FGF23, and nonalcoholic fatty liver disease, defined by elevated aspartate aminotransferase and alanine aminotransferase and hepatic fat infiltration on ultrasound of the liver with elastography.
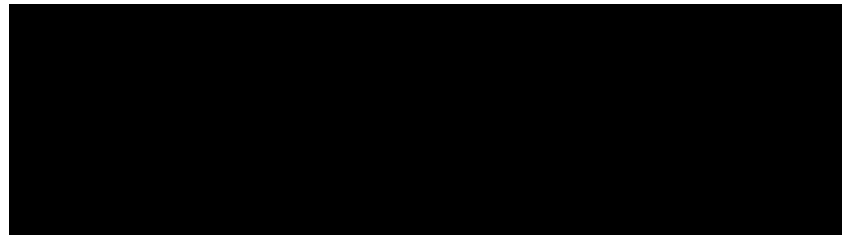
Statistical Plan

Summary statistics will include frequency distributions, mean with standard deviation, and median with interquartile range. Natural logarithmic transformation will improve continuous variable distributional characteristics as indicated. Chi-square and Fisher's exact tests, t-test, and Wilcoxon Rank-Sum test will evaluate between-group comparisons. Pearson or Spearman correlation coefficients will assess for continuous variable correlations. Bivariate analyses will identify potential confounders of the relationships between the predictors and the outcomes.

We will use general linear and logistic regression models to evaluate the relationships between the predictors and outcome measures. *A priori* covariates include sex and race given their relationships with the RAS and the outcomes. We will investigate potential effect modifiers by employing interaction terms and stratifying analyses. A potential confounder will be included in the multivariate models if i) there is an association between the predictor and outcome at p <0.2, or ii) there is a >10% change in the regression coefficient for the predictor-outcome relationship. Covariates will be retained in the final model if $p < 0.05$. A two-sided alpha of 0.05 will be considered statistically significant. We will use Enterprise Guide, Version 7.11 of the SAS System for Windows (SAS Institute Inc., Cary, NC) for all analyses.

Sample Size and Power

With 100 subjects we will have >90% power to detect a correlation as low as 0.4 between Ang II and Ang-(1-7) and blood pressure. Based on previous data, we estimate the prevalence of left ventricular hypertrophy is 40-60% before and 10-20% after treatment. We estimate the plasma ratio of Ang II to Ang-(1-7) is at least 7.7 (standard deviation 8.4). Therefore, we will have >90% power to detect an odds ratio of 1.15 or higher both at baseline and at one year. The prevalence of albuminuria is 20-60%; therefore the effect size and power we can achieve with 100 subjects will be similar to that achieved for left ventricular hypertrophy.

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