CRISP 3 PROTOCOL: RENAL IMAGING TO ASSESS PROGRESSION IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD): EXTENSION (CRISP III)

INTRODUCTION

The Division of Kidney Urology and Hematology Disease (DKUHD) of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) funded a cooperative agreement (UO1) for a consortium of participating clinical centers (PCCs) and a data coordinating and imaging analysis center (DCIAC) to develop and implement studies to test whether imaging techniques can provide accurate and reproducible markers of progression of renal disease in patients with polycystic kidney disease.

The awarded participating clinical centers are Emory University, University of Kansas, and Mayo Foundation (with a subcontract to the University of Alabama). The awarded DCIAC is the University of Pittsburgh.

PRIMARY GOAL

The goal of the CRISP Study is to conduct a prospective, longitudinal trial to evaluate the accuracy and validity of magnetic resonance imaging to determine disease progression in ADPKD defined as a change in both renal and renal cyst volumes and renal function over time.

SPECIFIC AIMS

CRISP is a uniquely characterized population of 241 ADPKD patients on which comprehensive clinical information, MR determined total kidney volume (TKV), liver cyst volume (LCV) and renal blood flow (RBF), and genotyping data are available. Longitudinal follow-up over an average of 7.3 years during CRISP I and II has established the exponential nature of TKV growth, but also illustrated the considerable variability seen within the population. Increasingly, as patients reach renal insufficiency endpoints, the predictive nature of baseline TKV in terms of severity of renal disease and other qualitative complications has been established. The predictive value of RBF has also been suggested.

CRISP III will build on the findings from this unique population with studies to address five specific aims as outlined below. These will further collect and analyze data and develop models to better utilize the predictive nature of TKV, RBF and LCV. The characteristics of individual cysts and the predictive value of the pattern of cyst development will be explored. Genetic and proteomic studies will be facilitated to aid the identification of genetic risk factors and biomarkers, and a pilot study will explore the role of sodium intake in influencing disease severity. The overall goal of this project is to maximize the use of the national resource that is CRISP to improve the predictive value of early imaging and other data, and hence, facilitate clinical trials of this common cause of ESRD.

Aim 1. Extend the serial quantification of total kidney (TKV) and liver (TLV) and of kidney (KCV) and liver cyst (LCV) volumes in order to develop and test new models for predicting the risk of developing renal insufficiency

> **Hypothesis 1a**. Baseline TKV and change in TKV predict loss of kidney function.
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> **Hypothesis 1b.** The progression of polycystic liver volume (LCV) will be similar to but distinct from that of TKV; baseline LCV, adjusted for covariates, will independently predict the rate of increase in LCV and complications arising within the liver.

Aim 2. Determine the extent to which age and sex-adjusted measurements of renal blood flow (RBF), determined by MR imaging, predict the rate of change in TKV and determine if RBF and TKV independently predict the risk of developing renal insufficiency.

Hypothesis 2. Baseline RBF predicts the rate of increase in TKV and, independent of and in addition to baseline TKV, predicts renal insufficiency.

Aim 3. Develop methods to quantify total cyst number, individual cyst volumes, and pattern of distribution of cysts in each kidney and apply these to analyze the influence of renal cyst number, volume, and topography at baseline on the subsequent course of TKV and GFR and the risk of developing renal insufficiency.

Hypothesis 3a. Renal cyst number and volume will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.

Hypothesis 3b. Renal cyst topography (medullary vs. non-medullary) will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.

Hypothesis 3c. Individual renal cyst growth is continuous and exponential (similar to the growth pattern in TKV) and patterns of renal cyst growth or involution will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.

Aim 4. Expand and analyze CRISP biological samples collected in NIDDK repositories to improve genotype/phenotype and biomarker studies, and facilitate independently funded ancillary studies.

Sub-aim 4.1 Collect and analyze DNA samples and clinical data from CRISP family members for enhanced genotype/phenotype studies and to facilitate other genetic studies.

Sub-aim 4.2 In conjunction with the NIDDK Biomarkers Consortium and ongoing ancillary studies utilizing CRISP samples, determine specificity and sensitivity of urinary and serological markers to: 1) detect severity of renal and extra-renal ADPKD manifestations (e.g., TKV and LCV); and 2) predict rates of their progression.

RESEARCH STRATEGY AIM 1

Extend the serial quantification of total kidney (TKV) and liver (TLV) and of kidney (KCV) and liver cyst (LCV) volumes in order to develop and test new models for predicting the risk of developing renal insufficiency.

Significance. CRISP I findings [1-3], independently validated [4], have made testing therapies aimed at reducing or slowing the growth of kidney cysts feasible. Due in part to CRISP findings, the number of clinical trials in ADPKD has increased from one in 1999 to 19 clinical trials, currently (PKD Foundation website PKDcure.org). CRISP II has shown a strong association between baseline TKV and both, decline in GFR measured by iothalamate clearance and patient reported outcomes (renal complications of ADPKD). Because enough patients have now developed renal function endpoints (CKD stages 3, 4 and 5, and ESRD), we can begin to prove that TKV, and specially TKV adjusted for height (to decrease the influence of body size, htTKV), predicts the development of renal insufficiency. We expect more patients will develop

renal endpoints during CRISP III, making the confirmation of the predictive value of htTKV and the development of more precise predictive models possible. Establishment of htTKV as a predictor of renal insufficiency by a decade or more before GFR declines to a clearly abnormal level would be a welcome new tool for exploring the potential benefits of new therapies offered to patients in the early stages of ADPKD.

It is not clear if TLV and LCV impact TKV or the relationship between TKV and renal endpoints. CRISP demonstrated a higher and earlier prevalence of liver cysts than previously thought, reaching 94 % by the third decade of life [5]. CRISPIII offers the opportunity to characterize hepatic cyst growth in relation to TKV and renal insufficiency and the association between LCV, patient reported outcomes (e.g. early satiety, abdominal pain, reflux, dyspnea, etc), and potential covariates (including genotype, age and TKV).

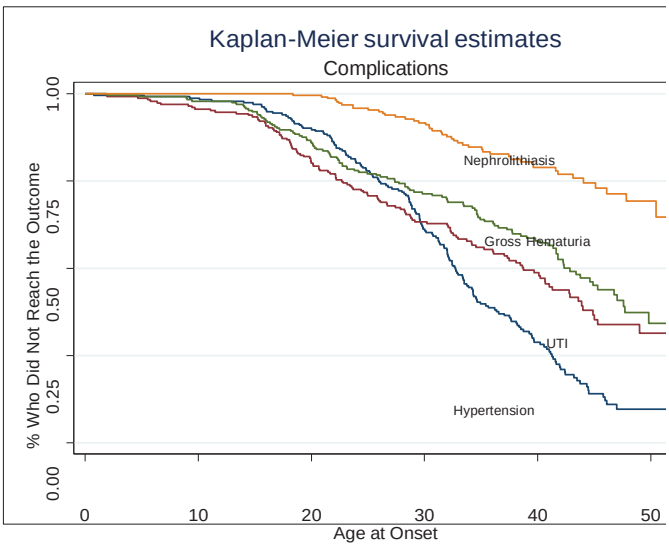
Innovation. This is the only longitudinal study documenting the associations between TKV and KCV, and the course of progressive renal disease from a period of 'normal renal function' to well defined renal end-points. MR methods have been developed or improved to achieve the goals of the study. Lessons learned from studying the evolution of ADPKD in the kidney are being applied to the liver and the relationship of both disease processes is being evaluated. Innovative modeling will provide the means to risk stratify patients for the purpose of counseling and establish surrogate endpoints for use in clinical trials.

Approach – Progress Report/Preliminary Studies. CRISP II Aim 1 proposed to extend the observations of CRISPI to ascertain the extent to which quantitative or qualitative structural parameters predict renal insufficiency and to develop and test new metrics to quantify and monitor disease progression. In 2006 we completed the initial survey in 241 ADPKD patients enrolled in CRISP and published a seminal report showing that kidney and cyst growth were quantifiable, bilaterally equal, exponential-like, and progressive at unique, relatively constant signature rates for each patient over decades [2-3]. Higher rates of kidney enlargement were associated with more rapid decrease in renal function. Each doubling of TKV from baseline was associated with a decline in GFR, whether measured by iothalamate clearance, equations based on serum creatinine concentration or measured creatinine clearance, but associations were strongest for iothalamate clearance [6]. The compounding effect of exponential-like growth was the most powerful determinant of long-term kidney expansion, but the cyst number was also important [7]. Cyst number, but not the rate of growth, accounted for the higher severity of PKD1 compared to PKD2 [8]. Rates of cyst growth much higher than those observed in CRISP had to occur at some point in their natural history to account for the cyst volumes observed in the study. In some cases, when renal cysts are detected by ultrasound in newborns, and possibly in all cases, these exuberant rates occur *in utero* [7].

We have retrospectively and prospectively analyzed the relation between baseline TKV (241 CRISP patients) and life-altering, renal complications [9] manuscript in preparation). These started in the first decade of life; only eight participants remained free of complications at the close of data collection on 12-01-09. HtTKV (cc/m) was strongly associated with the number of renal complications experienced by patients independent of age. Figures 1 and 2 demonstrate the % chance of surviving without certain clear-cut renal complications or declining renal function during CRISP I and II. Patient reported outcomes of hypertension, gross hematuria, urinary tract infection and nephrolithiasis occurred earlier and more often compared to renal insufficiency. Fifty per cent developed hypertension by age 30, urinary tract infection by 40, gross hematuria by 45 and nephrolithiasis > 50 yrs (Figure 1). One hundred thirty nine patients reached stage 2, of these, 58 reached stage 3 and, of these, 14 reached stage 4 of K/DOQI, illustrating the

progressive decline in renal function in this cohort (Figure 2). Plots of cumulative onset curves for hypertension and decreased iothalamate clearance were exponential-like, similar to that for htTKV, further suggesting a tie between cyst growth and renal complications. Indeed, baseline htTKV predicted the onset of hypertension (P<0.000), gross hematuria (P<0.006) and renal insufficiency defined by K/DOQI criteria (P<0.000) in multivariable analysis. A Cox regression model applied to receiver operator curves showed that baseline htTKV predicted K/DOQI stage 3 renal insufficiency (AUC 0.83, P<0.001) with high sensitivity (81.0%) and specificity (72.1%) at an htTKV cut-point of 600 cc/m.

We reported that the overall prevalence of hepatic cysts was 83%, rising to 94% in the oldest patients. The total hepatic cyst volume was significantly greater in women than in men [5]. Hepatic cyst prevalence and aggregate total hepatic cyst volume increased with age.



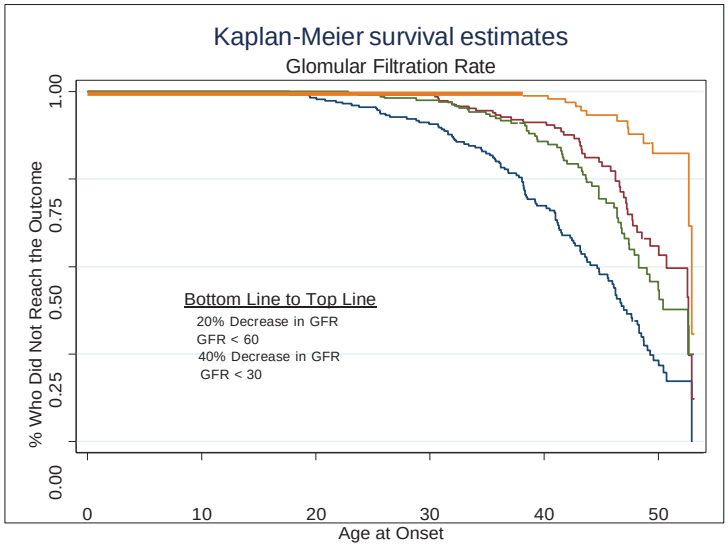


Figure 1

Figure 2

Approach / Plan. In CRISP III we will continue to collect data to extend the number of patients reaching the various renal insufficiency endpoints reflected by measured iothalamate clearance or serum creatinine based estimated GFR (eGFR). We have begun a similar analysis of LCV that will continue through CRISP III. Whereas CRISP II measured only LCV, MR image acquisition in CRISPIII has been modified to allow measurements of TLV. We anticipate that 194 CRISPII subjects are available to enroll in CRISPIII. CRISPIII will include all CRISPI and II volunteers including those enrolled in other clinical trials testing the effects of inhibition of the renin angiotensin aldosterone system (HALT n=60) and vasopressin V2 receptor inhibition (TEMPO, n=26). Importantly, the Principal Investigator (Dr. Ty Bae) and personnel for the Imaging Center for HALT and CRISPII are the same. The CRISP/HALT liaison committee has reviewed and approved dual participation in both CRISPII and HALT and the CRISP and HALT Steering Committees have approved the development of CRISPIII. Individuals who also participate in HALT and TEMPO complete the necessary studies of CRISPIII that are not included in HALT or TEMPO. Appropriate model adjustment factors will be included to account for the differential effects of being included in a clinical trial.

RESEARCH STRATEGY AIM 2

Determine the extent to which age and sex-adjusted measurements of renal blood flow (RBF), determined by MR imaging, predict the rate of change in TKV and determine if RBF and TKV independently predict the risk of developing renal insufficiency.

Significance. A reduction in RBF occurs early in the course of ADPKD, precedes the development of hypertension, and may contribute to disease progression and development of renal insufficiency [11-14]. Studies have shown marked media thickening, increased wall-to-lumen ratio, and reduced number of small arteries and arterioles in polycystic kidneys [15-18]. These along with the presence of global glomerulosclerosis suggest a role for ischemia in ADPKD progression [18]. Whether this is due to compression of renal arteries and parenchyma by expanding cysts, intrinsic vascular abnormalities linked to mutations in *PKD1* or *PKD2* (strongly expressed in vascular smooth muscle and endothelium) or both results is not known [19-24]. Activation of the renin-angiotensin system, increased sympathetic nerve activity, enhanced endothelin generation, and impaired nitric oxide production may contribute to renal vasoconstriction, remodeling of microcirculation, and development of renal insufficiency [25-32].

Ischemic damage has been proposed to be the final common pathway to end-stage kidney injury in other chronic kidney diseases (CKD) [33-35] . Vascular remodeling and/or peritubular capillary loss, reduced oxygen supply caused by interstitial expansion, and increased metabolic demand by tubular epithelial cells lead to apoptosis, epithelial-mesenchymal transdifferentiation, and progression of interstitial fibrosis. This provides a rationale for treatments aimed at protecting RBF and preventing hypoxia. Implementation and evaluation of these therapies is hampered by the lack of non-invasive methods to monitor RBF in diseased kidneys.

Para-aminohippurate (PAH) clearance has been used to estimate RBF in ADPKD [11-14]. This assumes normal extraction of PAH, unlikely in ADPKD, particularly in the presence of renal insufficiency. Doppler ultrasonography has been used in native kidneys and allografts to estimate the resistive index, a surrogate for renal vascular resistance (RVR) [36-39]. Prospective studies have shown a significant association between high resistive indices and poor outcome in CKD [40-41]. In ADPKD, resistive indices correlate with morphologic and functional indices of disease severity [42]. However, measurements of resistive index are not well standardized and are influenced by intrarenal (arterial compliance) and extrarenal (breath holding, bradycardia, pulse pressure) factors [43-44]. They reflect RVR only when arteries are compliant. Currently, breath-hold cine Phase Contrast-MRI imaging is the best method to directly and accurately measure RBF in diseased kidneys [45].

Innovation. The potential of MRI as a noninvasive method for measuring blood flow *in vivo* has long been recognized, but widespread clinical application has been limited by patient motion during imaging, poor signal-to noise ratio, and long imaging times [46-49]. RBF measurements are challenging because kidneys are subject to cardiac, respiratory, and bowel motion and renal arteries may be multiple (accessory arteries often being small and difficult to identify) and have oblique and variable orientation to the aorta. Limited success rate and poor reproducibility of RBF measurements in a study using uncompensated free-breathing methods underscores the importance of good respiratory motion control [50]. Technological innovations (oblique slice plane definition, cine flow techniques, fast segmented k-space imaging, phased-array receiver coil technology, vector electrocardiographic techniques) now allow high-resolution breath-held RBF imaging with improved signal-to-noise ratio and synchronization with cardiac cycle [51-59]. This requires only 10-15 min, including flow plane scouting.

Approach – Progress Report/Preliminary Studies. CRISP sought to determine whether 1: RBF can reliably be measured by MR, 2: RBF changes can be detected over short periods of time, and 3: RBF reductions predict structural and/or functional progression. Validation studies demonstrated absolute requirement for precise MR blood flow pulse sequence parameters, accuracy using steady and pulsatile flow phantoms, and intra-observer and inter-observer reproducibility in healthy volunteers and ADPKD patients, particularly when blood vessel diameter is 23mm [60-61]. MR measurements of RBF were performed at Mayo Clinic and Emory University during CRISP I. KUMC now acquires RBF images in CRISP patients participating in HALT-PKD. Images from the three PCCs are transferred via internet to the DCC for central measurements of RBF using commercially available semi automated flow measurement software and a standardized procedure. CRISP I longitudinal studies showed that RBF reduction and parallels TKV increase. Lower baseline RBF was associated with structural (TKV slope) and functional (GFR slope) disease progression [62]. Presence of hypertension and low serum HDL cholesterol levels independently associated with faster RBF decline [62].

During CRISP II, RBF continued to decrease more rapidly than GFR (Table 1). A preliminary analysis of CRISP II shows that baseline RBF does not add significantly to the predictive value of baseline lnTKV for BL-YR8 slope TKV (R squared 0.32 vs 0.31) or BL-YR8 lnTKV slopes (0.07 vs 0.06). This is not inconsistent with the independent association of baseline RBF and renal growth observed in CRISP I, since this was of borderline statistical significance (p=0.034) when baseline log10 TKV (the strongest predictor of TKV increase) was included as a covariate in the regression model [62]. On the other hand, in both, CRISP I and II, RBF significantly added to the predictive value of TKV on the rate of GFR decline [63] (Table 2). Higher TKV and lower RBF were significantly correlated with renal function decline, whereas higher GFR at baseline was associated with steeper GFR slopes because of regression to the mean.

Table 1. Baseline (BL) through YR8 visit RBF and GFR values

	RBF			GFR		
Visit	**N**	**mL/min/ 1.73 m^2**	**Avg % change from BL**	**N**	**mL/min / 1.73 m^2**	**Avg % Change From BL**
BL	118	737 ± 228		118	99 ± 23	
YR1	106	686 ± 168	-4.0*	106	98 ± 24	0.5
YR2	107	640 ± 191	-10.9*	107	99 ± 28	2.0
YR3	99	653 ± 197	-8.5*	99	94 ± 26	-3.4*
YR6	72	483 ± 236	-33.9*	72	80 ± 36	-18.1*
YR8	38	494 ± 346	-32.6*	38	72 ± 33	-22.0*

* significant difference from baseline (paired t-test)

Table 2. Relationship of BL TKV, GFR and mean CRISP I RBF with GFR slopes (r-squared)

Covariates	**GFR slope CRISP I**	**GFR slope all values**
lnTKV0	0.15*	0.22*
GFR0	0.07*	0.03
Mean RBF	0.06*	0.08*
lnTKV0, GFR0	0.33*	0.34*
lnTKV, Mean RBF	0.16*	0.23*
lnTKV, GFR0, Mean RBF	0.45*	0.43*

* significant predictor in the model.

Approach – Plan. During CRISP III we will continue to explore the association of TKV, RBF, and other covariates with structural and functional progression of the disease. The protocol for RBF measurements will be the same used in CRISP I and II. Breath-hold, oblique-coronal 2D true-FISP (FIESTA) with fat sat with 4mm fixed slice thickness at 2 mm spacing (i.e., overlap 50%) will be used to identify the aorta and renal arteries. Slices perpendicular to the renal arteries will be chosen to accurately measure velocity. Velocity encoding (VENC) values of 100 or 50 cm/sec will be used. Small FOV (14-16 cm) and large matrix (256x192 or 512x512) are important for an accurate measurement of the vessel size. Segmented, prospectively cardiac-triggered phase contrast flow measurements will be obtained to compute the mean and peak velocities, as well as the total mean flow, during the cardiac cycle.

RESEARCH STRATEGY AIM 3

Develop methods to quantify total cyst number, individual cyst volumes, and pattern of distribution of cysts in each kidney and apply these to analyze the influence of renal cyst number, volume, and topography at baseline on the subsequent course of TKV and GFR and the risk of developing renal insufficiency.

Significance. Evidence supports that formation and expansion of cysts causes the decline of GFR in ADPKD. The mechanism by which this occurs is not known. Kidneys have ~ 1 million nephrons, but cysts form in relatively few of them. The collecting system originates from the ureteric bud. Subsequently, in a series of dichotomous branchings during renal development, the collecting ducts unite with the distal ends of nephrons which form in the cortex from metanephric mesenchyme. Obstruction of a single proximal tubule in the cortex disrupts the filtration of a single glomerulus, whereas blockade of a single papillary collecting duct could reduce filtration in thousands of upstream glomeruli. Thus, cysts in medullary collecting ducts have greater potential to impair GFR than cysts in cortical collecting ducts and much greater than those arising from tubules proximal to collecting ducts. The ultimate impact of tubular obstruction by cyst formation would depend on location (medulla vs. cortex) and number of cysts formed. A quantitative analysis of cyst number, volume and topography may predict the pace of development of renal insufficiency.

Innovation. A quantitative analysis of cyst number, volume, and topography and their relation to renal function has never been reported in ADPKD. CRISP I, II and III offer a unique opportunity to explore these issues by taking advantage of imaging data accumulated over 12 years of follow up with concurrent measurements of GFR.

Approach – Progress Report/Preliminary Studies. This Aim is an extension of a sub-aim in Specific Aim 1 of CRISP II. CRISP has shown that the number of cysts detectable by MRI increases with age and that renal morphological (and associated phenotypical and clinical) differences between PKD1 and PKD2 are likely because more cysts develop earlier in PKD1, not because they grow faster [8] .

To explore further the importance of cyst number on the severity of the disease, standardization of the cyst counting process is necessary. Manual cyst counting is time consuming, but highly repeatable. The intra-class correlation coefficient was 0.99 when two radiologists counted the cysts

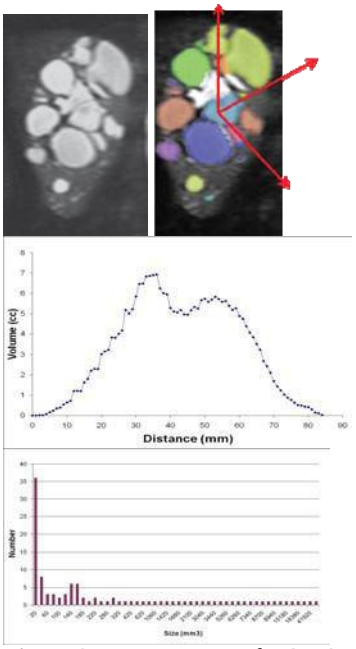


Figure 3. T2 MR image of a CRISP subject (top left), segmented renal cysts (top right), spatial distribution of cyst volume from the kidney centroid (middle), and cyst size distribution histogram (bottom).

in mid-sections of renal MRI in 238 patients [64]. Reproducibility was within ±10%. We have also performed a preliminary study of counting the total number of cysts in 8 sets of kidneys (Table 3). Cysts 2 3 mm in diameter were counted on gadolinium-enhanced T1 MR images (~40 – 50 coronal slices/kidney). Some of the cysts 3-5 mm in diameter may have been overlooked due to tangential imaging planes. The number of cysts was similar in left and right kidneys and, in 7 of the 8 cases, the number of cysts increased in 6 years. Increases in cyst number may be due to new formation of cysts or growth of cysts that were too small to detect at baseline but enlarged to the point they became visible. Inspection of histological sections of human polycystic kidneys shows that there are considerable numbers of cysts below 2 mm in diameter within the parenchyma that are separated from macroscopically visible cysts. We have also developed and tested a method of estimating the total number of cysts in a kidney from the cyst number measured on a mid-slice of MR images and will develop semi-automated methods to measure the total number of cysts so that cyst counting can be applied to larger numbers of cases. We have also developed a semi-automated computerized method for segmentation of renal cysts from MRI (Figure 3). With this method, we can compute the volume and topography within each kidney including the volumetric distribution of medullary vs non-medullary cysts.
We also reported that two sequential measurements of individual cyst volumes in CRISP patients over a span of 3 years showed growth rates up to 71%/yr, but most cysts grew approximately 10-15%/yr [7]. These preliminary measurements establish the feasibility of our proposed studies.
Table 3

		Change in total renal cyst count in 8 typical CRISP patients over 6 year interval									
			Baseline			Baseline			Year 6		
ID	Sex	Age	TKV	htTKV	GFR	Right	Left	Total	Right	Left	Total
		y	cc	cc/m	ml/m/1.73						
100505	f	41	802	501	127	193	202	395	367	340	707
113094	m	19	1602	942	95	287	344	631	454	494	948
124300	m	34	2878	1573	72	513	551	1064	1046	946	1992
124952	f	34	401	243	113	288	344	632	280	316	596
136055	f	17	655	423	117	295	285	543	294	408	814
143806	f	45	1154	721	98	238	272	510	385	328	713
188920	m	36	1339	732	76	568	576	1144	867	734	1601
193273	m	23	483	253	123	293	240	533	325	319	644

Approach – Plan. T1 (± gadolinium) and T2 MR images at baseline and follow-up years 1, 2 and 3 are available in 241 CRISPI participants, T1 and T2 MR images at year 5 and 7 are available in 200 CRISP II participants and we anticipate that T1 and T2 MR images will be available in 194 CRISP III participants. To determine to what extent renal cyst number and volume are associated with severity and progression of ADPKD (Hypothesis 3a), we will determine whether the total number of cysts and volume at baseline and their change from baseline to year 10 are associated with rates of change in TKV and GFR and renal function outcomes. To determine to what extent cyst topography is associated with severity and progression of ADPKD (Hypothesis 3b), we will determine whether the spatial and size distribution of renal cysts and their change from baseline to year 10 are associated with rates of change in TKV and GFR and renal function outcomes. Both objectives require methodologic improvements to standardize the measurements and computerized image processing programs

for quantitative analysis which we have developed during CRISP II (Figure 3). The first year of the new project will be invested in refining the strategy and developing additional tools for analysis. Computerized and manual cyst counting methods will be compared. The spatial and volume distribution of renal cysts in each kidney (Figure 3) will be analyzed and compared in terms of distribution skewness, kurtosis, and bilateral asymmetry. To determine cortical versus medullary topography of cysts we will examine their location and configuration (Figure 4). Cysts arising in the cortex often lack parenchyma between the cyst cavity and the renal capsule. Medullary derived cysts commonly push the cortex toward the capsule (in which case there is a rim of cortical tissue separating the cavity from the capsule) or are entirely contained within the central core of the kidney. We will develop additional criteria for defining cyst origin as we develop additional methods and analytic tools. Cyst distribution will also be defined as generalized (cysts distributed in cortex and medulla along full extent of the entire renal sections) or segmental (cysts distributed unevenly e.g. polar or central with unaffected poles) and volumes of non-cystic parenchyma and their change over time will be quantified.

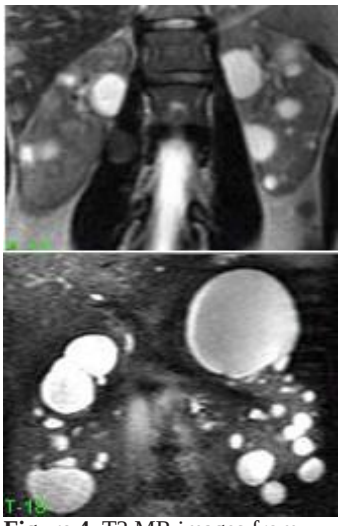


Figure 4. T2 MR images from two CRISP subjects: predominantly medullary (top) vs predominantly cortical and exophytic cyst distribution (bottom).

To determine whether individual cyst growth is continuous and exponential, similar to the growth in TKV (Hypothesis 3c), we will evaluate the patterns of individual cyst growth within and between individuals. We will select 10 well-defined cysts in each kidney (20 cysts per subject) from serial MR images (baseline and follow-up YR 3, 5, 8, 10, 12) of 60 PKD1 and 15 PKD2 subjects with high quality MR images selected to include 20 PKD1 and 5 PKD2 patients with low, 20 PKD1 and 5 PKD2 patients with intermediate, and 20 PKD1 and 5 PKD2 patients with high rates of renal enlargement. One-half of the cysts will be segmented from the baseline images as a reference and the operator will review T2 MR images from later years and segment cysts that correspond to those in the baseline images. The other half of the cysts will be segmented from the YR 10 or 12 images as a reference and the operator will review T2 MR images from previous years and segment cysts that correspond to those in YR 10 or 12. This will allow inclusion of cysts that might have appeared or disappeared during the 12 years of follow-up. The volume of individual cysts will be measured. We expect to identify different patterns of growth or involution of individual cysts that may have an effect on rates of kidney growth and functional outcomes. Some cysts may grow to the point where they make contact with surrounding cysts. We will measure these cysts even though they may not be clearly separated from surrounding cysts. The repeated cyst volume data over time (using appropriate spacing) will be assessed to determine departure from linearity using mixed-method repeated measures models, after log transformation. The cysts may show overall linearity for all cases, departure from linearity for all cases or differential departure from linearity. Groups of patients (large vs small kidneys, PKD1 vs PKD2, hypertensive vs normotensive) will be compared to determine if these important group differences show differential departure from linearity.

RESEARCH STRATEGY AIM 4

Expand and analyze CRISP biological samples collected in NIDDK repositories to improve genotype/phenotype and biomarker studies, and facilitate independently funded ancillary studies.

Sub Aim 4.1.

Collect and analyze DNA samples and clinical data from CRISP family members for enhanced genotype/phenotype studies and to facilitate other genetic studies.

Significance. CRISP is a uniquely characterized ADPKD population, clinically, in terms of imaging data, and genetically. Hence, it is ideally suited to identify and characterize factors that influence the severity of disease. *PKD2* mutation is typically associated with milder disease with ESRD occurring on average 20 years later than in PKD1 [65]. In CRISP, PKD2 patients had significantly smaller TKV and TCV, which were associated with the number of cysts rather than their rate of expansion [2, 8]. Although there is little evidence in ADPKD that allelic effects strongly influence the phenotype, recent studies indicate that specific missense alleles may not be fully penetrant (hypomorphic alleles) [66-67]. Alone, these alleles can result in mild PKD, homozygous or compound heterozygous cases are viable with typical to severe disease and a combination of an inactivating and hypomorphic allele can result in early onset ADPKD. Therefore, variants in the normal ADPKD allele haplotype may be a factor that influences intra-familial variation in disease severity. It is likely that genetic factors (quantitative trait loci; QTL) elsewhere in the genome also significantly influence disease severity. By collecting DNA samples and clinical/imaging information on CRISP family members, the degree of intra-familial variability will be much better characterized, correlations with genotype determined, and material will be available to identify modifying factors underlying this phenotypic diversity.

Innovation. The carefully formulated clinical and imaging protocols developed for CRISP will be employed to define the CRISP family members. State-of-the-art genetic methods will be employed to characterize this population, largely as ancillary studies. This will include analysis of common variants employing a genome-wide association study (GWAS), haplotype and sequence analysis of *PKD1* and *PKD2*, and next-generation sequencing approaches to characterize rarer variants that may influence severity.

Approach-Preliminary Data. Genetic analysis of the 202 CRISP families identified likely mutations in 91.1%, with 85.3% *PKD1* and 14.7% *PKD2* [68-69]. Truncating mutations (including 4% with larger rearrangements) were found in 63.9% of the total population and missense changes in 21.8%. CRISP is the best genetically characterized ADPKD population and was utilized to develop algorithms to predict the significances of substitutions that have been employed to evaluate all published mutations [66-70]. Family history information, including drawn pedigrees are available on most families and sample collection has begun at Emory and Mayo.

Approach-Plan. Since the germline mutation will be known from CRISP family members, this study will greatly increase the size of the CRISP genetically characterized population and allow questions about intrafamilial variability, correlations with genetic variables and ancillary genetic studies to be addressed in a large population. We estimate that the average CRISP family has 4 living affected relatives not participating in CRISP and so the potential size of the CRISP cohort is >1000 cases from the 202 families. Preliminary analysis indicates that the average age of these affected family members is 48 years, that ~25% have ESRD and a further 18% renal insufficiency with a GFR <60 mL/min/1.73m^2.

Approval from the local IRB has been obtained to collect clinical data and DNA samples from CRISP family members, including site specific procedures established to contact relatives. The family member will be asked to sign the consent form if interested in participating and to contact the study coordinator if they have any questions about the study. A DCIAC generated identifier will be assigned to each family member. After receipt of the signed consent, patients will be asked to complete a questionnaire detailing family history to confirm and expand the existing family information. Clinical information will be obtained by the study coordinator through interviews and by review of medical records. This will include renal function, imaging data and details of disease complications (hematuria, urinary tract infections, kidney stones and hypertension; see Family Member Questionnaire in Appendix). Whenever possible, the most recent CT or MR examination of the abdomen, or if not available the most recent abdominal ultrasound, will be reviewed and kidney information abstracted. Funding from the Genetic Modifier Ancillary Study will allow analysis of volume from these clinical imaging records. Two blood samples identified by the ID study number will be collected. A 10 ml blood sample in EDTA tubes will be collected for DNA isolation and sent to the NIDDK Cell Repository at Rutgers University. A second blood sample (3.5 ml) will be collected for measurement of serum creatinine and sent via the PCC to the Cleveland Clinic. All clinical and genetic information will be entered into the CRISP database that is maintained by the DCIAC. These data and samples will be available to CRISP and other authorized investigators.

The focus of this study is to characterize the variability of the disease phenotype in ADPKD, including intrafamilial variability, and to correlate phenotypic measures with genic and allelic effects. We envisage that data will be available on ~1000 patients from 202 ADPKD families. Of these, 157 families (~785 patients) will be PKD1, 27 families (~135 patients) PKD2 with the mutation unknown in 18 families (~90 patients). The clinical parameters to be employed include eGFR, age at onset of ESRD, TKV, age at onset of hypertension, the occurrence of hematuria, urinary tract infections or kidney stones, estimates of severity of liver disease and the occurrences of an ICA. These studies will be a complement to others performed with the Clinical Data Interchange Standards Consortium (CDISC) to characterize disease outcomes in ADPKD. Age and gender corrected values will be compared to the phenotypes in the PKD1, PKD2 and mutation unknown populations. In addition, within the genic groups comparisons will be made between and within families (taking into consideration the closeness of the relationship) and employing variance component analysis to estimate of the degree of variation due to genetic modifying factors. Within the PKD1 and PKD2 populations, the significance of mutation type (truncating or in-frame) will be correlated with the clinical endpoints. In addition, mutation position will be compared to the phenotypes, and for *PKD1* (where greater numbers are available) mutation position of truncating and in-frame changes determined. These studies, due to the large size and degree of phenotypic and genetic characterization, are likely to reveal new insights into the importance of genetic factors to determine the disease phenotype in ADPKD.

This expanded population will be utilized in a number of other genetic studies by the CRISP Investigators and others funded as Ancillary Studies to CRISP. Presently, a GWAS, which will employ the CRISP and CRISP family members as approximately 20% of the total population, has been funded (DK079856). This study will determine if common variants underlie a significant degree of the phenotypic variability in PKD1, and employ TKV and eGRF as phenotypic measures. The extent to which variation in the *PKD1* and *PKD2* haplotype, especially on the "normal" allele, influence the severity of disease has been questioned by the studies of hypomorphic alleles [66]. The CRISP Investigators plan to address this question by

sequencing *PKD1* and *PKD2* in ~500 family members of CRISP. This study was submitted as a CRISP Ancillary Study in October 2010, will address the importance of hypomorphic alleles and haplotypes to disease variability in ADPKD and provide much more information about genetic variation at these loci. A third study that is envisioned will follow-up on the GWAS study with a targeted sequencing approach employing next-generation sequencing methods. In this case, regions of genome highlighted as potential sites of modifiers will be sequenced in patients to look for rarer variants that may have a greater modifying effect. This Ancillary Study is likely to be submitted in 2012.

Sub Aim 4.2.

In conjunction with the NIDDK Biomarkers Consortium and ongoing ancillary studies utilizing CRISP samples, determine specificity and sensitivity of urinary and serological markers to: 1) detect severity of renal and extra-renal ADPKD manifestations (e.g., TKV and LCV); and 2) predict rates of their progression.

Significance. CRISPI and II have begun to establish TKV as a surrogate marker and predictor of ADPKD progression that surpasses in specificity and sensitivity serum creatinine or GFR [2]. Availability of appropriately collected and stored urine and serum samples from the CRISP study participants together with high quality longitudinal genetic, demographic, clinical, imaging and laboratory data provide a unique opportunity to investigate and establish additional biomarkers. For example, unlike structural or anatomical changes that may not be recognized on imaging studies until cyst diameter reaches dimensions at least 50 times greater than their initial size, urinary or serological biomarkers may already be elevated and more accurately reflect disease activity. Therefore, appropriately identified biomarkers may be superior to TKV measures as a marker of disease severity and allow for improved efficiency of ADPKD drug development, establish marker profiles that relate to the stage of ADPKD and aid in investigating discordant organ involvement. Ultimately, the use of these markers may facilitate the development of personalized care plans for individual ADPKD patients.

Innovation. Initial studies of candidate biomarkers including MCP1 and NGAL demonstrate important information regarding 1) associations with TKV and 2) longitudinal variation in the CRISP cohort. Recent technological advances in high throughput proteomic technologies allowed the identification of novel highly specific markers to diagnose and predict progression of several chronic renal disorders. For example, such markers have been identified by capillary electrophoresis-mass spectrometry (CE-MS) for diabetic and IgA nephropathy [71-72]. CE-MS was also used to identify ADPKD-specific urinary markers and has been utilized by ongoing ancillary CRISP study validating preliminary proteomic signatures identified in another similarly characterized population [73], Weiss and colleagues through ARRA funding (RO1DK082690) are establishing circadian and post-prandial variation in serum and urine metabolomic profiles in healthy ADPKD individuals. In conjunction with the HALT PKD networks and the CRISP-HALT liaison committee, validation and discovery studies in CRISP III are anticipated (see letter of support). Importantly, duplicate sample analysis from a single CRISP participant from both proteomic and metabolomic platforms will provide insight into unique serum and urinary signatures. A CRISP/ADPKD biomarker platform will facilitate the development of surrogate markers for monitoring the therapeutic efficacy of targeting specific ADPKD pathways. In particular, this area of development and validation of ADPKD biomarkers (with new and existing technologies) will be performed in conjunction with the NIDDK Biomarkers Consortium (see letter of support).

Approach. Samples of serum and urine (24-hr and freshly collected spot samples) have been collected from CRISPI/II participants at all visits. These samples have been carefully aliquoted and stored and are available with CRISP Steering committee approval from the Rutgers University Repository. Continuation of urine and serum sample collection throughout the CRISPIII is planned and will provide the material needed for the studies outlined above and will be particularly useful for the identification of anticipated ADPKD activity markers, predictors of ADPKD progression and characterization of the natural course of the disease progression. Selection of promising candidate biomarkers for testing on CRISP samples will be informed by the FDA guidelines for identification of valid biomarkers. Specifically, individual candidate markers will be prioritized based on established scientific framework or body of evidence that take into account the physiologic, pharmacologic or clinical significance of the these markers as well as established characteristics of the analytical system used for their measurement. This approach has been applied for selection of markers in completed [74-75] and ongoing ancillary and independently-funded studies utilizing the CRISP samples, e.g., CE-MS-based analyses (Dr. Andreas Kistler, Zurich University, Switzerland), evaluation of NGAL [76] and IL-18 (Dr. Chirag Parik, Yale), and evaluation of copeptin and pro-ANP (in collaboration with Dr. Ron Gansevort, University of Groningen, and Dr. Joachim Struck, Research Department B.R.A.H.M.S). We anticipate that additional ancillary or independently-funded ADPKD biomarker discovery studies will be initiated by both CRISP (e.g., urinary CD14; [77]) and non-CRISP investigators. A list of approved ancillary studies is provided in Appendix A.

The design of studies for evaluating biomarker specificity and sensitivity to detect and predict specific outcomes in the CRISP cohort, as well as all subsequent analyses will be performed and/or validated by the CRISP Data Coordinating Center. This approach will ensure proper adjustment of biomarker data for important demographic and clinical variables. Upon completion of specific biomarker studies, generated data will be integrated with the existing CRISP database. Available biomarker data will be assessed on prospective basis with multilevel modeling analyses to identify optimal combinations of biomarkers, demographic and clinical data for detection of disease activity and prediction of its progression.

RESEARCH DESIGN AND METHODS

Overview of Design: The CRISP III Study is a prospective, observational study that is an extension of CRISP I and CRISP II. CRISP I was a prospective, observational study that enrolled 241 ADPKD subjects between the ages of 15 and 45 years and was designed to determine if novel imaging techniques such as magnetic resonance (MR) imaging could reliably and accurately detect change in renal structure early in the course of APDKD. 201 CRISP I participants completed followup in CRISP II. It is anticipated that all CRISP I subjects not yet on dialysis or having received a transplant (n= 3) are eligible to enroll in CRISP III. CRISP III is designed to include all CRISP I individuals including those who enroll simultaneously in other clinical trials. In this respect, HALT, an ongoing interventional trial of the PKD Clinical trials network has 40 CRISP subjects in Study A (which includes MR imaging identical to that proposed in this submission) and 23 subjects in Study B (no MR imaging). In addition 25 CRISP participants are currently enrolled in interventional trials involving V2 receptor antagonists. Importantly, the co-Principal Investigators (Dr. Ty Bae, and Dr. James Bost) and personnel for the Imaging Center (now at the University of Pittsburgh) for both HALT and CRISP III are the

same. The CRISP/HALT liaison committee will review and approve dual participation in both CRISP III and HALT and the CRISP and HALT Steering Committees will approve the CRISP III protocol. To minimize subject burden and to maintain retention throughout CRISP III, those CRISP III individuals who also participate in HALT will not undergo duplicate imaging, blood pressure measurements or blood sampling. They will, however, complete the necessary studies of CRISP III that are not included in HALT.

General Protocol: Measurements of TKV, KCV, TLV, LCV, RBF, GFR and other laboratory evaluations (CRISP website, MOP) will be made twice during CRISP III (year 10 and 12 of the longitudinal study) in participants utilizing a similar approach to CRISP I and II [1-3]. Plasma creatinine concentration will be measured annually. Medical histories and ADPKD specific patient reported outcomes will be obtained every 3 months. The frequency of these visits are increased from CRISP II as patients are more likely to present with complications specifically related to TKV and TLV on a more frequent basis. Unique additions or changes to the parent CRISP III protocol, e.g. AIM 5 Pilot Study, are noted in association with each Specific Aim.

CRISPIII Timeline:

<u>April 2011-September 2011</u>
Protocol refinement, IRB approvals
Forms development, MOP development
External Expert group review and approval
Continued analysis of longitudinal data from CRISP I and II

<u>October 2011-March 2013 (Baseline or YR 1 VISIT)</u>
Initial PCC Visit 1
Quarterly contacts with participants via telephone for detailed review of medications, medical visits, and hospitalizations

<u>April 2013-March 2014</u>
Finish PCC Visit 1
Quarterly contacts continue
Acquisition of plasma creatinine (duplicate determination)

<u>April 2014-March 2015</u>
PCC Visit 2
Quarterly contacts continue

<u>April 2015-March 2016 (YR 4 VISIT)</u>
Finish PCC Visit 2
Quarterly contacts continue
Acquisition of plasma creatinine (duplicate determination)

<u>April 2016-March 2017</u>
Complete analysis and prepare for CRISP IV

Eligibility and patient recruitment for CRISP III:

All CRISP I participants not yet on dialysis or receiving renal transplantation will be invited to participate in CRISP III. At entry into CRISP I participants met a number of inclusion and exclusion criteria. Exclusion criteria for participation in CRISP III are:

1. Current psychiatric or addiction or non-compliance disorder that in the discretion of the principal investigator indicates that the subject will not successfully complete the study.
2. Current medical problem that in the discretion of the principal investigator would make unsafe the participation in the study
3. Unable to provide written informed consent
4. Currently receiving renal replacement therapy or having received a renal transplant

PCC visits and annual blood samplings for participants who are pregnant will be postponed until six months following the delivery of a child and termination of lactation.

CRISP I participants with new MRI incompatible clips or pacemakers or who have developed severe claustrophobia can be recruited into CRISP III, but will not undergo MR studies.

To enroll in CRISP III, individuals must provide written informed consent meeting the requirements of the local IRBs. A typical consent process will include at least two consent forms, one that covers the basic elements of the CRISP III study and a separate consent form requesting permission to contact family members. Consenting to the latter will not be required to participate in the study. Separate consent forms will be developed to obtain historical and clinical information and a blood sample from known affected family members and for site-specific studies not covered in the main study consent form.

The CRISP III protocol does not exclude participants that enroll in other interventional trials. If CRISP III participants are recruited into an interventional trial (e.g HALT clinical trial) that also requires imaging studies, the visits for CRISP III and for the interventional trial will be coordinated to avoid duplication of tests and undue burden on the participant. Only data from baseline visits in interventional trials will be initially used for CRISP III analysis. Analysis of the data obtained on subsequent visits will be held until the interventional trial is completed. The CRISP III coordinating center and the intervention trial coordinating centers will share tracking and data collection schedules so that data on images completed can be stored. We anticipate that most of the CRISP III biochemical, imaging and urinary data will be collected as part of the other trials. These include serum creatinine, urine albumin, BP measurements, weight, and kidney volume. Medical information related to CRISP III will in part be collected in other trials, but there will be some CRISP III specific information that may need to be acquired by the CRISP III coordinators. For example, measurements of the GFR by the iothalamate clearance may not be performed in the intervention trials but will be performed in CRISP III participants.

Study Visits:

Study visits will include PCC visits on years 1 and 3; annual visits on years 2 and 4 to either the PCC or a local physician's office/laboratory; quarterly telephone interviews; recruitment of family members, sample collection and DNA isolation.

PCC visits (years 1 and 3): These visits will be conducted at each PCC following the same standardized protocol. Participants will be admitted to the in-patient GCRC in the late afternoon or evening or in the morning prior to eating or taking medication. On admission, participants will meet with one of the investigators, sign the consent form and undergo a formalized medical history interview. Information regarding medications (prescribed and over the counter), quality of life, and level and quality of pain will be obtained using procedures identical to those used in CRISP I and II. A family history questionnaire will also be obtained. Quality of life (SF-36v2),

pain, and family history questionnaires can be completed at any time during the PCC visits. Subjects will undergo a complete physical examination with standardized blood pressure determinations. If indicated, a B-HCG qualitative urine pregnancy test will be performed.

Blood and urine samples will be collected in the morning, prior to morning hydration or taking medications or food. Blood will be collected for:

1. Serum Creatinine – Serum samples will be obtained in duplicate, one processed at the local lab and the other frozen and batch shipped to the Cleveland Clinic Laboratory.
2. Total Electrolyte Panel – Sodium, potassium, chloride, total CO2, blood urea nitrogen, glucose and creatinine (at PCC).
3. Lipid Panel – Total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol (at PCC).
4. Twenty mL will be collected in two SST tubes (tiger-top, 10 mL each) and 16 mL in two PST tubes (green/grey-top, 8 mL each). Samples are to be centrifuged (without decanting) and shipped refrigerated (on frozen cold packs) to the NIDDK Biosample Repository at Fisher Bioservices on the day of collection, where they will be aliquotted into 1 mL tubes and archived.

Urine will be collected for:

1. Urine albumin and creatinine (at PCC).
2. Freshly voided urine specimens will be centrifuged in 50 mL PP tubes at 500 g for 5 minutes as soon as possible, with volume, processing times, and voiding times noted (processing times should be no longer than 20-30 minutes from the time of acquisition). Tubes will be kept in ice throughout this process. The bottom 250 !JL pellet (sometimes barely- or non-visible) will be transferred with a 1.0 mL pipette to a 1.5 mL eppendorf tube previously prepared with 750 !JL of TriReagent (Molecular Research Center, Inc. Cincinnati, OH), and inverted several times and put on ice prior to freezing at -80°C for future RNA/DNA retrieval. The remaining urine sample will then be transferred to 10 mL polypropylene (not polystyrene) Falcon culture tubes, stored in six 5 mL aliquots, and sent to the NIDDK Repository at Fisher Bioservices. The NIDDK Repository will supply all tubes, labels and shipping materials.
3. Urine samples for MCP-1 analysis will be sent annually from the NIDDK Repository at Fisher Bioservices to KUMC.

Whether in-patients or out-patients, the participants will have been instructed to drink three 8 oz glasses of water between 9:00 p.m. and 10:00 p.m. on the evening before the testing and to remain fasting but free to drink water ad lib. They will be asked to go to bed at 10:00 p.m. In the morning between 6:00 a.m. and 8:00 a.m. they will be asked to drink six 8 oz glasses of water in preparation for the iothalamate clearance determination which will start at 8:00 a.m., according to the protocol outlined in Appendix 1. GFR determinations will be performed using the short non-radiolabeled iothalamate clearance with standardized conditions and monitoring of bladder emptying using a bladder scan to maximize accuracy. The concentrations of iothalamate in plasma and urine will be measured by capillary electrophoresis. The duration of the test for the iothalamate clearance is approximately 2 hours. The plasma and urine samples will be packaged in a "refrigeration specimen" transport box and mailed to Mayo Medical Laboratories. The measurements will be performed at Mayo Medical Laboratories.

After completion of the GFR determination, the participants will undergo an MR examination of the kidneys and liver and determination of renal blood flow. This should take approximately 30 minutes.

Prior to the visit to the PCC, participants will be mailed a family history questionnaire. During the PCC visit, the study coordinator will review the completed questionnaire and the information regarding the family history of ADPKD will be updated. For those participants who have previously completed the family history questionnaire, this will be reviewed at each PCC visit to

assure that no new changes to the family member affection or living status has changed. The study coordinator will ask the participants permission to contact their relatives and to sign a separate informed consent for this purpose.

Blood pressure measurements: The standardized HALT method for obtaining blood pressure will be used. These measurements will be obtained at the time of the PCC visits, annually for local patients or only at the year 1 (2012/2013) and year 3 (2014/2015) visits for the rest. Blood pressures will be determined in the morning prior to antihypertensive medication intake using automated or non-automated oscillometric techniques (Dinemap, Critikon) and devices maintained and calibrated at the CTSA clinical sites or PCCs. The non-dominant arm (in terms of handedness) will be used to obtain BP readings unless there is a reproducible (on at least three consecutive measurements) difference in systolic BP of 20 mm Hg or more between arms. If there is a reproducible difference in systolic blood pressure of 20 mm Hg or more between both arms, the arm with the higher blood pressure will be used. In all other cases, the non-dominant arm will be used. Participants will also be instructed to abstain from smoking and consuming caffeine for 30 minutes prior to taking their BP measurements. After sitting quietly for at least 5 minutes with the arm resting at heart level, three readings will be obtained at least 30 seconds apart. If there is a difference of more than 10 mm Hg (systolic or diastolic) between the second and third readings in one sitting, a fourth and fifth reading will be recorded for that sitting.

Serum creatinine measurements: Serum creatinine will be determined annually for all participants. Blood will be drawn at the PCC and serum samples will be obtained in duplicate. One sample will be for serum creatinine determinations at the PCC. The other will be batch shipped every three months to the Cleveland Clinic for validation. HALT participants will have the serum creatinine done at the annual HALT visit. For non-local participants who are unable to return to the PCC on years 2 and 4, a blood sample will be obtained in duplicate at a local facility. Duplicate serum samples will be shipped to the PCC, one for processing and creatinine measurement at the PCC and the other will be batch shipped annually to the Cleveland Clinic. For standardization purposes the local labs will be contacted directly with the procedure to be followed.

<u>MR Imaging:</u>

MR images will be obtained at each PCC using the procedures described below. After the acquisition, MR images will be reviewed locally at each PCC site and securely transferred via secure internet connection to the Image Analysis Center (IAC). The procedures for MR scanning of the heart (HALT study only), kidneys and liver are as follows:

Before each study, the MR scanner will be adjusted for proper shimming (i.e., correction of magnetic field inhomogeneity).

1. Breath-holding instruction will be provided, and the subject will be coached prior to MR scanning. Administration of oxygen via nasal cannula may help improve the breath-hold capacity, particularly for subjects with limited breath-hold capacity.
2. EKG pads will be placed over the chest. If EKG gating is not available or functioning, it may be replaced with a peripheral pulse gating.
3. Subject will be placed supine on the MR table with his or her arms to the side.
4. A phased-array surface coil will be positioned with its center over the inferior costal margin, i.e. over the expected location of the kidneys.
5. Scout scan to locate the scan range of the entire kidneys. A stack of axial images to cover the most anterocaudal and posterocranial aspects of the kidneys is highly recommended.

6. The field-of-view (FOV) should be kept as small as possible (30-35 cm) without producing wrap-around artifacts.
7. Breath-hold, coronal T2 scan ('SSFSE': Single-Shot Fast Spin Echo or 'HASTE': Half-Fourier Acquired Single-shot Turbo spin Echo) with fat sat at 9mm fixed slice thickness, usually achievable in a single breath-hold. Please make sure both kidneys are imaged completely without missing any anterior or posterior portions. This coverage assurance is critical for the following T1 imaging.
8. Coronal T1 scan (3D 'VIBE': Volumetric Interpolated Breath hold Exam or 'LAVA': Liver Acquisition Volumetric Acceleration) without fat sat at 3mm fixed slice thickness (acquisition will be performed at 6mm thickness and then the slice will be interpolated at 3mm, i.e., in GE, ZIP =2 in the slice direction). Keep the flip angle ::15^{o}. To improve signal-to-noise ratio (SNR), keep the Bandwidth low (62 kHz or 42 kHz) and/or increase the number of phase-encoding steps (be aware, the acquisition time will increase). In GE LAVA sequence, turning off "optimize flip for contrast-to-noise ratio (CNR)" will allow to change the flip angle or bandwidth. Do NOT use parallel imaging (no 'SENSE', 'ASSET', 'iPAT' or 'GRAPPA').
9. Breath-hold coronal T2 scan (SSFSE/HASTE) with fat sat of the kidneys at 3mm fixed slice thickness, which would require 1-4 breath-holds depending on the kidney size. Use as few breath-holds as possible. The first scan should cover the posterior aspect of the kidney. Neighboring image groups should be overlapped by a single 3mm slice. To determine correct table position choose the "shift-mean (starting point in GE)" of the second scan for example: the first shift-mean = -60mm, the number of slices in the first set =23, (23-1)x3=66mm, new shift mean =-60+66=6mm.
10. Breath-hold coronal T2 scan (SSFSE/HASTE) without fat sat of the kidneys with adjusted slice thickness, 3-6 mm, i.e. the slice thickness best attainable with a single breath-hold (The adjusted slice thickness may not remain the same in a follow-up MR scan if there is a change in the subject's breath-hold capacity or kidney size.) Repeat the scan over the liver with the same slice thickness. This scan and the scan for the kidney should share one overlapping liver slice (i.e., the most posterior slice of the liver scan should be identical to the most anterior slice imaging the liver in the kidney scan. If more than two scans are required to cover the anterior liver, again the neighboring scans should be overlapped by one slice.
11. Breath-hold coronal 2D true-FISP ('FISP': Fast Imaging Steady-state Precession or 'FIESTA': Fast Imaging Employing Steady-state Acquisition) without fat sat at 3mm fixed slice thickness, which would require 1-2 breath-holds depending on the kidney size. Use as few breath-holds as possible. The first scan should cover the posterior aspect of the kidney. Neighboring image groups should be overlapped by a single 3mm slice. To determine correct table position choose the "shift-mean (starting point in GE)" of the second scan for example: the first shift-mean = -60mm, the number of slices in the first set =23, (23-1)x3=66mm, new shift mean =-60+66=6mm.
12. (For renal blood flow measurement) Breath-hold, oblique-coronal 2D true-FISP (FIESTA) with fat sat with 4mm fixed slice thickness at 2mm spacing (i.e., overlap 50%) over the aorta and renal arteries. See the figure below for the orientation of the image plane. Typical parameters: 192x256 matrix, 75^{o} flip angle, 125 kHz BW, 15-sec scan.
13. (For renal blood flow measurement) Breath-hold, phase-contrast technique of renal blood flow measurement. From the FIESTA images, the renal arteries will be identified. To accurately measure velocity, it is important to choose the imaging slice perpendicular to a vessel. Velocity encoding (VENC) value of 100 or 50 cm/sec will be used. Small FOV (14-16 cm) and large

matrix (256x192 or 512x512) are important for an accurate measurement of the vessel size. Segmented, prospectively cardiac-triggered phase contrast flow measurements will be obtained to compute the mean and peak velocities, as well as the total mean flow, during the cardiac cycle. Please, see the renal artery figures below

For image transfers, images will be pushed from the local PCC MR scanner to the PC workstation. For participant confidentiality, participant names and identifiers will be removed and replaced with CRISP-ID numbers and accession numbers prior to image transmission to the IAC. A virtual private network (VPN) client has been installed on the PC workstation to encrypt the data for secure transmission via the Internet. The IAC will review the images and generate quality control reports for PCCs. Images determined to be inadequate for measurement must be reacquired.

The stereology method, a quantitative morphology by statistical analysis of the structures of random sections, is widely used in cytopathology and medical imaging analysis. A point- counting stereologic technique involves a simple, fast method of segmenting an object by counting the number of intersections of a randomly oriented and positioned grid over the object. This method does not require border tracing or threshold determination, but relies on the operator's decision of selecting each point that intersects the object. The areas of the whole kidney in each image can be calculated from the collection of points, and volume measurements can be made from a set of contiguous images. Analysis software, written by the Mayo Foundation, will be utilized for making stereology measurements. Each volumetric measurement will be made by a trained analyst at the DCC, and will be reviewed by a radiologist for quality control. Agreement between the radiologist and technician in the CRISP Study was very high (97%). The result from the radiologist's review of stereology measurements will be used to calculate the whole kidney volume.

Annual fasting sample collections:

On off years, participants will have blood samples collected either at the PCC or at their respective clinics for the determination of creatinine concentrations (see above).

Quarterly telephone interviews:

During the interviews information regarding medication changes, hospitalizations, doctor visits and outpatient procedures will be recorded. A standardized symptom check list aimed at identifying both renal and extra-renal complications, symptoms and diagnostics will be administered. Requests for birth certificates or official birth weight documents will be obtained from the CRISP participants. A follow-up study form will be completed after each telephone interview. Any physician who has examined/treated the participant since the last visit or telephone interview will be contacted to obtain information about the participant's health.

Plans to address participants who are lost to follow up:

Participants who have consented into CRISP but have not completed all study visits and no further information is available are considered lost to follow up. For these participants we will utilize the National Death Index (NDI) and the United States Renal Data System (USRDS) to gather potential endpoint data. As previously mentioned in this protocol we expect more participants to reach endpoints during CRISP III. Endpoints such as death and ESRD can be found in the listed sources. Applications and data use agreements (DUA) will be sent by individual PCCs to the NDI and USRDS. Upon approval from the NDI and USRDS, the PCCs will provide the following data; name, date of birth (four-digit year), social security number, sex, and date of death if available. Patients will be assigned a unique identifier (no duplicates). The direct patient identifiers will be sent to the USRDS via secure email or with an encrypted CD/DVD by mail with the password sent separately. The USRDS will upload the file to a host site called M+Box. M+Box is a partnership

between the University of Michigan and M+Box that offers an easy and safe way to store and share files. The USRDS will put the file on the M+Box site in a password protected folder and provide a link to that folder to the study team to download the data. This folder will expire after a set amount of time. Similarly the listed patient data excluding date of death will be sent to NDI via encrypted CD/DVD. NDI will return acquired data to PCCs on an encrypted CD/DVD. This data will also be resubmitted (if necessary) to the NDI and USRDS at the end of the study.

Plans to collect additional data from USRDS for ESRD participants:

We will utilize the described process above to not only collect LTFU information from USRDS but to collect information for all CRISP participants who have reached ESRD (both transplant and dialysis). The USRDS is a wealth of information containing data such as serum creatinine values within 45 days of ESRD and the date it was measured. Information such as this is crucial to our analysis and will allow us to more accurately model the rate at which GFR declines and the shape of the trajectory, which we are using to develop predictive models of kidney function in PKD.

4. Recruitment of family members, sample collection and DNA isolation:

CRISP III will collect and analyze DNA samples and clinical data from CRISP family members for enhanced genotype/phenotype studies and to facilitate other genetic studies. A major component of CRISP II (Aim 3) was to collect more exhaustive family histories of all CRISP I patients and draw an electronic pedigree for each family (Progeny); CRISP III (sub Aim 4.1) will be a continuation of this goal. Identified affected family members who agree to participate will be consented into the study and clinical and imaging data from the patient retrieved from clinical records. A blood sample will be collected for a determination of serum creatinine at the Cleveland Clinic laboratory (unless the participant is on dialysis or has received a transplant) and for DNA extraction and the establishment of EBV transferred lymphoblastoid cell-lines, employing the NIDDK Center for Genetic Studies, Rutgers University Cell and DNA
Repository. Samples will be sought from all traceable individuals from each of the families with proven ADPKD by established imaging criteria. We estimate from preliminary analysis of the CRISP families that approximately four further affected individuals over 18 years of age will be traceable in each family making a total of 800 family members. Analysis of known family data predicts that they will have an average age of ~53 years, that 53% will have ESRD and a further 11% renal insufficiency measured by a serum creatinine 21.4mg/dl, females and 21.6mg/dl males. Participants will also be asked to complete a lifestyle questionnaire (to assess smoking history, caffeine exposure, estrogen exposure and levels of physical activity) and a family history questionnaire to further extend the traceable family. When possible, the most recent CT or MR examination of the abdomen, or if not available, the most recent ultrasound images will be reviewed and renal volume estimated using established formulae. Kidney volume will be calculated by the ellipsoid formula: Volume = length x width x thickness x pi/6, using maximum length in longitudinal plane and for width and thickness in the transverse plane perpendicular to the longitudinal axis of the kidney at the level of the hilum. If only coronal plane films are available, the kidney depth may be assumed to be equal to the width of the hilum so that the formula becomes: Volume = length x (width) squared x pi/6. Although not as accurate as the MR data available from CRISP I patients, it will be a relatively reliable means to assess renal disease severity in all patients. All of this clinical and lifestyle information, plus the available genetic information on the family, will be stored in the CRISP database that is maintained by the DCIAC.

ANALYSES

Aim 1. Extend the serial quantification of total kidney (TKV) and liver (TLV) and of kidney

(KCV) and liver cyst (LCV) volumes in order to develop and test new models for predicting the risk of developing renal insufficiency

Hypothesis 1a. Baseline TKV and change in TKV predict loss of kidney function.

To characterize the development of renal insufficiency, we will employ a battery of GFR measurements based on iothalamate clearance and serum creatinine concentration (MDRD equation). For example, we will evaluate the pattern of GFR decline over time to determine if individual participants slopes are appropriate or not. In conjunction with our evaluation of slopes, we will employ a repeated measures mixed model (hierarchical model) using all time points to assess the effects of our predictors, time, and the interactions between predictors, such as TKV and time, to predict decline in GFR We will also assess hard endpoints such as GFR thresholds defined by K/DOQI stages 3, 4 and 5, 4) 50% decrease in GFR from the CRISP I baseline, 5) dialysis, transplantation, and death from ESRD.

Multivariable analysis will be extended to include more individuals with renal insufficiency outcomes predicted to occur in CRISP III in order to obtain more rigorous testing of the hypothesis that TKV predicts renal insufficiency. ROC analysis will be used to determine the sensitivity and specificity of TKV to predict renal insufficiency. By including continuous changes in GFR we will demonstrate trends of change relatively early in the course of individual patients, and thereby determine more precisely the earliest clear indication of renal insufficiency in relation to TKV and change in TKV. To measure annual change over time for TKV, iothalamate clearance and plasma creatinine concentration we will calculate within participant intercepts and slopes, transforming the measurement if appropriate. For continuous outcomes (slope of GFR) we will use linear regression, for dichotomous outcomes (50% decrease in iothalamate clearance) logistic regression and for ordinal outcomes (K/DOQI stage) ordinal logistic regression. During our model building process we will also test the benefit of 1) inclusion of other variables, known to associate with TKV that occur in advance of the development of renal insufficiency (hypertension, pain, gross hematuria) to determine if they enhance the identification of patients destined to develop renal insufficiency beyond what htTKV provides, and 2) the addition of other variables explored in Specific Aim 2, 3, and 5) in empirical models utilizing new candidate biomarkers.

Preliminary hierarchical linear modeling [10] of iothalamate clearance trajectories indicates that: 1) the decline of renal function over time varies among individuals, and 2) the rate of decline can be predicted from patient gender and baseline TKV values (e.g., based on the CRISP baseline visit data, this model predicts iothalamate clearance at the third year follow up with $r=0.76$ for training and $r=0.71$ for validation datasets, both $p<0.0001$). We will further develop these trajectory models using extended iothalamate clearance data and inclusion of time-varying predictors (e.g., TKV). These analyses will be complemented by Generalized Estimation Equations (GEE) modeling.

Hypothesis 1b. The progression of polycystic liver volume (LCV) will be similar to but distinct from that of TKV; baseline LCV, adjusted for covariates, will independently predict the rate of increase in LCV and complications arising within the liver.

We have determined previously in this cohort that LCV and TKV increase with age. In CRISP III we will determine the correlation of change in LCV and TKV in individual patients adjusting for appropriate covariates such as gene and mutation type. Our analytic approach will be similar to that used for the kidneys when the outcome is continuous.

Aim 2. Determine the extent to which age and sex-adjusted measurements of renal blood flow (RBF), determined by MR imaging, predict the rate of change in TKV and determine if RBF and TKV independently predict the risk of developing renal insufficiency.

Hypothesis 2. Baseline RBF predicts the rate of increase in TKV and, independent of and in

addition to baseline TKV, predicts renal insufficiency.
Our analytic approach to RBF will be to first determine the patterns of decline in RBF over time using repeated measures ANOVA testing for linear or quadratic trend and to see whether these patterns change over time. Next, we will add baseline RBF to the existing baseline analyses and models developed in Aim 1 and assess whether adding baseline RBF improves the ability to predict loss of renal function. Potential RBF and TKV or TCV interaction (potentially using ROC cutpoints) will also be considered. Lastly, we will add all RBF values to the longitudinal / hierarchical models developed in Aim 1.
Aim 3. Develop methods to quantify total cyst number, individual cyst volumes, and pattern of distribution of cysts in each kidney and apply these to analyze the influence of renal cyst number, volume, and topography at baseline on the subsequent course of TKV and GFR and the risk of developing renal insufficiency.
Hypothesis 3a. Renal cyst number and volume will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.
First, we will perform Pearson or Spearman correlations comparing cyst number and volume with TKV and GFR at each time point. Second, we will perform correlations of cyst number and volume at each time point with the slopes of appropriately transformed TKV or GFR values, which represent average change. Third, we will regress the slopes on baseline cyst number and volume including appropriate covariates, genotype, or clinical markers. Finally we will use mixed models or GEE including cyst number and volume at each time point, a time effect and appropriate interactions.
Hypothesis 3b. Renal cyst topography (medullary vs. non-medullary) will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.
For this hypothesis we will assess the following predictors: 1) The degree of cortical cyst distribution (CCD) on a scale from 1 to 5 (1: mostly medullary, 3: diffuse, 5: mostly cortical) at baseline; 2) The ratio of medullary to cortical cyst area percentages (MPCP). A ratio of 1 implies a diffuse distribution; values above 1 imply that the percentage of cysts occupying the medullary area is greater at baseline; and 3) The ratio of number of cysts in the medullary region to the number of cysts in the cortical area (MNCN) at baseline. We will use GEE based regression models with GFR as our dependent measure. Baseline MPCP (MNCN) will be included in the model at every time period to adjust for possible regression to the mean. The model will also contain a time variable (for which we will explore alternative correlation structures) and MPCP (MNCN). From this model we can evaluate how much GFR changes with a one unit change in baseline MPCP (MNCN) and how much the addition of time and subsequent MPCP (MNCN) values impacts that change. We can also test whether baseline MPCP (MNCN) values affect GFR differently over time by including the time by MPCP (MNCN) interaction. The model will be refined by considering whether inclusion of total cyst volume vs. total kidney volume improves the model (the extent of multicolinearity will be evaluated first), whether the addition of baseline covariates and modifiers improves the model, and whether the addition of other time varying covariates improves the model. Similar models will be built using CCD but this predictor will have 5 or 2 (if we dichotomize as primarily medullary vs non-medullary) levels so a baseline adjustor would not be necessary. For
Hypothesis 3c. Individual renal cyst growth is continuous and exponential (similar to the growth pattern in TKV) and patterns of renal cyst growth or involution will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.
First, we will plot the cyst growth patterns over time for each cyst and assess whether the pattern

is linear or exponential or some other trend. Second, we will create slopes for each cyst based on the appropriate transformation. Third, we will use GEE modeling to see if genotype, age, gender or other factors predict cyst growth using the slopes as our outcome measure. GEE is necessary because we have multiple cysts per person. We will also need to adjust for whether or not the cyst merged with another at a particular time point and account for whether or not the participant is potentially receiving treatment due to their participation in HALT-PKD or TEMPO.

Aim 4.1. Collect and analyze DNA samples and clinical data from CRISP family members for enhanced genotype/phenotype studies

Hypotheses 4a. This large cohort of genetically and phenotypically characterized CRISP families will clarify the role that genic and allelic factors play in determining phenotype. The range of intra and inter-familial variability will shed light on the importance of other genetic factors.

We estimate we will have ~1000 cases from the 202 families for analysis with an average age of 48 years. Approximately 25% will have ESRD and a further 18% renal insufficiency. On average, each family will have 5 affected members but there will be a great deal of variability. The majority are PKD1 families (157) with 27 PKD2 and no mutation detected (NMD) in 18. Mutation studies have shown in the characterized cases that ~70% of families have a truncating mutation and ~30% in-frame. Genetic factors analyzed will be the gene involved and the type of mutation. Mutation studies will analyze mutation type (truncating or in-frame) and mutation position. The major phenotypic end-points will be TKV and eGFR (MDRD equation), but the secondary endpoints of age at onset of hypertension, the occurrence of hematuria, urinary tract infections or kidney stones, estimates of severity of liver disease and the occurrences of an ICA, will also be analyzed.

As part of our ongoing assessment of TKV we will use height adjusted TKV and log transform to more approximate normality (lnhTKV). This should account for gender differences. We will explore a similar transformation for eGFR if needed. InhTKV and eGFR will be compared between the PKD1, PKD2 and NMD populations at each time period using ANOVA and by repeated measures ANOVA or mixed models including a time effect. Potential covariates, such as age, will be added as needed. We will also create indicators of severity for lnhTKV and eGFR (most and less severe 10 and 25%, for instance) and use chi-squared analysis to see how much these groups overlap across our three genetic groups. Multinomial GEE modeling will be used to assess whether family membership is significant and we will assess the differences between within and between family variability. If there is a within family effect, family id will be included as a random effect in the above mixed model assessment of the relationship of our primary outcomes to gene type. This analysis will allow quantification of the amount of phenotypic variability due to genetic factors beyond the gene and environmental influences. In addition, the secondary outcomes will be compared between the genic groups using similar methodology if they are continuous or GEE modeling if binary (eg kidney stones Yes/No)

Comparison of mutation type will be analyzed in a similar fashion. Expected outcomes are that some in-frame mutations will consistently be associated with milder disease (in families), indicating that some mutations are incompletely penetrant. Analysis of the extreme populations and the intra/inter familial comparison are likely to be particularly informative. This may lead to the identification of a group of hypomorphic alleles. Further analysis of specific mutation types, such as splicing mutations, for instance, may also be informative. Comparison with mutation position will be done by plotting the position of the mutation against the age/gender corrected primary end-points to determine if some areas of the gene are associated with more severe disease and using mixed models to evaluate whether the position is significantly different. To analyze position independent of hypomorphic alleles the analysis could also be done for just

truncating mutations and with the likely hypomorphic alleles excluded. Similar analysis will be done with the gene divided into 10-20 equal units for both the primary and secondary outcomes. Overall this analysis will provide the most comprehensive data on the influence of gene and mutation type on various outcomes, with the influence of mutation type being particularly interesting. This population with many larger families will also provide a much clearer view of how much other genetics factors influence the outcome and start to provide data on how the environment may be important in ADPKD.

SUPPLEMENT TO CRISP III FOR ADDITIONAL/EXTENDING DATA COLLECTION

During data analysis of CRISP III and preparation for CRISP IV PCCs will collect additional data/extend data collection by contacting participants via phone every 6 months +/- 2 months. Participants will also have one blood draw during this time period to measure creatinine. If blood draw is performed clinically and the information is in the participant's medical record then the test does not have to be performed however if it is not available in the medical record then blood will be drawn either at the PCC or at a local lab. Participants will be re-consented using an addendum consent form for study procedures. The phone calls will be conducted in the same manner as the quarterly telephone interviews that were conducted throughout CRISP III utilizing the same forms and entering the information on the CRISP website. The additional data collection efforts will serve to create a bridge between CRISP III and subsequent funding possibilities for CRISP IV; maintaining contact with, and data collection from, the CRISP participants over the next year is critical to successful implementation of CRISP IV if funded by NIDDK (with a potential start date of June 1, 2017).

APPENDIX A: CRISP ANCILLARY STUDIES

June 2011

IN PROGRESS

Perrone

Modeling Disease Progression in ADPKD: Total Kidney Volume as a Clinical Trial Endpoint. *Approved 12-27-11*

PROGRESS: Our primary research objective is to develop a predictive model for ADPKD progression using TKV, and to estimate the performance characteristics of that model. It is already known, qualitatively, that TKV predicts progression; however our goal is to explicitly quantify the predictive validity of TKV. More specifically, we want to elucidate the quantitative prognostic implications of a given change in TKV in the context of patient covariates such as gender, age, weight, blood pressure, and genotype, and also in the context of clinical event histories (i.e., frequency of gross hematuria, cyst infection, kidney stones, abdominal/flank pain). This quantitative model will be utilized to generate scientific consensus—among clinicians and scientists from academia, medical product development, and regulatory agencies—on the utility and reliability of changes in TKV as a biomarker and clinical endpoint for the progression of ADPKD.

The work is being funded by the PKD Foundation and the Critical Path Institute has been subcontracted to build the TKV database which will include several data sources including CRISP I and eventually CRISP II. Data collection is ongoing at this point and a master data dictionary has been built.

Torres

Seeks permission to measure copeptin and ANP in baseline or Year 1 plasma samples of CRISP patients as a surrogate for vasopressin. *Approved 12-09-09*

ABSTRACT: Wendy E Boertien*[1], Esther Meijer[1], Jie Li[2], James E Bost[2], Joachim Struck[3], Michael F. Flessner[4], Ron T Gansevoort[1] and Vicente E. Torres[5]. *Copeptin, a Surrogate Marker for Vasopressin, is Associated with Disease Progression in the CRISP cohort of ADPKD patients.* in *American Society of Nephrology, Renal Week 2010 (submitted).* 2011.

Experimental studies suggest a detrimental role for vasopressin in the pathogenesis of ADPKD. The significance of vasopressin in human ADPKD, however, is yet unclear. We therefore investigated whether vasopressin is associated with disease progression in a cohort of ADPKD patients.

Baseline plasma copeptin, a reliable surrogate for vasopressin, was measured in 241 ADPKD patients who participated in the CRISP study (a longitudinal, observational study). Patients were followed for 3 years. Every year total kidney volume (MRI) and renal function (iothalamate clearance) were measured. In these 241 patients (age 32.4 ±8.9 years, 40% male, GFR 97.8 ±24.7 ml/min/1.73m^2), median copeptin level was 2.9 (IQR 1.8 −5.0) pmol/L. Copeptin concentration was higher in males than in females. Remarkably, baseline copeptin concentration was not associated with plasma osmolality ($p = 0.29$), urine osmolality ($p = 0.16$) and 24 hour urine volume ($p = 0.17$). In contrast, baseline copeptin concentration was significantly associated with change in total kidney volume during follow-up (std. $B = 0.24$, $p < 0.01$). This association remained significant after adjusting for gender, age, cardiovascular risk factors and baseline TKV (std. $B = 0.14$, $p = 0.03$). Baseline copeptin concentration was also

significantly associated with change in GFR after adjusting for gender, age cardiovascular risk factors and baseline GFR (std. B = -0.15, p = 0.03). These data show that in ADPKD patients, copeptin levels, as a marker for vasopressin, are not correlated with normal physiologic parameters as plasma nor urinary osmolality or urine volume. Most importantly, high copeptin levels are independently associated with disease progression in ADPKD patients, confirming experimental studies suggesting a detrimental role for vasopressin.

Kistler/Serra

Seeks permission to examine their battery of putative prognostic markers in the urine of a select cohort of CRISP patients to determine if the markers predict those will progress to larger kidneys and reduced GFR at faster rates than those who do not express the markers at high levels. Partnered with Dr. Arlene Chapman. *Approved 2/23/09 and remains an active project.*

ABSTRACT: Andreas D. Kistler[1], Harald Mischak[2], Justinya Siwy[2], Arlene B. Chapman[3], Rudolf P. Wuthrich[1] and Andreas L. Serra[1]. *A unique urine proteomic pattern in autosomal dominant polycystic kidney disease.* in *American Society of Nephrology, Renal Week 2010 (submitted).* 2010.

Using capillary electrophoresis coupled to mass spectrometry (CE-MS), we have previously found a unique urinary polypeptide profile characteristic for autosomal dominant polycystic kidney disease (ADPKD). Here, we validated these findings from a Swiss ADPKD cohort in a large US American ADPKD cohort and tested whether urinary biomarkers correlate with volume progression of polycystic kidneys. Spot urine samples of 48 patients from the control arm of the SUISSE ADPKD study, 124 patients from the CRISP cohort and of 86 healthy controls were analyzed. All ADPKD patients were followed up by serial MRI based kidney volumetry in 6-months (SUISSE ADPKD study) or annual (CRISP) intervals for 18-36 months. Our previously published diagnostic biomarker model for ADPKD that was based on a training sample of 17 Swiss ADPKD patients performed well in the CRISP cohort, achieving 91.9% sensitivity and 90.7% specificity (AUC 0.964). A refined diagnostic model based on proteomic data of all 48 analyzed samples from the SUISSE ADPKD study achieved an even higher performance upon validation in the CRISP cohort (sensitivity 93.5%, specificity 94.2%, AUC 0.974). Using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) we were able to identify 30% of these diagnostic biomarkers. Most of them represent collagen fragments mirroring drastic changes of extracellular matrix turnover in polycystic kidneys. We then used 110 patients from both studies as a training and 62 CRISP patients as a validation cohort to identify urinary biomarkers that correlate with TKV growth expressed in percent or ml per year. Although a few markers showed significant correlation with volume progression after adjustment for multiple testing, the correlation was weak and not sufficient to establish a clinically useful prognostic biomarker model. In conclusion, we were able to validate and refine our previously established diagnostic urinary biomarker model for ADPKD in an independent cohort. We could not find prognostic biomarkers, which may be due to insufficient follow up time. [1]

1. Kistler, A.D., H. Mischak, J. Siwy, A.B. Chapman, R.P. Wuthrich, and A.L. Serra. *A unique urine proteomic pattern in autosomal dominant polycystic kidney disease*. in *American Society of Nephrology, Renal Week 2010 (submitted).* 2010.

Sandford

Seeks permission to use anonymised data to carry out power calculations for the design of autosomal dominant polycystic kidney disease specific clinical trials using MR renal volume measurement as a clinical end-point. *Approved 7-27-08; Dr. Bost has sent de-identified data for the analysis*.

PROGRESS: The MRC and the United Kingdom are very interested in establishing registries of well phenotyped ADPKD individuals for which to then identify potential participants in randomized clinical trials. To do so, the Cambridge Renal Unit has requested use of the deidentified CRISP dataset to perform power calculations to determine the number of participants needed for interventional studies.

Parikh

Study of NGAL in CRISP subjects. The study is underway and the PI is working with Dr. Bost and Dr. Chapman to obtain the appropriate samples from the NIH repository.
Approved. Study and analysis completed. Manuscript is under review in the journal kidney international.

MANUSCRIPT: Chirag R. Parikh, Neera K. Dahl, Arlene Chapman, James E. Bost, Charles L. Edelstein, Diane M. Comer; Raoul Zeltner[1]; Xin Tian; Jared J. Grantham; Stefan Somlo. *Evaluation of Urine Biomarkers of Kidney Injury in Polycystic Kidney Disease: From Bench to Bedside.*(*Manuscript* submitted to Kidney International)
Introduction: Progressive disruption of renal tubular integrity in the setting of increased cellular proliferation and apoptosis is a feature of ADPKD. The effect of these processes on expression of two markers of tubular injury, IL-18 and NGAL, was examined in murine models and in cyst fluid and urine of patients with ADPKD. Methods: NEED A DESCRIPTION OF MICE AND CYSTS. In four annual serial urine samples from 107 ADPKD Consortium for Radiologic Imaging for the Study of Polycystic Kidney Disease (CRISP) participants, NGAL and IL-18 excretion rates were determined in conjunction with measures of total kidney volume (TKV) and estimated GFR (eGFR) utilizing the MDRD equation. Results: *Pkd2*$^{WS25/-}$ mouse and Han:SPRD rat kidneys showed prominent expression of NGAL and IL-18/IL-18R, respectively, in epithelial cells lining kidney cysts. In human ADPKD cyst fluid, biomarker levels were elevated (140-4939 ng/ml for NGAL and 100-588 pg/ml for IL-18). In CRISP participants, mean % TKV increase was 5.4 %/year and mean decline in eGFR was -2.4 mL/min/year. The trend of mean urine NGAL and IL-18 over three years was statistically significant, however, there was no association between biomarker tertiles for IL-18 and quartiles for NGAL and change in TKV or eGFR over three years of follow-up. In summary, NGAL and IL-18 are abundantly expressed in the tubular epithelial cells lining cysts in murine polycystic kidneys and are elevated in human cyst fluid. Urinary NGAL and IL-18 excretion are mildly and stably elevated in ADPKD, but do not correlate with changes in TKV or kidney function. This may be due in part to the lack of communication between individual cysts and the urinary collecting system in this disorder.

Wallace

A Novel Biomarker of ADPKD Activity in Serum and Urine. The study is underway and Dr. Wallace is determining levels of periostin in serum and urine samples from patients in the KUMC cohort that have been stored on site. Access to repository samples will only be necessary if the results of the preliminary study indicate that wider applicability would be of value. This ancillary study is supported by Dr. Wallace's sub-project of a NIH-sponsored PKD Center grant, Dr. James P. Calvet, Principal Investigator DK057301. *Work in progress*

PROGRESS: Dr. Wallace is in the process of evaluating several reagents that are needed to increase sensitivity and accuracy of this inquiry. This will require an unpredictable length of time to complete.

Harris

Identifying genetic modifiers of severity in ADPKD DK079856). This NIH funded R01 is an ancillary study to the CRISP study.

Grant Application ABSTRACT:
The phenotype associated with the Mendelian disease autosomal dominant polycystic kidney disease (ADPKD) is highly variable. Of paramount importance clinically is the severity of the renal cystic disease. Previous studies have shown that genic (PKD1 or PKD2) and (to a less extent) allelic factors influence the phenotype, but one of the most important modulating factors is genetic background. Identifying quantitative trait loci (QTL) that significantly influence the severity of disease would help understand pathogenesis, be of prognostic importance and may guide therapeutics. The development of high-density SNP arrays provides a means to map these QTL in large, clinically and genetically well-characterized populations, employing a genome-wide association study (GWAS). The NIDDK-funded CRISP and HALT PKD studies have a combined cohort of >1,000 ADPKD patients who are highly characterized clinically and genetically; >700 have renal MR imaging data. MR calculated total kidney volume (TKV) has been shown to be a good measure of disease severity, informative before a decline in GFR is detectable. Additionally, well characterized PKD1 populations are available at UCHSC, Emory, Mayo, KUMC, Toronto, Cambridge and Oxford to aid the discovery, replication and verification steps that are required to differentiate genuine QTL from false positive associations. Here, we propose a GWAS employing the Illumina Human660w-quad Genotyping BeadChip (658,000 SNPs), in 1600 PKD1 Caucasians (Aim 1). Total kidney volumes (TKV) data will be available in ~1100 of these patients and informative eGFR data in ~900 cases. The 7600 most likely associated SNPs detected with these endpoints will be further assayed using a customized array in a replicate population of 1600 PKD1 patients with the TKV and eGFR phenotypic endpoints, as above (Aim 2). A final verification step will analyze the 30 most promising loci in a population of 1216 patients with the same endpoints (Aim 3). The 30 loci will each be tested with ~12 SNPs (total of 348) to refine the QTL and highlight possible causative genes. To maximize the power of the study we will analyze the data as a combined population and family-based association study. In summary, this consortium of groups will perform the first GWAS in ADPKD to identify modifiers of disease severity. A three-stage design; discovery, replication and verification steps are to be employed to maximize the chance to identify QTL and minimize false positives.

Harris

Genetic Analysis of the CRISP ADPKD Population and Correlations with the Rate of Disease Progression. *Approved and funded Work in progress*

Grant Application ABSTRACT: Assessment of the influence of the PKD1 haplotype on the severity of ADPKD. Submitted as an R01 but did not get a fundable score. Dr. Harris plans to resubmit.
Autosomal dominant polycystic kidney disease (ADPKD) is a progressive, genetic kidney disease that often results in end-stage renal disease (ESRD). The severity of disease is highly variable with some patients developing clinically significant disease *in utero*, while others escape ESRD even in old age. Effects at the genic, allelic, genetic background and environmental level influence this variability. The gene involved, *PKD1* or *PKD2*, is known to be a major factor determining severity, but significant intragenic variability indicates that other non-genic factors are also important. Recent studies have highlighted that allelic effects can radically influence the phenotype and, in particular, indicate a role for incompletely penetrant (hypomorphic) alleles. These can alter the dosage of the functional ADPKD protein, polycystin-1 (PKD1) or polycystin-2 (PKD2), and are a significant factor determining the extremes of the disease phenotype. Hence, if an inactivating allele is inherited *in trans* with a hypomorphic variant, severe early onset disease can result, while the hypomorphic allele alone can cause mild ADPKD, without renal failure. In this study we will analyze the importance of variants on the "normal" *PKD1* allele to modulate the severity of disease in the typical, adult onset, PKD1 population. The populations that will be analyzed are those involved in the observational study, CRISP, and the clinical trial, HALT PKD, due to the wealth of clinical, imaging and genetic data available; plus other families collected at Mayo, Emory and UCHSC. In this study, two types of variants will be considered, common variants (minor allele frequency >1%) and haplotypes of such variants, and rare variants that might alone be hypomorphic alleles. Genetic information about *PKD1* and *PKD2* already available from the study participants will be combined with new sequence data for these genes obtained from a family member, so that the full haplotype, including rare variants and the disease causing mutation, will be available from 720 PKD1 families. Haplotyping of additional family members will result in available information on common variants in 1800 patients. In Aim 1, correlations will be made between disease severity measured by total kidney volume (TKV) and eGFR and the common variants and haplotypes of variants found on the "normal" allele. In Aim 2, similar correlations will be made between rare alleles inherited *in trans* with the disease causing mutation and the clinical and imaging endpoints. The final aim will determine if next-generation sequencing methods, including Single Molecular Real Time (SMRT) sequencing, will be an alternative means to rapidly generate this haplotyping data for analysis. Overall the study will determine the extent to which variants within the *PKD1* gene influence disease presentation and progression and, hence, are a major modifier of the PKD1 phenotype.

COMPLETED

Athena

Direct DNA Sequencing of Research Samples from CRISP Study. *Completed*

PROGRESS: DNA sequences are completed and data available for future analyses.

Brummer

Validation of Renal Blood Flow Measurements by MRI. *Completed*

ABSTRACT: Brummer, M., S. Dambreville, B. King, A. Chapman, J. Glockner, V. Torres, A. Wallin, K. Bae, D. Frakes, A. Yoganathan, and CRISP. *Renal Flow Measurement by Breath-Hold Phase-Velocity MRI: A Validation Study*. in *International Society of Magnetic Resonance in Medicine 13th Scientific Meeting*. 2005. Miami Beach, Florida.
Torres, V.E., B.F. King, A.B. Chapman, M.E. Brummer, K.T. Bae, J.F. Glockner, K. Arya, J.P. Felmlee, J.J. Grantham, L.M. Guay-Woodford, W.M. Bennett, S. Klahr, C.M. Meyers, X. Zhang, P.A. Thompson, J.P. Miller, and CRISP, *Magnetic Resonance Measurements of Renal Blood Flow and Disease Progression in Autosomal Dominant Polycystic Kidney Disease.* Clinical Journal of American Society of Nephrology, 2007. 2: p. 112-120.

Chapman

To Determine if Plasma or Urinary TGF-B is a Marker of Disease Severity and Progression in Early ADPKD. *Completed*

PROGRESS: Chapman TGF-B systems may be activated in ADPKD patients. CRISP participants had urinary latent and total TGF-B and serum TGF-B levels measured from their baseline visit. No differences from control populations were seen with regard to serum TGF-B levels. Total urinary TGF-B levels were elevated in CRISP participants. however, no association was found at baseline with total kidney volume. Further analyses will be done to determine the association with change in kidney function and kidney volume over time.

Chapman

The Role of the Renin-Angiotensin-Aldosterone System in Renal and Cyst Growth in Early ADPKD. *Completed*

PROGRESS: Measures of the renin-angiotensin system including plasma renin activity and plasma aldosterone levels were completed at baseline. PRA increased and PAC was decreased in those taking inhibitors of the renin-angiotensin system. Plasma aldosterone levels were directly correlated to levels of renal function. Further analyses will be done to determine the association with change in kidney function and total kidney volume over time.

Grantham

A Prospective Examination of the Relation Between MCP-1 and Disease Status in Non-Azotemic Patients with Autosomal Dominant PKD. *Preliminary analysis is completed. Additional analysis awaits collection of CRISPII baseline samples. Completed June, 2010 and ASN abstract prepared.*

ABSTRACT: Grantham, J.J., V.E. Torres, A.B. Chapman, K.T. Bae, L.M. Guay-Woodford, P.C. Harris, M. Mrug, W.M. Bennett, M.M. Moxey-Mims, and J.E. Bost. *Urinary monocyte chemotactic protein-1 (MCP1) is a biomarker of progression in autosomal dominant polycystic kidney disease (ADPKD)*. in *American Society of Nephrology, Renal Week 2010 (submitted).* 2010.
The progression to ESRD begins in ADPKD long before changes in GFR are clearly recognizable, complicating prognosis counseling and impeding the implementation of novel treatments targeted to specific molecular pathogenetic mechanisms. Inflammation and fibrosis begin relatively early and have been associated with renal chemokines and cytokines. In ADPKD patients with normal GFR and varying degrees of renal insufficiency, MCP1 was excreted in the urine to a greater extent in those with advanced disease than in those with normal GFR (Zheng, D. et al Kid Int 14:2588, 2003). MCP1 is produced by cyst epithelial cells, moves into the urine and accumulates to high levels within cysts. The Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) has followed 241 subjects (15-46 y.o. and eGFR at entry >70 ml/min) between Jan 5, 2001 and December 1, 2009 in a prospective study including measurements of total kidney volume relative to height (htTKV) by MR and GFR by iothalamate clearance. Urine MCP1 (pg/mg creatinine) was measured at baseline to determine the extent to which the chemokine predicted progression of htTKV increase and GFR decrease over the next 7.3 years. Baseline urine MCP1 in women and men was 687 641 pg/mg, n=138 and 465 403 pg/mg, n=89, respectively (P=0.0016) and GFR was 99 26 and 96 22 ml/min/1.73 m^2, respectively (P=0.2792). Urine MCP1 (pg/mg) was greater in PKD1 (655 610, n=175) than in PKD2 (365 283, n=34) (P<0.001). Baseline urine MCP1 correlated positively with baseline htTKV (R=0.436, P<0.0001, n=227) and inversely with baseline GFR (R=-0.184, P=0.0059, n=222). In a multivariable model including baseline htTKV, age, BMI, gender, race, htTKV slope, and GFR, baseline urine MCP1 associated significantly with declining GFR (OR 1.13, P=0.000) and K/DOQI Stage 1-4 (OR 1.11, P<0.001). On the basis of these findings we conclude that urinary MCP1 predicts disease progression in patients with ADPKD.

Levey and Stevens

Use of CRISP Data Base for Evaluation of GFR-Estimation Equations Using Serum Creatinine and Cystatin C. *Completed*

PROGRESS: The CRISP data were used to validate other larger collections of iothalamate-cystatin-creatinine-based GFR measurements in patients with a variety of progressive renal diseases. There were no stand alone abstracts or publications featuring the CRISP data.

Torres

Urinary eCAMP and Aquaporin 2 Levels as Predictors of Disease Progression in ADPKD.
Completed

ABSTRACT: Torres, V.E., X. Wang, C.J. Ward, J.J. Grantham, A.B. Chapman, L.M. Guay-Woodford, K.T. Bae, P.A. Thompson, J.P. Miller, and CRISP. *Urine Cyclic AMP and Aquaporin2 in Early Autosomal Dominant Polycystic Kidney Disease*. in *J Am Soc Nephrol*. 2004.

A urine concentration defect is common to all renal cystic diseases. Cyclic AMP likely promotes cystogenesis by increasing fluid secretion and cell proliferation. Renal cAMP and aquaporin 2 (AQP2) mRNA levels are increased in animal models orthologous to human ADPKD, ARPKD, and nephronophthisis. Treatment with a vasopressin V2 receptor antagonist corrects these abnormalities and inhibits cystogenesis (Nature Medicine 9:1323, 2003; 10:363, 2004). Urine cAMP (anion exchange and fluorimetric detection) and AQP2 (quantitative immunoblotting) were measured in random urine samples from ADPKD CRISP participants and healthy controls to determine whether they can be used as surrogate markers of disease progression.

		n	Age (years)	cAMP nmol/mosm	U-AQP2 U/mosm	OSM mosm/L
ADPKD	M	87	32.9±8.5	5.11±2.37	0.57±0.55	377±205
	F	126	32.6±9.0	7.72±7.20	0.84±1.21	345±193
	Both	213	32.7±8.8	6.65±5.86	0.72±1.00	358±198
Control	M	15	33.7±5.9	5.06±1.82	0.48±0.24	773±174
	F	35	35.8±5.8	4.92±2.42	0.39±0.20	398±184
	Both	50	35.2±5.9	4.96±2.24	0.42±0.21	510±249
P-value	Sex		ns	ns	ns	<0.001
(ANOVA)	ADPKD		ns	ns	ns	<0.001

Random urine osmolalities in the ADPKD patients were lower than in controls. Differences in urine cAMP and unglycosylated (U-) AQP2 were not significant. No correlation was detected between urine cAMP or AQP2 and total kidney volume (TKV) at baseline or change in TKV over time. Previous studies in rodents showed increased cAMP levels in kidney tissue. If renal levels of cAMP were also elevated in human ADPKD, this is not reflected in the urine. This would be consistent with the known predominant proximal tubular derivation of urine cAMP and with the marked cAMP accumulation in renal papillae without changes in urine cAMP known to occur following vasopressin administration (AJP 237:F218, 1979).

RESPONSE TO REVIEWERS

1.1 SPECIFIC AIMS

CRISP is a uniquely characterized population of 241 ADPKD patients on which comprehensive clinical information, MR-determined total kidney volume (TKV), liver cyst volume (LCV) and renal blood flow (RBF), and genotyping data are available. Longitudinal follow-up over an average of 7.3 years during CRISP I and II has established the exponential nature of TKV growth, but also illustrated the considerable variability seen within the population. Increasingly, as patients reach renal insufficiency endpoints, the predictive nature of baseline TKV in terms of severity of renal disease and other qualitative complications has been established. The predictive value of RBF has also been suggested.

CRISP III will build on the findings from this unique population with studies to address five specific aims as outlined below. These will further collect and analyze data and develop models to better utilize the predictive nature of TKV, RBF and LCV. The characteristics of individual cysts and the predictive value of the pattern of cyst development will be explored. Genetic and proteomic studies will be facilitated to aid the identification of genetic risk factors and omic biomarkers, and a pilot study will explore the role of sodium intake in influencing disease severity. **The overall goal of this project is to maximize the use of the national resource that is CRISP to improve the predictive value of early imaging and other data, and hence, facilitate clinical trials of this common cause of ESRD.**

Aim 1. Extend the serial quantification of total kidney (TKV) and liver (TLV) and of kidney (KCV) and liver cyst (LCV) volumes while integrating genomic and other biomarkers in order to develop and test new models for predicting the risk of developing renal insufficiency

Hypothesis 1a. Baseline TKV and change in TKV predict loss of kidney function.

Hypothesis 1b. The progression of polycystic liver volume (LCV) will be similar to but distinct from that of TKV; baseline LCV, adjusted for covariates, will independently predict the rate of increase in LCV and complications arising within the liver.

C1. They now have 7.3 yrs of follow up for this purpose. A key goal is that the investigators feel that now they can just begin to test if TKV can predict renal dysfunction, since now some patients have CKD stages 3, 4, 5 and ESRD. By measuring more dysfunction in more patients, the Principal Investigators can get more precise estimates of the predictive value of TKV. This aim largely overlaps with aims of CRISP I and II, so it is beneficial that there is consistency. On the other hand, it appears that the authors' data is quite convincing already about TKV and other parameters that lead to renal function deterioration. It would have been helpful to distinctly identify what additional information will be obtained from >10 years follow-up.

C2. With respect to the first two aims, the application itself however does not justify how much additional follow up is needed or would add to the power that they already have. The investigators present information on how the growth of cysts is exponential. They do not however present specific data to provide the reviewer with a sense for what they are able to show now and how the additional follow up period will add either in a significant way or perhaps in a non-significant way to what they are able to show. The application would be

strengthened tremendously by a better description of the adequacy of power afforded by the additional follow up time.

We are in the process of publishing a paper showing how CRISP II data significantly contributes to furthering the cause of using TKV as a surrogate endpoint. We have enclosed the latest submitted draft for your review (see appended paper by Chapman, et al. Kidney Volume Strongly Predicts Renal Insufficiency in Autosomal Dominant Polycystic Kidney Disease). The major findings were: that TKV more highly correlates with GFR measured 6 to 8 years later than to GFR measured at the same time, and height adjusted TKV is a better predictor of stage 3 disease than baseline age, serum creatinine, blood urea nitrogen, urinary albumin or MCP1 excretion. We feel the additional of 5 more years of TKV and renal outcome measures will allow us to:

1. **Evaluate TKV, TCV and Parenchymal Volume PV as predictive variables using baseline and continuous data from CRISP I and end-points for renal insufficiency from CRISPI, II and III.**

2. **Develop a non-linear model of GFR sequential change in order to determine when in the disease course:**

    a. **GFR over-compensation may occur,**
    b. **Compensated GFR begins to decline, and to explore more refined linkage between GFR and TKV, onset of hypertension, hematuria and albuminuria.**
3. **Develop a prognosis model based on baseline data and outcomes after 3, 8 and 12 years incorporating a battery of pathologic elements including: TKV, BMI, BSA, cyst count, eGFR, HDL cholesterol, Ualb, pain, gender, genotype, age, hypertension and hematuria organized as dichotomous variables using ROC cut-offs to define thresholds for continuous variables and presence/absence of non-continuous variables. Use Cox logistic regression to develop graded risk depending on the presence at baseline of the number of combined elements versus the chance (%) of baseline patients who reached a CKD Stage 3 end-point in 3, 8 and 12 years (Can also explore eGFR- and creatinine change-endpoints). Results from Aim 4 will be included in the model where appropriate.**

C3. The investigators have found that it is useful to index total kidney volume by height. This seems practical. It is not clear, but they should have also simply included height and weight in the multivariable model as simple covariates which might account for nonlinearities of using height as the index variable.

We will certainly consider adding height and weight as covariates in the subsequent model building exercises. We explored several transformations and results are presented in APPENDIX A.

C4. It should be noted that the population for CRISP III is smaller than the previous two studies and will be confounded in part by including the subjects who are enrolled in HALT and

TEMPO. It does beg the question as to whether GFR measurements will be altered by renin-angiotensin antagonism?

Very good point. We anticipate that 40% of the CRISP III participants will be enrolled in HALT or TEMPO. We can certainly include an indicator variable in models to account for participation in a trial. We will also look at trends over time in TKV and GFR for individuals in trials versus those not. Of course, we will not know if they are in the control or treatment groups which will make adjustments a bit of a challenge.

Aim 2. Determine the extent to which age and sex-adjusted measurements of renal blood flow (RBF), determined by MR imaging, predict the rate of change in TKV and determine if RBF and TKV independently predict the risk of developing renal insufficiency.
Hypothesis 2. Baseline RBF predicts the rate of increase in TKV and, independent of and in addition to baseline TKV, predicts renal insufficiency.

C5. RBF measures are difficult, and the investigators seem ambiguous if this measurement is helpful or not. It appears marginal compared to the dominant effect of TKV.

We have shown using phantoms and repeat studies in volunteers that current breath-hold MRI technology in CRISP allows accurate and reproducible direct measurements of RBF. These are not per se difficult, but require proper training and adherence to a standardized protocol. Like other measurements of physiological parameters, determinations of RBF are very sensitive and their precision is affected by day-to-day variability and physiological noise. While the nephrologic community generally accepts the value of direct measurements of GFR, it has been less accepting of real-time measurements of RBF, despite its recognized importance in the progression of chronic kidney disease. We believe that direct MR measurement of RBF is a helpful and underutilized tool to understand and monitor the progression of ADPKD (and likely other types of CKD). The following observations in CRISP I and CRISP II support this view:

a) **The decline in RBF precedes the decline in GFR (Table 1).**
b) **The percent decline in RBF is greater than the percent decline in GFR (Table 1).**

Table 1. Absolute and percent changes in eGFR (MDRD), iothalamate clearance, RBF and TKV during CRISP I and CRISP II.

Visit	BL	YR1	YR2	YR3	YR6	YR8
eGFR, ml/min/1.73 m^2	86.5±27.5	85.7±23.3	84.3±26.0	80.6±23.0	73.2±26.4*	69.8±26.5*
eGFR, % of BL	---	100.4±14.0	98.3±15.9	94.6±16.1*	86.0±27.9*	84.6±28.3*
Cioth, ml/min/1.73 m^2	99.3±22.9	98.5±23.8	98.9±27.1	96.0±26.8	81.6±35.5*	75.2±35.2*
Cioth, % of BL	---	101.2±23.0	101.5±23.6	97.9±25.7	83.1±31.4*	78.8±34.8*
RBF,ml/min/1.73 m^2	732±224	691±176	645±201†	656±202†	497±231*	477±214*
RBF, % of BL	---	96.7±22.3	89.2±21.9*	92.8±24.4*	67.8±27.1*	65.6±20.0*
TKV, ml	1041±630	1095±673	1210±773	1216±778	1510±1103*	1629±1246*

TKV, % of BL	**---**	**104.2±6.5***	**112.3±9.5***	**115.4±13.2***	**139.3±40.5***	**154.0±41.4***
vs *p<0.0001; † p<0.01						

c) **Stratification of patients by HtTKV and RBF identifies subsets at high risks for GFR decline better than HtTKV or RBF alone (Table 2).**

Table 2. Iothalamate clearance values during CRISP I and CRISP II in participants stratified by HtTKV and RBF at baseline

			Iothalamate Clearance (ml/min/1.73 sq.m.)				
BL HtTKV	**BL RBF**	**N[&]**	**BL**	**YR1**	**YR2**	**YR3**	**YR6-8**
<450	**>850**	**20**	**112.0±17.6**	**115.1±15.9**	**117.3±18.6**	**117.0±23.4**	**109.0±24.1**
	600-850	**20**	**97.7±17.6**	**103.5±15.8**	**104.7±23.2**	**103.7±18.7**	**89.3±22.9**
	<600	**8**	**111.9±23.0**	**112.3±22.5**	**105.5±21.3**	**115.5±20.6**	**92.8±16.8‡**
450-750	**>850**	**11**	**119.6±23.3**	**115.3±27.4**	**124.4±19.7**	**114.7±23.6**	**107.9±35.0**
	600-850	**11**	**102.1±29.4**	**94.5±14.3**	**92.1±16.6**	**97.0±23.7**	**75.7±25.2‡**
	<600	**12**	**81.7±9.6**	**79.1±20.4**	**84.1±9.6**	**78.6±11.5**	**67.5±26.5**
>750	**>850**	**3**	**94.4±36.6**	**113.6±23.7**	**91.6±20.3**	**100.3±26.2**	**57.7±29.7**
	600-850	**6**	**104.9±21.3**	**97.2±16.6**	**89.9±21.0**	**91.1±16.6**	**53.7±14.0†**
	<600	**19**	**74.9±9.5**	**67.8±15.0‡**	**63.9±20.5‡**	**57.4±15.4†**	**37.2±19.8***
[&]Only patients with RBF measurements at YR6 and/or YR8 are included							
vs *p<0.0001; † p<0.01; ‡ p<0.05							

d) **lnHtTKV and RBF at baseline are strongly correlated with GFR at year 6 and/or 8 (Figure 1). During CRISP III we will determine whether incorporation of baseline TKV and RBF into a single model will have a better predictive value than either alone.**

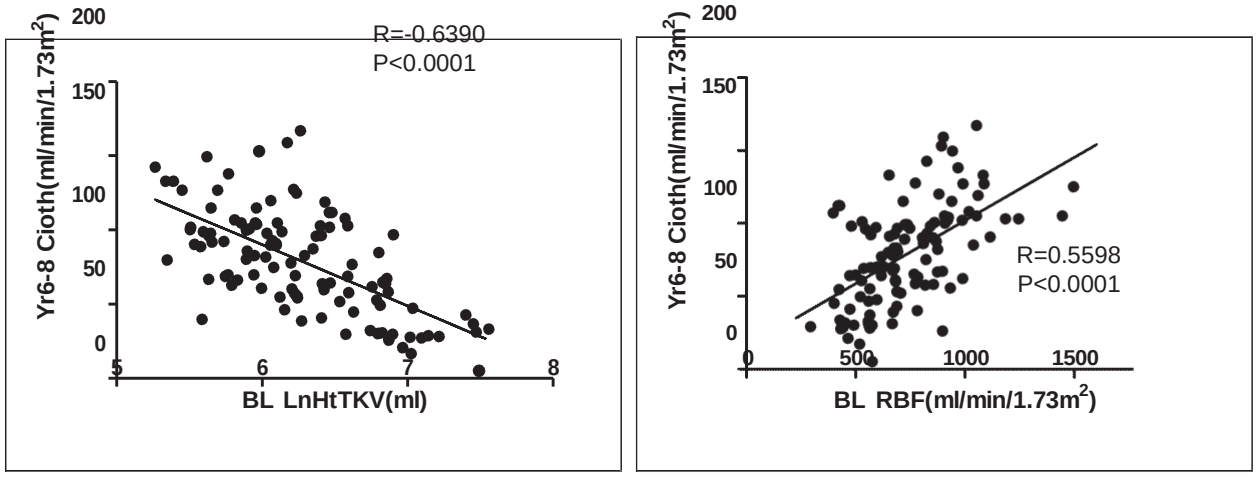


Figure 1.

C6. With Aim 2, the investigative team provides more information on how the relationships between RBF and TKV and the decline in kidney function varied between CRISP I and II. Clearly additional information is necessary to put these variations in relationships in context; however, the team should justify that the additional information to be gained from CRISP III will be sufficient to clarify these associations. The information provided in table 1 is particularly compelling. It would be more demonstrative of the need for additional follow up if it were stratified by length of available follow up, in light of the fact that the change from baseline really accelerates as the number of people with follow up to that time point drop off. I don't see any evidence that supports the potential for informative censoring, but given it is a possibility, examining the data in this manner would be helpful toward supporting the contention of the team.

Since RBF declines earlier and to a greater extent than GFR, we predict that stability of RBF over time will be strongly associated with stability of GFR. This will be addressed during CRISP III. If confirmed, RBF could become an earlier marker of disease progression which could be used in clinical trials (based on preliminary observations from CRISP, RBF is a secondary endpoint in HALT-PKD Study A). Longer periods of observation in a larger number of patients planned in CRISP III will help to clarify the potential of RBF as an outcome measure and possibly a therapeutic target in ADPKD.

From a statistical perspective we will have to explore various options to account for different lengths of follow-up for CRISP participants. We could add length of follow-up as a term in the model, explore imputation techniques such as last value carried forward or use some other imputation technique.

Aim 3. Develop methods to quantify total cyst number, individual cyst volumes, and pattern of distribution of cysts in each kidney and apply these to analyze the influence of renal cyst number, volume, and topography at baseline on the subsequent course of TKV and GFR and the risk of developing renal insufficiency.

Hypothesis 3a. Renal cyst number and volume will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.

Hypothesis 3b. Renal cyst topography (medullary vs. non-medullary) will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.

Hypothesis 3c**.** Individual renal cyst growth is continuous and exponential (similar to the growth pattern in TKV) and patterns of renal cyst growth or involution will be associated with rates of change in TKV and GFR and risk of developing renal insufficiency. These relationships may vary by genotype.

C7. The results of the objectives of Aim 3 (quantify total cyst number, individual cyst volume, and pattern of distribution of cysts) have never been reported in ADPKD. I had the impression that this was a goal of the prior I and II studies. The investigators indicated they developed and tested a method of estimating the total number of cysts based on a mid-slice MRI. This method is not presented. They also developed methods to semi-automatically measure the number of cysts. This is also not presented.

C8. With respect to Specific Aim 3, the team proposes a fascinating hypothesis that the location of the cyst may reflect the number of nephrons that were affected by its growth. Within this aim, the team proposes the development of a method to count cysts. They provide a discussion that presents the manual process as well as the process by MRI and a discussion of how the automated process will be refined. However, with respect to topography, the discussion of the ability to make such determinations and the process by which they will refine the determination is in considerably less depth.

With the additional cyst count and longitudinal cyst size assessments we hope to:

1. **Develop a method to fast-track the determination in MR scans of the total number of cysts in a polycystic kidney.**

2. **Develop a method to fast-track the quantification of the predominant intra-renal distribution of cysts within a polycystic kidney (cortex vs medulla).**

3. **Develop a method to fast-track the determination of individual cyst growth kinetics over 3, 8 and 12 years.**

4. **Develop an iterative analytic model in which nominal values for HtTKV, age of cyst formation, total number of cysts at age, cyst distribution at age, rate of total cyst growth, rate of cyst growth (cortex/medulla) operating conjointly predict age-related changes in GFR.**

5. **Use preliminary data developed by morphometric analysis of a subset of CRISP MR scans and histologic sections of pathologic specimens of early, intermediate and late-stage ADPKD kidneys to determine the total cyst burden (visible by MR scan and invisible to MR scan).**

6. **Use preliminary data from 5 to create and test the iterative model in 4.**

7. **Upon completion of 1-3, analyze CRISP cases to determine the extent to which the analytic model predicts the decline in GFR .**

Aim 4. Expand and analyze CRISP biological samples collected in NIDDK repositories to improve genotype/phenotype and biomarker studies, and facilitate independently funded ancillary studies.

Sub-aim 4.1 Collect and analyze DNA samples and clinical data from CRISP family members for enhanced genotype/phenotype studies and to facilitate other genetic studies.

Sub-aim 4.2 In conjunction with the NIDDK Biomarkers Consortium and ongoing ancillary studies utilizing CRISP samples, determine specificity and sensitivity of urinary and serological markers to: 1) detect severity of renal and extra-renal ADPKD manifestations (e.g., TKV and LCV); and 2) predict rates of their progression.

C9. I would have liked to see more details on future plans the biological specimens and how ancillary studies using these specimens will be prioritized or otherwise handled in the future, when many of the specimens have already been distributed.

As with CRISP I, CRISP II biological samples will be made available to requesting investigators through the data sharing plan once the CRISP II dataset is finalized and all major primary papers related to the CRISP II population are published. The ancillary studies group chaired by Dr. Grantham, reviews ongoing requests for use of biological samples from active CRISP protocols that could include biological samples from CRISP I and II but really reflect requests from the CRISP III time period. The prioritization of approval for sample request has been determined based on the scientific quality and rationale of the submitting investigator as well as the volume and number of samples requested. Given that all of the future is not possible to see, we have not restricted the use of these samples and wanted to make sure that they were well used. Given that this is an observational cohort, the need to protect the baseline samples for future use is somewhat less critical than baseline samples from interventional studies.

C10. With respect to Specific Aim 4, the team presents their approach toward the genetic analysis of family members that are affected. They provide a good discussion of how these family members will be recruited, specifically highlighting the manner in which their rights as human subjects will be protected. They do not however discuss the potential impact or lack of impact that selection bias for the more severe cases (which is a distinct possibility given that they will approach relatives who know they have the disease) may have on their findings.

We plan to collect samples just from affected individuals, but that does not mean that we will not evaluate all at-risk family members. We are very aware that there is considerable intra-familial variability in ADPKD, and, indeed, is one of the major motivations for doing this study. Our general approach will be to contact all at risk family members over 18 years, for instance, sibs, children and/or parents of an affected individual. We will interview the patient to determine if they consider they have ADPKD and, more importantly, request access to clinical records. These will be evaluated, and imaging that may provide a firm diagnosis will be requested and analyzed by an experienced nephrologist/radiologist. If no images that provide a clear diagnosis are available, the patient will be counseled to have kidney imaging to obtain a diagnosis, with the advantages and possible disadvantages of obtaining a diagnosis of ADPKD explained. If the patient is not interested in obtaining imaging for diagnostic purposes but is prepared to provide a blood sample, research mutation testing will be performed in families where the mutation is known. The results form this testing will not be employed for diagnostics, not communicated to the patient, or added to the patient's clinical record, but could be the basis for providing further guidance to the patient about the value of obtaining renal imaging. We understand that this scheme is not perfect but we do feel that we will be able to analyze most affected members of families that wish to participate even if they have a mild manifestation of the disease.

C11. In the discussion of the candidate biomarkers, the justification for the biomarkers chosen (MCP1, NGAL) is not lengthy. Given the value of stored specimens from such a unique cohort, a greater consideration of pilot testing samples before utilizing samples for testing in

the entire cohort is justified unless the evidence is so strong that it makes sense to test the entire cohort.

We agree with this approach. In previous ancillary study submissions, applicants have been required to provide both analytical plans and power calculations to justify the number of samples requested. In addition, if indicated small pilot studies have been required prior to utilizing the entire or a large component of the CRISP cohort for study. In addition, normative data has often been requested to determine the feasibility of addressing biomarker measurements in this moderately sized (n=241 maximum) study population. We have also taken into consideration the eventual potential decay of candidate markers after decades of storage even under ideal conditions. This precious resource should be used for marker discovery and validation purposes, now and in the future. All of these issues are considered at the time of ancillary study submission.

C12. Aim 4 has some intriguing possibilities with the first real broad-scale genotype/phenotype assessment beyond the baseline genetics of this disease. The analysis and, indeed, the biomarker assessment part of this aim both seem underdeveloped.

Current Funded Studies

Torres' ancillary study on copeptin and ANP- Preliminary analysis revealed that copeptin and ANP are significant predictors of disease progression. A manuscript will be prepared.
Parikh NGAL Ancillary study – A manuscript has been prepared for submission based on the results of the NGal biomarker study in CRISP cohort.
Potential Ancillary study on acute kidney Injury - Dr. Joseph Bonventre provided a letter of support for CRISP III submission. He is interested in collaborating with CRISP investigators looking at acute kidney injury biomarker panels.
Kistler/Serra urine biomarkers - Completed first round of urinary proteomic analysis. The CRISP data helped them improve and validate their initial results. Currently analyzing PKD2 urine samples in order to look for fingerprint specific for PKD1 and separate from PKD2.
Metabolomics analysis – Dr. Robert Weiss at UC Davis. Initial data completed in 13 ADPKD patients and 13 matched controls to establish day to day and within individual variability as well as metabolites uniqe to ADPKD. Use of future CRISP III urinary samples from CRISP III participants not receiving medications for metabolomic analysis.

The descriptions of the genotype/phenotype studies in this aim are brief, as dictated by the new format of R01 grants, but we feel with the wealth of data available in the CRISP population and to be collected from family members that this will be an extremely strong population for analysis. In addition, we have a good deal of experience in analysis of this type of data as illustrated in Ref 8, 66 and 67 of the grant and Rossetti et al 2002 JASN 13(5):1230-7 and Rossetti 2003 Lancet 13(5):1230-7 and so we believe that we are in a strong position to maximally understand the significance of this data.

<u>Aim 5</u>. Determine whether a program of intensive dietary counseling and intervention in a small group of CRISP participants not participating in other clinical trials is successful in modifying the relatively fixed pattern of sodium intake observed in CRISPI and reducing the rate of growth of the polycystic kidneys.

C13. Aim 5 (evaluate diet/ salt intake effect on cyst/ kidney volume), is good innovative. Unfortunately the patient population is now fairly diseased – it seems like a less affected population might have been more suited to this aim?

C14. Here, the main consideration is if historical controls are adequate, and if the rate of change of TKV changes for that individual compared to their historical control. Overall, this aim is not very appealing for several reasons. First, it appears reasonable that all subjects should be advised of the importance of reduced sodium intake. If that has been done, then historical controls are unlikely to be sufficient for comparison. If the changes in the kidney volume are non-linear, then comparison of the patient to his/ herself is also insufficient. It seems more appealing scientifically to compare an intensive control group to a usual care group that is matched. Unfortunately, that may not be possible given the number of study subjects available.

C15. Finally with respect to Aim 5, the evidence that they present supporting the dietary intervention is not substantial. Given that this dietary intervention is of minimal risk, this is not a safety issue as much as it is an issue of justified use of resources in this grant. This section claims a cause effect relationship between high salt intake and cyst growth where it is observational. They plan on providing the dietary intervention but will not have a group against which they can compare the rate of cyst growth. This is a little incongruous with the fact that the early aims require additional follow up to characterize the growth pattern. This would imply that they do not have adequate historical data against which to compare how cyst growth is altered by lower dietary sodium.

We have decided not to include this Aim in the CRISP III protocol. Dr. Torres will consider resubmitting as a separately funded study.

Appendix A

Mean total kidney volume, standard deviations and coefficients of variation for male and female CRISP participants with and without adjusting for measures of body size.

Adjusted	Visit	Male		Female		Overall		P
		M (SD)	Coefficient of variation	M (SD)	Coefficient of variation	M (SD)	Coefficient of variation	
Not adjusted	0	1161.1 (731.8)	0.63	1014.1 (609.3)	0.60	1072.6 (663.3)	0.62	0.105
	1	1203.7 (741.0)	0.62	1067.2 (653.8)	0.61	1123.0 (692.4)	0.62	0.142
	2	1335.7 (848.6)	0.64	1169.6 (737.5)	0.63	1239.9 (788.8)	0.64	0.128
	3	1388.4 (884.3)	0.64	1202.4 (780.0)	0.65	1276.6 (826.3)	0.65	0.100
	6	1728.6 (1141.9)	0.66	1404.8 (1062.1)	0.76	1534.7 (1103.5)	0.72	0.043
	8	1966.6 (1361.5)	0.69	1410.7 (1036.1)	0.73	1644.0 (1209.6)	0.74	0.021
Baseline Height Adjusted TKV (cc/m)	0	633.9 (392.1)	0.62	611.1 (361.5)	0.59	620.2 (373.4)	0.60	0.643
	1	657.5 (396.4)	0.60	641.6 (386.3)	0.60	648.1 (389.7)	0.60	0.762
	2	729.1 (454.0)	0.62	702.4 (435.3)	0.62	713.7 (442.5)	0.62	0.664
	3	758.1 (476.0)	0.63	723.3 (460.9)	0.64	737.2 (466.2)	0.63	0.586
	6	943.3 (608.3)	0.64	845.3 (631.8)	0.75	884.6 (622.7)	0.70	0.280
	8	1077.5 (727.7)	0.68	854.2 (628.0)	0.74	947.9 (677.6)	0.71	0.085
Baseline Weight	0	13.2 (7.6)	0.58	14.9 (9.1)	0.61	14.2 (8.6)	0.60	0.120

Adjusted	Visit	Male		Female		Overall		P
		M (SD)	Coefficient of variation	M (SD)	Coefficient of variation	M (SD)	Coefficient of variation	
Adjusted TKV (cc/kg)	1	13.8 (8.0)	0.58	15.7 (9.7)	0.62	14.9 (9.1)	0.61	0.117
	2	15.2 (9.2)	0.60	17.1 (10.9)	0.64	16.3 (10.3)	0.63	0.192
	3	15.9 (9.7)	0.61	17.5 (11.2)	0.64	16.8 (10.6)	0.63	0.288
	6	19.9 (12.6)	0.63	20.6 (15.5)	0.75	20.3 (14.4)	0.71	0.762
	8	22.1 (14.0)	0.63	21.2 (16.5)	0.78	21.6 (15.4)	0.72	0.753
Baseline BSA Adjusted TKV (cc/m2)	0	549.0 (325.7)	0.59	574.7 (338.8)	0.59	564.5 (333.2)	0.59	0.560
	1	571.8 (336.0)	0.59	603.5 (361.2)	0.60	590.5 (350.7)	0.59	0.502
	2	633.5 (385.4)	0.61	659.4 (408.3)	0.62	648.5 (398.1)	0.61	0.639
	3	659.9 (404.8)	0.61	676.8 (425.0)	0.63	670.1 (416.2)	0.62	0.767
	6	823.6 (521.1)	0.63	794.3 (591.0)	0.74	806.1 (562.8)	0.70	0.722
	8	929.3 (604.8)	0.65	810.5 (607.7)	0.75	860.4 (606.6)	0.71	0.309
Baseline BMI adjusted TKV (cc/m2)	0	44.1 (26.5)	0.60	41.0 (25.6)	0.62	42.2 (26.0)	0.61	0.373
	1	46.1 (27.9)	(33.1)		0.61	43.3 (27.5)		(31.8)
	2	51.1 (31.9)			0.63	47.3 (31.0)		
	3	53.3			0.62	48.2		

44.5 (27.6)	0.62	**Male**	**Female**	**Overall**
48.9 (31.4)	0.458			
50.2 (32.3)	0.64			
	0.390			
	0.64			
	0.250			

Adjusted	Visit	Male		Female		Overall		P
		M (SD)	Coefficient of variation	M (SD)	Coefficient of variation	M (SD)	Coefficient of variation	
	6	66.9 (44.1)	0.66	56.9 (43.9)	0.77	60.9 (44.1)	0.72	0.118
	8	73.3 (48.2)	0.66	57.7 (45.0)	0.78	64.3 (46.8)	0.73	0.082
BMI adjusted TKV for every visit (cc/m2)	0	44.1 (26.5)	0.60	41.0 (25.6)	0.62	42.2 (26.0)	0.61	0.373
	1	45.2 (26.9)	0.60	43.0 (28.0)	0.65	43.9 (27.5)	0.63	0.540
	2	50.1 (31.5)	0.63	46.7 (31.3)	0.67	48.1 (31.3)	0.65	0.424
	3	52.0 (32.4)	0.62	46.8 (31.8)	0.68	48.9 (32.1)	0.66	0.232
	6	63.6 (41.8)	0.66	54.8 (45.4)	0.83	58.3 (44.1)	0.76	0.173
	8	68.7 (44.8)	0.65	52.3 (43.3)	0.83	59.2 (44.5)	0.75	0.054
Baseline Height-squared	0	346.8 (211.5)	0.61	368.8 (216.1)	0.59	360.0 (214.1)	0.59	0.435
Adjusted TKV (cc/m2)	1	359.8 (213.7)	0.59	386.3 (229.8)	0.59	375.4 (223.3)	0.59	0.378
	2	398.7 (244.7)	0.61	422.5 (258.8)	0.61	412.4 (252.6)	0.61	0.496
	3	414.8 (258.2)	0.62	435.8 (274.2)	0.63	427.4 (267.5)	0.63	0.566
	6	515.7 (326.4)	0.63	509.5 (378.0)	0.74	512.0 (357.3)	0.70	0.906
	8	591.7 (392.8)	0.66	518.0 (382.7)	0.74	548.9 (386.9)	0.70	0.322

CRISP Publications
June 2011

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