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

CRISP Consortium.

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 Washington University in St. Louis. Due to the relocation of the DCIAC P.I. from Washington University to the University of Pittsburgh, the DCIAC for CRISP II is located at 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

Specific AIM 1: Extend the preliminary observations of CRISP-I to ascertain the extent to which quantitative (kidney volume and hepatic and kidney cyst volume) or qualitative (cyst distribution and character) structural parameters predict renal insufficiency and develop and test new metrics to quantify and monitor disease progression.

This aim will address four hypotheses:

a) Increased renal volume in general and all renal volumes > 750 ml adjusted for age and other significant covariates in CRISP I predict rate of loss of renal function as well as progression to specific endpoints, e.g. KDOQI Stage IV, ESRD, and/or death.
b) Baseline medullary vs. non-medullary cyst volume and cyst number in CRISP I predict loss of renal function over time.
c) Prediction models (formulas) utilizing age and renal volume at baseline in CRISP I will effectively predict loss of renal function over time.
d) Baseline liver cyst volume adjusted for the appropriate variables predicts rate of increase in liver cyst volume in CRISP I participants.

Specific AIM 2: Extend the preliminary observations of CRISPI to ascertain the extent to which age and sex-adjusted measurements of renal blood flow by MR technology predict the rate of renal growth; and, renal blood flow and kidney volume predict the rate of renal function decline in ADPKD.

This aim will address three hypotheses:

a) Baseline renal blood flow predicts the rate of increase in renal volume in CRISP I participants
b) Baseline renal blood flow, independent and in addition to baseline renal volume, predicts loss of renal function in CRISP I participants
c) Combining longitudinal measures of renal blood flow and renal volume may enhance the capacity to predict loss of renal function in CRISP I participants

Specific AIM 3: Collect DNA samples and clinical information from CRISP family members known to have ADPKD for use to examine genotype-phenotype relationships and by independently funded studies to identify genetic modifiers.

This will address two hypotheses:

a) Genetic heterogeneity and mutation type and/or location affect disease severity in the CRISP population.
b) Genetic factors that modify the renal and hepatic phenotypes will be detected by a genome-wide association study employing a high resolution SNP array (this hypothesis will be examined using the CRISP population by an ancillary study to be submitted as a separate RO1 application in February 2007).

Specific AIM 4: Maintain and expand a database of uniformly and accurately collected information including renal structural and functional parameters and a repository of biological samples which can be used by ancillary or independently funded studies initiated by CRISP or non-CRISP investigators.

An ancillary study that during CRISP I began to examine whether urine MCP1 (a product of cyst formation and growth excreted in increased amounts in baseline urine collections) concentrations predict clinical renal imaging patterns and disease course will continue during CRISP II. This study will address the following hypotheses:

a) The pattern of urinary excretion of MCP1 in individual patients remains consistent over time.
b) Baseline urinary excretion of MCP-1 predicts total kidney volume and total cyst volume and number, loss of renal function, and progression to specific endpoints, e.g. KDOQI Stage IV, ESRD, and/or death.
c) Urinary excretion levels of periostin and other potential markers identified by micro-array screening of human ADPKD tissues will also predict total kidney volume and total cyst volume and number, loss of renal function, and progression to specific endpoints, e.g. KDOQI Stage IV, ESRD, and/or death.

BACKGROUND AND SIGNIFICANCE

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is a major cause of disabling morbidity and is the fourth leading cause of end-stage renal failure in the world, affecting more than 500,000 U.S. citizens and millions more worldwide. It is the most common single-gene disorder that is potentially lethal. Annual costs to treat ADPKD exceed 1 billion dollars for dialysis, renal transplantation and regular management of other complications secondary to the disease[1].

ADPKD is caused by mutations within either of two genes, *PKD-1* and *PKD*-2. Both genotypes are characterized by the progressive enlargement of innumerable cysts derived from tubules that lead to an overall progressive increase in the size of the kidneys. Over the last 5 years knowledge of the molecular and cellular pathogenesis of ADPKD has significantly increased. The *PKD* genes have been identified and the functions of their protein products have been defined[2].

ADPKD is a bilateral condition, although the absolute changes in kidney size may be asymmetric. The progressive increase in kidney size is associated with considerable morbidity throughout the life of individual patients, and include 1) abdominal pain, 2) gross hematuria, 3) hypertension at an early age, 4) renal stones, 5) renal infections, 6) cosmetic deformity of the abdomen and 7) renal insufficiency in those older than 50 years of age[3-11]. ADPKD is in fact a systemic illness. Cysts are frequently found in the liver, pancreas, arachnoid and less frequently in the spleen and testis. Aneurysms of the cerebral arteries occur in approximately 5 - 10 % of patients with ADPKD, and abnormalities of the heart valves are detected in approximately one-fourth of patients.

The hallmarks of ADPKD are innumerable fluid-filled cysts scattered throughout both kidneys that cause the total renal size to increase many times greater than normal. In affected individuals, enlargement of the kidneys

generally progresses steadily culminating in renal insufficiency in more than 50 %, although the age of onset of renal failure is highly variable. Examples of end-stage renal disease (ESRD) in the first year of life have been reported, but it is also common knowledge among nephrologists that patients with well-developed ADPKD may live beyond 80 years of age without serious impairment of renal function. Consequently, it is impossible to predict from clinical information the long term course of the disease in young, asymptomatic patients.

In patients with a positive family history, the diagnosis of ADPKD is established by the demonstration of bilateral renal cysts defined by ultrasound, computed tomography, magnetic resonance imaging or direct surgical inspection. The disease exhibits a dominant mode of genetic transmission with complete penetrance. Genetic linkage to markers on chromosomes 16 and 4 have been used in relatively large families to determine those without renal cysts who may be at risk for ADPKD and more recently mutation analysis has become commercially available.

Although all patients who inherit ADPKD develop cysts within the kidneys, there is substantial variability in the occurrence of renal failure. Several groups of investigators in North America and Europe have explored the age of onset of ESRD[12-19]. Patients with ADPKD most commonly develop ESRD in the sixth decade of life. In the Modification of Diet in Renal Disease study (MDRD), ADPKD subjects with GFR values between 25 and 55 ml/min per 1.73 m^2 lost GFR at a rate of 5.8 ml/year, whereas in non-ADPKD participants (chronic glomerulonephritis, hypertensive renal disease, etc) GFR decreased 3.1 ml/min per year. Once the GFR begins to decrease the typical course is one of inexorable decline in filtration culminating in death from uremia, unless the patient is rescued with dialysis and/or renal transplantation. The rate of functional decline can be highly variable among unrelated individuals with ADPKD as well as between members of the same family. This suggests that factors in addition to the inherited mutations determine the rate of functional decline.

As for many other chronic, progressive disorders, GFR is a poor marker of renal function in ADPKD. GFR levels remain at levels well within the normal range for many years during which time renal cysts occupy progressively increasing fractions of total renal volume[12-19]. Compensatory adjustments in glomerular filtration and tubular reabsorption help to maintain the GFR on a nearly even keel until the loss of filtering units falls below the minimum required to maintain the filtration rate normal. At this juncture, GFR falls in a linear, precipitous decline.

Pathology. Cysts have been found in the kidneys of first trimester fetuses who carry one of the ADPKD mutations. More typically, the disease goes unnoticed until it is discovered in the course of a physical examination or by ultrasound or computed tomographic testing. Dissection studies of kidneys in the early stage of disease development indicate that the cysts may arise in all segments of the nephron and collecting ducts. More recent studies using immunohistochemistry or hormonal responsiveness in cyst-derived cultured cells suggest a predominant origin from collecting ducts. Close examination of the cysts by light and electron microscopy has revealed evidence that adjacent parenchyma is compressed along with infiltration into the interstitium of mononuclear cells in association with fibrosis. It is important to emphasize that the cysts appear to develop in only a small fraction of the nephrons and collecting ducts, perhaps fewer than 1%[20]. The distribution of the cysts may be highly asymmetric within and between the kidneys.

The tubule basement membrane surrounding the individual cysts are typically thickened and laminated. In early stages of the disease examined by light microscopy, the adjacent renal parenchyma appears to be uninvolved. On the other hand, studies of cell proliferation and apoptosis markers indicate that the adjacent non-cystic renal tubule cells may respond to a proliferative stimulus similar to that observed within the epithelial cells lining the cysts.

As the disease progresses, the size of the individual cysts increases, but whether the number of cysts increase is not known. There is a progressive decrease in non-cystic parenchyma which has led researchers to suggest that the enlarging cysts crowd out the normal parenchyma in the same way that solid neoplasms displace and erode tissues in which they arise. As the cysts enlarge and the total kidney size increases, the volume of non-cystic parenchyma, on which the function of the kidneys depends, declines. There is evidence of accelerated apoptosis in the renal cysts and the adjacent non-cystic parenchyma. In later stages the interstitium expands owing to the accumulation of collagenous material and frank fibrosis together with foci of mononuclear cells. The distortion of the interstitium involves the peritubular capillaries, veins and arterioles, in association with the sclerosis of small and medium-sized arteries[20].

In the terminal stages of the disease, glomeruli are commonly globally sclerotic which more typically reflects an antecedent scarring process within afferent arterioles in contrast to the focal sclerosis pattern of glomeruli subjected to abnormal transcapillary hydrostatic pressure. Non-sclerotic glomeruli appear enlarged, reflecting compensatory hypertrophy. Mild to moderate proteinuria is observed in ADPKD and appears to be a harbinger of poor prognosis for overall renal function. At the end-stage, polycystic kidneys are typically enlarged, sometimes more that 10 times greater than normal. The end-stage polycystic kidney is comprised primarily of fluid trapped in cysts varying in size from a few microliters to more than 100 ml. The surface of the kidney is typically laced with bands of fibrotic material. On the cut surface, the cysts stand out as distinct cavities between strands of scar tissue. Normal parenchyma is rarely seen.

Alterations in the interstitium adjacent to cysts can be observed early in the course of the disease in human patients. Several studies have suggested that tubulo-interstitial changes may be important in the development of renal insufficiency in human ADPKD[21-23]. ADPKD is associated with polycystic liver in the majority of patients. The liver cysts are usually not detected until late in the course of the disease, but in some women the livers may reach a very large size. In these unfortunate patients partial hepatectomy or liver transplantation may be required to achieve an acceptable quality of life.

Future approaches to therapy of ADPKD: Signal transduction pathways and pathophysiologic mechanisms have been defined to the point that therapeutic trials are being planned to investigate the potential effects of novel molecules to slow the rate of disease progression[24]. The use of these new compounds is dependent upon the development of accurate measures of disease progression that can be used for prospective studies.

In the broad field of Nephrology, the preservation of GFR is held to be the major goal of treating most progressive renal disorders. It is important to note, however, that a disease like ADPKD has morbidities that diminish the quality of life of patients long before kidney function declines to the point that requires renal replacement therapy. Several major morbidities (hypertension, pain, gross hematuria, stone, abdominal distension, renal infection) appear to be linked to the progressive enlargement of the kidneys due to the cysts. Consequently, goals of ADPKD therapy include relieving the suffering caused by enlarged kidneys by limiting the growth of cysts. As noted previously, verifiable changes in GFR occur relatively late in ADPKD after major damage has been done by the cysts and fibrotic mechanisms have been activated. Thus, GFR is not a useful indicator of therapeutic effectiveness in the early stages of the disease if a major goal of therapy is to prevent the growth of the cysts to prevent their secondary effects to destroy renal structure and ultimately, reduce renal function. In order to treat the disease before irreversible damage is done, a more sensitive and pertinent marker of disease progression is needed.

Significance of CRISPI and CRISPII: In 2000, PKD researchers at the University of Alabama, Emory University, University of Kansas, Mayo Clinic and Washington University St. Louis joined together to create the Consortium for Radiologic Studies of Polycystic Kidney Disease (CRISPI). This consortium of Participating Clinical Centers (PCCs) and a Data Coordinating and Imaging Analysis Center (DCIAC) developed and implemented studies to test whether imaging techniques could provide accurate and reproducible markers of progression of renal disease in patients with PKD. The Steering committee, comprised of principal investigators from the PCCs and DCIAC, developed initial study protocols for the imaging studies and proceeded to collect and analyze radiologic and clinical data over the last 5.5 years.

The primary objectives of this investigation were to: (1) to develop and test the accuracy and reproducibility of imaging techniques to monitor changes in renal cyst size and parenchymal involvement in well characterized cohorts of patients with PKD to assess their utility as surrogate markers of disease progression, (2) to establish and maintain a database of uniformly and accurately collected information including renal functional parameters and other selected markers of disease progression identified by the DCIAC and the PCCs, to correlate parenchymal involvement with renal functional changes in PKD patients with various rates of progression, and (3) to maintain and make available such data to facilitate the planning and implementation of clinically appropriate interventions in the near future.

The goals of CRISPII are to extend the observations of CRISPI in order to: 1) draw unequivocal linkage between the rate of kidney/cyst enlargement and qualitative (signs and symptoms) and quantitative (declining renal function reflected in iothalamate clearance and albuminuria) end-points; 2) to provide a marker of disease progression (kidney volume) sensitive and accurate enough to be used as a primary outcome marker

in clinical trials aiming to forestall disease progression; 3) to develop and test other biomarkers of disease progression.

PRELIMINARY STUDIES/PROGRESS REPORT

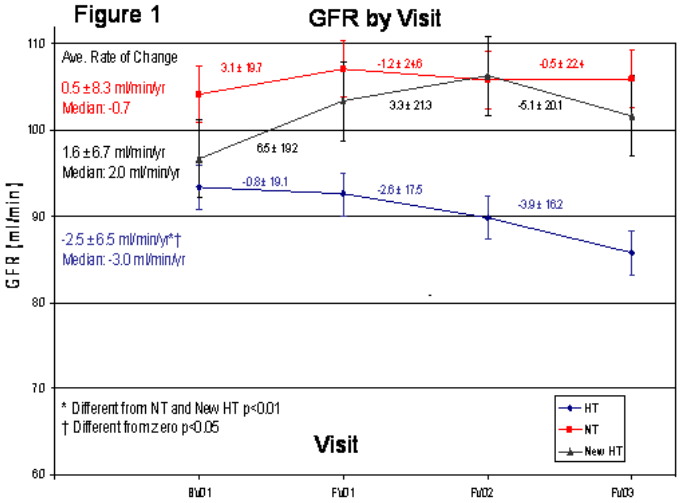
Researchers from the University of Alabama, Emory University, University of Kansas, Mayo Clinic and Washington University developed a consortium that has produced interesting results in a cohort of 241 individuals (145 women, 96 men) with ADPKD screened from a total of 289 eligible subjects; 235 subjects remained in the study at the end of the 3rd year of study, a remarkable rate of retention. The cooperation and synergies evinced among investigators of diverse scientific backgrounds in the Patient Coordinating Centers (PCCs) and Data Coordinating and Image Analysis Center (DCIAC) has been remarkable. Accomplishments of the group effort are listed below:

Development and testing of a method to determine total kidney and total cyst volumes. A novel MR-based method to measure and to quantify total renal volume (TKV) and total renal cyst volume (TCV) was developed and rigorously tested in subjects with ADPKD[25, 26]. MRI-based morphometric methods were shown to reproduce total kidney and cyst volumes in phantoms with reliability coefficients of 99.9 % and 89.2 %, respectively. The coefficient of variation of total kidney volume measurements by stereology in 4 subjects studied at each of the 4 PCCs was 3.5 %. Statistical models for describing the changes over time have been developed and used for analytic purposes. The longitudinal measurements can, and have, been used for planning intervention trials with imaging endpoints, allowing quantitative information about the tradeoffs of the number of participants, the length of follow-up and the frequency of assessment.

MR is superior to ultrasound for precisely determining total kidney and total cyst volumes. Ultrasound was determined to be sufficiently accurate to determine renal cystic involvement for screening and enrollment into CRISP I and could determine very large differences in total renal volume utilizing both the ellipsoid formula and longitudinal length measurements. Within and between observer variability of total renal volume measures were significantly greater with ultrasound vs. MR. Ultrasound was of insufficient accuracy for longitudinal measurements of change in renal volume in contrast to MR. Thus, although ultrasound will be an important tool for screening individuals at risk for ADPKD prior to enrollment in therapeutic trials, it is not useful as a measurement tool to quantify progression of renal volume over relatively short intervals of time.

Asymmetry of renal enlargement. Cyst development was frequently found to be asymmetric, although on the whole the average left kidney volume exceeded right kidney volume by 19.3 %. The greatest asymmetry was 163 %, left > greater than right; by contrast the right kidney volume maximally exceed that of the left by 48 %. The median Left vs. Right ratio of 1.091 reflects the fact that 163 left kidneys were larger than the matched right kidney. The biologic implications of this renal volume asymmetry are not clear, but the finding does suggest that the germ cell mutation, which is found in all of the renal cells, is probably not the sole determinant of how fast kidney cysts may enlarge.

Hypertensive subjects have larger kidneys and lower GFRs than normotensives. Sixty four participants were normotensive at enrollment into CRISPI. Twenty two have subsequently developed hypertension at a mean time 2 years after enrollment into CRISPI. Age, body mass index, weight and serum creatinine concentrations were significantly greater in those who developed hypertension in comparison to those who remained normotensive. At this time we have insufficient power to determine if renal or cyst volume enlargements are greater in those who develop hypertension. Hypertensive subjects demonstrated a significant increase in total renal volume, cystic volume and % change in renal volume from baseline that was not detected in the normotensive or newly hypertensive CRISP participants (Figure 1). Systolic and diastolic blood pressure levels measured throughout the three year follow up of CRISP I were directly related to the rate of renal enlargement in both treated hypertensive ($r=0.21$, $P<0.03$) and untreated normotensive ($r=0.38$, $P<0.02$) individuals. Hypertensive subjects demonstrated a significant decline in renal function determined by both iothalamate clearance (Figure 2) and serum creatinine measurements (Figure 3), while no change in renal function occurred in the normotensive individuals or those who became hypertensive in the course of the study.



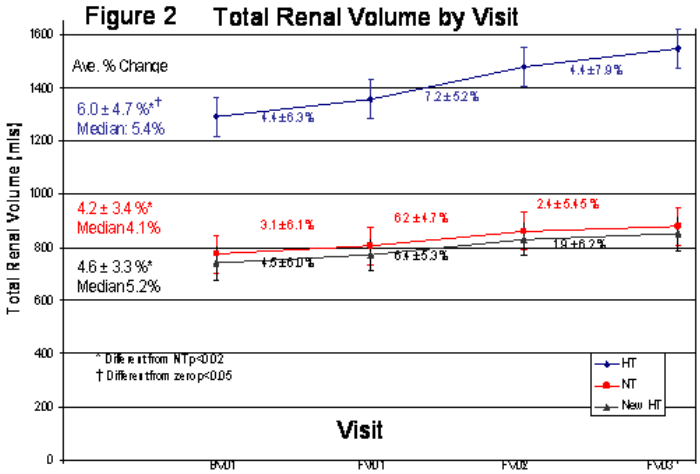


Figure 1. Annual rate of change in glomerular filtration rate measured by iothalamate renal clearance in normotensive (squares), new hypertensives (triangles) and hypertensives (diamonds) in CRISPI. A significant decline in glomerular filtration rate was found in the hypertensive (-3.0 ml/yr/1.73m^2) subjects that was significantly different from both normotensives and new hypertensives.

Figure 2. Annual rate of change in renal volume in normotensive (squares), new hypertensives (triangles) and hypertensives (diamonds) in CRISPI. A significant increase in renal volume from baseline was found the hypertensive subjects and a significantly greater rate of change in renal volume than in normotensive subjects.

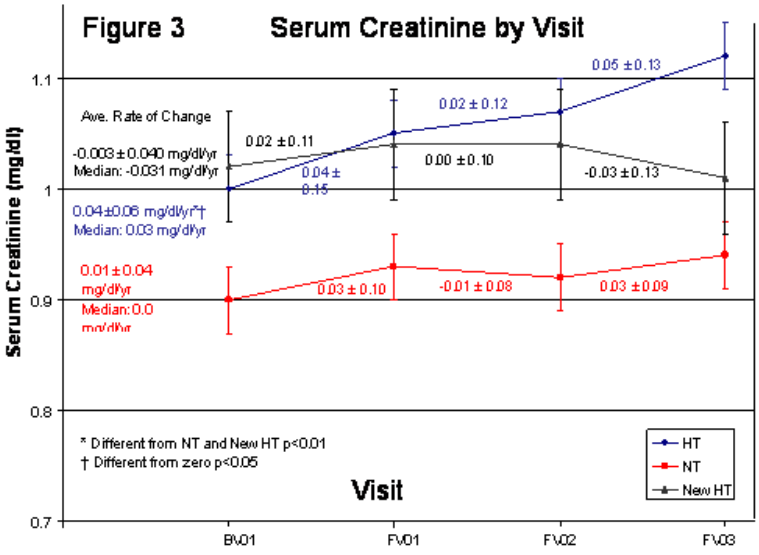
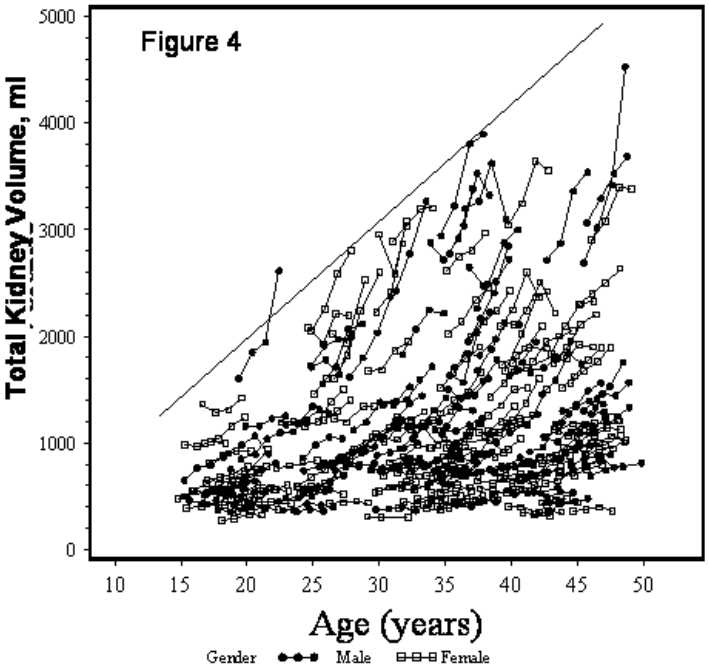


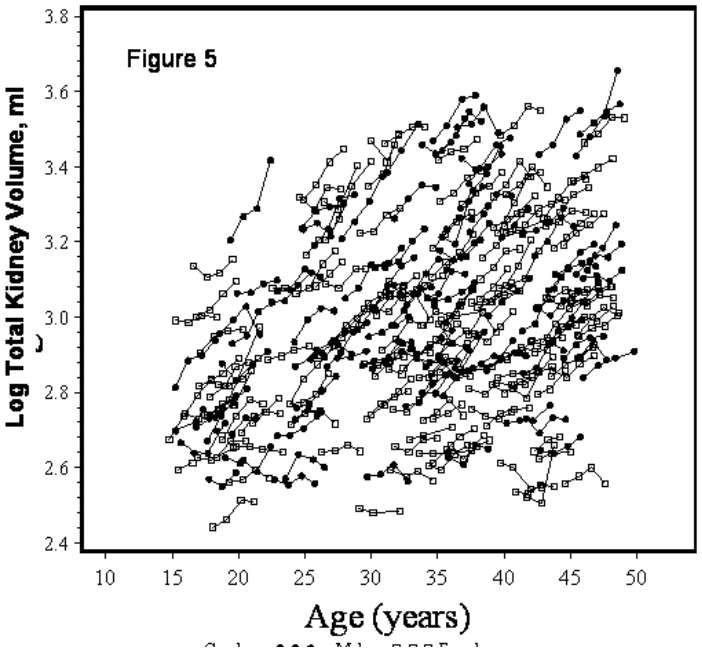
Figure 3. Annual serum creatinine levels in normotensive (squares), new hypertensives (triangles) and hypertensives (diamonds) in CRISPI. The average annual rate of change was significantly increased only in the subjects who were hypertensive at baseline.

Kidney and cyst volumes increase continuously in ADPKD. There was an increase in total kidney and cyst volume from year to year in over 80 % of ADPKD subjects although the apparent rate of increase varied widely from subject to subject[27]. This is illustrated in Figure 4. Shown are TKVs (in milliliters) for individual subjects who were female (open squares) or male (closed circles). Four sequential measurements of TKV were available for most of the individuals. As shown in Figures 4-6 , some individuals demonstrated rapid rates of increase in TKV, whereas in others renal volume increased by only a few per cent over a period of 4 years. This striking data set illustrates the clinical course of ADPKD in dramatic terms. Total kidney volume was generally less in the younger subjects than in those over age 30. The line in Figure 4 represents an

approximation of the upper limit of total kidney volume (TKV) in this cohort (slope =slope 100 ml/year; intercept = 0. It is important to add that total kidney volume measurements in all nineteen ADPKD subjects from the Mayo and Kansas CT volumetric studies[28, 29] fell within the maximal limit of the CRISPI cohort. A random coefficient model on $\log_{10}$ TKF gives a mean (SD) intercept (baseline visit) of 2.96 (0.25) and a slope of 0.022 (0.014) corresponding to 910 ml and an average 5.2% growth.

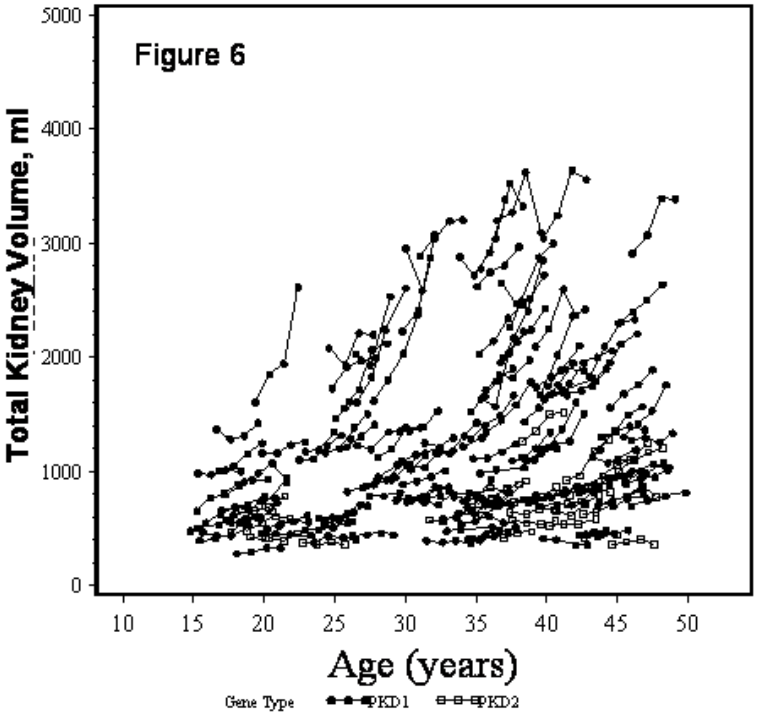
Patterns of renal volume change. The general pattern of kidney and cyst enlargement in ADPKD patients gave the impression that the rate of kidney enlargement was non-linear (Figure 4). Indeed, in a semi-log plot total kidney volume appeared to increase as a logarithmic function of age (Figure 5) in those cases with the most rapid rates of volume increase. This observation is consistent with the view that mural epithelial cell proliferation within cysts progressively increases the potential cyst volume. Fluid accumulates within this potential volume by net trans-epithelial secretion of salt and water to fill and further expand the potential space created by proliferation of the mural epithelial cells. Pumping of fluid into closed compartments leads to hydrostatic pressures within the cysts that are higher than the surrounding parenchyma, a factor that may be of importance in the crowding of adjacent non-cystic parenchyma and for the propensity of these cysts to rupture and to cause hemorrhage.





It is interesting to find that each patient seemed to follow a prescribed rate of kidney volume increase from year to year. It seems reasonable to suppose that each person may exhibit a "signature" rate of kidney and cyst growth that reflects the underlying germ-line and acquired mutations. Fitting jointly a random coefficient model for each kidney provides an estimated correlation of .91 between the rate of growth on the right kidney to that in the left kidney. If this observation is confirmed over a longer time period, then more refined analysis might yield an instrument of great clinical utility for determining prognosis in individual patients relatively early in the course of the disease and in the selection of subjects for clinical trials.

There was considerable overlap between males and females in respect to the rate of kidney enlargement



(Figures 4 and 5), although males overall had larger kidneys at baseline.

Cyst Number but Not the Rate of Cystic Growth Is Associated with the Mutated Gene. The significance of gene type to disease progression is analyzed in this study of the CRISP cohort. Gene type was determined in 183 families (219 cases); 156 (85.2%) had PKD1, and 27 (14.8%) had PKD2. PKD1 kidneys were significantly larger (Figure 6), but the rate of cystic growth (PKD1 5.68%/yr; PKD2 4.82%/yr) was not different (P = 0.24). Cyst number increased with age, and more cysts were detected in PKD1 kidneys (P < 0.0001). PKD1 is more severe because more cysts develop earlier, not because they grow faster, implicating the disease gene in cyst initiation but not expansion [30].

Renal volume and kidney function. Most nephrologists who work in the field think that the expanding cysts cause secondary structural and functional changes in polycystic kidneys. To examine this possibility we separated individuals in the CRISPI cohort by gender and sorted them into 5 renal volume categories at baseline (in ml.): < 500, 501 - 1000, 1001 - 1500, 1501 - 2000, and >2000 in order to survey the potential effects of renal enlargement on outcomes (Table 1).

Mean age tended to increase in both male and female cohorts in association with kidney volume. There was a striking trend in kidney volume in subjects with the PKD2 genotype. No PKD2 individual had kidneys larger than 1500 ml and most were less than 1000 ml. This fact is also illustrated in Figure 6. The PKD2 subjects (open squares) clearly exhibited lower values for TKV than age-matched PKD1 subjects. Approximately 90% of the CRISPI cohort has been successfully genotyped. It has been established that PKD2 patients develop ESRD approximately 16 years later than patients with PKD1[31]. This fact, together with the new data in Figure 3 that PKD2 subjects have smaller (less cystic) kidneys than age-matched PKD1 subjects strongly supports the hypothesis that total kidney and total cyst volume have dominant roles in promoting ESRD in these patients.

Table 1

Males TKV	Age	%PKD2	% Hypertensive	Corrected IOTH base	Change in GFR Baseline - Year 3 Ioth	Coc-Gault	cr UV/P	Ualb ug/d	UMCP-1 ng/mg crea
<500	22.9	40.0	35.7	116.1	8.5	-15.5	0.8	39.6	223
501-1000	32.1	34.5	45.9	123.3	12.6	-3.0	-11.1	23.6	347
1001-1500	35.1	0.0	80.0	109.0	-7.7	-11.2	-2.5	44.0	456
1501-2000	32.0	0.0	92.3	108.5	-2.9	-10.4	-2.4	58.7	799
>2000	37.6	0.0	100.0	101.7	**-24.2**	**-25.6**	**-20.8**	120.9	711
Females									
<500	29.5	25.0	15.4	112.3	2.8	3.0	0.0	21.5	376.3
501-1000	31.6	23.1	60.3	103.4	-4.4	-2.5	-3.0	34.1	487.6
1001-1500	33.5	10.0	76.9	94.9	-6.3	-4.8	12.2	37.6	771.0
1501-2000	37.9	0.0	75.0	83.4	-11.1	**-12.7**	-8.2	63.7	1264.1
>2000	35.0	0.0	92.9	80.6	**-23.2**	-9.6	**-8.9**	68.5	1185.3

Bold changes P < 0.05

The data in Table 1 are averages. Age, %PKD2, % hypertensive, Corrected Iothalamate Clearance, Ualbumin and UMCP-1 were measured at enrollment. (Baseline). Changes in GFR (Iothalamate Clearance, Cockcroft-Gault creatinine clearance, and measured creatinine clearance over a 24h interval are differences between Baseline and Year 3 (an interval of 3 years).

At enrollment, GFR (Iothalamate) appeared to correlate inversely with TKV in males and females, a finding that was reported in a previous publication from this study[32] and is illustrated in Figure 1. Table 1 also indicates that clear-cut changes in GFR, reflected by significant decreases in Iothalamate, Cockroft-Gault and measured creatinine clearances, occurred in relation to the increase in renal volume. In the >2000 ml TKV groups, significant paired decreases in iothalamate, Cockroft-Gault estimated creatinine clearance and measured creatinine clearances were observed in the fourth year of observation. Declines in relation to increasing renal volume were also found in relation to urinary albumin excretion and the excretion of the chemokine, Monocyte

Chemotactic Protein-1 (MCP-1). Urine albumin and MCP-1 excretion appear to rise above normal levels (> 26 ug/d; > 263 pg/mg creatinine, respectively) relatively early in the course of the disease and may be alternative markers of disease progression before changes in GFR can be detected.

These preliminary findings suggest that further refinement of the renal volume indicator of disease progression may yield even more powerful predictive tools for managing this disease. Moreover, based on the CRISPI and the combined Mayo-Kansas University CT studies reported previously[28, 29], it is clear that sequential measurements of Total Kidney and Total Cyst volumes reliably portray disease progression.

Developing a marker of disease severity. We have made a step toward the goal of developing an age-adjusted index of total renal volume progression (Progression Severity Index, PSI). In preliminary calculations, the TKV of each subject in the CRISPI cohort was compared on enrollment to that of the most advanced cases in the combined CRISPI and Mayo-Kansas University cohorts[28, 29]. The PSI was determined from the ratio of the measured TKV (subject)/ Maximal TKV in the CRISPI cohort (estimated from the equation for the line defining Age vs Maximum TKV in Figure 1) determined for each subjects age at baseline. When multiplied by 100, the PSI is the percentage of maximal kidney volume for the stated age of the subject. We found in a preliminary analysis that PSI was directly correlated with a) declining GFR, b) increased urine albumin excretion and c) onset of hypertension.

This, or an index based on a regression on age, is a promising new way to select subjects with minimal, moderate or severe ADPKD for clinical trials and possibly to judge prognosis.

Disease severity in African Americans. African Americans (n=28) demonstrated significantly smaller renal (896 vs. 1178 ml) and cyst volume (423 vs. 565 ml) than their non-African American (n=215) counterparts. AA and non-AA were similar with regard to age, gender distribution, weight, body mass index, and age of diagnosis of ADPKD. A similar inverse relationship between total renal volume and GFR was present: AA: r= -0.43, P<0.004, non-AA: r=-0.40, P<0.0001). In those with confirmed PKD1 and PKD2 mutations, PKD1 AA demonstrated significantly smaller renal and cyst volumes than their non-AA counterparts. Two findings need to be explored further: 1) the prevalence of the PKD2 genotype appears to be greater in AA than in non-AA and mutation identification needs to be completed in all participants and 2) measurements of the renin-angiotensin-aldosterone system (Approved Ancillary Study in CRISPI and proposed in this application) to determine if activation of the RAAS is relatively suppressed in this African American cohort. Further studies are needed to clarify this potentially important racial difference in disease severity.

MR determination of renal blood flow. Validation Studies for MR Flow Measurement: An MR-based method to measure renal blood flow was developed and validated in phantoms, healthy controls and ADPKD individuals at Mayo College of Medicine and Emory University. Steady-flow measurements with a PVA phantom that has mechanical and magnetic properties reflecting those of vessel wall and internal diameters ranging from 3 to 11 mm demonstrated close agreement between actual and MR estimated flows (r=0.991) with an average overestimation of 0.9±4.9%. Pulsatile-flow measurements showed 0.6–4.1% errors of estimated flow rates, using 14 or 20 cm FOVs, a 5 mm tubing and actual flow rates of 315 or 540 mL/min. Reproducibility was evaluated through blinded repeated analysis by two radiologists of data sets from 19 patients. Average intra-reviewer CVs were 1.4% and 1.2%. Intra-class correlation coefficients were 0.987 and 0.983. The average inter-reviewer CV was 2.5% with a reliability coefficient of 0.983[31]. Further validation studies have been performed in healthy volunteers to assess the reproducibility of the measurements using independent acquisitions and the effect of gadolinium administration. Immediate repetition of a flow scan showed a standard deviation of 17.5 ml/min on average, corresponding to a mean CV of 2.9%. Repetition of the scan including the plane scouting process showed a standard deviation of 34.2 ml/min on average, corresponding to a mean CV of 6.0%. The mean flow following gadolinium administration was on average 6.64 ml/min higher than pre-contrast flow.

Cross-sectional study of RBF. 127 participants, forty-six male and eighty-one female (32.9±8.2 years of age) had MR RBF measurements at baseline at the Mayo Clinic or Emory University. Forty of them (31.5%) had multiple renal arteries. Left kidneys were larger than right kidneys and had more severe disease. RBF was lower in the left kidneys. Right and left kidney volumes, cyst volumes, and percent cyst volumes were inversely correlated with the ipsilateral RBF. Iothalamate clearances were inversely correlated with age and kidney volume and positively correlated with RBF. When considered alone, age, diagnosis of hypertension, kidney

volume and RBF were all significant predictors of GFR. In the multiple-variable model, however, only age and RBF were significant independent predictors[31].

Longitudinal analysis and predictive value of RBF. To determine whether RBF changes over time, participants at Mayo Clinic and Emory University underwent determinations of RBF at 1, 2, and 3 years after the baseline studies. After 3 years of follow-up, RBF had significantly declined and TKV and TCV had significantly increased, while GFR had remained stable. Correlation and multiple regression analysis were used to examine the effects of age, gender, body mass index, hypertension status, mean arterial pressure (MAP), TKV, RBF, GFR, serum uric acid, HDL and LDL cholesterol, urine sodium excretion (UNaE) and UAE on GFR and TKV slopes. TKV, TCV, RVR, serum uric acid, UAE, UNaE, age, BMI, MAP, and estimated protein intake were positively and RBF and GFR negatively correlated with TKV and TCV slopes. TKV, TCV, RBF, RVR, UNaE, and UAE were independent predictors of TKV and TCV slopes. TKV, TCV, and MAP were negatively and RBF positively correlated with GFR slopes. Regression to the mean confounded the analysis of GFR slopes. TKV, TCV, and RBF were independent predictors of GFR decline. These results suggest that RBF reduction a) parallels TKV increase, b) precedes GFR decline, and c) predicts the structural and functional disease progression of ADPKD[33].

Monocyte Chemotactic Protein-1, a disease severity marker. Urinary MCP-1 excretion appeared to be a marker of disease severity (Table 1). This chemokine is synthesized by renal cyst epithelial cells and may reflect a phenotypic transformation in tubular epithelium that becomes cystic[34]. The CRISPI study confirms that urinary MCP-1 may increase above normal levels early in the course of the disease and may be a marker of inflammation or interstitial irregularities that are a serious consequence of cyst expansion. Since MCP-1 is synthesized by the mural epithelial cells and accumulates to very high levels in cyst fluid. To find its way into the final urine, however, cysts must be in direct communication with the urinary collecting system. Since most macroscopic cysts larger than a few millimeters in diameter have no connections to the urinary collecting system, the major source of MCP-1 in the final urine may be relatively small cysts that remain hydraulically connected to the tubules from which they derived. Thus, it is tempting to speculate that urinary MCP-1 may reflect the contributions of relatively small cysts that may have been newly formed. If that hypothesis can be confirmed, MCP-1 might be useful as a surrogate marker of disease activity early in the course of the disease in individuals with relatively small cysts.

Liver cysts. Hepatic cysts were found at greater prevalence than previously reported in all age groups: 83% overall, and 58% in 15 to 24, 85% in 25 to 34, and 94% in 35 to 46 age groups; 85% in women (57%, 91%, and 95% from the younger to the older subgroups, respectively); and 79% in men (60%, 75%, and 93%, respectively). The high prevalence of hepatic cysts in the current study cohort of relatively preserved renal function indicates that the relatively late onset of the liver abnormality in some subjects is not the consequence of a uremic environment. The detection of cysts in relatively young subjects exemplifies the superiority of MRI over ultrasound for imaging small cysts, and probably accounts for the larger prevalence in early stage disease than published previously. The prevalence of hepatic cysts was directly related to renal volume ($\chi^2 = 4.30$, $P = 0.04$) and to renal cyst volume ($\chi^2 = 5.59$, $P = 0.02$). A wide range of hepatic cyst burden was observed (0 to 4673 mL, a logarithmic transformation mean of 3.20 mL). Furthermore, we found that hepatic cyst volume was significantly greater in women than in men (5.27 vs. 1.94 mL) (P=0.003). The average hepatic cyst volume was 0.25, 5.75, and 22.78 mL in sequential age groups, respectively ($P < 0.0001$). Hepatic cyst volume and renal volume correlated ($r = 0.22$, $P = 0.001$). Mean renal volume was greater in subjects with than those without hepatic cysts (1004 vs. 712 mL) (P =0.0005)[35].

Significance of Complex Renal Cysts. Complex renal cysts, a marker of renal complications including cyst hemorrhage, developed in over 80% of the CRISPI cohort and were significantly associated with the total renal volume (r=0.67) and renal cyst volume (r=0.66). These findings demonstrate a potential renal imaging marker to predict structural disease severity. In our preliminary study of 70 subjects with complex cysts, we found the mean complex cyst volumes were 5 mL in 15 to 24, 21 mL in 25 to 34, and 21 mL in 35 to 46 age groups. The youngest age group was significantly different from the other two groups. Women had larger complex cyst volume than men (mean 20 vs 12 mL), but without statistical significance. No statistically significant difference (p=0.47) in complex cyst volume was observed between the subjects with and without a history of hematuria.

Comparison between GFR methods. To study the natural history of ADPKD, accurate assessment of changes in GFR over time (GFR slope) are needed. A study in patients with baseline moderate to severe hypertensive chronic kidney disease (GFR < 65 mL/min per 1.73 m^2) found equivalent results between iothalamate clearance and the MDRD equation (33) However, subjects in CRISP had normal or near normal renal function at baseline (creatinine clearance >70 mL/min). Furthermore, several recent studies have suggested that estimated GFR with serum creatinine based equations is not accurate in populations with predominantly normal renal function (34,35) To investigate this further, we compared GFR slope by different methods with respect to baseline predictors in the CRISP cohort (n=241)[36]. Each subject had up to four annual GFR measures by three different methods: a 2 hour iothalamate clearance, a 24 hour creatinine clearance, and the abbreviated MDRD equation. For each individual, Iothalamate GFR was regressed on time from baseline to generate a percent slope (annual percent change in GFR). A decline in GFR was defined as a slope of -5% or lower annually. Predictors for a decline in GFR were compared between methods. These baseline predictors included kidney cyst volume, hypertension, urine albumin to creatinine ratio (ACR), and age. As shown in the following table, associations were stronger between predictors and a decline in GFR by iothalamate clearance slope than by the MDRD equation slope. There were no statistically significant associations by creatinine clearance slope. Based on these findings, continued measurement of GFR by iothalamate clearance is needed to understand the natural history of ADPKD. Changes in muscle mass or dietary protein over time may confound a serum creatinine based equation slope and lead to erroneous conclusions.

	Odds ratio for a decline in GFR (-5% or lower annually		
Predictor	Iothalamate Clearance	MDRD Equation	Creatinine Clearance
Cyst Volume			
> 500 mL	4.1 (2.3 to 7.4)*	2.3 (1.3 to 4.0)*	1.5 (0.8 to 2.6)
≤ 500 mL	1	1	1
Hypertension			
Present	3.5 (1.9 to 7.1)*	2.7 (1.5 to 5.1)*	1.1 (0.6 to 2.0)
Absent	1	1	1
ACR			
> 30 mg/g	3.1 (1.7 to 5.6)*	1.9 (1.1 to 3.4)*	1.4 (0.8 to 2.6)
≤ 30 mg/g	1	1	1
Age Group			
> 40 years	2.6 (1.1 to 6.0)*	1.3 (0.6 to 2.9)	1.2 (0.5 to 2.7)
25 to 40 years	1.5 (0.7 to 3.2)	1.4 (0.7 to 2.9)	1.1 (0.5 to 2.3)
< 25 years	1	1	1

Genotyping Studies of CRISP subjects. Mutation screening has been completed on 239 CRISP patients (including inferred information on two family members from which we do not have samples) from 202 families, 32 of which are multiplex within the study. It involved amplifying the coding regions of PKD1 and PKD2 as 82 fragments and analysis of the products by DHPLC. Mutation negative samples, ones with missense, in-frame deletions or atypical splicing changes, and controls were sent to Athena Diagnostics for sequencing (total 150). Large deletions were also screened in persistent mutation negative cases. An algorithm was developed to predict the pathogenicity of missense and atypical splicing changes including the chemical significant of substitutions, evolutionary conservation in orthologs to fish and in homologous proteins, and population data, including segregation in pedigrees and analysis of normal controls.

Using this comprehensive screening approach, mutations were determined in 182 pedigrees (90.1%), representing 213 patients. Linkage identified this disease gene in three further families (8 patients). One hundred and fifty seven families are PKD1 (85.2%) and 27 PKD2 (14.8%), similar ratios to previous studies of clinical ADPKD populations.

For the PKD1 population, 107 (66.5%) have truncating mutations (frame shifting [49], nonsense [38] or splicing [16]), 43 (27.7) were missense changes and 9 (5.8%) in-frame deletions/insertions. In the PKD2 families, 22 (84.6%) were truncating, 3 (11.1%) missense, and 2 in-frame deletions (7.4%). Although the majority of changes were unique to a single family, 53 (29.1%) were due to a recurrent mutation.

Comparisons of PKD1 and PKD2 patients showed that baseline kidney and cyst volumes are significantly larger in PKD1 than PKD2. However, the rate of growth of kidney and cyst volume as measured at a Log10 was not significantly different between the two genotypes. Counting of cysts shows that PKD1 kidneys have

more cysts and so indicate that the milder disease in PKD2 is due to less cyst development rather than slower cyst growth.

DCIAC Infrastructure. To support the achievement of the scientific goals outlined above, the DCIAC has implemented an integrated system which allows PCC staff to de-identify electronic MR records and transfer them securely to the DCIAC where they are processed to yield measurements used in the analyses described above. A web-based data entry system allows the PCC staff to enter and track administrative, clinical and laboratory information about each participant. This information is then integrated into an analysis database which is used by the statisticians at the DCIAC for analyses and for study report generation. This database is in the process of being packaged for transport to the NIDDK Data Repository to support the DNA and biologic specimens that are being transferred to the NIDDK repositories.

Statistical analyses are primarily conducted using SAS. Mixed-model repeated-measures analyses have been used for analyses of the longitudinal data and was used for the planning of Study A of the large scale, NIDDK sponsored HALT PKD intervention trial.

RESEARCH DESIGN AND METHODS

Overview of Design:

The CRISP II Study is a prospective, observational study that is an extension of CRISPI. CRISPI was also 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. It is anticipated that 220 CRISPI subjects are available to enroll in CRISPII. CRISPII is designed to include all CRISPI individuals including those who enroll simultaneously in other clinical trials. In this respect, HALT, an ongoing interventional trial of the PKD Clinical trials network may maximally enroll up to 105 subjects in Study A (which includes MR imaging identical to that proposed in this submission) and 32 subjects in Study B (no MR imaging). Importantly, the Principal Investigator (Dr. Ty Bae) and personnel for the Imaging Center (now at the University of Pittsburgh) for both 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 CRISPII.

To minimize subject burden and to maintain retention throughout CRISPII, those CRISPII 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 CRISPII that are not included in HALT.

CRISPII Timeline:

<u>April 2006-March 2007</u>

- Protocol refinement, consent form development for CRISP II, local IRB approval
- Forms and MOP development
- Center expansion to include University of Kansas and University of Alabama in Birmingham for measurement of renal blood flow
- Submission of all renal blood flow measures done in CRISP I to Washington University for central review
- Quality Assurance protocol for renal blood flow acquisition at all PCC's
- Review and concept approval of CRISPII protocol by the EAC of CRISPI.
- Continue analyses of longitudinal data initiated in CRISPI
- Complete transfer of data and biologic samples to NIDDK repositories

<u>April 2007-March 2008 (Baseline or YR1 visit)</u>

- First PCC visit for CRISP II participants

Semi-Annual contact with participants via telephone for detailed review of medications, medical visits, hospitalizations
Annual acquisition of plasma creatinine (duplicate determination)

April 2008-March 2009 (YR2 visit)

Annual acquisition of plasma creatinine (duplicate determination)
Semi-Annual contact with CRISP extension participants for detailed review of medications, medical visits and hospitalizations
Initiate analyses of combined CRISPI and CRISPII longitudinal data

April 2009-March 2010 (YR3 visit)

Second full PCC visit of CRISP II participants
Semi-Annual contact with participants via telephone for detailed review of medications, medical visits and hospitalizations
Continue analyses

April 2010-March 2011 (YR4 visit)

Annual acquisition of plasma creatinine (duplicate determination)
Semi-Annual contact with CRISP extension participants for detailed review of medications, medical visits and hospitalizations
Data analysis, close out visits, transfer of CRISPII data and samples to NIDDK repositories, completion of ancillary studies.

Eligibility and patient recruitment for CRISPII:

CRISPI participants will be invited to participate in CRISPII. At entry into CRISP I participants met a number of inclusion and exclusion criteria. Exclusion criteria for participation in CRISP II 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

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 II, but will not undergo MR studies.

To enroll in CRISP II, 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 II 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 II protocol does not exclude participants that enroll in other interventional trials. If CRISP II participants are recruited into an interventional trial (e.g HALT clinical trial) that also requires imaging studies, the visits for CRISP II 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 II analysis. Analysis of the data obtained on subsequent visits will be held until the interventional trial is completed. The CRISP II 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 II 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 II will in part be collected in other trials, but there will be some CRISP II specific information that may

need to be acquired by the CRISP II 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 II 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 physicians office/laboratory; semi-annual telephone interviews; recruitment of family members, sample collection and DNA isolation.

1. 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 CRISPI. 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 (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 µL 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 µL 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. 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 2007 and 2009 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 GCRCs 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.

MR Examination: The imaging protocol for CRISPII has been revised from the MR imaging protocol used in CRISPI. The rationale of this revision is as follows. In December 22, 2006, the FDA issued a Public Health Advisory notifying healthcare professionals of 90 reports of Nephrogenic Systemic Fibrosis or Nephrogenic Fibrosing Dermopathy (NSF/NFD) in patients who have moderate to end-stage kidney disease and received gadolinium-based contrast agents for MRI and MRA. Further information may be found at the following websites:

http://www.fda.gov/medwatch/safety/2006/safety06.htm#Gadolinium
http://radiology.rsnajnls.org/cgi/content/full/2423061640v1

Although a causative relationship between gadolinium contrast medium and NSF/NFD has not been definitely established, published data raised the suspicion that there may be an association between NSF/NFD and gadolinium contrast medium in patients with compromised renal function. In view of these concerns, we will stop using gadolinium contrast medium in our revised MR imaging. Gadolinium-enhanced MR imaging facilitates the process of measuring the kidney volume and identifying the renal arteries, however, is not absolutely required. Instead, an additional fast imaging sequence, 2D true-FISP (FIESTA) without fat sat, will be obtained to image the kidneys just as T2 imaging. This will provide an additional cue to help delineate the kidney border on T1 images. We will acquire 2D true-FISP (FIESTA) with fat sat to depict the renal arteries prior to the phase-contrast RBF measurement sequence.

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.

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/HASTE with fat sat) with 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/FMPSPGR/LAVA without fat sat) with 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 $\leq 15^{o}$. To improve 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 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) with 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 (FIESTA) without fat sat with 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.

2. 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).

3. Semi-annual telephone interviews:

During the interviews information regarding medication changes, hospitalizations, doctor visits and outpatient procedures will be recorded. 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.

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

A major component of CRISP II (Aim 3) is to collect more exhaustive family histories of all CRISP I patients and draw an electronic pedigree for each family (Progeny). 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 ≥1.4mg/dl, females and ≥1.6mg/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. In approximately 200 of these family members we plan to obtain MR analysis to determine kidney volume as part of an ancillary study to CRISP II conducted by the CRISP investigators (an R01 grant that will be submitted February 5, 2007) which will try to map modifier loci in this population. The severity of the cystic liver disease will also be estimated (grades 0-4: 0, no cysts; 1, <5%; 2, 2-20%; 3, 20-50%; 4, >50% of liver volume made up of cysts). 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.

Analytical methods:

All data are entered into a database maintained by the DCIAC and undergo a variety of quality control procedures to insure its validity. Prior to analysis, simple descriptive statistics and graphic displays will be examined to insure the integrity of the data. In all analyses issues of scaling and distributional assumptions will be carefully monitored.

Specific AIM 1: Extend the preliminary observations of CRISP-I to ascertain the extent to which quantitative (kidney volume and hepatic and kidney cyst volume) or qualitative (cyst distribution and character) structural parameters predict renal insufficiency and develop and test new metrics to quantify and monitor disease progression.

<u>Hypothesis 1a</u>: Increased renal volume in general and all renal volumes > 750 ml adjusted for age and other significant covariates in CRISP I predict rate of loss of renal function as well as progression to specific endpoints, e.g. KDOQI Stage IV, ESRD, and/or death.

We will use the following variables:

Dependent Variable: GFR as measured by a 2 hour iothalamate clearance and
death. GFR will be analyzed as a continuous measure and will be used to define KDOQI Stage IV and ESRD.

Independent Variable: Total kidney volume (TKV) as a continuous measure and using the >750 ml cut point.

Planned covariates will include: gender, age, race, baseline GFR, hypertension status, urine albumin (UAE) excretion, baseline and follow-up average systolic, diastolic and mean arterial blood pressures and site. Potential modifiers to be investigated will include body mass index (BMI), serum uric acid, HDL and LDL cholesterol, 24 hour urine volume, dietary sodium and protein intake, estimated use of tobacco and class of antihypertensive medication use. We will also explore the addition of parenchyma volume as a modifier (however, it is calculated from total volume so we will need to assess multicolinearity first). Due to the documented co linearity and dependence between total kidney volume and total cyst volume (Grantham J, 2006) we cannot use this variable as a covariate or modifier but may explore additional analyses using total cyst volume as our primary independent variable. There is a hypothetical possibility that the non cystic volumes (parenchyma volume) may play an important role in disease progression. It will not be possible to determine this effect in this study due to the increased variability of measurement of cyst volume. The hypothesis would be either that parenchyma volume remains constant and cyst and renal volume are totally dependent or that at some point parenchyma volume goes down and the co linearity between cyst and renal volume disappears. It

is important to note that parenchyma volume can vary greatly from individual to individual although it represents only a small fraction of TKV. In fact, clinicians make a semi quantitative assessment of parenchyma tissue in imaging studies when they discuss prognosis with individual patients.

Measures: For both our independent and dependent variables we will have one baseline measure, three follow-up measures from CRISP I and two from CRISP II.

GFR and TKV as a continuous measure:

STEP 1: We will use scatter plots and Pearson and Spearman correlation coefficients to assess the relationship between GFR and TKV at each time period. We expect to see a curvilinear relationship (Bae et al, 2006). This relationship appears to be curvilinear based on iothalamate/MR data as well as other ultrasound and creatinine data (Fick et al, 2001), and we will assess this with the use of log transformations, amongst others, as has been done in prior publications (Grantham, 2006).

STEP 2: We will use repeated measures ANOVA to test the relationship between GFR and time specifically focusing on tests for linear and quadratic trends.

STEP 3: We will use GEE based regression models with GFR as our dependent measure. Recent advances in GEE research and updates to statistical packages such as STATA allow use of GEE for Gaussian based continuous dependent variables as well as dichotomous or count data. However, GEE is sensitive to missing data and we may use a generalized mixed models approach instead. We will have five follow-up measures per person. Baseline TKV will be included in the model at every time period to adjust for possible regression to the mean (Barnett 2005). The model will contain a time variable (for which we will explore alternative correlation structures) and TKV. From this model we can evaluate how much GFR changes with a one unit change in TKV, whether or not the TKV at follow-up significantly predicts the GFR at follow-up above and beyond the baseline TKV value, and how much the addition of time influences that change. We can also test whether time behaves linearly and whether the slope changes over time by including the time x TKV interactions.

Because we are developing the model based on the premise that TKV predicts GFR we will explore whether or not use of TKV at the same time point GFR is measured or use of TKV lagged one follow-up time period is appropriate. Given that there is a one year difference it is possible that TKV at the same time period could act as a predictor but it is more likely that the lag effect is most appropriate. What we expect is that the baseline values primarily predict GFR and that subsequent time point measurements only marginally add to the predictive power of the model. Use of time-lagged GEE models will be tested in all subsequent analyses where appropriate.

The model will be refined by assessing whether the addition of baseline covariates and modifiers improve the model, and whether the addition of other time varying covariates and modifiers improve the model. Since GEE is preferred for prediction models it is our first choice. We will explore use of mixed models instead if the GEE model does not converge appropriately (e.g. due to missing data). While GEE is the typical approach when the dependent measure is dichotomous or count data we prefer to utilize GEE in the context of continuous dependent measures as well. We will also explore random coefficient mixed models as needed. Our guiding reference for this analysis will be “Applied Longitudinal Data Analysis for Epidemiology” by Jos W. R. Twisk, 2003, Cambridge Publishing.

Power: Starting with the simplest case where we run separate regression analysis at each time point to assess how TKV predicts GFR, a sample size of 220 achieves 80% power to detect an R-Squared of 0.03 assuming no covariates. Using an alpha of 0.01 (Bonferroni correction for doing five models (one for each time point) increases the detectable R-squared to 0.05. The addition of baseline TKV, covariates and moderators will decrease the amount of R-squared we can detect. Using all time points will reduce this even further. The baseline correlation between TKV and RBF was r=-0.344 which corresponds to an R-squared of 0.11.

GFR and TKV as a dichotomous cut point:

STEP 4: At each time point we will compare whether the mean GFR is different for our two TKV groups using independent group t-tests.
STEP 5: GEE will be used with GFR as our dependent measure. Our between subjects factor will be TKV Group status, our within subject factor will be follow-up number and baseline TKV will be a covariate to adjust for regression to the mean. From this model we can evaluate how much GFR changes with TKV Group status and how much the addition of time influences that change. We can also test whether time behaves linearly with the inclusion of TKV in the model. The model will be refined by considering whether there is a time x TKV interaction, whether the addition of baseline covariates improves the model, and whether the addition of other time varying covariates improves the model. Because our covariates and modifiers are not necessarily equal for the two TKV groups we will explore use of propensity scores instead of the traditional approaches to covariate adjustments. As an alternative to GEE we may explore use of repeated measures mixed models as needed.

Power: A repeated measures design with TKV cut point (<= or > 750) as the between factor and day as the within factor has 2 groups. We assume 64% in the > 750 group with the remaining 36% in the ≤ 750 group based on an extrapolation of baseline CRISP I data. Each subject is measured five times. This design achieves 80% power to detect a decrease of 2.7 in GFR assuming the GFR in the low volume group is 98.2 mL/mm/1.73 m^2 with a SD of 24.9 using a Geisser-Greenhouse Corrected F Test with a 5% significance level. This analysis also assumed an autoregressive correlation structure across the 6 time periods with an initial correlation of 0.3. Using log transformed GFR would result in an even smaller detectable change.

While there are no standard approaches to power assessment for GEE, in a simulation study, Jung and Ahn (2003) showed they would need 167 subjects to detect a time x group interaction beta coefficient of 0.1 with a 30% / 70% distribution for the group variable, 80% power, alpha=0.05 and an autocorrelation structure with initial correlation of 0.6. Like us, the simulation assumed one baseline and 5 follow-up measures.

Time to Event:
STEP 6: Cox proportional hazards regression will be used to determine whether total kidney volume predicts time to development of KDOQI Stage IV, ESRD, and/or death. We expect 18% of individuals will have CKD Stage 4 and 8% to have ESRD by study end. Actual time of event (day) may not be available. Rather we will know the event most often within ± 3 weeks. Consequently the time of event for our subjects will be measured in overlapping intervals. In this case the model will need to incorporate interval censoring. We will build the model first with only baseline TKV and second with the TKV values at each time point prior to the event by including the TKV by time interaction. We will build separate models with TKV as a continuous measure and as a dichotomous grouping variable. Finally, other covariates will be added as appropriate.

Event status at end of study:

STEP 7: Since each individual will be followed for eight years we will assess whether or not increasing TKV is related to whether or not the individual was at KDOQI Stage IV, ESRD or dead by year eight. We will follow the same modeling stages as STEP 6 but use logistic regression instead of Cox. We will assess whether increasing TKV increases the probability of the event occurring when TKV is continuous and, separately assess whether or not TKV values above 750 ml increase the probability of having the event. We may also incorporate the time period where the event occurred. IN this case GEE where our independent variable is dichotomous and there are clustered (fixed time points) observations within each subject indicating trends up to the point the subject was classified as having the adverse outcome. Although, the number or time points may vary (i.e. subjects experiencing an event at varying times relative to baseline), so it we may use a generalized linear mixed model approach instead.

Power: Table 1 shows a range of detectable hazards ratios of TKV on CKD Stage 4 and ESRD in a Cox regression model at alpha=0.05 and 80% power for different levels of r-squared where r-squared denotes the estimate of explained variability of outcomes based on the model with all covariates but TKV status. It also

shows the corresponding odds ratios for the logistic regression model to assess the events at the end of the study. The Model assumptions were that 18% of the 220 subjects will have CKD Stage 4 by study end and 8% will have ESRD. The continuous measure of TKV is in log10 units and the TKV group variable is based on the 750 cut point.

	CKD Stage 4				ESRD			
	TKV Continuous		TKV Two Groups		TKV Continuous		TKV Two Groups	
R^2	HR	OR	HR	OR	HR	OR	HR	OR
0.0	1.17	1.63	2.53	2.51	1.27	2.01	4.02	3.30
0.1	1.18	1.68	2.66	2.63	1.28	2.08	4.33	3.49
0.2	1.19	1.73	2.82	2.78	1.30	2.18	4.74	3.72
0.3	1.21	1.80	3.03	2.95	1.33	2.30	5.27	4.00
0.4	1.23	1.89	3.31	3.20	1.36	2.46	6.03	4.41

<u>Hypothesis 1b</u>: Baseline medullary vs. non-medullary cyst volume and cyst number in CRISP I predict loss of renal function over time.

With the focus narrowing to assess medullary cyst volume we will use the following variables:

Dependent Variable: GFR as measured by a 2 hour iothalamate clearance and death. GFR will be analyzed as a continuous measure.

Independent Variables:

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.

3) The ratio of number of cysts in the medullary region to the number of cysts in the cortical area (MNCN) at baseline.

Potential covariates and modifiers: same as hypothesis 1a

GFR and MPCP or MNCN as a continuous measure:

STEP 8: 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 (Barnett 2005). 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 or 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.

GFR continuous and CCD:

STEP 9: GEE modeling will be used with GFR as our dependent measure. Our between subjects variable will be CCD (5 levels or 2 levels if we dichotomize as primarily medullary vs. not) and our within subjects variable will be time. From this model we can evaluate how much GFR changes as a function of baseline cyst distribution and how much the addition of time influences that change. We can also test whether there is a change in the effect of baseline CCD on GFR over time by including the time by CCD interaction.

The model will be refined by considering whether inclusion of total cyst volume or 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.

Power: Since there is no baseline or prior study reporting of any of our independent measures we were unable to do specific power calculations. However, with 220 subjects we can detect a Pearson correlation coefficient between cyst volume and renal function of 0.16 at alpha=0.05 and 80% power.

Hypothesis 1c: Prediction models (formulas) utilizing age and renal volume at baseline in CRISP I will effectively predict loss of renal function over time

While we have established with CRISP I data that TKV is a predictor of adverse outcomes and have established that its predictive power remains after statistical adjustment for age, gender, race and other clinical and laboratory measurements, it needs to be translated into guidelines that can be applied by the clinician to provide quantitative prognostic information.

To explore this hypothesis we have developed an age and gender-adjusted indicator of volume severity. The polycystic kidney severity index (PSI) is the ratio of the measured TKV (subject)/ Maximal TKV in the CRISP-I cohort (estimated from the equation for the line defining Age vs. Maximum TKV) determined for each subject's age at enrollment. When multiplied by 100, the PSI is the percentage of maximal kidney volume for the stated age of the subject.

GFR and PSI continuous

Step 10: From a statistical perspective developing a linear formula to predict GFR from PSI would be an obvious first step. We will explore this approach but experience shows that doctors tend not to use formulas in the clinical setting. Consequently, we expect that the most clinician-friendly use of the PSI will be to develop ranges of the PSI which will define individuals into groups with relatively homogenous prognosis. For example, if a patient has a PSI of 0.5 and an initial GFR of 98 it is projected to increase by 20% in two years. We will use PSI percentiles and GEE to determine how granular to make our groupings in order to maximize the predictive ability of PSI. We will first divide PSI into two groups based on the median, then three groups based on the tertiles (using two dummy coded variables), then four groups based on the quartiles etc. and continue the process until we have non-significant pair wise differences. GFR will be our dependent measure, PSI groupings our between-subjects factor and time our within subjects variable. We will add the PSI group and time interaction to see if baseline PSI predicts GFR also as a function of time. We will then incorporate age and gender into the model and see if they are significant (our hypothesis being that they will not be as these variables were incorporated into the PSI calculations). Finally, we will asses whether the incorporation of information about the baseline cyst volumes or distribution, genotype, or other covariates substantially improve the clinical utility of using the PSI.

ESRD and PSI

Step 11: Chi-Squared Automated Detection (CHAID) will be used to determine the PSI cut point that best distinguishes who will achieve ESRD by the end of the study. CHAID will look at all possible splits of PSI and determine the split that best predicts ESRD. The same staged modeling approach used in Step 10 (first adding time then age and gender then other covariates) will be done to assess how baseline knowledge of these variables improves the predictability of our PSI cut point.

NOTE 1: We will attempt to determine the appropriate groups of PSI in steps 10 and 11 with half of our sample (or 2/3) and use the remaining subjects to validate the chosen groupings.

NOTE 2: Should PSI not prove a successful predictor we will explore use of Classification and Regression tree algorithms with TKV, age and gender to develop a decision tree algorithm to predict ESRD at study end. For example, the tree may predict that if you are a female African American you are most likely to develop ESRD when your baseline TKV is xx.

Hypothesis 1d: Baseline liver cyst volume adjusted for the appropriate variables predicts rate of increase in liver cyst volume in CRISP I participants.

We will use the following variables:

Dependent Variable: Liver cyst volume (LCV).

Independent Variables: Liver cyst volume at baseline

Potential covariates will include gender, age, race, hypertension, blood pressure and site. Potential modifiers to be investigated will include total cyst volume (TCV), total kidney volume, age of menarche if possible, progesterone use (if data are reliable), alkaline phosphatase, GFR, number of pregnancies, and years of estrogen use. We will also explore the possible inclusion of caffeine use and LDL and HDL cholesterol depending on the reliability of the data..

STEP 11: We will use GEE regression models with LCV as our dependent measure. Our independent variables will be LCV at baseline, and time (baseline as the reference group). From this model we can evaluate how much LCV changes over time and the influence of our value at baseline. We can also test whether time behaves linearly with the inclusion of baseline LCV in the model by assessing the interactions. The model will be refined by considering whether the addition of baseline covariates and modifiers improve the model. We are particularly interested in whether the slope of the line changes for age and gender.

Power: Starting with the simplest case where we run separate regression analysis at each time point to assess how baseline LCV predicts LCV, a sample size of 220 achieves 80% power to detect an R-Squared of 0.03 assuming no covariates. Using an alpha of 0.01 (Bonferroni correction for doing five models (one for each time point) increases the detectable R-squared to 0.05. The addition of covariates and moderators will decrease the amount of R-squared we can detect. Using all time points will reduce this even further.

A repeated measures design with follow-up time period as the within factor has 1 group with 220 subjects and data on LCV collected 5 times. This design achieves 82% power to detect an increase in log10 LCV of 0.2 from baseline to the final follow-up measure assuming an overall SD of log10 LCV of 2 (based on CRISP I data) using a Geisser-Greenhouse Corrected F Test with a 5% significance level. This analysis assumed a correlation of 0.3 between the baseline and final follow-up value.

Specific AIM 2: Extend the preliminary observations of CRISP I to ascertain the extent to which age and sex-adjusted measurements of renal blood flow by MR technology predict the rate of renal growth; and renal blood flow and kidney volume predict the rate of renal function decline in ADPKD.

Hypothesis 2a: Baseline renal blood flow predicts the rate of increase in renal volume in CRISP I participants

We will use the following variables:

Dependent Variable: TKV Total Renal Volume (continuous)

Independent Variables: RBF Renal Blood Flow at baseline (continuous)

Planned covariates will include gender, age, and race. Potential modifiers include total kidney volume, body mass index (BMI), hypertension status, specific class of antihypertensive medication use, statin use, serum uric acid, HDL and LDL cholesterol, urine sodium (UNaE) and albumin (UAE) excretions, and estimated use of tobacco. Another potential modifier will be the average mean arterial blood pressure during the study to reflect blood pressure control.

TKV and RBF as continuous measures:

STEP 12: We will use GEE regression models with TKV as our dependent measure. Our independent variables will be baseline RBF (and functions of RBF if the relationship is not linear), and time (baseline as the reference group). From this model we can evaluate how much TKV changes with a one unit change in baseline RBF and how much the addition of time influences that change. We can also test whether baseline RBF values affect TKV differently over time by including the time by RBF interaction.

The model will be refined by considering whether inclusion of total cyst volume improves the model (the extent of multicolinearity will be evaluated first), whether the addition of baseline covariates and modifiers improves the model.

Power: Starting with the simplest case where we run separate regression analysis at each time point to assess how baseline RBF predicts TKV, a sample size of 220 achieves 80% power to detect an R-Squared of 0.03 assuming no covariates. Using an alpha of 0.01 (Bonferroni correction for doing seven models (one for each time point) increases the detectable R-squared to 0.05. The addition of covariates and moderators will decrease the amount of R-squared we can detect. Using all time points will reduce this even further.

A repeated measures design with follow-up time period as the within factor has 1 group with 220 subjects and data on TKV collected 5 times. This design achieves 82% power to detect an increase in 67 from baseline to the final TKV follow-up measure assuming an overall SD of 670 (based on CRISP I data) using a Geisser-Greenhouse Corrected F Test with a 5% significance level. This analysis assumed a correlation of 0.3 between the baseline and final follow-up value.

Hypothesis 2b: Baseline renal blood flow, independent and in addition to baseline renal volume, predicts loss of renal function in CRISP I participants

The analytical approach will basically be to add RBF to the existing **baseline** analyses and models developed in Aim 1 and assess whether adding RBF improves the ability to predict loss of renal function. Potential RBF and TKV or TCV interaction will also be considered.

Hypothesis 2c: Combining longitudinal measures of renal blood flow and renal volume may enhance the capacity to predict loss of renal function in CRISP I participants

The analytical approach will basically be to add RBF to the longitudinal analyses and models developed in Aim 1 and assess whether adding RBF improves the ability to predict loss of renal function. Potential RBF and TKV or TCV interactions will also be considered.

Specific AIM 3: Collect DNA samples and clinical information from CRISP family members known to have ADPKD for use to examine genotype-phenotype relationships and by an independently funded study to identify genetic modifiers.

Hypothesis 3a: Genetic heterogeneity and mutation type and/or location affect disease severity in the CRISP population.

This analysis will be similar to previous studies of ADPKD populations[30,39]. The questions to be asked concern the extent to which genic and allelic effects are associated with the phenotype, defined by the imaging and biochemical measures of renal function available in this population. For gene type (*PKD1* and *PKD2*) comparisons will be made with the renal volume data and GFR (and last GFR prior to ESRD) using mixed-model ANOVA and t-test analysis, adjusting for age and gender[30]. Within family correlations will be taken into account using generalized estimating equations (GEEs). Data analysis concerning mutation type and position will initially be limited to the *PKD1* gene, as there will be far less *PKD2* data. As many different mutations to *PKD1* cause disease and none is common, to maximize the likely significance of the results, *PKD1* mutations will grouped according to type. As we have not found mutation type significant in previous studies[30,40]we will restrict the analysis to two groups, truncating, compared to in-frame, that mechanistically are most likely to be relevant.

Using all identified individuals with PKD1 from the CRISP families' population (n~800 individuals) we will test for an association of mutation type with renal disease severity. Renal volume, calculated GFR and age at onset of ESRD will be used as the measurements of disease severity. As renal severity differs significantly within families, and use of family averages limits the power of the analysis, this variable will be analyzed per individual. Multiple hierarchical linear regression will be used to model current individual GFR levels as a function of mutation type, age, and gender; taking into account potential within-family correlations using GEEs.

Other endpoints include age at onset of RI or ESRD, age at onset of ESRD alone, and at onset of Stage 3 or Stage 4 RI. Life table methods (Kaplan-Meier, log rank test) will be used to analyze age at onset of the combined endpoint of Stage 3 or 4 RI or ESRD by mutation type. Individuals without the event of interest will be censored at age of their last renal evaluation. The Cox proportional hazards model will be used to assess and test the effects of type of *PKD1* mutation on survival while controlling for gender. Data will be presented using median survival estimates, and Cox model hazard ratios and 95% confidence intervals. As survival times for individuals within a family may not be entirely independent, the robust variance (with family as the cluster variable) will be used when testing for effects with the Cox model. We will also explore use of unordered multi-event survival methods to handle the clustering affect of family members.

Power: We estimate that 65% of 800 patients from typical families will have the combined endpoint of ESRD or RI; renal volume measurements will be available in all cases, and mutation data in 90%. With 650 events, the survival analysis will have 80% power to detect hazard ratios as small as 1.4 when comparing mutation groups (truncating [~65% of patients] vs. in-frame [~35%]). With renal events beginning at about age 30 and median renal survival occurring at about age 50, a hazard ratio of 1.4 translates to being able to detect a difference in median survival as small as 5.7 years between mutation groups. When comparing GFR levels between mutation groups, a difference of 5.6 ml/min can be detected with 80% power, assuming a standard deviation of 30 ml/min. While these power calculations do not consider the effect of within family correlations in disease severity, our experience has been that these correlations are quite small, and as such have little impact on the power estimates. Overall this analysis will show whether there is a correlation between mutation type and the severity of renal disease, with small hazard ratios detectable. The other reason for collecting this information, and especially the DNA samples, is to allow analysis for modifier genes that will be undertaken in the CRISP II ancillary studies R01 grant application to be submitted February 5, 2007 by the CRISP investigators

Hypothesis 3b: Genetic factors that modify the renal and hepatic phenotypes will be detected by a genome-wide association study employing a high resolution SNP array. (Note: This hypothesis will be examined using the CRISP population by an ancillary study to be submitted as a separate R01 application in February 2007).

In the modifier study a genome-wide association study (GWAS) employing a high resolution SNP array (Illumina, 317,000 SNPs) will be employed to look for genetic factors that modify the renal and hepatic phenotypes in the CRISP cohort and their families. This ancillary study will first screen the CRISP cohort, using the baseline and longitudinal imaging data as the primary end-points. A second phase of the study will complete a GWAS of the CRISP families' samples employing renal volume and GFR as the primary endpoints. Loci positive in both populations at a level of P=<0.001 or in either group independently at P=<10^{-6} will be screening specifically in an additional ADPKD population. ANOVA will be the primary analysis tool for this study using appropriate transformation to identify a data scale under which ANOVA assumptions are met. Important co-variants for this study are age, genotype, gender and some lifestyle factors such as smoking

history and BMI. Identification of one or more modifying locus that significantly influences the clinical phenotype outcome in ADPKD will be of prognostic importance.

Specific AIM 4: Maintain and expand a database of uniformly and accurately collected information including renal structural and functional parameters and a repository of biological samples which can be used by ancillary or independently funded studies initiated by CRISP or non-CRISP investigators.

An ancillary study started during CRISP I began to examine whether urine MCP1 concentrations, a product of cyst formation and growth excreted in increased amounts in baseline urine collections, predict clinical renal imaging patterns and disease course. This study will continue during CRISP II with the specific aims to determine whether the pattern of urinary excretion of MCP1 in individual patients remains stable from year to year and whether baseline urinary excretion of MCP-1 predicts total kidney volume and total cyst volume and number, loss of renal function, and progression to specific endpoints, e.g. KDOQI Stage IV, ESRD, and/or death. Urinary excretion levels of periostin and other potential markers identified by micro-array screening of human ADPKD tissues will also be studied. Much of the development work for this ancillary study has been done under the auspices of another NIH project by Dr. D.P. Wallace at the NIH funded Polycystic Kidney Disease Center at the University of Kansas Medical Center. Affymetrix gene chip screening has identified several candidate products that are especially well-suited for exploration in CRISP patients as biomarkers of disease progression. Among them, periostin was markedly over expressed in ADPKD cyst epithelial cells compared to tubule cells from normal human kidneys. The goal of this ancillary study is to apply those new findings to the CRISP cohort in which the disease has been fully characterized. For the most part, the statistical analysis will follow the plans for Specific AIMS 1 and 2.

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