AASK Cohort Study

Protocol

March 25, 2004

AASK COHORT PROTOCOL

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Section 1. SUMMARY

Despite excellent blood pressure control and despite use of reno-protective antihypertensive medication, hypertension-related renal disease commonly progresses. The factors that determine the progression of this condition remain poorly understood. The primary objective of the AASK Cohort Study is to determine prospectively the long-term course of kidney function and risk factors for kidney disease progression in African-Americans with hypertension-related kidney disease that receive recommended antihypertensive therapy. A secondary objective is to determine the occurrence of cardiovascular disease and assess its risk factors in the setting of hypertension-related kidney disease.

The AASK Cohort Study is a prospective, observational study that is an extension of the AASK clinical trial. The AASK trial was a randomized, clinical trial that tested the effects of 3 different medications used as first line antihypertensive therapy (ramipril, metoprolol and amlodipine) and 2 levels of blood pressure control (usual control and more aggressive control). Of the 1,094 randomized participants in AASK, it is anticipated that 650-750 individuals who have not reached ESRD will enroll in the Cohort Study. In addition, those individuals who reached ESRD during the AASK trial will be invited to attend one visit for collection of DNA. For those who enroll in the Cohort Study, twice each year, approximately every 6 months, exposures will be collected. Exposures will include environmental, genetic, physiologic, and socio-economic factors. The primary renal outcome will be a clinical outcome defined by doubling of serum creatinine, ESRD or death. Appropriate antihypertensive treatment (medications and target BP level as determined in the AASK trial) will be provided to all participants who do not have ESRD. In this fashion, the cohort will directly control two of the major 'known' determinants of kidney disease progression (treatment of hypertension and use of reno-protective, antihypertensive medication) and will therefore address its research objectives in the setting of recommended antihypertensive care. We anticipate a minimum of 4 contacts and maximum of 6 contacts for BP control per participant per year. The anticipated duration of follow-up in the Cohort Study will be 5 years (total of 9-12 years, including the period of the AASK trial).

It is anticipated that the AASK Cohort Study will provide data that enhance our understanding of the processes that determine progression of renal disease. Furthermore, data from this study might ultimately lead to new prevention strategies that delay or prevent the onset of ESRD.

Section 2. OBJECTIVES AND RESEARCH QUESTIONS

The primary objective of the AASK Cohort Study is to determine prospectively the long-term course of kidney function and risk factors for kidney disease progression in African-Americans with hypertension-related kidney disease that receive recommended antihypertensive therapy. A secondary objective is to determine the occurrence of cardiovascular disease and assess its risk factors in the setting of hypertension-related kidney disease.

Research questions that will be addressed are as follows:

1. What is the long-term course of kidney function in this population?
2. What are the environmental, genetic, physiologic, and socio-economic factors which predict the progression of kidney disease?
3. What are the long-term effects of the AASK trial interventions on the progression of kidney disease?
4. Does the development of proteinuria predict the progression of kidney disease?
5. What is the impact of recommended blood pressure therapy, as determined by the AASK trial, on the progression of kidney disease in comparison to usual care in the community? (Note: this question might be addressed using a corresponding subgroup of the CRIC cohort.)
6. What comorbidities, particularly cardiovascular disease, occur in the setting of hypertension-related kidney disease?
7. What risk factors predict the occurrence of cardiovascular disease?
8. What are the patterns of change in metabolic variables and cardiovascular-renal risk factors during the transition from pre-ESRD to ESRD?

Section 3. BACKGROUND AND RATIONALE

During the past three decades, there has been a progressive decline in mortality from cardiovascular and cerebrovascular disease. In contrast, no such reduction in the mortality from end-stage renal disease (ESRD) has been observed and, in fact, in the past decade (1989-1998) the number of patients entering the ESRD program in the United States has doubled. Consequently, there are now more than 300,000 patients receiving renal replacement therapy in the US at an annual cost to the Medicare ESRD Program of ~ $12 billion or about $43,000/patient/year (USRDS 2000). It has been well established that the leading causes of ESRD are diabetes mellitus and primary hypertension, accounting for nearly 70 percent of all ESRD in the United States (diabetes 43%; primary hypertension 24%) (USRDS 2000). Demographic data suggest that those most susceptible to ESRD are the elderly (> 65 years) whose ranks have increased more than seven-fold in the last 2 decades, those of lower socio-economic status, and minority groups, especially African Americans, who like the elderly, are disproportionately represented in the ESRD population when compared to their numbers in the general population (38.2% vs.13%).

Recent data from the USRDS (USRDS 2000) support older studies (Rostand, 1982) and show incidence rates of ESRD in African Americans that are about 4.5 fold greater than corresponding rates in white Americans of European ancestry. While this increased risk is found for nearly all forms of renal disease, the most important increased risks are from primary hypertension and diabetes mellitus because they affect the largest number of patients with ESRD. When compared to whites, African Americans have a nearly 5 fold greater incidence of ESRD due to diabetes mellitus, largely due to type II diabetes, and a 7 fold greater incidence of ESRD associated with primary hypertension, with the greatest difference seen in those under age 65 years. As a result, primary hypertension represents 34 percent of all ESRD in African Americans and when present in African Americans accounted for 9 percent of all ESRD in the US between 1995-1998 (USRDS 2000). In addition, data from the MRFIT and MDRD studies suggest a significantly greater rate of loss of renal function in African Americans with hypertension than in whites (Walker, 1992; Klahr, 1994; Hebert, 1997). Taken together these data suggest a unique susceptibility of African Americans to renal disease, especially from primary hypertension.

Blood Pressure: The relationship between blood pressure and kidney disease is graded, continuous and progressive, such that the risk of ESRD increases throughout the range of blood pressure. Perhaps the most convincing evidence comes from the MRFIT study that recorded blood pressure in 332,544 men who were then followed for occurrence of ESRD over 16 years of follow-up (Klag, 1996). Relative to the category of optimal BP (SBP < 120 and DBP < 80 mmHg), the adjusted relative risk of developing all-cause ESRD was 1.2 for normal, 1.9 for high normal, 3.1 for Stage 1 hypertension, 6.0 for Stage 2 hypertension, 11.2 for Stage 3 hypertension, and 22.1 for Stage 4 hypertension. However, because of the high prevalence of Stage 1 and 2 hypertension, over 50% of ESRD cases occurred among persons with Stage 1 or 2 hypertension and < 10% among persons with Stage 4 hypertension. In subsequent analyses from MRFIT, a similar pattern was present in African-American and white men; however, at any given blood pressure level, the risk of all-cause ESRD and hypertension-related ESRD was greater in African-Americans than whites (Klag, 1997). The differential between African-Americans and whites persisted after adjustment for blood pressure, cholesterol, income, diabetes and prior myocardial infarction.

One explanation for the susceptibility of African Americans to renal damage from hypertension may be that they have a greater duration or body burden of hypertension. African American children have higher blood pressure than their white counterparts (Gutgesell, 1981), and elevated blood pressure in childhood is associated with adult hypertension (VanLente, 1994). In addition, there is a high prevalence of non-dipping hypertension in African Americans (Gretler, 1994). Since an impaired nocturnal fall in blood pressure may be a risk factor for renal deterioration (Timio, 1995), impaired circadian blood pressure rhythms together with a long duration of high blood pressure may contribute to progressive damage. Another factor that may increase the body burden of blood pressure is inadequate hypertensive therapy due to either no treatment (e.g., from limited access to health care or failure to seek medical attention) or inadequate treatment (e.g., from less intensive or effective therapies).

Still, some individuals believe that renal failure due to primary (essential, non-malignant) hypertension does not exist or else that it is overstated as a cause of renal failure (Schlessinger, 1994; Siewart-Delle, 1998). Data from the USRDS (USRDS 1997) and NHANES (Burt 1995)

suggest that the annual rate for developing ESRD in the hypertensive population ranges from 1/500 to 1/1100 depending on whether all hypertensives or just treated hypertensives are examined. Thus, unless a very large population of hypertensives is studied, or a high-risk population, such as hypertensive African Americans, is enrolled, few patients will develop renal failure and therefore ESRD will not be considered an important consequence of hypertension. In contrast, the feasibility study for the study for the AASK Trial, using strict clinical criteria for hypertension-related kidney disease, found only arterio- and/or arteriolonephrosclerosis as the primary lesion in 38/39 renal biopsies performed in these subjects (Fogo, 1997).

The mechanisms by which hypertension might damage the kidney are numerous. Translational and shear forces produced by hypertension damage the vascular endothelium causing vascular growth; vascular hypertrophy; local release of ANG II, TGFß1, inflammatory cytokines; mesangial cell proliferation; fibroblast transformation; matrix accumulation; and glomerular scarring (O'Callaghan, 2000, Border, 1998). Of interest, TGFß1 has been demonstrated to be over expressed in African Americans with chronic renal failure (Suthanthiran, 1998).

Sustained glomerular hypertension, in part a result of systemic hypertension, may produce glomerular hyperfiltration, increased mesangial cell activity and subsequent scarring by intermediary processes similar to those noted above (Brenner, 1985). In the case of African Americans, the process of glomerular damage may be greater than in whites since data suggest greater hyperfiltration in African Americans in response to a solute load (Parmer, 1994). Also, increased wall:lumen ratios from pre-puberty through adulthood has been reported either because of an intrinsic alteration or a consequence of a more exuberant response to the effects of blood pressure (Tracy, 1990). This may result in increased renal vascular resistance, reduced renal blood flow and renal ischemia (Frolich, 1990). The importance of these molecular effects of hypertension is underscored by the finding that certain antihypertensives, especially ACEI, show renoprotective effects, which might be independent from their antihypertensive effects (Agodoa, 2001).

Management of Hypertension: Despite aggressive blood pressure control and despite use of reno-protective antihypertensive medications, hypertension-related renal disease commonly

progresses. Among participants enrolled in the MRFIT clinical trial, blacks experienced deterioration of renal function (as measured by reciprocal creatinine slope) despite conventional blood pressure control, i.e. an average diastolic BP < 95 mmHg (Walker, 1992). In contrast, non-blacks in MRFIT with similarly controlled blood pressure had stable renal function. Initial published results from the AASK trial (Agodoa, 2001) confirm this observation, that is, among the 436 persons assigned to ramipril, all of whom received conventional or aggressive blood pressure control, there were 47 cases of ESRD and 44 instances of substantial GFR decline (either a 50% decline in GFR or reduction of 25 ml/min/1.73 m^2 from baseline). Final but as yet unpublished results from AASK confirm that even those patients assigned to the most effective therapy, on average, had substantial declines in GFR (see Section 4). The focus of the AASK Cohort study is to determine the factors that explain this decline in renal function that occurs despite control of blood pressure to recommended levels.

Environmental and Socio-Economic Factors: As a group, African Americans, like other minority groups in the US, often have less education and lower incomes than their white counterparts. They also may live in high stress environments and have less secure employment. This situation has important health implications and may produce reduced access to medical care, poor understanding of medical problems, less aggressive health seeking behavior and as a result less effective, minimal or no medical care (Feldman, 1992). An ecologic analysis of patients with ESRD has revealed a strong positive association between the percentage of families below the poverty line by each zip code and the number of patients with ESRD (Rostand,1992) suggesting an important role for socioeconomic factors in the genesis of chronic renal failure. In contrast, Byrne and colleagues (1994) found that at all levels of income, African Americans had a significantly greater prevalence of ESRD than whites; this finding suggests that in addition to socioeconomic factors, other factors account for the higher prevalence of ESRD in African-Americans than in non-African-Americans.

It is difficult to tease apart the effects of socio-economic from the effects of environmental factors on the progression of renal disease. In any group of people, social structure and culture have major economic and health consequences, the latter resulting from changes in diet, physical activity, and other exposures, that may cause increased body mass, diabetes, hypertension,

atherosclerosis, and low birth weight. In the case of African Americans, a large percent have a blunted rate of renal sodium excretion and evidence of blood pressure that is salt sensitive (Weinberger, 1986). This has led to an hypothesis (Wilson, 1991) suggesting that as a consequence of the African Diaspora and exposure to Western cultural practices, these people, acclimated for millennia to a low salt intake, became exposed to high salt diets that could not be excreted effectively producing not only high blood pressure but also hyperfiltration, glomerular hypertension and attendant renal damage. A similar construct has been postulated by Brenner et al (1982) for the glomerular sclerosis resulting from high protein diets.

In addition to salt and protein, other dietary factors such as obesity and dyslipidemia may promote renal damage. In this regard, restricting calories has, at least in animal studies, been shown to prevent glomerular sclerosis and may in part explain the glomerular changes associated with obesity (Maeda, 1985; Kasiske, 1990; Verani, 1992). African Americans consume diets low in potassium that have been demonstrated to increase renal vascular wall: lumen ratios thereby contributing to hypertension and renal damage (Ford, 1998; Tobian, 1984). Because of a high prevalence of milk intolerance African Americans consume diets low in calcium that may also be associated with hypertension (Fruedenheim, 1991; Appel, 1997). Low dietary calcium together with altered vitamin D production, and, perhaps, parathyroid function may also promote vascular growth (Rostand, 1999). Such deficient diets may also contribute to alterations in cellular ion transport (Weder, 1984; Ferrannini, 1989) that may play a role in hypertension and type II diabetes mellitus found at increased frequency in African Americans (Cowie, 1989). A tendency to obesity in African Americans, coupled with low potassium diets, may impair glucose tolerance, produce insulin resistance and may contribute not only to the high prevalence of hypertensive renal disease but also the production of advanced glycation end products that have also damaging effects on the kidney (Tanji, et al. JASN, 2000).

Environmental exposures may be important contributors to progressive deterioration of renal function. Among these are employment as a laborer (Rostand, 1989), occupational exposure to numerous toxic substances (Nuyts, 1995), and high risk behaviors including smoking, excess alcohol consumption and use of illicit substances such as cocaine.

Genetic Factors: Renal disease (from a variety of causes including hypertension, diabetes, focal glomerulosclerosis and AIDS) is more prevalent in the African American population than in the non-African-American population, suggesting a genetic susceptibility to renal injury (USRDS 2000). Studies have also demonstrated a markedly increased risk of renal dysfunction in relatives of patients with end-stage renal disease (odds ratios for renal disease as high as 9-fold if a first-degree relative has ESRD), that is, findings that lend strong support to the concept of nephropathy susceptibility genes in the African American population (Freedman, 1993; Freedman, 1997; Klag, 1997). Still, the role of environmental factors cannot be completely excluded, because of residual confounding from geographic location, education, income and socioeconomic status. At present, no susceptibility gene for this increased incidence of nephropathy has been clearly defined, although several candidates exist.

Relationship of Change in Proteinuria to Incident ESRD: Over the past 20 years, there have been hundreds of studies that show a relationship between reductions in proteinuria and slowed declines in either actual or calculated GFR. More recently, a number of clinical trials in people with nondiabetic renal disease, including the AIPRI and REIN trials, have shown that a reduction in proteinuria is associated with a delay in the time to doubling of serum creatinine and decline in GFR. Moreover, data from long term clinical studies as well as clinical trials like AASK and the recently completed IDNT trial (diabetes) demonstrate that failing to reduce proteinuria, in spite of blood pressure reduction, while not directly harmful, does not provide for optimal preservation of renal function. Moreover, the optimal degree of proteinuria reduction that correlates with slowing of kidney disease is not known. Additionally, it is unclear whether antihypertensive agents that do not reduce proteinuria, when used with agents that reduce proteinuria, preserve kidney function to the same degree as other agents all known to reduce proteinuria.

Cardiovascular Disease (CVD) in the Setting of ESRD: Cardiovascular diseases are the leading causes of death in ESRD patients accounting for nearly 50 percent of all deaths. The incidence of CVD may even be 10 times the rate in the general population (Foley, 1998). The increased prevalence of cardiovascular disease in ESRD populations can be related to: 1) demographics; 2) the diseases causing ESRD; 3) factors associated with renal disease; and 4)

factors associated with renal replacement therapy (RRT).

Demographics: The median age of RRT patients now is about 64 years. USRDS data (1999) also show that the number of comorbid cardiovascular conditions, the onset of RRT, and the development of subsequent CVD events rise with increasing age.

Diseases Causing ESRD: The two leading causes of ESRD, diabetes mellitus and hypertension, are strongly associated with the development of cardiovascular disease and its consequences. Patients with progressive renal insufficiency have been shown to be at increased risk for the development of cardiovascular disease. Jungers, et al. (1999) have shown pre-ESRD patients have a 3 fold greater likelihood of a cardiovascular event than those with normal renal function. This observation has been recently confirmed by results from the Hypertension Optimal Treatment (HOT) Study showing that subjects with GFR < 60 ml/min were at increased risk for major cardiovascular events and mortality (Ruilope, 2001).

Factors Associated with Renal Disease: Progressive renal dysfunction is associated with the occurrence of traditional and non-established risk factors for CVD. Dyslipoproteinemia is commonly seen in ESRD and the extent of lipid abnormalities depends on the duration and the severity of renal failure (Attman, 1991). The contribution of hypertension to atherosclerosis and to left ventricular hypertrophy (LVH) is well known. LVH is the most common cardiac structural change seen in ESRD and its prevalence increases as renal function deteriorates. LVH is well known to be an independent risk for sudden cardiac death. The anemia that develops with progressive renal deterioration may also contribute to LVH and increased LVMI which compromise coronary vasodilator reserve and which, together with a mismatch of cardiomyocyte mass and capillaries, contribute to a tendency to myocardial ischemia (Levin, 1999; Amann, 1998). Independent of diabetes mellitus, renal insufficiency is associated with insulin resistance and glucose intolerance that may produce vascular endothelial damage as a result of the production of advanced glycation end products (Schmidt, 1999). Secondary hyperparathyroidism and altered vitamin D metabolism seen with developing renal

failure may be associated with dyslipidemia, glucose intolerance, LVH, and, together with associated alterations in calcium and phosphorus metabolism, accelerated athero- and arteriosclerosis and vascular calcification (Rostand, 1999).

Other factors that may be important include: early menopause in women, hyperhomocysteinemia, and inflammation (Stehman-Breen, 1999; Chaveau, 1993; Zimmerman, 1999). Attempts to modify these risk factors in ESRD subjects have not been successful in reducing cardiovascular events or mortality. However, a recent study (Meier-Kreische, 2001) reported that during the past decade, CVD mortality in dialysis patients awaiting transplant and in transplant recipients has decreased significantly suggesting that either invasive intervention, risk factor modification, and/or changes in the dialysis prescription may have ameliorated CVD mortality. It remains to be determined which risk factors and treatment interventions are the most important clinically.

Summary of Rationale and Significance: The incidence and prevalence of hypertension-related ESRD are relentlessly increasing, despite evidence from national surveys that rates of blood pressure-related cardiovascular disease are declining. In view of the substantial public health burden of hypertensive kidney disease, particularly among African-Americans, and evidence that the condition is progressive, even among persons with well-controlled and appropriately treated hypertension, efforts to understand the determinants of disease progression should be a high national priority. In this setting, the most prudent strategy for determining risk factors for hypertension-related ESRD in African-Americans is to establish a cohort of individuals with early evidence of renal disease but with variable rates of progression.

The AASK trial is extremely well positioned to accomplish this task. First, the AASK cohort is a unique, established cohort, one that could never be assembled again. Second, the AASK cohort is extremely well-characterized. Baseline data on many relevant exposures, including extensive medical history, detailed medication records and numerous laboratory measurements, are already available. A bank of biological specimens has already been established. A major ancillary study of genetic factors is underway. Third, the AASK cohort is enriched with

individuals who have progressive renal disease. To date, over 150 individuals are on dialysis or have received a renal transplant. Hence, the cohort is well-positioned to characterize distinct phenotypes (progressors and non-progressors). If another 150 cases of ESRD occur in AASK over the next year, there will be a total of 300 ESRD cases, a number which vastly exceeds the number of ESRD cases from all causes in most population based cohort studies, few of which enrolled large numbers of African-Americans. Fourth, the additional follow-up of AASK participants should allow us to identify and characterize individuals with slow, but clinically important, renal disease progression. To date, ~15% of AASK participants who reached ESRD comprise a group of individuals with rapid disease progression. From the standpoint of prevention, the distinction between slow and rapid progressors is important because the largest fraction of hypertension-related ESRD likely occurs from slow rather than rapid progression of disease. Finally, the cohort is well-positioned to directly control, rather than statistically adjust for, two of the major known determinants of kidney disease progression, that is, treatment of hypertension and use of reno-protective, antihypertensive medication.

Section 4. AFRICAN AMERICAN STUDY OF KIDNEY DISEASE AND HYPERTENSION (AASK) TRIAL

Background: The optimal strategy to prevent hypertension-related kidney failure is uncertain. Two strategies that might be effective are (1) selection of antihypertensive medications that have reno-protective effects beyond blood pressure control and (2) aggressive blood pressure control beyond conventional recommendations.

Trials of angiotensin converting enzyme inhibitors (ACEI) in patients with diabetic and proteinuric non-diabetic kidney disease have documented significant benefits from ACEI. The impact of ACEI on progression of renal disease in African-Americans has been unknown since all published trials had too few African-Americans randomized to such agents. Although animal studies have demonstrated prevention of glomerulosclerosis by calcium channel blockers (CCB), human studies have not consistently confirmed their renoprotective effects. Likewise, preliminary evidence suggested that aggressive blood pressure control might retard the progression of renal disease (Klahr, 1994; Hebert, 1997; Toto, 1995).

In this setting, the African American Study of Kidney Disease and Hypertension (AASK) was designed to evaluate the impact on progression of hypertensive kidney disease of two different blood pressure (BP) goals and three treatment regimens initiated by a ß-blocker (BB, metoprolol); a dihydropyridine (DHP) CCB (amlodipine), or an ACEI (ramipril). Recruitment into the full-scale trial began in February 1995, with planned treatment through October 2001. However, in September 2000, the amlodipine arm was terminated at the recommendation of the Data and Safety Monitoring Board (DSMB). The DSMB recommendation was based on safety concerns that arose because interim analyses showed a slower mean GFR decline and a reduced rate of clinical endpoints (rapid decline in renal function, ESRD or death) in the ramipril and metoprolol groups relative to the amlodipine group in proteinuric participants. Participants originally assigned to amlodipine remained in the trial in order to test the effects of the blood pressure goals on renal disease progression. However, they were provided open label medication, typically ramipril, instead of amlodipine that was discontinued. On September 30, 2001, data collection in the AASK trial ended. Participants were provided blinded

antihypertensive medication until the end of October 2001 when investigators became unblinded. Thereafter, as part of closeout, participants were provided unblinded treatment.

Participants: Participants were self-identified African-American hypertensives (n=1,094), aged 18-70 years, with GFR between 20-65 ml/min/1.73m^2, and no other identified causes of renal insufficiency. Inclusion/exclusion criteria were as follows:

Inclusion Criteria for the AASK Randomized Trial:

- African-American men and women (including Black individuals born in the Caribbean, Africa, Canada, etc.)
- Age 18-70 years.
- Hypertension was defined as a sitting diastolic blood pressure of 95 mmHg or more. The average of the last two of three consecutive readings on a random zero sphygmomanometer machine at any visit was used. Hypertensive participants on antihypertensive therapy needed only one qualifying clinic visit. Those not currently on medications at baseline qualified on each of two consecutive clinic visits.
- Reduced renal function, defined as a pre-randomization (G1 visit) ^{125}I-iothalamate glomerular filtration rate between 20-65 ml/min/1.73m^2.
- Willingness and ability to cooperate with the protocol.

Exclusion Criteria for the AASK Randomized Trial:

- History of malignant or accelerated hypertension within 6 months prior to study entry; previous chronic peritoneal or hemodialysis or renal transplantation.
- Known secondary causes of hypertension.
- Any known history of diabetes mellitus type I and II, or fasting (8-12 hrs.) glucose > 140 mg/dl on two occasions, or glucose > 200 mg/dl on one occasion prior to randomization.
- A ratio of urinary protein (mg/dl) to creatinine (mg/dl) exceeding 2.5 in a 24-hour urine sample collected shortly before the initial GFR visit. (This ratio is used as an estimate of > 2.5 g/day proteinuria without needing to factor for validity of the collection.)
- Clinical or renal biopsy evidence of any renal disease other than hypertensive

nephrosclerosis. Persons with arteriographically documented renal arterial atherosclerotic disease less than 50% stenosis of the renal artery were considered eligible for the study if the PI at the center felt the disease was not clinically significant.

- History of drug abuse in the past 2 years, including narcotics, cocaine or alcohol (> 21 drinks per week).
- Serious systemic disease that might influence survival or the course of renal disease. (Chronic oral steroid therapy was an exclusion, but steroid-containing nasal sprays were not. Inactive sarcoidosis was not an exclusion.)
- Clinical evidence of lead intoxication.
- Arm circumference > 52 cm, which precluded measuring blood pressure with the "thigh" blood pressure cuff. Arm length such that if the cuff circumference extended into the antecubital space so that the cuff interfered with placement of the stethoscope over the brachial artery for blood pressure measurement.
- Clinical evidence of congestive heart failure, current or within the preceding six months. Ejection fraction below 35% measured by any method. Heart block greater than first degree or any other arrhythmia that contraindicated the use of any of the randomized drugs.
- Reactive airway disease, current or in the preceding six months requiring prescribed treatment for more than two weeks.
- Impairment or difficulty in voiding, precluding adequate urine collections.
- Intake of non-steroidal anti-inflammatory agents (NSAIDs) more than 15 days/month, excluding aspirin. Inability to discontinue NSAIDs or aspirin for 5 days prior to GFR measurement.
- History of severe adverse reaction to any of the randomized drugs required for use in the protocol or contraindication of their use.
- Pregnancy or likelihood of becoming pregnant during the study period; lactation.
- Serum potassium level > 5.5 mEq/L at the SV2 and confirmed at G1 for those not on ACE inhibitors during Baseline, or serum potassium level > 5.9 mEq/L at the SV2 and confirmed at G1 for those on ACE inhibitors during Baseline.
- Leukopenia < 2,500/mm3 at SV2 and confirmed at the end of Baseline.

- Medically-indicated need for any of the randomized drugs for any other reason (including angina pectoris, migraine, arrhythmia).
- Allergy to Iodine.
- Suspicion that the participant was unable to adhere to medications or comply with the protocol visit schedule.
- Participation in another intervention study.

Participant enrollment began in August 1994 and ended in September 1998. Table 2 displays baseline characteristics of all randomized participants and characteristics of participants who are likely to enroll in the Cohort Study.

Core Design of the Trial: The AASK trial had a 3 X 2 factorial design (Table 1). Participants were randomized to a usual mean arterial pressure (MAP) goal of 102-107 mm Hg or to a low MAP goal of < 92 mmHg, and to treatment with one of three antihypertensive study drugs, a sustained-release ß-blocker (BB), metoprolol (Toprol XL), an ACEI, ramipril (Altace), or the DHP-CCB, amlodipine (Norvasc). Doses were 50-200 mg/day, 2.5-10 mg/day, and 5-10 mg/day, respectively. If the BP goal was not achieved on the study drug, additional unmasked drugs were added in the following recommended order: furosemide, doxazosin, clonidine, hydralazine, and minoxidil. The dosage of each drug was increased to the maximum tolerated dose before the addition of a subsequent agent.

Table 1: Overview of AASK Trial Design with Enrollment (n) by Randomized Group

	Randomized Medication			
	ACEI	**BB**	**CCB**	**Total**
Low MAP < 92	215	215	110	540
Usual MAP 102-107	221	226	107	554
Total	436	441	227	1094

The Usual Goal (MAP 102 to 107 mmHg) corresponds to a blood pressure of approximately 140/90 mmHg and reflects traditional blood pressure recommendations. The Low Goal of MAP

< 92 mmHg was a more aggressive goal with uncertain benefit. The lower limit of 102 in the Usual Goal (MAP 102 to 107) group provides a minimum targeted separation between the two MAP groups of 10 mmHg. The mean 10 mmHg difference allowed for clear differentiation between the two blood pressure groups.

A randomization scheme that resulted in a 2:2:1 (metoprolol:ramipril:amlodipine) ratio was used because AASK pilot data revealed an early increase in GFR in the DHP-CCB group compared to the ACEI and BB groups. This increased the projected statistical power for the DHP-CCB vs. BB comparison, allowing a smaller sample size for the amlodipine group. Study drug assignment but not BP goal was double masked.

Table 2: Characteristics of All Randomized Participants at Baseline and Characteristics of Active Participants (as of 9/14/2001) at Baseline and at Most Recent Visit.

		All Randomized Participants	Active Participants as of 9/14/2001	
Characteristic	**Units**	**Baseline Value**	**Baseline Value**	**Most Recent Value**
		(n=1094)	(n=682)	(n=682)
Age @SV2	years	54.5 ± 10.7	54.9 ± 10.1	59.8
% men		61.2%	61.9%	
Systolic BP	mmHg	150 ± 23.9	149 ± 23.8	131 ± 17.7
Diastolic BP	mmHg	95.5 ± 14.2	95.2 ± 14.2	78.4 ± 10.9
Mean Arterial Pressure	mmHg	114 ± 16.0	113 ± 16.1	96.2 ± 11.6
Body weight	Kg	89.5 ± 20.7	90.4 ± 20.1	92.7 ± 22.2
BMI	kg/m^2	30.6 ± 6.6	30.9 ± 6.4	31.7 ± 7.1
Income Group				
0-14,999		47.6%	46.0%	
15,000-39,999		25.6%	27.6%	
40,000-100,000+		8.2%	8.7%	
Not available		18.6%	17.7%	
Education				
Not a HS grad		40.6%	40.6%	
HS grad		29.8%	30.1%	
College or beyond		29.4%	29.2%	
Smoking				
Never		42.1%	42.1%	
Current		29.3%	26.0%	
Past		28.5%	32.0%	
LVH by EKG		35.4%	37.1%	
GFR	$ml/min/1.73m^2$	45.7 ± 13.0	48.6 ± 11.9	43.6 ± 18.3
Serum Creatinine				
Men	mg/dL	2.18 ± .76	2.00 ± 0.58	2.26 ± 1.13
Women	mg/dL	1.77 ± .57	1.65 ± 0.48	1.95 ± 1.0
Urine Protein				
Men	g/d	.61 ± 1.1	.36 ± 0.72	.52 ± 0.93
Women	g/d	.41 ± .73	.31 ± 0.61	.50 ± 1.0
Urine Protein/Creatinine				
Men	mg/mg	.33 ± .51	.19 ± .34	.32 ± .55
Women	mg/mg	.33 ± .53	.24 ± .41	.41 ± .80
Glucose	mg/dL	95.0 ± 18.5	95.0 ± 18.5	101 ± 37
Total cholesterol	mg/dL	212 ± 45.5	211 ± 43.3	198 ± 41.6
LDL cholesterol	mg/dL	136 ± 41.0	137 ± 38.5	120 ± 35.7
HDL cholesterol	mg/dL	48.3 ± 16.1	48.3 ± 15.7	50.5 ± 16.1
Triglycerides	mg/dL	141 ± 80.9	134 ± 69.4	141 ± 90.5

Data Collection: At each visit, three consecutive seated blood pressures were measured using a Hawksley MKII random zero sphygmomanometer after at least 5 min rest, with the mean of the last two readings calculated. All personnel measuring blood pressures were centrally trained and certified annually. During the six-month period following randomization, antihypertensive drugs were adjusted at monthly protocol and interim visits to achieve the BP goal. Subsequent protocol visits occurred at two-month intervals. GFR was assessed by ^{125}I-iothalamate clearance at baseline twice, then at 3, 6 and every six months thereafter. Serum and urine creatinine and urine protein were measured by a central laboratory at 6 month intervals. Specimens of blood and urine from each annual visit were stored. Fasting lipid profiles and quality of life measurements were collected annually. Throughout follow-up, adherence by pill count was assessed at each protocol visit; medication usage, both antihypertensive agents and other medications, was collected at each visit.

Trial Outcome Variables: The primary analysis of renal function was based on the rate of change in GFR (GFR slope). GFR slope was determined separately over the first three months after randomization (acute phase) and during the remainder of follow-up (chronic phase), because previous studies indicated that drug interventions could result in acute changes in GFR that differ from long-term effects on renal disease progression. The analytic plan called for determining both i) the mean chronic slope, and ii) the mean total slope from baseline to end of follow-up, including both phases, and for inferring a definitive beneficial effect on renal function of an intervention that significantly reduces the magnitude of both the chronic and total mean slopes. The mean total slope assesses the effect of interventions on renal function during the study period, while the chronic slope is interpreted as the parameter more likely to reflect long-term disease progression.

The protocol also designated a secondary clinical-outcome analysis, based on the time from randomization to any of the following endpoints: i) a confirmed reduction in GFR by 50% or by 25-ml/min/1.73m^2 from the mean of the two baseline GFRs, ii) ESRD, defined as need for renal replacement therapy, or iii) death. The clinical endpoint analysis was identified as the principal assessment of patient benefit. In contrast to the analysis of GFR slope, which addresses the mean drug effect on renal function in all patients including those with little or no GFR decline,

the clinical endpoint analysis is based on events of clear clinical impact, either large declines in renal function or death. Urinary protein excretion, expressed as the urine protein to creatinine ratio (UP/Cr), was also specified as a secondary outcome variable.

Conduct of the Trial: Trial results have high internal validity as measured by adherence to interventions and retention of participants. On average, a 10 mmHg separation in MAP between the low and usual MAP goals was achieved throughout the follow-up period. After the initial 6 month titration period, the average MAP was 93.7 in the low MAP group and 103.7 in the usual MAP group. The average number of medications used to achieve the low MAP goal was 3.1; the corresponding number for the usual MAP group was 2.4. The percent of participant visits without cross-over to the other 2 drug groups was 92.0% in the BB group, 89.6% in the ACEI group, and 93.1% in the CCB group.

Retention of participants was excellent. As of October 17, 2001, vital status was known on 1,082 (99%) of randomized participants. Over the course of the trial, 103 persons died, and another 159 (14%) reached ESRD. Of the remaining 820 participants who were eligible for a close-out GFR, 733 (89%) had a close-out measurement. A poll of trial participants about possible participation in the cohort study suggests that ~ 675 participants will enroll in the AASK cohort, not including the ESRD patients who will be asked to provide DNA.

Analysis Plan of the Trial: The protocol specified three primary comparisons (ACEI vs. beta-blocker, DHP-CCB vs. beta-blocker, and low vs. usual MAP goal). The ACEI vs. DHP-CCB comparison was designated as a secondary rather than a primary comparison because the DHP-CCB and ACEI interventions were expected to produce acute slopes in opposite directions, complicating the comparison of these two groups.

The primary renal function analysis was based on a mixed effects model with random intercepts and random acute and chronic slopes. The mean acute, chronic, and total slopes were estimated by restricted maximum likelihood for each treatment group; total mean slopes were estimated as time-weighted averages of the acute and chronic slopes. The effects of the treatment interventions were estimated by appropriate contrasts of these mean slopes. The model included

clinical center and the following prespecified baseline factors as covariates: proteinuria (expressed as the log transformed UP/Cr to account for positive skewness), history of heart disease, mean arterial pressure, gender, and age. Analyses of the clinical outcome events and other designated events were performed by Cox regression with adjustment for the same covariates as the analysis of GFR slope.

During the trial, members of the Steering Committee became aware of external clinical studies published after the initiation of the AASK that indicated a slowing of renal disease progression by ACEI in patients with elevated proteinuria, as well as studies suggesting DHP-CCBs may increase the level of proteinuria and not slow renal disease progression. Accordingly, an extension of the primary renal function model was analyzed which incorporated interaction terms between log baseline UP/Cr and each treatment group comparison. Subsequently, subgroup analyses were performed in participants with baseline UP/Cr above and below 0.22 (a value corresponding to ~300 mg/ day, which suggests the presence of microalbuminuria). The baseline UP/Cr > 0.22 subgroup includes one-third of the study participants, with the remaining two-thirds belonging to the baseline UP/Cr < 0.22 subgroup. The UP/Cr cut point of 0.22 was post-hoc but was selected independently of the AASK data.

Summary of AASK Interim Results: Among participants with proteinuria > 300 mg/day, those assigned to ACEI had a 36% slower mean decline in GFR to three years ($p<0.006$) and 48% reduced risk of the clinical endpoints vs. the DHP-CCB group ($p =0.003$). In the whole cohort, there was no significant difference in mean GFR decline from baseline to three years between treatment groups. However, ACEI group had a 38% reduced risk of clinical endpoints ($p = 0.005$), 36% slower mean decline in GFR after three months ($p=0.002$), and less proteinuria ($p < 0.001$) than the DHP-CCB group. On the basis of these results, the AASK investigative group concluded that ACEI retards renal disease progression compared to DHP-CCB in patients with hypertensive renal disease and proteinuria and may offer benefit to patients without proteinuria (Agodoa, 2001).

Summary of AASK Main Results: Main results of the AASK trial were presented at the Annual Scientific Sessions of the American Heart Association on November 14, 2001. In brief,

the presence of even small amounts of proteinuria at baseline (urinary protein to creatinine ratio [UP/C] of > 0.22 or ~300mg of protein/day) was associated with rapid progression of kidney disease. In most analyses, the level of proteinuria also influenced the effects of AASK treatments. The separation of MAP between the low and usual BP groups was 10 mmHg, which is greater than that achieved in any previous trial. Still, those on the lower BP goal had similar renal disease progression as those on the usual goal.

For the comparison of medications, results were not definitive because the total GFR slopes and chronic slopes (3 months to end of follow-up) did not reach statistical significance in the same direction in all patients. However, important findings were evident. Blood pressures during follow-up were similar in the three randomized groups. Ramipril as compared to metoprolol reduced the rate of decline in GFR over 4 yrs by 25% and the rate of composite clinical events by 22% in all patients. In patients with baseline UP/C of > 0.22, ramipril reduced the risk of clinical events by 46% as compared to amlodipine, while metoprolol reduced the risk of clinical events by 37% as compared to amlodipine. The analysis of GFR slope after 3 months favored metoprolol over amlodipine, while both the chronic and total GFR slopes were significantly better with ramipril as compared to amlodipine. In patients with UP/C < 0.22, there was no suggestion of a benefit of metoprolol as compared to amlodipine.

In summary, a lower than usual BP goal was not associated with additional slowing of the progression of hypertensive renal disease in African Americans. Ramipril as compared to metoprolol appears to slow renal disease progression independent of protein level, while ramipril and metoprolol slow progression as compared to amlodipine in patients with baseline UP/C >0.22 (roughly > 'dipstick positive' proteinuria).

Implications of AASK Trial Results for Cohort Study: The main results from AASK, in combination with published interim results, have implications for the AASK Cohort Study. First, the incidence of clinical endpoints and the progression of kidney disease was high, even in the group that receive the most effective therapy. Specifically, in the ramipril group, the cumulative incidence of clinical outcomes was ~30% over 5 years, and the average annual decline in GFR (total mean slope) was 1.9 ml/min/1.73m^2/yr. This documented decline in renal

function, which is roughly twice the average age-associated decline in GFR in the general population (~1 ml/min/1.73m^2/yr) highlights the importance of identifying factors other than blood pressure that predict, if not determine, progression of hypertensive kidney disease. Second, of the three medications tested in AASK, ramipril had the most beneficial effects on kidney function. These results support provision of ramipril therapy to all participants in the AASK Cohort. Third, among patients with proteinuria, metoprolol appeared to be more reno-protective than amlodipine. Accordingly, beta-blocker therapy follows ramipril and diuretic therapy in the recommended treatment algorithm.

Section 5. DESIGN OF THE AASK COHORT

Overview: Despite excellent blood pressure control and despite use of reno-protective antihypertensive medication, hypertension-related renal disease commonly progresses. The factors that determine the progression of this condition remain poorly understood. The overall objective of the AASK Cohort Study is to determine prospectively the long-term course of kidney function and risk factors for kidney disease progression in African-Americans with hypertension-related kidney disease that receive recommended antihypertensive therapy. A secondary objective is to determine the occurrence of cardiovascular disease and assess its risk factors in the setting of hypertension-related kidney disease.

The AASK Cohort Study is a prospective, observational study that is an extension of the AASK clinical trial. The AASK trial was a randomized, clinical trial that tested the effects of 3 different medications used as first line antihypertensive therapy (ramipril, metoprolol and amlodipine) and 2 levels of blood pressure control (usual control and more aggressive control). Of the 1,094 randomized participants in AASK, it is anticipated that 650-750 individuals who have not reached ESRD will enroll. In addition, those individuals who reached ESRD during the AASK trial will be invited to attend one visit for collection of DNA. Exposure data is collected annually. Exposures include environmental, genetic, physiologic, and socio-economic factors. The primary renal outcome is a clinical outcome defined by doubling of serum creatinine, ESRD or death. Antihypertensive treatment recommended by current treatment guidelines will be provided to all participants who do not have ESRD. The ACE inhibitor, Ramipril, will be included in the antihypertensive regimen. While no difference in renal outcome was seen in participants randomized to the two BP levels in the AASK trail, current treatment guidelines recommend a BP goal < 130/80 mmHg in those with CKD. Thus, this will be the treatment goal in the cohort. In this fashion, the cohort directly controls two of the major 'known' determinants of kidney disease progression (treatment of hypertension and use of reno-protective, antihypertensive medication) and therefore addresses research hypotheses in the setting of recommended antihypertensive care. We anticipate a minimum of 4 contacts and maximum of 6 contacts for BP control per patient per year. The anticipated duration of follow-up in the Cohort Study is 5 years (total of 9-12 years, including the period of the AASK trial).

There will be 3 periods for analyses, depending on the timing of measurements. Period 1 covers just the AASK trial extending from randomization to the end of September, 2001. Period 2 covers both the AASK trial and cohort periods. Period 3 covers just the AASK cohort, extending from the end of the trial to end of the cohort June, 2007. Table 3 below provides an overview of the Cohort study in relation to the AASK Study.

The AASK Cohort Study provides the core infrastructure for epidemiologic investigations that elucidate potential risk factors for kidney disease progression. Data is collected and stored in a fashion that permits future analyses when preliminary evidence warrants such analyses and when resources become available. For instance, fingernail clippings for heavy metals and blood for inflammatory markers will be collected and stored; assays will not be performed immediately. In contrast, ambulatory blood pressure is obtained at baseline in the cohort and then analyzed.

Timeline: Baseline data collection in the AASK Study commenced in February 1995 with the start of recruitment. Follow-up data collection in the trial ended on September 30, 2001. Baseline data in the cohort phase began in April 2002. Follow-up data collection will end 5 years later. Table 3 displays these key dates.

Table 3: Key Dates Relevant to the Design of the AASK Study and the AASK Cohort Study

February 1995	Start of the AASK Study Recruitment
September 1998	End of the AASK Study Recruitment
September 30, 2001	End of Data Collection in the AASK Study
April 2002	Start of the AASK Cohort Recruitment
June 30, 2007	Anticipated End of Data Collection in the AASK Cohort
July 1, 2007	Start of AASK Cohort Close Out
December 31, 2007	End of AASK Cohort Close Out

Population: Participants in the AASK Cohort will include all participants in the AASK trial who are not on renal replacement therapy. If participants develop ESRD during the cohort phase, they will continue to be followed. (In addition, those individuals who have already been

placed on renal replacement therapy during the AASK trial will be invited for one visit at which DNA is collected; otherwise, because these participants reached ESRD at variable intervals before the start of the cohort and because there would be the potential for extreme selection bias, further data will not be collected.) Table 2 in Section 4 displays characteristics of all randomized participants in AASK as well as the subset of participants who were active in follow-up and who are likely to enroll in the Cohort Study.

Inclusion Criteria: The only inclusion criteria for the AASK Cohort is prior randomization in the AASK Study and provision of informed consent, specific for the Cohort Study. In this fashion, the Cohort will retain those participants who subsequently developed diabetes and other illnesses that might lead to renal disease and participants who moved away from a local clinical center. While such participants would have been excluded from the trial, their inclusion in the Cohort study is appropriate both to describe the long-term progression of kidney disease and its complications and to minimize the potential for bias.

Recruitment: Upon the conclusion of the AASK trial, each participant is invited to participate in the Cohort Study. The primary benefits of the AASK Cohort study include provision of antihypertensive medications (which will be free of charge) and routine management of hypertension. There are no major risks associated with participation in the Cohort Study.

Contact Pattern and Data Collection Elements: The purpose of the study visits are to collect exposure data, ascertain clinical outcomes and manage antihypertensive therapy. Data collection for exposures are collected at baseline and annually thereafter. Management of antihypertensive therapy occur at baseline and every 6 months and at an additional 2-4 visits per patient per year. For those requiring just 2 additional visits (total of 4 visits each year), the visit interval will be quarterly. The basic nomenclature for the data collection visits is "C" followed by a number that corresponds to months after enrollment (e.g., C0, C0.1, C3, C6, C9, C12, C15, C18, etc.). Additional blood pressure management visits may occur in between these visits. Note that during the baseline visit window (C0), participants are asked to have a second serum creatinine measure (labeled the C0.1 serum creatinine); baseline creatinine is the average of two serum creatinine measurements labeled C0 and C0.1. Clinical outcomes are ascertained at each contact.

The types of data to be collected include questionnaire responses (exposures and clinical event surveillance), blood pressure, weight, electrocardiogram, blood, urine and finger nails clippings. Ambulatory blood pressure monitoring and echocardiography are obtained at baseline and every other year (total of 3 times). At the 2 semi-annual data collection visits and 2 other visits, hypertension management occurs. An additional 2 visits may be required to achieve blood pressure control. While participants are encouraged to receive their antihypertensive medical care through the AASK Cohort, some persons may decide not to accept such care. In this case, they are asked just to attend the semi-annual data collection visits.

For those persons who have not reached ESRD, Table 4 of this section displays the data collection items and procedures by visit during the first two years. The pattern of data collection items and visits during all subsequent years will be similar to that of year 2, except that ambulatory blood pressure monitoring and echocardiography occurs every other year.

For those persons who reach ESRD during the Cohort Study, Table 5 of this section displays the data collection items and procedures by visit during the first two years. Data collection visits occur once each year. Again, the pattern of data collection items and visits during all subsequent years are similar to that of year 2 except that the echo will occur every other year. These post ESRD data collection visits occur at the same time the routinely scheduled cohort visits would have occurred.

For those persons who reached ESRD before enrolling in the Cohort Study, there will be one visit at which blood will be collected for DNA.

Table 4: Data Collection Items and Activities by Visit During the First 2 Years of the AASK Cohort Study for Participants who have Not Reached ESRD

	C0	C0.1	C3	C6	C9	C12	C15	C18	C21	C24
Informed Consent	x									
Contact Information	x		x	x	x	x	x	x	x	x
Enrollment (Form 81)	x									
Blood Pressure Measurement (Form 110)	x	x	x	x	x	x	x	x	x	x
Visit Form (Form 111)	x	x	x	x	x	x	x	x	x	x
Weight (Form 110)	x	x	x	x	x	x	x	x	x	x
Exposures Questionnaire (Form 85)	x					x				x
Other Questionnaires (Forms 180, 186, 187, 190, 191)	x					x				x
Sleep Questionnaire – C24 & C48 (Form 174)										x
Demographic / Medical History (Form 84)	x									
Medication Questionnaire (Form 140)	x	x	x	x	x	x	x	x	x	x
Fasting for: (Form 122) - Creatinine, Lipids, Glucose, Insulin, Routine Chemistry CBC	x					x				x
Creatinine (non-fasting) (Form 122)		x		x				x		
CBC (Form 113)	x					x				x
Frozen Plasma – C12, C24, C36, C48, C60 (Form 127)						x				x
Stored Specimens	x			x		x		x		x
DNA (Form 120)	x									
Local Labs Re: BP Rx	o	o	o	o	o	o	o	o	o	o
24-Hr Urine (Forms 123, 125)	x					x				x
Finger Nail Clippings (Form 168)	x					x				x
Central Electrocardiogram - C0, C24 & C48 (Forms 114, 115)	x									x
Local Electrocardiogram – C12, C36 & C60 (Form 116)						x				
Ambulatory BP – C0, C24 & C48 (Forms 170, 171, 173)	x									x

	C0	C0.1	C3	C6	C9	C12	C15	C18	C21	C24
Echocardiogram – C0, C24 & C48 (Forms 117, 119)	x									x
Hospitalization (Forms 144, 145)	-	-	-	-	-	-	-	-	-	-

x = expected per protocol
o = optional, related to BP management, per discretion of PI
- = PRN

Table 5: Table of Data Collection Items and Activities by Visit for Participants who Reach ESRD after Enrolling in the Cohort Study

	Visit*					
	0	**6**	**12**	**18**	**24**	**30**
Contact Information (collected locally)	x		x		x	
Q'naire – Exposures (Form 85)	x		x		x	
Visit Form (Form 111)	x		x		x	
Start of Dialysis/Transplant (Form 128)	x					
Status of Dialysis/Transplant (Form 129)			x		x	
Medication Q'naire (Form 140)	x		x		x	
Labs (Form 122) - Lipids, Glucose, Insulin - Routine Chemistry, including Ca and PO4	x		x		x	
- CBC (Form 113)	x		x		x	
Frozen Plasma (Form 127) – *collected only if not done prior to dialysis.*	x					
Finger Nail Clippings (Form 168)	x		x		x	
Central Electrocardiogram – C0, C24 & C48 (Forms 114, 115)	x				x	
Local Electrocardiogram – C12, C36 & C60 (Form 116)			x			
Echocardiography (Forms 117, 119)	x				x	
ESRD Hospitalization (Form 141)	-	-	-	-	-	-

x = expected per protocol
- = PRN

*Visit schedule will follow original schedule set during pre-ESRD phase. For example, the initial visit will occur at the time when the yearly data collection for the Cohort would have occurred.

For those patients on dialysis, the appropriate tubes can be given to the dialysis unit staff. The staff at the dialysis unit can draw the patient's serum for the clinical center. Blood should be drawn pre-hemodialysis; however, patients supported with peritoneal dialysis or a kidney transplant can have their blood drawn at any time. The center can then send the tubes to the CBL for processing (central measure) or process the sample at the center (local measure).

Informed Consent

To enroll in the AASK Cohort Study, individuals must provide written informed consent. Sample consent forms will be developed. Clinical center PIs can adapt these forms as needed to meet the requirements of local IRBs. For participants who have not reached ESRD, a typical consent process would include two consent forms, one that covers the basic elements of the cohort study (contact pattern and data collection elements except for DNA) and a separate consent form for collection of DNA. For participants who have already reached ESRD, only the consent form for DNA will be used. Additional consent forms may be required to cover additional procedures that are not covered in the original consent.

Blood Pressure

Blood pressure will be measured in a standardized fashion by trained, certified observers using the Tycos Classic Hand Aneroid device. These measurements will be used to guide antihypertensive drug therapy for those patients who have not reached ESRD. While the general approach to blood pressure measurement will be identical to that used in the AASK trial, we decided against continuation of the Hawksley MKII random-zero device in the Cohort because the American Hospital Association and the Environmental Protection Agency have proposed to eliminate mercury from hospitals. We also decided against electronic devices, because these devices use an oscillometric technique. In contrast, aneroid devices use the auscultatory technique that was used during the trial.

BP Management

AASK participants who have not reached ESRD will be encouraged to have their blood pressure managed by AASK Cohort investigators and staff. The target blood pressure is based upon prevailing guideline (specifically, JNC VII guidelines), while selection of first line therapy is based on the results of the AASK trial. An algorithm for stepwise blood pressure control has been developed, along with specific suggestions for replacement of medications used in the AASK trial for medication to be used in the Cohort Study. Drugs listed as part of the algorithm

will be offered free of charge to the participant. However, there will be investigator latitude. If other drugs are used to control blood pressure, the investigator, patient's insurance company or the participant will need to cover these costs. Once a participant reaches ESRD, blood pressure management will be the responsibility of the patient's nephrologist rather than the AASK team. The AASK team may continue to provide drugs listed as part of the algorithm free of charge on a compassionate care basis. However, the provision of medications is not mandatory, because the investigators at that point will not have primary responsibility for blood pressure control.

Provision of antihypertensive care to the AASK Cohort Study participants has scientific, practical, and ethical roles. The scientific role is to directly control two of the major 'known' determinants of kidney disease progression (treatment of hypertension and use of reno-protective, antihypertensive medication). In this fashion, we will test our research hypotheses in the setting of recommended antihypertensive care. The practical role is to promote retention of individuals who otherwise might not participate in the Cohort Study after the trial ends. The ethical role is to avoid the situation of studying the impact of inadequately treated hypertension among individuals who received excellent care in the trial yet have inadequate resources to cover their own care after the trial ends. Note that the Cohort Study is neither designed nor powered to compare the effects of different antihypertensive agents on renal function or cardiovascular disease outcomes.

The recommended blood pressure goal during the cohort phase of AASK is a systolic blood pressure < 130 mmHg and a diastolic blood pressure < 80 mmHg. This goal corresponds to national guidelines (JNC VII, 2004 and NKF, 2003) published after the initiation of the AASK Cohort Study.

The recommended approach to antihypertensive drug therapy is based upon the results of the AASK trial, which documented that:

- ACEI-based therapy was superior to CCB-based therapy, at least in patients with proteinuria (> 0.22 mg protein/mg creatinine), and perhaps among those with lesser degrees of proteinuria,
- ACEI-based therapy appears to be superior to beta-blocker therapy, irrespective of

proteinuria, and

- Beta-blocker therapy appears to be superior to CCB-based therapy among individuals with proteinuria.

Table 6 (below) summarizes the use of medications in both the AASK trial (completed) and the recommended approach to medications in the AASK Cohort Study. In contrast to the AASK trial, which rigidly controlled and monitored the BP management protocol, investigators will have more discretion. Still, investigators will be encouraged to follow broadly the approach outlined below and to follow general prescribing recommendations for these medications.

Table 6: Drug Titration Protocol in the AASK Study and Recommended Approach in the AASK Cohort Study

AASK Study (Order Prescribed by Protocol)	**AASK Cohort (Recommended Algorithm)**
Randomized Drug	Ramipril (Altace)* (maximum dose of 20 mg/day) [ARB*** for those who develop an ACEI cough]
Furosemide	Furosemide*** or HCTZ***
Doxazosin	Verapamil (Covera-HS)* or Beta blocker [Carvedilol (Coreg)]**
Clonidine	If beta blocker used: Amlodipine (Norvasc)*** If beta blocker not used: Clonidine pills*** or Clonidine TTS*** or Reserpine*** If beta blocker nor Verapamil used: Diltiazem (Tiazac)**
Hydralazine or Minoxidil	Hydralazine***, Doxazosin/other α-blocker***, or Minoxidil***

*Provided by manufacturer with financial support
**Provided by manufacturer
***Purchased

Specific considerations might influence the selection of medication. The following is a partial list of such considerations that generally should be based on well-accepted principles of care as outlined in JNC VII:

For participants who had developed an ACEI-related cough in the trial or who subsequently

develop an AECI-related cough in the Cohort study, an angiotensin-receptor blocker (ARB) should be used. [Note: Use of an ARB should not be used in persons who develop angioedema.]

For participants who have had a myocardial infarction or who develop a myocardial infarction, a beta-blocker should be used.

For patients with heart failure or with impaired left ventricular function, doxazosin should be avoided.

Participants are offered free management for their hypertension as part of the AASK Cohort study. Hypertension management will include provision of antihypertensive medications and visits with AASK staff and investigators. Quarterly visits (one every three months) are anticipated. An additional two visits for blood pressure management (total of 6 visits per year) could be offered in order to achieve or maintain blood pressure control. Additional visits beyond these six visits are at the discretion of the local investigator who might, for example, refer the participant back to his/her personal physician for a work-up of secondary hypertension.

The medications listed in Table 6 of this section are offered free to the participants. Sources of medications include donations from pharmaceutical companies (e.g. ramipril from Monarch) and purchase of other medications (e.g., Lasix, HCTZ) from clinical center funds. Reimbursement of other medications may be pursued, potentially through insurance company reimbursement if the participant has health insurance that covers medication costs.

At a practical level, the following is a reasonable approach to transition patients from AASK trial medications:

Patients Assigned to Beta-Blocker. Taper beta-blocker per PI's instructions while simultaneously adding Ramipril.

Patients Assigned to Ramipril. Provide open-label Ramipril.

Questionnaires

Questionnaires are administered annually that focus on potential exposures of interest and on surveillance for outcomes (ESRD and cardiovascular outcomes). Exposures include health habits (alcohol, smoking, analgesic use, drug use), exposure to IV contrast, and psycho-social factors. Instruments to be used will include, where possible, questionnaires used in the AASK trial (SF-36) and standardized instruments used in other studies, including the Jackson Heart Study. Psychosocial questionnaires will include the SF36, the Approach to Life, the Beck Depression Inventory II, and the Diener Satisfaction of Life Form. A sleep questionnaire will be administered twice.

Medications

At each visit, types of antihypertensive medications and types of other concurrent medications are collected using procedures developed in AASK.

For patients who reach ESRD during the Cohort Study, types of antihypertensive medications and types of other concurrent medications are collected at each visit. Due to the large number of common medications that many ESRD patients take, the transplant/dialysis facility will be requested to fax a copy of the patient's most recent medication sheet as a secondary data collection tool. This will include the dose and route of IV/Subq medications such as EPO/aranesp, vitamin D (Calcijex, Zemplar, Hectoral).

Fasting Blood

On an annual basis, fasting lipids [total cholesterol, LDL cholesterol (calculated), HDL cholesterol and triglycerides], glucose, insulin, routine chemistry panel and CBC will be measured. Other analytes will include C-reactive protein (CRP), and potentially other measures of inflammation, measures of oxidative stress and novel lipid risk factors damage, e.g. Lp(a).

Homocysteine will be measured at C0 and C48 or at the end of the study (which ever is sooner). From each collection, aliquots of serum and plasma will be stored for future analyses. To facilitate comparisons of data collected during the trial, the Central Laboratory of the Cleveland Clinic will perform most analyses, including measurements of creatinine and lipids.

To monitor the effects of antihypertensive drug therapy, local laboratories (e.g. electrolytes) will be obtained as needed at the discretion of the clinical center PI.

DNA

DNA will be collected once. Blood will be shipped to the AASK Genetics Core Laboratory at Mt. Sinai Hospital by overnight mail. When received, blood will be divided into 3 aliquots: 10 ml will be used to isolate genomic DNA; 10 ml will be used either to immortalize lymphocytes or for controlled freezing of 4 aliquots of purified PBMC; approximately 50 ul will be spotted onto IsoCode Stix (Schleicher & Schuell) and dried as an archive for future DNA isolation/sample identification/quality control.

24-Hour Urine Collection

Once each year, a 24-hour urine collected will be obtained. Analytes will include creatinine, protein, albumin, sodium and potassium. From each collection, aliquots will be stored.

Finger Nails

Finger nails will be collected once each year. Participants will be asked to trim each of their 10 fingers with a chromium-free nail clipper (to be provided) and will be asked to put the clippings in a labeled plastic bag. The bags will be stored at room temperature and then shipped to the Central Biochemistry Laboratory at the Cleveland Clinic. The Laboratory for Instrumental Neutron Activation Analysis, part of the Interfaculty Reactor Institute of Delft University of Technology (Delft, The Netherlands) can perform these analyses, as it has done for other cohort studies. The neutron activation analyses provides measurements of 50 heavy metals, including

elemental mercury, chromium, and lead.

Electrocardiogram

A central ECG will be obtained at C0, C24 and C48. A copy will be retained for local reading and the original sent to the Cardiovascular Procedures Core Laboratory at Lenox Hill Hospital for central coding. Specific codes of interest are the presence of LVH and myocardial infarction. A local ECG will be obtained at C12, C36 and C60.

Echocardiography

At baseline (C0) and at years 2 and 4 (C24 and C48) a "limited" echocardiogram will be obtained to measure left ventricular mass. This 2-dimensional-directed, M-Mode echocardiogram will record LV septal thickness, LV posterior wall thickness and LV dimensions (separately, during systole and diastole). The Cardiovascular Procedures Core Laboratory at Lenox Hill Hospital will serve as central reading facility for the study.

Ambulatory BP Monitoring

At baseline (C0) and at years 2 and 4 (C24 and C48), 24-hour ambulatory blood pressure recordings will be obtained. The study will use the SpaceLabs™ 90217 Ultralite or SpaceLabs™ 90207 devices. For each 24-hour recording, measurements will be obtained every 30 minutes through the day and night, from which awake and asleep averages will be calculated, along with other variables including dipping status.

Method of Renal Replacement Therapy (RRT)

For participants who reach ESRD, data will be recorded at each annual visit to verify and update the method of RRT (cadaveric transplant, living related donor, hemodialysis and its frequency, peritoneal dialysis (CCPD, CAPD, other)).

Renal Outcomes: The primary clinical outcome for analyses that include the period of the AASK Cohort Study will be a composite outcome defined by the occurrence of:

Confirmed doubling of serum creatinine (as measured centrally on specimens from 2 visits) or

ESRD (dialysis or transplantation) or

Death

The inclusion of deaths as part of the composite outcome will reduce the risk of informative censoring. In most instances, parallel analyses will be performed in which deaths are not included as part of the composite outcome. For analyses that focus on the outcomes during the trial period when GFRs were collected (Period 1), the composite outcome will be based on a 25 ml/min/1.73m^2 or 50% reduction in GFR from baseline (rather than a doubling of serum creatinine).

For mechanistic analyses of longitudinal change in kidney function, the primary outcome will be the slope of GFR change in which GFR is estimated from the 3-variable AASK prediction equation that includes serum creatinine, sex, and age.

For analyses of proteinuria, the outcomes will include a continuous outcome defined by the urine protein/urine creatinine ratio (UP/Cr) and 2 binary outcomes [a UP/Cr > 0.22 (roughly 300 mg/d of proteinuria) and a UP/Cr > .66 (roughly 1 gm/d of proteinuria)].

Note that confirmation of a doubling of serum creatinine is required. That is, once a follow-up creatinine measurement documents a doubling of creatinine, a repeat creatinine must confirm the initial 'doubling'.

Cardiovascular Outcomes: Clinical cardiovascular outcomes is classified as either 'definite' or 'probable' as defined below. The category of 'total' cardiovascular outcomes is defined as the occurrence of either a 'definite' or 'probable' cardiovascular outcomes. In most analyses, cardiovascular outcomes are grouped together as a composite outcome; however, in some instances, cause-specific cardiovascular events are outcomes of interest.

A ‘definite’ cardiovascular outcome measure will be defined as the occurrence of:

- Cardiovascular death OR
- Cardiac revascularization procedure OR
- Non fatal myocardial infarction (non fatal myocardial infarction is defined as a clinical report of myocardial infarction from the investigator and the presence of one of the following:
  - Elevation of CPK > 2 times the upper limit of normal for the given hospital supported by the elevation of cardiac specific enzyme above the normal range such as MB fraction or cardiac troponin, OR in the absence of cardiac specific enzymes determination of a typical evolutionary pattern defined as an elevation of CPK to 2 times the upper limit of normal for the given hospital followed by a fall of at least 50% or the appearance of new pathological Q-waves in two or more contiguous leads, OR the appearance of a R-wave with R/S ration in lead V1 > 1.0 in the absence of another explanation for these or a loss of progression of R-waves V2 through V5 OR
- Heart failure requiring hospitalization and therapy with either an inotropic agent, vasodilator or ACE inhibitor or required an increase dose of a diuretic or required ultra filtration or dialysis OR
- Permanent neurological deficit of at least 24 hours duration attributed to a stroke and requiring hospitalization and confirmation by radiographic imaging.

A ‘probable’ cardiovascular outcome will be defined by any of the following:

- Non-fatal MI and documentation by a clinical report of myocardial infarction from the investigator but lacking confirmation of elevated enzymes or EKG changes OR
- Permanent neurological deficit of at least 24 hours duration attributed to stroke requiring hospitalization but lacking confirmation by radiographic imaging OR
- A centrally read ECG that documents a new MI in comparison to the baseline ECG OR
- A centrally read wall motion defect on echocardiogram in comparison to the baseline echocardiogram .

The Cardiovascular Outcome Committee will review potential cardiovascular hospitalizations using discharge summary and laboratory reports from these hospitalizations. Clinical centers are required to query patients about hospitalizations. All hospitalizations are reported. If the clinical center determines that cardiovascular event may have occurred during a hospitalization, a

discharge summary will be obtained from that hospitalization and forwarded to the DCC for distribution to the Cardiovascular Outcome Committee for their review.

Each hospitalization that is potentially cardiovascular in nature are reviewed by two members of the Cardiovascular Outcome Committee. If the two members of the Cardiovascular Outcome Committee are in agreement as to whether a cardiovascular outcome has been met, the case will be classified as such. If the two members of the Cardiovascular Outcome Committee are in disagreement as to whether a secondary or tertiary outcome has been met, the case will then come before the full Outcome Committee for review and adjudication.

Section 6. ANALYSIS SECTION

Statistical Analyses: The analysis plans for the AASK Cohort are based on three periods of follow-up:

Period 1: The randomized trial phase, including data from February 1995 through September 30, 2001.

Period 2: The complete data collection period, including both the randomized trial and continued follow-up in the AASK Cohort. This period extends from February 1995 though June 30, 2007.

Period 3: The AASK Cohort period, from February 1, 2001 through June 30, 2007.

Even though many analyses for Period 1 will be carried out in conjunction with the randomized trial, other Period 1 analyses, particularly those based on new measurements obtained in afterthought serum and urine specimens, will be performed by the AASK Cohort investigators. Certain analyses for Periods 2 and 3 will be carried out prior to the end of AASK Cohort data collection in 2007. These will be conducted using the same methods as analyses for the full follow-up periods, but with earlier administrative censoring dates. We first review the main outcomes and summarize the general analytic approach for relating the renal outcomes to baseline factors for the three periods. Subsequently, we outline the specific analyses to be used to relate renal and cardiovascular outcomes to baseline and follow-up factors as appropriate for each of the eight primary research questions specified in Section 2.

Basic Renal Analytic Approach

Period 1

Renal Outcomes: Two primary renal clinical outcomes for Period 1 are defined as the time from randomization to the following composite events:

G1 - First confirmed GFR event (defined by a 25 ml/min/1.73m^2 or 50% reduction in GFR from baseline), renal failure, or death.

G2 - First confirmed GFR event (defined by a 25 ml/min/1.73m^2 or 50% reduction in GFR from baseline), or renal failure.

Outcome (G1) was the main secondary clinical outcome for the randomized trial. This outcome includes death primarily to avoid bias that can result if deaths are censored. Because inclusion of deaths may obscure purely renal effects, the second renal composite outcome (G2) is defined by confirmed GFR events and renal failure alone, censoring deaths. Additional secondary renal clinical outcomes include a composite of a confirmed GFR event, renal failure and cardiovascular deaths (censoring other deaths), and a "hard-endpoint" composite including renal failure and deaths of all causes.

The primary mechanistic renal analysis in Period 1 is based on the mean rate of change in GFR (GFR slope). A key secondary renal outcome is the change in the urine protein/creatinine ratio. This section describes methods for analyses designed to relate renal outcomes to baseline factors while controlling for the randomized study interventions.

Time-to-Event Analyses: The association of baseline factors with the clinical renal outcomes will be evaluated with Cox regression models (Klein 1997) including both the baseline factors of interest and indicator variables for the six cells of the 2x3 factorial trial design to control for the randomized treatment interventions. For patients randomized to the calcium channel blocker arm, a time-dependent indicator variable will be used to distinguish between the periods before and after September 22, 2000 when the calcium channel blocker intervention was terminated. Cox regressions of outcome (G1) will be administratively censored on September 30, 2001 or the date of final loss of contact with the patient; Cox regressions of (G2) will be censored for death, final loss of patient contact, or September 30, 2001.

Analyses of GFR Slope: The association of baseline predictor variables with GFR slope will be analyzed with mixed effects models (Laird 1982) containing fixed effects terms for the predictor variables of interest and their interaction with follow-up time along with additional fixed effects terms to control for differences in the mean rates of GFR decline among the six cells of the 2x3 factorial design. For the four cells within the ACE inhibitor and Beta Blocker arms, the fixed effects terms will express a 2-slope spline model with separate slopes prior to and after 3 months follow-up. For the Calcium Channel Blocker arm, the fixed effects terms will express a 4-slope spline model, with one slope in months 0-3 after randomization, a second slope from month 3 to termination of the Calcium Channel Blocker arm on September 22, 2000, a third slope from September 22, 2000 to January 1, 2001, and a fourth slope thereafter. The third slope will control for a reversal of the acute effect observed in months 0-3. The random effects terms in the mixed

models will include random intercepts, initial slopes (slopes prior to 3 months) and chronic slopes (slopes after 3 months) for each patient.

A potential complication of the slope-based analyses is the risk of informative censoring due to loss-to-follow-up due to death, dialysis, or dropout. If censoring is informative, the standard mixed effects models may give biased estimates of the effects of predictor variables that are associated with early loss-to-follow-up. Therefore, during the initial months of the Cohort Study, the results of the standard mixed effects models will be compared to extensions of these models that account for informative censoring. In particular, the selection model of Schluchter (1992) and DeGrutollo and Tu (1994) provides a natural extension of the standard mixed effects model used in the randomized trial. Alternative pattern mixture models will also be considered (Little 1995). This approach will be used to assess the bias due to informative censoring in the regression coefficients associated with a wide range of predictor variables. If substantial bias is identified for important predictor variables, informative censoring models will be routinely used in place of the standard mixed effects models.

Period 2

Renal Outcomes: The clinical and mechanistic outcomes for Period 2 (with up to 12 years of follow-up) will be analogous to those for Period 1 (with up to 6 years of follow-up), but will be based on serum creatinine rather than GFR. The primary renal clinical outcomes for Period 2 are defined as the time from randomization to the following composite events:

S1 - First doubling of serum creatinine from baseline, renal failure, or death.

S2 - First doubling of serum creatinine from baseline or renal failure.

Other key secondary renal clinical outcomes include the first doubling of serum creatinine, renal failure, or cardiovascular death, and the combined hard endpoints of renal failure or death.

The primary mechanistic renal analysis in Period 2 is based on the rate of change in predicted GFR using the following regression model derived from AASK enrollees at baseline (Lewis, 2001):

$$\text{Predicted GFR} = 329 \times (\text{Pcr})^{-1.096} \times (\text{age})^{-0.294} \times (0.736 \text{ for women}).$$

The predicted GFR from this equation is approximately proportional to the inverse of the serum creatinine, with calibration for age and gender. In general, Period 2 analyses will be based on the data of all 1,094 randomized patients, regardless of whether they provided data during the AASK Cohort.

Time-to-Event Analyses: The association of baseline factors with the clinical renal outcomes will be analyzed in Period 2 using Cox regression models similar to those used for Period 1, but with modified definitions of the terms used to control for the randomized treatment groups. Specifically, the Cox models will include indicator variables defining the patient's randomized group, as well as separate time-dependent indicator variables specifying the time periods that the patient was actually assigned to the randomized intervention (randomization to September 22, 2000 for the Calcium Channel Blocker group, and randomization to September 30, 2001 for the ACE inhibitor and Beta Blocker groups). Cox regressions of the first composite (G1) will be administratively censored at the end of The AASK Cohort on June 30, 2007 or at the date of loss of contact with the patient; analyses of (G2) will be censored at death, June 30, 2007, or loss of patient contact.

Analyses of Predicted GFR Slope: Similarly to Period 1, the association of baseline factors with predicted GFR slope in Period 2 will be investigated with mixed effects models including terms for the baseline factors of interest and their interaction with follow-up time plus additional terms to control for the randomized treatments. Multi-slope linear spline models will be used to account for different mean rates of change over different periods in the respective randomized treatment groups. The time-points for the changes in mean slope under these models will be determined after examination of the data, but can be expected to include changes in slope at the same time points as the Period 1 mixed effects models. Due to the long follow-up period several alternatives for the random effects component of the mixed models will be considered, including models which allow for higher correlations between measurements spaced closer together than for measurements further apart.

Period 3

Renal Outcomes: The outcomes of Period 3 will be defined in terms of creatinine similarly to Period 2, but time 0 will be the time of the initial AASK Cohort protocol visit. The baseline serum creatinine for Period 3 analyses will be taken as the serum creatinine measured at this visit. Period 3 analyses will be restricted to patients who attended the initial AASK Cohort

protocol visit. Data from the randomized trial may be used in the definition of baseline factors for Phase 3 analyses, including assessments of the rate of disease progression such as GFR slope or change in proteinuria. Adjustment for prior progression rates should substantially increase the power of Period 3 analyses.

Analyses of Clinical Renal Events: Cox regressions similar to those used for Phase 1 and 2 will be used to relate the clinical renal event outcomes to baseline factors. Even though patients will no longer be on their randomized interventions, indicator variables defining the original randomized groups will be used to control for any carryover effects of the randomized treatments. Period 3 analyses of the composite (G1) will be administratively censored on June 30, 2007 or at final loss of contact with the patient; analyses of (G2) will be censored at death, June 30, 2007, or loss of patient contact.

Analyses of Predicted GFR Slope: Analyses of predicted GFR slope in Phase 3 will be conducted with mixed effects models similar to those of Phases 1 and 2, with terms for the baseline factors and their interaction with follow-up time plus additional terms to account for the patients previous randomized group during the trial.

Analysis Plans for Specific Research Questions Listed in Section 2 of this Protocol

Research Question 1. What is the long-term course of kidney function in this population?

The long-term course of kidney function will be characterized in Period 3 by investigating a) the rates of the composite outcomes (S1) and (S2) and other secondary renal event endpoints as a function of follow-up time, b) the distributions of long-term average rates of predicted GFR decline among patients belonging to relevant subgroups, and c) the pattern of change in predicted GFR over time within individual patients.

For (a), cumulative incidence curves will be constructed for each renal clinical outcome variable to characterize the proportions of AASK patients reaching renal events at different follow-up times. In addition to considering the full study group as a whole, Cox-regression techniques will be used to estimate rates of renal events as a function of follow-up time for patients with specific combinations of baseline characteristics. For (b), "smooth" density function estimates will be obtained for the distribution of long-term mean GFR slopes (after controlling for the randomized

groups as described in the Basic Renal Analytic Approach) for the full cohort and for relevant patient subgroups. This will allow evaluation of many different aspects of the distributions of long-term rates of decline. Of particular interest are the proportions of patients who are nonprogressors as defined by a long-term average GFR slope greater than –1 ml/min per $1.73m^2$/yr (corresponding to normal aging), or who are rapid progressors, defined by an average GFR slope less than –4 ml/min per $1.73m^2$/yr, say. To address (c), parametric and nonparametric extensions of the basic linear mixed effects model will be used to determine whether individual patient's long-term GFR declines follow constant linear paths or more complex nonlinear trajectories with different slopes during different periods of time.

Research Question 2. What are the environmental, genetic, physiologic, and socio-economic factors which predict the progression of kidney disease?

Research Question 2 will be addressed with analyses for each of the Periods 1, 2 and 3. For the renal clinical event outcomes, unadjusted risk ratios for baseline factors will first be obtained using Cox models with the baseline factors and indicators for the randomized groups (see Basic Renal Analytic Approach) as predictor variables. Subsequently, adjusted risk ratios will be obtained by adding other baseline factors (such as clinical center, demographic factors, or previously identified predictors of renal progression) to the models. Nonlinear relationships between a factor and risk of the clinical outcomes will be evaluated using splines or step functions (Therneau 2000). The possibility that a factor may have different risk ratios at different times (nonproportional hazards) will be investigated with residual plots and by interaction terms with follow-up time (Therneau 2000B). Interactions of baseline predictor variables with each other and with the treatment groups will be investigated by adding interaction terms to the Cox models. In particular, it will be of interest to test whether the association of renal events with baseline factors is modified by the initial level of GFR and/or proteinuria.

The association of follow-up factors with renal events will be investigated with Cox models that include time-dependent terms for the follow-up factors plus additional terms to adjust for the treatment groups and relevant baseline factors (see Basic Renal Analytic Approach). Depending on the application, the hazard rate at a particular time may be modeled as a function of

- The cumulative mean of the factor being investigated (averaging all measurements from baseline to the current time),

- The most recently observed value of the predictor variable,
- An earlier (lagged) value of the predictor variable.

As for the renal events, the association of GFR decline (or predicted GFR decline) with specific baseline factors will be evaluated first with adjustment only for the randomized treatment groups, and then with adjustment also for relevant baseline factors. The adjustment for baseline factors will usually be accomplished by adding these factors and their interactions with follow-up time to the mixed effects models.

Several strategies will be used to relate the decline in GFR (or predicted GFR) to factors measured during follow-up. For simplicity, in many cases all follow-up measurements of a factor will be averaged to obtain a single mean value for each patient that can be entered in mixed effects models along with its interaction with follow-up time. Weighted averages may be used to account for the spacing between successive measurements. This type of model evaluates the cross-sectional association between the follow-up factor and GFR decline, and does not account for the temporal order of the measurements. Alternative models will also be considered in which the mean rate of GFR (or predicted GFR) decline between months t and t + 6 is related to the cumulative mean of the predictor variable up to month t or to the most recent measurement of the predictor variable prior to time t. Further terms will be added to distinguish between short-term acute effects and long term effects for factors whose changes are expected to cause hemodynamic changes in GFR.

Comparisons of Change in GFR to Change in Serum Creatinine

The analysis plans for Research Questions 2 and 3 depend on the validity of using serum creatinine in place of GFR in the definition of the renal outcomes. Therefore, during the initial months of the AASK Cohort Study, the data from the clinical trial phase of the study will be used to clarify the relationship between GFR and creatinine-based outcomes. The timing of GFR-based outcomes (G1 and G2) will be compared to creatinine-based outcomes (S1) and S2). Joint mixed effect models including both the change in GFR and the change in the AASK-predicted GFR from serum creatinine will be used to evaluate the association of changes in GFR with changes in predicted GFR. The model of Schluchter will be used to compare the correlation of GFR slope with time-to-renal failure or death to the correlation of predicted GFR slope with time-to-renal failure or death. This will provide insight into the validity of GFR slope and

predicted GFR slope as surrogates for the hard endpoints of renal failure or death. The possibility that certain factors may have different relationships with predicted GFR slope than with actual GFR slope will be investigated using longitudinal mixed models relating the factors the difference between predicted and actual GFR.

Research Question 3. What are the long-term effects of the AASK trial interventions on the progression of kidney disease?

As described in the General Renal Analytic Approach, long-term effects of the AASK interventions on the renal clinical outcomes will be tested by time-dependent indicator variables for the randomized treatment groups in the Cox models which distinguish between the effects of the interventions during the randomized trial and the long-term effects following the end of the trial. The long-term effects of the interventions on predicted GFR slope will be evaluated by adding linear spline terms to the mixed effects models to allow comparison of mean predicted GFR slopes between the original randomized groups after the termination of the trial.

Research Question 4. Does the development of proteinuria predict the progression of kidney disease?

As an outcome variable, change in proteinuria will be analyzed using methods similar to those used for change in GFR. Proteinuria will be expressed as a urine protein/creatinine ratio as in the randomized trial, and log transformed due to positive skewness. Since the mean changes in the log urine protein/creatinine ratio are nonlinear, the mixed effects models will include spline terms allowing different slopes over each 6- or 12-month interval between scheduled urine protein/creatinine measurements.

The effect of proteinuria on subsequent renal clinical events will be evaluated first by Cox models relating baseline proteinuria to each of the renal outcomes during the follow-up period of the analysis (either Period 1, Period 2, or Period 3). Subsequently, models examining the association of the change in proteinuria during the first 6 months (or 12 months for Period 3 analyses) of the period of analysis will be related to rates of the clinical events after the first 6 (or 12) months. As for other risk factors, these models will control for the randomized treatment groups, and will be carried out both with and without adjustment for other potential risk factors. Due to interactions between proteinuria and the randomized treatment comparisons identified in

the randomized trial, relevant interaction terms between proteinuria and the treatment groups will be considered. A similar approach will be used to relate baseline proteinuria and initial changes in proteinuria to subsequent GFR slope (Period 1) and to predicted GFR slope (Periods 2 and 3). Further longitudinal analyses, with proteinuria modeled as a time-dependent covariate, will be used to investigate whether GFR slope changes over time for individual patients following increases in proteinuria.

Research Question 5: What is the impact of recommended blood pressure therapy, as determined by the AASK trial, on the progression of kidney disease in comparison to usual care in the community?

Rates of renal clinical events and predicted GFR slope will be compared during Period 3 between the AASK cohort participants and African Americans with hypertensive kidney disease enrolled in the Chronic Renal Insufficiency Cohort Study. Relevant baseline factors will be included as covariates to control for initial differences between the two groups. We expect that these comparisons will be carried out using Cox models and mixed effects models similar to those described above, but the specific analyses will developed jointly by the AASK Cohort and the Chronic Renal Insufficiency Cohort investigators.

Research Question 6: What comorbidities, particularly cardiovascular disease, occur in the setting of hypertension-related kidney disease?

Incidence of cardiovascular events will be recorded, and expressed per-patient year of follow-up.

Research Question 7: What risk factors predict the occurrence of cardiovascular disease?

Cox regression models analogous to those employed for the renal outcomes will be used in Periods 1, 2, and 3 to relate baseline and follow-up predictor variables to the incidence of various classes cardiovascular events defined by the Outcome Committee. Analyses will typically be structured to determine the additional predictive values of non-traditional cardiovascular risk factors (e.g., homocysteine and CRP) beyond those of traditional cardiovascular risk factors (smoking, diabetes, blood pressure, LDL and HDL). Left ventricular mass will be evaluated both as a risk factor and as an outcome variable for Period 3 analyses. When treated as an outcome variable, mixed effects models will be used to relate the slope of left ventricular mass

vs. time to potential predictor variables.

Research Question 8: What are the patterns of change in metabolic variables and cardiovascular-renal risk factors during the transition from pre-ESRD to ESRD?

Standard techniques will first be used to evaluate changes in quantitative and categorical factors between their final pre-ESRD measurement and post-ESRD assessments for those patients who initiate dialysis during the cohort study. Assessment of these changes between the last pre-ESRD measurement and post-ESRD measurements is complicated by the possibility that the parameters of interest may change after the last pre-ESRD measurement but prior to the transition to ESRD. Thus, we will also consider longitudinal analyses relating the metabolic and cardiovascular-renal risk factors to follow-up time, with initiation of ESRD entered as a time dependent covariate. In this way, the effect of initiation of dialysis can be evaluated after controlling for the rate of change in the factors being analyzed prior to ESRD.

Projected Follow-up and Power

Methods of Projecting Numbers of Future Events: The statistical power for the main time-to-event analyses in Periods 1, 2, and 3 depends on the numbers of events for the respective composite outcomes during these periods.

For Period 1 (the AASK trial through September 30, 2001), the number of events were calculated based on the actual reported numbers of events during the trial.

For Period 3 (the AASK Cohort period from February 1, 2001 though June 30, 2007), the numbers of events were estimated using a 2-stop process. In the first step, Weibul models were used to estimate event probabilities for each composite outcome as a function of the initial pre-randomization baseline urine protein/creatinine and baseline GFR for AASK patients in the ACE and Beta Blocker Groups. These models were then applied to the most recent urine protein/creatinine and GFR values to project future event rates for likely AASK Cohort participants in all three treatment groups. Likely AASK Cohort participants were identified as those patients who were alive and had not reached renal failure as of September 14, 2001 and who Clinical Centers indicated were not lost to follow-up and were likely to agree to participate in the AASK Cohort. An additional 3% loss-to-follow-up was assumed for each outcome.

Projection of future events for the Period 2 analyses had to account the definition of the creatinine-based events from a doubling from the pre-randomization baseline creatinine rather than from a new creatinine at the beginning of The AASK Cohort. To deal with this complication, the 2-step process used for the Period 3 power analyses was modified as follows for the composite outcomes which including a creatinine doubling component. In the first step, separate Weibul models were developed for the composite outcomes with creatinine events defined by increases from baseline by factors of either 1.333, 1.667, 2.0, 2.5, 3.0, or 4.0. In the second step, the likely AASK Cohort participants who had not previously had a doubling of serum creatinine were stratified into six groups depending on their most recent serum creatinine value. Then, the appropriate Weibul model from the first step was used to project the number of patients in each strata whose latest creatinine would increase during the AASK Cohort period to a value equal to at least two time the patients pre-randomization baseline creatinine or who would reach one of the other events defining the composite outcomes. An additional 3% loss-to-follow-up was assumed for each outcome during the AASK Cohort period.

Power Calculations

Based on the responses of AASK centers to a questionnaire on enrollment of patients for AASK Cohort, it is projected that 677 of the initial 1094 randomized patients will be alive and not on dialysis and will agree to participate in the AASK Cohort Study. Table 7 gives the projected numbers of events for each composite outcome, as the associated projected minimum detectable treatment effects (with 80% or 90% power based on level 0.05 2-sided tests) for increases in risk associated with 1) a dichotomous risk factor with 50% prevalence in the AASK patients, 2) a dichotomous risk factor with 20% prevalence in the AASK patients, and 3) a 1-standard deviation change in a quantitative risk factor which is linearly related to the log-transformed relative risk. The power calculations correspond to unadjusted risk ratios, do not account for potential use of covariates which might be correlated with the risk factors in multivariate models.

To illustrate the power calculations, consider analyses done in Period 3 comparing non-dippers to dippers based on ambulatory blood pressure monitoring. Assuming (conservatively) that 20% of AASK patients are non-dippers, the AASK Cohort will have 80% power to detect a 62% increase in the rate of composite endpoint of doubling of serum creatinine, ESRD, or death for non-dippers compared to dippers. The study will have 90% power to detect a 75% increase in

the event rate. If the proportion of non-dippers turns out to be 50%, the AASK cohort will have 80% and 90% power to detect 47% and 56% increases in event rates for this composite outcome. As a second example, consider an analysis relating serum total cholesterol to the composite endpoint of doubling of serum creatinine, ESRD, or death over the full Period 3. The standard deviation of total cholesterol at baseline in the randomized trial was 45 mg/dL. The Period 3 analysis would have 80% and 90% power to detect 21% and 25% increases, respectively, in the event rate corresponding to a difference of 45 mg/dL in total cholesterol.

Table 7: Minimum Detectable Increases in Relative Risk of Primary and Key Secondary Events for AASK and After-AASK Time-to-Event Analyses Based on ESRD, Serum Creatinine, and Death

Phase	Analysis	Number Of Events	Minimum Detectable Effect Sizes					
			80% Power			90% Power		
			Risk Factor With 50% Prevalence	Risk Factor With 20% Prevalence	1 SD Δ in Quantitative Variable	Risk Factor With 50% Prevalence	Risk Factor With 20% Prevalence	1 SD Δ in Quantitative Variable
Period 1 AASK Trial Only	ESRD, GFR Evt or death	340	35%	46%	16%	42%	55%	19%
	ESRD, GFR Evt	263	41%	54%	19%	49%	64%	22%
	ESRD or death	249	43%	56%	19%	51%	67%	22%
Period 2 AASK + After AASK	ESRD, Scr Evt or death	530	28%	36%	13%	33%	42%	15%
	ESRD, Scr Evt	413	32%	41%	15%	38%	49%	17%
	ESRD or death	452	30%	39%	14%	36%	46%	16%
Period 3 After AASK Only	ESRD, Scr Evt or death	210	47%	62%	21%	56%	75%	25%
	ESRD, Scr Evt	173	53%	70%	24%	64%	85%	28%
	ESRD or death	176	53%	69%	23%	63%	84%	28%

Assumptions: Phase 1 extends from 1994 through Sep 30, 2001. After-AASK extends from February 1, 2002 through June 30, 2007. Of 1,094 originally randomized patients for the AASK trial, 263 were lost to death or dialysis by September 30, 2001. The AASK centers indicate that an additional 154 patients are lost to follow-up or unlikely to consent to After-AASK, leaving 677 active patients on September 30, 2001. For Phase 3, the numbers of events during After-AASK were estimated by first fitting Weibul models to estimate event probabilities for each outcome as a function of baseline urine protein/creatinine and baseline GFR for patients in the ACE and Beta Blocker Groups. These models were then applied to the most recent urine

protein/creatinine and GFR values to project future event rates for patients in all three treatment groups. Extensions of these models were used to account for the differences between the patient's most recent serum creatinine value and their initial baseline serum creatinine when evaluating rates of doubling of serum creatinine from the original pre-randomization baseline serum creatinine for Phase 2 analyses. A 3% annual loss-to-follow-up was assumed for the period from October 1, 2001 though June 30, 2007.

Quality Assurance and Quality Control

Quality assurance focuses on steps taken prior to data collection to assure accuracy and minimize errors. Quality control focuses on steps taken after data are collected to examine quality, measure reproducibility and identify errors. Due to finite resources, quality assurance and control efforts must be concentrated on key procedures and measurements, typically, those related to the primary outcomes and exposures of interest.

Quality Assurance

Primary steps to assure high quality data are recruiting and retaining highly motivated staff, training (and retraining) of data collectors, and observing staff during mock or actual visits. Local monitoring is vitally important and can identify and correct problems weeks or months before such problems become apparent on quality control reports. Specific activities that lead to high quality data collection and that standardize procedures within and across field centers include 1) preparation of a well-documented protocol and a manual of operations, 2) centralized training and certification of technicians, 3) proficiency requirements before initial certification of technicians, 4) requirements for a minimum number of procedures to maintain certification, 5) routine observation of technicians, and 6) routine calibration of equipment.

Quality Control

The principal goals of quality control in a multi-center study such as the AASK Cohort study are to identify problems in data collection with sufficient time to institute appropriate corrections

and to quantify the quality of data collected over the course of the study so as to provide information necessary to interpret study results. To accomplish the first goal, adequate data must be accumulated to enable valid analyses to be performed within a brief period after initiation of data collection. To accomplish the second goal, sufficient data must be compiled throughout the study to detect any drift or deterioration in data quality over time.

For quality control pertaining to field center operations, the coordinating center can provide considerable information from simple analyses. Bimonthly reports of such analyses should include 1) counts of completed tests, 2) distributions of test results by technician and center over time, 3) displays of reproducibility by technician and center, 4) counts of clinical events and of medical record retrieval, 5) distributions of lag time in data entry, 6) distribution of data entry errors, and 7) response time to queries from the coordinating center. Investigators and staff will review these reports carefully to identify areas of concern and take corrective action, if appropriate.

Section 7. ORGANIZATION AND ADMINISTRATION

Overview

The AASK Cohort will include 21 clinical centers, a coordinating center and several central laboratories. In addition to these centers, there will be several committees and subcommittees, comprised of AASK cohort investigators and staff, to provide guidance and oversight of the study. To minimize confusion and to take advantage of the existing infrastructure of AASK, the organizational structure will be similar to that of the AASK trial.

The following is a list of participating centers:

- The 21 AASK Clinical Centers from the AASK Trial - University of California at San Diego, Case Western Reserve University, Emory University, University of California at Los Angeles - Harbor Hospital, Howard University, Johns Hopkins University School of Public Health, Martin Luther King Medical Center, Medical University of South Carolina, Meharry Medical College, Harlem Hospital, Morehouse Medical College, Lenox Hill Hospital, Ohio State University, Rush Medical Center, University of Alabama, University of Florida, University of Miami, University of Michigan, University of Southern California, University of Texas - Southwest and Vanderbilt University. These centers will be responsible for retention of participants and collection of data.

- The AASK Coordinating Center at the Cleveland Clinic. This center will be responsible for coordinating all activities of the study, including subcommittee and committee activities; preparation of the Manual of Operations; data collection and management procedures; data analyses; and preparation of study reports for the EAC, Steering Committee and NIDDK.

- The National Institute of Diabetes, Digestive and Kidney Diseases of the National Institutes of Health. The NIDDK will provide scientific, administrative and fiscal input into the conduct of the study.

- A Central Laboratory at the Cleveland Clinic. This unit will receive all specimens, perform core laboratory analyses, and store all archival specimens.

- A Genetics Core Laboratory at Mount Sinai Hospital (NYC).

- A Cardiovascular Procedures Core Laboratory at Lenox Hill Hospital.

- Principal Investigators, Co-Investigators, Fellows, and Senior Staff from these centers will be eligible to present study data, participate in writing groups, and prepare manuscripts. In addition to the above centers, those pharmaceutical companies that provide substantive financial support for the AASK Cohort Study can attend Steering Committee meetings and can provide input on the study protocol and its amendments. These companies are not voting members of the Steering Committee.

Committees and Subcommittees

The following are standing committees and subcommittees of the study. At the request of the Steering or Executive Committee, ad hoc groups will be formed to address specific, time-limited issues.

External Advisory Committee (EAC) - The EAC will advise the NIDDK and the AASK Cohort Steering Committee on matters related to the design and conduct of this study. During the study, the committee will serve as a Data Safety and Monitoring Board (DSMB) and will monitor data accrual, data analyses and participant safety.

Steering Committee - The Steering Committee is the governing body of the study. It will approve the protocol and all major amendments; will provide scientific guidance; will delegate responsibilities to the Executive Committee and Subcommittees; will review Subcommittee recommendations; and will resolve any disputes, such as authorship. Its voting members will include the Principal Investigators of each clinical center, the Principal Investigator of the Coordinating Center, and the Project Officer from NIDDK. The Steering Committee will meet

at least annually and will vote on interim protocol changes, typically by FAX or email.

Executive Committee - The Executive Committee will act on behalf of the Steering Committee to oversee conduct of the study between Steering Committee meetings. Its members will include the Chair of study, Vice-Chair of the study, the NIH Project Officer, the DCC PI, and Chairs of each Standing Subcommittee. The Executive Committee will meet regularly by phone, typically monthly, to monitor conduct of the study, deliberate on major scientific and operations issues, and consider those items that might require Steering Committee approval. It will review all proposed publications, presentations and new substudies that have been initially reviewed by Publications and Ancillary Studies Committee.

Measurements/Quality Control Subcommittee - This subcommittee will recommend to the Steering Committee measures, processes, and procedures for assuring quality control of the study, including training, certification, quality control measures and procedures, and other activities directed at ensuring that data are valid and reliable.

Blood Pressure Management Subcommittee - This subcommittee will recommend to the Steering Committee the blood pressure goal; the approach toward use of antihypertensive medications; and adherence measurements in the cohort study. During the Cohort, it will track blood pressure management at the centers.

Cardiovascular Outcomes Endpoint Subcommittee - This subcommittee will recommend to the Steering Committee the procedures for measurement of relevant cardiovascular variables including, but not limited to, echocardiography, ambulatory blood pressure monitoring and electrocardiography. It will be responsible for developing the Manual of Operations chapters related to these outcomes.

Publications/Ancillary Studies Subcommittee - This subcommittee will develop presentation and publications procedures; will assemble writing groups; will review new paper proposals; will monitor publication progress; will advise the Executive and Steering Committees on all matters related to publication and presentation of study results; and will review ancillary studies. This

subcommittee will also serve as an ancillary studies subcommittee and will recommend to the Steering Committee the basic design components of the study and recommend changes in and additions to the protocol.

Genetics Subcommittee - This subcommittee will propose genetic analyses to be done in the cohort, will track the accrual of specimens, and will evaluate new proposals related to family and/or genetic substudies.

Study Coordinators/Retention Subcommittee - This subcommittee, comprised of the Study Coordinators from each Clinical Center, will develop procedures to enhance retention of participants; will monitor the work load of staff; and will evaluate the impact of proposed data collection procedures.

Protocol Changes and Amendments

As a clinical trial, the AASK trial appropriately discouraged protocol changes. In contrast, the AASK cohort study is an observational study in which additional data collection, either new exposures or outcomes, are encouraged. These proposals should be proposed as substudies (to be done by all clinical centers) or ancillary studies (to be done by a few centers). Still, new data collection must be justified in terms of scientific importance, participant burden, and staff burden; fiscal implications will also be a major consideration. To facilitate review, investigators that propose substudies or ancillary studies must provide a synopsis of their proposal, typically 3-5 pages, that provides the hypothesis, background and methods, as well as a detailed description of the data collection procedure, frequency of collection, power analysis, participant burden, staff burden and budget, if appropriate.

Approval of new data collection items will require two or three steps, depending on the type of data collection proposed. First step: initially, the Publications and Ancillary Studies Subcommittee will review all proposals which request either collection of new data items or new analyses of stored specimens. Second step: if the PASC approves the study, the Executive Committee must then approve the request. Simple majorities of the PASC and the Executive

Committee are required for the first and second steps. Third step (contingent upon the type of data collection): if the additional data collection substantially increases participant burden OR substantially increases staff burden OR substantially increases costs OR obtains sensitive information, the entire Steering Committee must approve the new data collection item. In this case, the vote will require a 3/4 majority of the Steering Committee.

Section 8. REFERENCES

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