Polymorphisms in the Nonmuscle Myosin Heavy Chain 9 Gene (MYH9) Are Associated with Albuminuria in Hypertensive African Americans: The HyperGEN Study

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Abstract

Background: MYH9 is a podocyte-expressed gene encoding nonmuscle myosin IIA that is associated with idiopathic and human immunodeficiency virus-associated focal segmental glomerulosclerosis (FSGS) and hypertensive end-stage renal disease in African Americans. **Methods:** Four single nucleotide polymorphisms comprising the major MYH9 E1 risk haplotype were tested for association with estimated glomerular filtration rate (eGFR) and urine albumin:creatinine ratio (ACR) in 2,903 HyperGEN participants (1,458 African Americans (AA) in 895 families and 1,445 European Americans (EA) in 859 families) to determine the role of MYH9 in subclinical nephropathy. Association analyses employed general linear models in unrelated probands and generalized estimating equations in families. Adjustment was performed for age, sex, diabetes, BMI, medications, and mean arterial pressure separately in each race. **Results:** Mean (SD) eGFR and ACR were 74.3 (16.0) ml/min/1.73 m² and 20.3 (119.9) mg/g in EA, and 88.6 (20.9) ml/min/1.73 m² and 76.8 (394.5) mg/g in AA (both p < 0.0001 across ethnicities). Urine ACR was associated with rs3752462 (p = 0.01) and rs4821481 (p = 0.05) in unrelated AA and with rs4821481 (p = 0.03), rs2032487 (p = 0.04) and the E1 3224 haplotype (p = 0.013) in AA families. Single nucleotide polymorphisms and the haplotype were not associated with ACR in EA or with eGFR in either ethnic group. **Conclusions:** MYH9 variants are associated with albuminuria in hypertensive AA. The strength of the association was weaker than that in FSGS and hypertensive end-stage renal disease. MYH9 risk variants appear to be associated with primary FSGS with secondary hypertension, although nephrosclerosis may develop in response to hypertension in subjects homozygous for the MYH9 E1 risk haplotype.

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Names of primary centers and investigators of HyperGEN are given in the appendix.

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Introduction

High blood pressure is strongly associated with the progression of chronic kidney disease (CKD) to end-stage renal failure. However, the role of systemic hypertension in the initiation of arteriolar or hypertensive nephrosclerosis remains controversial [1]. African Americans (AA) are diagnosed with hypertension-associated end-stage renal disease (H-ESRD) far more often than European Americans (EA) [2]. Many AA labeled with H-ESRD present to nephrologists late in their course, at a time when it is difficult to determine whether a primary kidney disease initiated the renal failure [3]. Unfortunately, few of these individuals receive kidney biopsies. Many nephrologists code the diagnosis 'hypertensive-ESRD' in nondiabetic AA subjects with unknown cause for CKD, since long-standing secondary hypertension is nearly universally present [4]. Although cross-sectional studies reveal graded relationships between severity of hypertension and CKD, it is not possible to tell which syndrome was causative [5].

Lack of access to adequate healthcare and lower socioeconomic status are associated with risk for nephropathy in sporadic cases of ESRD [6]. In contrast, analysis of geocoded data in incident dialysis patients demonstrated that median family income and level of education were not associated with familially clustered ESRD, suggesting a role for non-socioeconomic status factors, possibly inherited [7]. More than 30% of AA and 12–15% of EA with common, complex forms of ESRD have first and/or second degree relatives with ESRD and many more have relatives with silent or unrecognized nephropathy [8].

The recent identification of *MYH9* gene associations in idiopathic and human immunodeficiency virus (HIV)-associated focal and segmental glomerulosclerosis (FSGS) and H-ESRD in AA has been a major breakthrough in our understanding of these etiologies of nephropathy [9, 10]. The *MYH9* gene association had an odds ratio (OR) of 4–5 in AA with FSGS and HIV-associated nephropathy (HIVAN), and ORs of 1.5–3.4 in H-ESRD [25]. In addition, replacing the *MYH9* risk haplotype with a protective haplotype would reduce nondiabetic ESRD by 70% in AA. The *MYH9* association may account for the failure of antihypertensive therapy, including the use of ACE inhibitors, to substantially slow progression of 'hypertension-associated kidney disease' in AA [11–13] and the clustering of different etiologies of ESRD in single AA families (including FSGS, HIVAN and H-ESRD) [14]. Since the risk haplotype is observed in 60% of AA and 4% of EA, polymorphisms in this gene could account for a portion of the ethnic disparity in nondiabetic forms of H-ESRD, HIVAN and FSGS [10].

To date, the *MYH9* gene has not been tested for association with markers of nephropathy in hypertensive subjects. We evaluated 4 single nucleotide polymorphisms (SNPs) comprising the major *MYH9* risk haplotype (the E1 haplotype) for association with subclinical nephropathy in HyperGEN study participants.

Materials and Methods

Study Population

HyperGEN study methods and participants have previously been reported [15]. In brief, HyperGEN is a multicenter cross-sectional study charged with identifying and characterizing genes that contribute to hypertension and related phenotypes as part of the National Heart Lung and Blood Institute-sponsored Family Blood Pressure Program (FBPP). HyperGEN consists of five field centers: Framingham (Mass.), Minneapolis (Minn.), Salt Lake City (Utah), Forsyth County (N.C.) and Birmingham (Ala.). Study variables assessed included blood pressures, serum and urine chemistries, anthropometrics, echocardiographic measurements, and basic demographic information including questions about family and personal history of hypertension, medication, diet, physical activity, and lifestyle. DNA extraction was performed.

We analyzed data in all EA and AA HyperGEN participants recruited from hypertensive sibships (including mild and severe hypertension) selected from families with two or more hypertensive siblings diagnosed before 60 years of age, as well as their adult offspring (offspring were not necessarily hypertensive or taking antihypertensive medications). Mild hypertension was defined as a systolic blood pressure (SBP) between 140 and 160 mm Hg or diastolic blood pressure (DBP) between 90 and 100 mm Hg, or taking one antihypertensive medication. Severe hypertension was defined as having an SBP greater than 160 or DBP greater than 100 mm Hg, or using two or more antihypertensive medications. Renal replacement therapy was an exclusion criterion.

Phenotypes

The urine albumin:creatinine ratio (ACR) was measured from an overnight urine collection. The distribution of urine ACR was highly skewed, so a logarithm-transformed variable was used for the association analyses. Estimated glomerular filtration rate (eGFR) was computed using the abbreviated MDRD equation, and creatinine clearance calculated from timed urine collections [16].

Statistical Analyses

Adjustment was performed for age, sex, diabetes, BMI, blood pressure medications, and mean arterial blood pressure, separately in each ethnic group. General linear models were used to test for association when analyzing one subject from each family and generalized estimating equations were used for analyses of all subjects to account for familial relationships. Haplotypes were estimated from PHASE separately for AA and EA [17]. Spacing of the four SNPs was taken from the NCBI website using the base pair starting position of each SNP. Because the study by Kopp et al. [10] identified the at-risk haplotype (3224 or GCCT for

Table 1. Demographic characteristics of the HyperGEN study population

Variable	AA				EA				p value (AA vs. EA)
	n	mean	median	SD	n	mean	median	SD	
Age, years	1,485	50.8	50.0	11.1	1,536	61.1	62.0	8.7	<0.0001
SBP, mm Hg	1,467	132.0	130.0	22.4	1,463	125.4	123.0	20.6	<0.0001
DBP, mm Hg	1,467	75.2	74.5	11.8	1,463	68.4	67.5	10.9	<0.0001
Mean arterial pressure, mm Hg	1,467	94.1	92.5	14.1	1,463	87.4	86.5	12.7	<0.0002
Serum creatinine, mg/dl	1,485	1.0	1.0	0.4	1,536	1.0	0.9	0.3	0.1
Body mass index	1,483	32.4	31.2	7.6	1,534	29.8	29.0	6.0	<0.0001
eGFR, ml/min	1,485	88.6	87.9	20.9	1,536	74.3	73.3	16.0	<0.0002
Urine ACR, mg/g	1,458	76.8	6.0	394.5	1,445	20.3	4.6	119.9	<0.0001
Log urine ACR	1,458	2.3	1.8	1.5	1,445	1.7	1.5	1.1	<0.0001
Cr Cl, ml/min/1.73 m^2	1,226	93.4	95.1	30.0	1,408	88.0	83.4	23.7	<0.0002

Cr Cl = Creatinine clearance; SD = standard deviation.
p values computed from the Wilcoxon two-sample test.

rs4821480, rs2032487, rs4821481, and rs3752462, respectively), this haplotype was tested against all other haplotypes combined. We put special emphasis on the recessive model, previously shown to be the most likely mode of inheritance model for this haplotype. In the original analysis of 17 *MYH9* SNPs and their haplotypes, the E1 haplotype was observed to have the strongest association [10]. This was confirmed by a sliding window analysis. More than 100 SNPs in this region of *MYH9* have now been genotyped, including virtually all haplotype tagging SNPs in the associated region. There is high linkage disequilibrium between associated SNPs and a causal SNP has not yet been identified, possibly due to the high degree of linkage disequilibrium. Although a few of these new SNPs demonstrated increased significance with somewhat lower p values compared to the 4 SNPs in the E1 haplotype, these new SNPs fell within the E1 haplotype block and their ORs and 95% CIs were nearly coincident. Statistically, it would be extremely difficult to separate these effects. The main association effect appears to come from the E1 haplotype in both EA and AA, as the OR for the E1 haplotype association is 7.7 in EA with idiopathic FSGS and 4.7 in AA [10]. This demonstrates that the E1 haplotype is powerfully associated with kidney disease in both ethnic groups. Genotyping additional SNPs in this region would not be likely to provide significant additional information.

Genotyping

The 4 SNPs evaluated were rs4821480, rs2032487, rs4821481 and rs3752462. SNP genotyping was performed on a TaqMan machine (Applied Biosystems, Foster City, Calif., USA) using standard methods.

Results

The study population consisted of 2,903 HyperGEN participants, 1,458 AA from 895 families and 1,445 EA from 859 families. Clinical and demographic character-

istics of the study population are presented in table 1. Although mean (SD) arterial blood pressures were fairly well controlled, they were lower in the EA (87.4 ± 12.7 mm Hg) than the AA participants (94.1 ± 14.1 mm Hg; p < 0.0001). Mean (SD) eGFR and ACR, respectively, were 74.3 (16.0) ml/min/1.73 m^2 and 20.3 (119.9) mg/g in EA subjects, and 88.6 (20.9) ml/min/1.73 m^2 and 76.8 (394.5) mg/g in AA subjects (p < 0.0001 for both ACR and GFR across race groups). Diabetes was present in 260 EA and 354 AA.

The results of the single SNP analyses in unrelated cases and in members of HyperGEN families are presented in tables 2 and 3, respectively. In the unrelated AA cases, urine ACR was significantly associated with rs3752462 (p = 0.01) and rs4821481 (p = 0.05), and a nonsignificant trend toward association was observed for rs2032487 (p = 0.08). In the AA families, urine ACR was also significantly associated with rs4821481 (p = 0.03) and rs2032487 (p = 0.04), with a nonsignificant trend for rs3752462 (p = 0.07). No SNPs were associated with urine ACR in EA and no significant associations with eGFR were observed in participants of either race (EA data not shown).

Of note, 3 of the 1,445 EAs in HyperGEN were homozygous for the rs2032487 susceptibility variant. These subjects had a very low mean eGFR = 30.3 ml/min/1.73 m^2 (vs. 87.4 and 89.1 ml/min/1.73 m^2 for heterozygotes and those homozygous for the resistant variant, respectively).

The GCCT (3224) haplotype of rs4821480, rs2032487, rs4821481, and rs3752462 was found in 3.9% of EA (three haplotypes were present in >1% of the population) and

Table 2. Single SNP associations in unrelated AA, using generalized linear models

SNP	p value for eGFR	p value for urine ACR	Mean log urine ACR		Mean urine ACR (original scale mg/g)	
			risk homozygotes	protective homozygotes	risk homozygotes	protective homozygotes
rs4821480	0.6	0.6	2.30	2.17	12.2	10.2
rs2032487	0.8	0.08	2.36	2.18	12.9	10.0
rs4821481	0.8	0.05	2.38	2.17	13.0	9.8
rs3752462	0.7	0.01	2.36	2.22	12.6	10.3

Table 3. Single SNP associations in all AA, using GENMOD

SNP	p value for eGFR	p value for $\log_e$ urine ACR	$\log_e$ urine ACR (SE)		Mean urine ACR	
			risk homozygotes	protective homozygotes	risk homozygotes	protective homozygotes
rs4821480	0.5	0.15	2.32 (0.06)	2.12 (0.11)	12.37	9.19
rs2032487	0.9	0.04	2.36 (0.06)	2.15 (0.11)	12.8	9.28
rs4821481	0.9	0.03	2.37 (0.06)	2.13 (0.11)	12.8	9.07
rs3752462	0.4	0.07	2.33 (0.06)	2.16 (0.14)	12.25	8.84

62.9% of AA (five haplotypes were present in >1% of the population). Among HyperGEN participants, 38.88% of AA and 0.13% of EA were homozygous for the 3224 E1 risk haplotype. Forming the E1 risk haplotype improved the significance of the urine ACR association results. Table 4 reveals that urine ACR was associated with having two copies of the E1 risk haplotype in AAs [p = 0.013; log urine ACR (standard error, SE) = 2.37 (0.06) for risk haplotype homozygotes vs. 2.18 (0.05) for nonrisk), corresponding to urine ACR values of 12.8 mg/g and 10.7 mg/g, respectively. Table 5 reveals the lack of association of *MYH9* E1 SNPs with type 2 diabetes mellitus by ethnicity (χ^2 test, 2 d.f.).

Discussion

We sought to determine whether SNPs in the E1 risk haplotype of the *MYH9* gene were associated with subclinical nephropathy in a large sample of EA and AA hypertensive sibling pairs and their offspring in the Hyper-GEN study. Siblings were concordant for high blood pressure and had concomitant measures of albuminuria and kidney function. After adjusting for diabetes and the se-

verity of hypertension, albuminuria was significantly associated with several SNPs comprising the *MYH9* E1 risk haplotype in AA participants, and the haplotype had a p value of 0.013 for association with urine ACR. This suggests that some AA in HyperGEN may have had a primary kidney disease, possibly FSGS with secondarily elevated blood pressure, or that high blood pressure directly caused albuminuria in a subset of genetically predisposed individuals. It is clear that not all HyperGEN participants had essential hypertension, as subjects with mild to moderate kidney disease not requiring renal replacement therapy were eligible for recruitment [15].

Identification of the *MYH9* association with FSGS, HIVAN, and H-ESRD, as well as other podocyte genes encoding podocin, nephrin, α-actinin 4, CD2-associated protein and *TRPC6* as causes of complex kidney disease, has altered our understanding of the importance of the podocyte and its cytoskeleton in forming a nearly protein-free urine and maintaining normal renal function and histology [9, 10, 18–21]. The strong *MYH9* association with what has been clinically labeled 'hypertension-associated ESRD', coupled with the failure of strict blood pressure control and use of ACE inhibitors to forestall ESRD in hypertensive AA with CKD, suggests that segmental

Table 4. Comparison between mean urine ACR, eGFR and creatinine clearance, by haplotype and ethnicity

Ethnicity	Haplotype	Mean urine ACR			eGFR		Creatinine clearance	
		$\log_e$ scale	mg/g	p value[1]	mean	p value	mean	p value
AA	3224	2.37 (0.06)	12.8	0.013	88.8 (0.69)	0.45	94.9 (0.96)	0.41
	Not 3224	2.18 (0.05)	10.7		88.0 (0.87)		93.4 (1.16)	
EA	3224	1.6 (0.09)	4.8[2]	0.49	29.0 (1.19)	0.31	29.8 (1.65)	0.31
	Not 3224	1.7 (0.03)	3.8		74.6 (0.45)		88.5 (0.58)	

eGFR in ml/min/1.73 m^2, creatinine clearance in ml/min. Figures in parentheses indicate SEs.
[1] Computed from the log-normal model, not the original scale. [2] This haplotype was observed in only 2 EA individuals.

Table 5. *MYH9* SNP associations with diabetes (χ^2; 2 d.f.) by ethnicity

SNP	Genotypes	AA				EA			
		T2DM		χ^2	p value	T2DM		χ^2	p value
		no	yes			no	yes		
rs2032487	CC	511	164	0.69	0.7	2	0	0.41	0.8
	CT	467	148			96	20		
	TT	133	36			1,149	238		
rs3752462	GG	75	18	1.20	0.5	579	118	1.75	0.4
	GT	419	131			550	119		
	TT	630	204			136	21		
rs4821481	CC	490	160	0.67	0.7	2	0	0.44	0.8
	CT	505	157			94	20		
	TT	134	37			1,173	238		
rs4821480	CC	584	178	0.76	0.7	2	0	0.44	0.8
	CT	393	132			93	20		
	TT	128	37			1,155	237		

and global forms of glomerulosclerosis may be primary renal diseases that lead to progressive nephropathy [11–13]. The *MYH9* association with albuminuria in AA HyperGEN participants, after adjustment for severity of hypertension, suggests that elevated blood pressures in participants could have been a secondary phenomenon related to an intrinsic kidney disease. The renal microvascular changes previously felt to be the sine qua non of hypertensive kidney disease, arteriolar and arterial nephrosclerosis do not correlate with systemic blood pressures in African American Study of Hypertension and Kidney Disease participants, and the vast majority of nondiabetic AA with hypertension and CKD had renal biopsies revealing global and/or segmental forms of glomeruloscle-

rosis with interstitial fibrosis [22]. Patients with classic clinical scenarios for hypertensive nephrosclerosis have also been shown to have FSGS on kidney biopsy [23, 24].

These results suggest that AA subjects in the Hyper-GEN study who are genetically susceptible to the development of a primary kidney disease based upon the *MYH9* genotype have significantly higher levels of urinary albumin excretion. Although the observed differences in urinary albumin excretion were statistically significant based on genotype, the differences were not clinically significant. Therefore, individuals with *MYH9* risk genotypes and slightly high urine ACR will need to be followed longitudinally in order to determine whether renal insufficiency or higher levels of proteinuria ultimately develop.

It is possible that excessive albuminuria, independent of the presence of systemic hypertension, is due to idiopathic FSGS or the early stages of what has been labeled H-ESRD, based on the strong *MYH9* association with these renal diseases. However, it remains possible that high blood pressure causes subsequent kidney disease or nephrosclerosis mainly in genetically susceptible individuals.

The strength of the *MYH9* association with albuminuria was markedly reduced in this study sample, as it was not enriched for severe forms of nephropathy. For example, the E1 risk haplotype association with nondiabetic ESRD in AA residing in the southeastern U.S. was on the order of $p = 10^{-15}$, with an OR of 2.38 [10, 25]. Similarly, nondiabetic ESRD subjects in the Family Investigation of Nephropathy and Diabetes revealed p values for disease association of 10^{-15} for several individual *MYH9* SNPs, with similar ORs. Since severe kidney disease was infrequent in HyperGEN participants, we could not detect associations with either eGFR or creatinine clearance. In total, the *MYH9* association with FSGS, HIVAN and H-ESRD has been replicated in 5 distinct cohorts containing individuals with advanced nephropathy [9, 10, 25]. *MYH9* E1 risk haplotype frequencies in EA and AA participants from HyperGEN were similar to those in the general populations, despite enrichment for the presence of high blood pressure.

In conclusion, the *MYH9* gene is the proximate cause of many cases of FSGS, HIVAN and H-ESRD in the AA population, an effect likely due to alterations in the podocyte cytoskeleton [26]. This gene has shown pronounced evidence of association in common, complex forms of nephropathy [9, 10, 25]. The current cross-sectional analysis assessed for *MYH9* SNP and E1 haplotype associations with albuminuria and kidney function in a large biracial cohort of essential hypertensives. This is the first analysis to search for associations with quantitative measures of subclinical kidney disease in a sample not enriched for nephropathy. In contrast to prior reports, the *MYH9* E1 haplotype was not as strongly associated with albuminuria as it was with severe kidney diseases and ESRD. These results suggest that most hypertensive AA are not at extreme risk for developing subsequent nephropathy, a result that has clearly been demonstrated in the Multiple Risk Factor Intervention Trial and Hypertension Detection and Follow-Up Program cohorts [27, 28]. Longitudinal studies in AA subjects with *MYH9* risk alleles will be required to determine whether high blood pressure is the result of a primary kidney disease such as FSGS or whether nephrosclerosis with albuminuria develops in hypertensive subjects homozygous for *MYH9* risk SNPs and haplotypes.

Appendix

Primary Centers and Investigators of HyperGEN

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