Common Charge-Shift Mutation Glu65Lys in K⁺ Channel β_1 -Subunit *KCNMB1*: Pleiotropic Consequences for Glomerular Filtration Rate and Progressive Renal Disease

Yuqing Chen^{a, g} Rany M. Salem^a Fangwen Rao^a Maple M. Fung^{a, d}
Vibha Bhatnagar^{a, d} Braj Pandey^{a, d} Manjula Mahata^a Jill Waalen^e
Caroline M. Nievergelt^{a, d} Michael S. Lipkowitz^f Bruce A. Hamilton^{a, c}
Sushil K. Mahata^{a, d} Daniel T. O'Connor^{a–d}

Departments of ^aMedicine, ^bPharmacology, and ^cInstitute for Genomic Medicine, University of California at San Diego, ^dVA San Diego Healthcare System, and ^eThe Scripps Research Institute, La Jolla, Calif., and ^fMount Sinai School of Medicine, New York, N.Y., USA; ^gRenal Division, Department of Medicine, Peking University First Hospital, Beijing, China

Key Words

Glomerular filtration rate • *KCNMB1* • Hypertensive nephrosclerosis • African-American Study of Kidney Disease and Hypertension

Abstract

Background: Glomerular filtration rate (GFR) is a heritable trait, and hyperfiltration (GFR increment in remnant nephrons) may accelerate renal functional decline in chronic kidney disease (CKD). Mesangial and vascular smooth myocytes control GFR by contraction, dependent on voltage-gated Ca²⁺ influx, which is controlled by the regulatory β_1 -subunit (*KCNMB1*) of large-conductance heteromeric K⁺ ('BK') channels. *KCNMB1* gain-of-function variant Glu65Lys results in generalized vasorelaxation and thus protection against systemic hypertension. Here we asked whether the Glu65Lys variant influences GFR, in the basal state or during progressive renal decline. **Methods:** We explored Glu65Lys effects on GFR in three populations spanning two ethnicities and two diseases (hypertension and nephrosclerosis). GFR was

either estimated (eGFR from serum creatinine) or directly measured (iothalamate clearance). **Results:** The 65Lys variant was relatively common, occurring on ~5–10% of chromosomes in different biogeographic ancestry groups, and 65Lys carriers exhibited higher eGFR in two primary care populations: extreme BP values in Kaiser clinics ($p = 0.029$, accounting for ~0.2% of trait variance), or treated hypertensives in VA clinics ($p = 0.017$, accounting for ~0.9% of trait variance). In blacks with progressive renal disease (NIDDK AASK), 65Lys carriers displayed a steeper slope in GFR chronic decline ($p = 0.030$, accounting for ~0.4% of trait variance), and Glu65Lys genotype also predicted time of onset of renal failure (log rank $p = 0.019$). **Conclusions:** Common *KCNMB1* gain-of-function variant Glu65Lys influences GFR, and 65Lys carriers exhibit not only elevated baseline GFR, but also more rapid GFR decline (and consequent development of renal failure) in CKD. The results suggest that profiling patients at Glu65Lys can assist in gauging renal prognosis as well as selection of rational therapy in hypertension with progressive renal disease.

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Introduction

Glomerular filtration rate (GFR) is a central element of renal function; judicious measurement of GFR allows timely detection of early renal impairment to track the course of established renal disease. Paradoxically, hyperfiltration (elevated GFR in 'remnant' nephrons) may accelerate progression of chronic kidney disease (CKD). GFR displays substantial genetic control in twin [1] and family studies [2], with heritability (h^2) approaching ~75–80% of trait variance [1], though the specific genes or even the number of contributing loci remain elusive. End-stage renal disease (ESRD) also displays familial aggregation [3, 4], and there is substantial interest in the potential impact of GFR-determining on progressive CKD. However, familial linkage (co-segregation) analyses of the GFR trait have yielded inconsistent results [2, 5–8], and genome-wide association (GWA) studies in >20,000 individuals have identified variants accounting for only <1% of population GFR variance [9]. Thus, the search for genes influencing GFR continues.

Ca^{2+} -activated K^+ channels of large conductance (known as 'Maxi-K', 'Big-K', 'BK', or 'Slo-K' channels) are widely expressed across mammalian tissues, where they influence a variety of physiological processes, including smooth muscle tone, neurosecretion, and hearing. At the molecular level, the heteromeric BK channel is formed by an ion-conducting (ion-pore) α -subunit in non-covalent contact with a trans-membrane regulatory β -subunit. A prominent form of the heteromeric BK channel in vascular smooth myocytes is an α_1 ion pore (KCNMA1) regulated by/interacting with a β_1 -subunit (KCNMB1). In excitable cells, plasma membrane depolarization occurs by opening of voltage-gated cation (Na^+ and Ca^{2+}) channels; plasma membrane re-polarization then proceeds by subsequent opening of voltage-gated K^+ channels, such as BK. The β_1 -subunit has a 'negative feedback' effect on BK ion fluxes, increasing channel sensitivity to cytoplasmic Ca^{2+} [10, 11]. Heteromeric KCNMA1/KCNMB1 BK channels are expressed in vascular smooth myocytes and mesangial cells [12], and the role of BK in regulation of GFR has been explored using a *Kcnmb1*-null mouse model [12], in which *Kcnmb1*($-/-$) mice display blunted GFR response to volume expansion [13].

The *KCNMB1* gene has been systematically explored for genetic variation [14], as well as the consequences of such variation. A single nucleotide substitution (G352A) in exon-3 of *KCNMB1* creates a Glu65Lys substitution in the extracellular loop, resulting in a gain-of-function (en-

hanced K^+ efflux) mutation [15] that is associated with a diminished prevalence of hypertension.

Based on the mesangial expression of *KCNMB1* [12], coupled with the electrophysiological and clinical/BP consequences of the Glu65Lys variant [15], we questioned whether this variant might influence GFR as well as its chronic decline in progressive renal disease. In this study, we explored the role of the Glu65Lys variant on GFR in three population samples, spanning two biogeographic ancestries (European and African) and two diseases (hypertension and CKD).

Methods

Subjects

Primary Care (Kaiser) Population. A population-based cohort with extreme blood pressures consisted of 516 male and 636 female white subjects. These participants were selected based on DBP in the upper or lower most extreme (5th) percentiles of DBP distribution in 25,599 males and 27,479 females in a primary care practice at Kaiser-Permanente of Southern California [16]. Individuals in the upper DBP percentiles were diagnosed as hypertensive, and 48% consumed antihypertensive medications; they were age-matched to individuals with DBP values in the lower extreme 5th percentiles. Serum creatinine was ≤ 1.5 mg/dl. GFR was estimated (eGFR) by the NIDDK MDRD formula: $eGFR = (186) \cdot (\text{serum creatinine} - 1.154) \cdot (\text{age} - 0.203) \cdot (1.21 \text{ if black}) \cdot (0.742 \text{ if female})$ (table 1a), as well as by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) algorithm [17]. Since the Kaiser study was observational, proteinuria data were not systematically obtained.

Primary Care (VA) Hypertension Population. 530 males and 17 females were recruited from the primary care outpatient clinics at the VA San Diego Healthcare System [<http://www.sandiego.va.gov/>]. The ancestry was white (European, by self-identification), diagnosed as hypertension. 90.1% of the subjects were treated with antihypertensive drugs, with 47.5% on 1–2 medications and the rest on additional (from 3–7) medications. GFR was calculated by the NIDDK-MDRD formula, as given above (table 1b) and also calculated with the CKD-EPI algorithm [17]. Since the VA study was observational, proteinuria data were not systematically obtained.

CKD (NIDDK AASK) Population. The NIDDK AASK (African-American Study of Kidney Disease and Hypertension) trial of non-diabetic CKD has been described previously [18, 19]. Briefly, participants were self-identified African-Americans with hypertension who were aged 18–70 years with a GFR between 20 and 65 ml/min/1.73 m², urinary protein/creatinine ratio <2.5 g/g, and no other identified causes of renal insufficiency. Based on a 3 × 2 factorial design, participants were randomized to either a usual mean arterial pressure goal of 102–107 mm Hg or to a lower mean arterial pressure goal of ≤ 92 mm Hg, and to treatment with 1 of 3 antihypertensive drugs: β -adrenergic antagonist metoprolol, 50–200 mg/day; ACE inhibitor ramipril, 2.5–10 mg/day; or dihydropyridine calcium channel antagonist amlodipine, 5–10 mg/day. GFR was assessed by renal clearance of [¹²⁵I]-iothalamate

Table 1. Subject characteristics**a** Characteristics of the primary care (Kaiser) population

	Lower BP	Higher BP	All
Male/female, n	251/349	265/287	516/636
Age, years	56.7 ± 0.6	59.9 ± 0.4	58.3 ± 0.4
SBP, mm Hg	107.8 ± 0.6	154.5 ± 0.6	129.9 ± 0.8
DBP, mm Hg	56.3 ± 0.1	99.2 ± 0.2	76.9 ± 0.6
GFR, ml/min/1.73 m ²	74.3 ± 0.6	74.1 ± 0.7	74.1 ± 0.5
Biogeographic ancestry	all white/European		

b Characteristics of the primary care (VA) hypertension population

		GFR ml/min/1.73 m ²
<i>Demographic</i>		
Male/female, n	530/17 (96.9%/3.1%)	
Biogeographic ancestry	all white/European	
Age, years	66.3 ± 0.5	
SBP, mm Hg	140.1 ± 0.8	
DBP, mm Hg	75.4 ± 0.5	
GFR, ml/min/1.73 m ²	71.8 ± 1.0	
<i>Antihypertensive drugs, % on each class at entry</i>		
ACEI	54.7	74.5 ± 1.1
ARB	7.3	73.7 ± 3.1
α ₁ -Antagonist	18.3	72.8 ± 2.0
α ₂ -Agonist	5.4	75.4 ± 3.6
β-Antagonist	43.5	74.2 ± 1.3
Calcium channel antagonist	30.6	72.0 ± 1.5
Diuretic	46.9	74.3 ± 1.2
None	9.9	71.6 ± 2.6
Other(s)	26.4	73.1 ± 1.6
<i>Number of antihypertensive drugs, % at entry</i>		
0	9.9	71.6 ± 2.6
1	20.1	73.8 ± 1.9
2	27.4	74.0 ± 1.6
3	22.2	74.8 ± 1.7
4	13.6	77.8 ± 2.3
5	5.6	66.4 ± 3.4
6	1.2	59.4 ± 8.6
7	0.1	62.9 ± 24.3

c Characteristics of the genotyped hypertensive nephrosclerosis (NIDDK AASK) cohort

<i>Demographic</i>	
Male/female, n	466/310
Biogeographic ancestry	all African-American
Age, years	66.6 ± 0.4
Blood pressure goal, usual/low	391/385
Antihypertensive drugs, randomized to:	
ramipril/metoprolol/amlodipine	325/304/147
<i>Physiological</i>	
Baseline GFR, ml/min/1.73 m ²	46.7 ± 0.5
Urine protein/creatinine, g/g	0.29 ± 0.17
GFR slope, ml/min/1.73 m ² /year	-1.93 ± 0.83

Because of multiple medications in some individuals, the total equals >100% in 'Antihypertensive drugs'. Since each individual was tallied once, the total equals 100% in 'Number of antihypertensive drugs'.

at baseline twice, then at 3, 6, and every 6 months thereafter. Serum and urinary levels of creatinine and protein were measured by a central laboratory at 6-month intervals. In the AASK trial, despite exclusion of subjects with substantial proteinuria (urinary protein/creatinine ratio <2.5 g/g), initial/entry protein excretion rate correlated inversely with baseline GFR, and predicted accelerated chronic decline of GFR (AASK JAMA 2001 and 2002). At the conclusion of the randomized portion of the study, AASK subjects who had not yet reached ESRD were invited to enroll in a cohort study for up to 10 years after initial enrollment [20].

The analysis is based on the rate of change in GFR (GFR slope). The GFR slope was determined separately during the first 3 months following randomization (acute slope) and after 3 months (chronic slope). The chronic slope was used as outcome in this study. The acute and chronic phases were distinguished because previous studies indicated that the AASK interventions have acute effects on GFR that may differ from their long-term effects on disease progression [18, 21]. GFR slope for each individual was calculated from measured GFRs by linear mixed-effects model.

Subjects as well as biological samples for DNA were ascertained with informed written consent and IRB approval.

Genotyping

The *KCNMB1* Glu65Lys (E65K, rs11739136, C/T) sequence was obtained from the dbSNP database [http://www.ncbi.nlm.nih.gov/SNP]. Genomic DNA was obtained from blood, or from buccal cells (Gentra Puregene Buccal Cell Kit [http://www1.qiagen.com/], yielding ~10–20 μg/individual), and typed using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry system developed by Sequenom according to a published protocol [22], or by Pyrosequencing [23]. Reproducibility of diploid genotypes was verified with blind-replicate samples, indicating 98.8% concordance.

Statistics

GFR and Progressive Decline in GFR. Since the frequency of minor allele homozygosity (Lys/Lys) is quite low (~0.25%) in European and African populations, meaningful numbers of Lys/Lys subjects were not available for statistical tests; we therefore combined Glu/Lys heterozygotes and Lys/Lys homozygotes for marker-on-trait analyses, thus effectively adopting a dominant model for 65Lys effects. Statistical analyses were performed with SPSS version 17. ANOVA with covariate (age, sex) adjustment, determined gene effects on GFR in the VA and Kaiser populations. Two-way ANOVA probed the interactions of gene-by-sex, gene-by-drug or, gene-by-BP status on GFR in the Kaiser population. In AASK, one-way ANOVA and linear regression determined the significance of genotype effects on baseline GFR and chronic (after 3 months on drug) GFR decline slope (ml/min/1.73 m²/year). Kaplan-Meier survival analysis investigated gene effects on the progression towards renal failure (composite endpoint: doubling of serum creatinine, or development of ESRD requiring dialysis), based on 10-year follow-up data in the NIDDK AASK population. To quantify the effect of a particular genetic variant on a renal trait, and normalize such values across traits, we estimated the coefficient of determination (adjusted R²) in general linear models (ANOVA or regression), in the presence or absence of the genotype; the difference in adjusted R² (full model minus model without genotype) represents the contribution of the genotype to the trait; multiplication by 100 yields the coefficient of determi-

Table 2. *KCNMB1* variant Glu65Lys: diploid genotype and allele frequencies across four biogeographic ancestry groups (ancestry determined by self-identification)

Biogeographic ancestry	Individuals n	Chromosomes (2n)	Glu65Lys diploid genotype			Allele, %		HWE p value
			Glu/Glu	Glu/Lys	Lys/Lys	Glu65	65Lys	
White	452	904	398	54	0	94.0	6.0	0.177
Black	342	684	310	32	0	95.3	4.7	0.364
Asian	150	300	125	24	1	91.3	8.7	0.896
Hispanic	120	240	98	20	2	90.0	10.0	0.417
Global	1,064	2,128	931	130	3	93.6	6.4	0.490

The genotype frequencies differed across populations. $\chi^2 = 18.4$, d.f. = 6, $p = 0.005$.

nation as a % of trait variance, where variance = (standard deviation) [2].

Exact (Permutation) Tests. In an exact test, a reference distribution is obtained by calculating all possible values of the test statistic under rearrangements of the labels on the observed data points. Resampling was performed with 'Resampling Stats Excel add-in version 3.2' [http://www.resample.com]. GFR decline rate values were independently permuted 1,000 times to generate an empirically derived distribution of test statistics. The observed test statistics were compared with the empirically derived distributions of test statistics to derive p values.

Population Admixture. African-Americans represent an admixed population with genetic contributions from both African and European biogeographic origins [24]. In order to confirm that AASK individuals with or without trait-associated genotypes were of comparable overall genetic background, and the observed associations were not simply an artifact of differential admixture between higher and lower GFR decline rate, GAMOVA (Generalized Analysis of Molecular Variance) [25] was used to test for and quantify the relationship between the overall genetic background of the subjects and quantitative phenotype GFR decline rate (chronic GFR slope), with an IBS (identity-by-state) distance matrix based on genotypes at 126 bi-allelic markers. GAMOVA thus assesses genetic background diversity using a genetic similarity matrix constructed from the allelic profiles of individuals under study.

Results

KCNMB1 Glu65Lys Allele Frequencies across Populations

The frequency of Glu65Lys variation was probed in 1,064 individuals (i.e. 2,128 chromosomes) arising from four different biogeographic ancestry groups (table 2). The minor (Lys) allele was found in each ancestry group. Frequencies differed across groups ($\chi^2 = 18.4$, $p = 0.005$), with the highest frequencies in subjects of East Asian or Hispanic ancestry (at ~9–10%), and the lowest in subjects

of European or African ancestry (at ~5–6%). Diploid genotypes were in Hardy-Weinberg equilibrium in each population group. The frequency of minor allele homozygosity (Lys/Lys diploid genotype) is quite low (~0.25%) in European and African populations, precluding meaningful numbers of subjects for statistical analysis; we therefore combined Glu/Lys heterozygotes and Lys/Lys homozygotes for marker-on-trait analyses, thus effectively adopting a dominant model for 65Lys effects.

KCNMB1 Glu65Lys: Effect on eGFR

Primary Care Population No. 1 (Kaiser). Here we studied 1,152 individuals from the most extreme (upper and lower 5th percentile) BP values in a primary care population. When we divided the sample into higher versus lower BP groups, BP status itself did not predict eGFR (lower BP at 74.3 ± 0.7 vs. higher BP at 74.1 ± 0.7 ml/min/1.73 m², $p = 0.817$). The effects of age, sex, antihypertensive drug, BP status and *KCNMB1* genotype on eGFR were tested by linear regression; a model combining *KCNMB1* genotype, age and sex predicted eGFR ($p < 0.001$) (table 3), while *KCNMB1* genotype itself accounted for ~0.2% of eGFR variance ($p = 0.03$). 65Lys allele carriers exhibited higher GFR than major allele homozygotes, by ~2.7 ml/min/1.73 m², or ~3.7% (76.4 ± 1.1 vs. 73.7 ± 0.5 ml/min/1.73 m², $p = 0.017$; fig. 1a), and the effect was also captured by permutation test ($p = 0.043$). After this global analysis, we studied the effects of Glu65Lys in subgroups (fig. 1b): the lower BP group (none on antihypertensive drugs), and higher BP group with or without drug treatment. GFR tended to be higher ($p = 0.052$) in the elevated BP group, and this effect was blunted by antihypertensive drug treatment ($p = 0.01$). 65Lys allele carriers displayed higher eGFR in both normoten-

Table 3. Regression: fractional effects of KCNMB1 variant Glu65Lys on GFR in three independent populations

	Population		
	Kaiser	VA	AASK
Biogeographic ancestry	white	white	black
Source	primary care	primary care	NIDDK nephrosclerosis trial
Dependent variable	GFR	GFR	GFR slope
<i>Overall MLR model (independent variables)</i>			
Predictors	gene, age, sex	gene, age	gene, initial GFR, sex, urine protein/creatinine
F	80.5 [300.8]	6.15 [21.26]	35.3
P	<0.001 [<i><0.001</i>]	0.002 [<i><0.001</i>]	<0.001
Adjusted R ²	0.17 [0.34]	0.019 [0.069]	0.151
<i>Model component (independent variable) p values</i>			
Gene	0.03 [0.04]	0.02 [0.02]	0.04
R ² for gene	0.2% [0.3%]	0.9% [1.0%]	0.4%
Age	<0.001 [<i><0.001</i>]	0.007 [<i><0.001</i>]	>0.5
Sex	0.001 [<i>>0.05</i>]	>0.05 [<i>>0.05</i>]	0.037
Urine protein/creatinine	N/A	N/A	<0.001
Baseline GFR	dependent	dependent	<0.001

GFR was calculated with NIDDK-MDRD formula as well as the CKD-EPI formula. Values in brackets reflect analyses with GFR calculated by the CKD-EPI algorithm.

MLR = Multiple linear regression, by stepwise regression method; Kaiser = primary care (Kaiser) population; VA = primary care (VA) hypertension population; AASK = hypertensive nephrosclerosis (NIDDK AASK) population. GFR: ml/min/1.73 m²; GFR slope: ml/min/1.73 m²/year. N/A = Not available. Values in italics indicate significance.

sives and hypertensives ($p = 0.029$), but the eGFR difference was abrogated by drug treatment (fig. 1b). Males exhibited higher GFR than females ($p = 0.027$), and 65Lys allele carriers exhibited higher eGFR than Glu/Glu homozygotes in both sexes ($p = 0.027$), while no gene-by-sex interaction was noted (fig. 1c).

Primary Care Hypertension Population No. 2 (VA). All individuals in this population were diagnosed with hypertension, and 90.1% consumed antihypertensive medications (table 1). In this population, effects of sex and BP status could not be explored, since 96.9% were male and all (100%) had a diagnosis of hypertension. By regression, we analyzed the effects of several variables on eGFR: *KCNMB1*, age, number of drugs, and different drug groups; among these, *KCNMB1* and age jointly predicted eGFR ($p < 0.002$; table 3), and the genotype effect alone accounted for $\sim 0.9\%$ of trait variance ($p < 0.02$). 65Lys allele carriers had ~ 7 ml/min/1.73 m² (or $\sim 10\%$) higher eGFR than wild-type Glu/Glu homozygotes (77.7 ± 2.8 vs. 70.6 ± 1.0 ml/min/1.73 m²; fig. 2); a permutation test

confirmed the difference ($p = 0.027$). Antihypertensive treatment did not demonstrate an independent effect on GFR, perhaps because only 9.9% were untreated.

Since renal function was not diminished in the majority of individuals in the primary care populations No. 1 (Kaiser) and No. 2 (VA), eGFR was also evaluated by the newer CKD-EPI formula [17]. Results were essentially unchanged from those obtained by MDRD-eGFR (table 3, CKD-EPI-eGFR values in brackets; table 4).

KCNMB1 Glu65Lys: Effect on GFR Decline in CKD (NIDDK AASK)

Baseline GFR. Participants in the AASK trial [19] had hypertension with progressive nephrosclerosis, and at baseline 97% were already taking antihypertensive drugs. Glu65Lys did not predict baseline/entry GFR ($F = 0.363$, $p = 0.574$) in this group with limited GFR range, though entry GFR did vary by urine protein/creatinine ratio and sex, being lower in subjects with greater proteinuria, and higher in males versus females.

Fig. 1. *KCNMB1* Glu65Lys: Effect on BP and GFR in a primary care (Kaiser) population. GFR was estimated with the MDRD algorithm and mean ± 1 SEM is shown. Numbers in parentheses (n) indicate the n for that observation. **a** Effects presented overall. The gene effect on GFR was determined by one-way ANOVA. **b** Role of BP status (higher vs. lower) and antihypertensive drug treatment. GFR was presented in 3 subgroups, high BP with/without antihypertensive medicines and low BP group. **c** Role of gene and sex. Effects were determined by two-way ANOVA.

Chronic GFR Decline Rate. The AASK trial was designed to determine factors contributing to progression of hypertensive nephrosclerosis, so here we tested whether *KCNMB1* genetic variation influenced chronic GFR decline rate (by serial iothalamate clearance). A regression model including *KCNMB1* Glu65Lys, urine protein/creatinine ratio, initial/baseline GFR, and sex predicted chronic GFR slope ($p < 0.001$; table 3), and *KCNMB1* itself accounted for $\sim 0.4\%$ of trait variance ($p = 0.04$). 65Lys (minor) allele carriers displayed $\sim 31\%$ faster GFR decline rate than

Glu/Glu (major allele) homozygotes (-2.46 ± 0.25 vs. -1.88 ± 0.08 ml/min/1.73 m²/year, $p = 0.030$; fig. 3a); the effect was confirmed by permutation test ($p = 0.017$).

Progression to Renal Failure during 10-Year Follow-Up. After conclusion of the AASK randomized trial, the AASK cohort was then followed for 10 years after initial enrollment [20]. Kaplan-Meier analysis indicated that the 65Lys allele, which is associated with more rapid decline of GFR, also predicted truncated renal survival as compared to Glu/Glu homozygotes (fig. 3b; Cox regression

Table 4. *KCNMB1* Glu65Lys (E65K) effects on GFR in primary care populations: eGFR calculated by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) algorithm

	Population	
	Kaiser	VA
Biogeographic ancestry	white	white
Source	primary care	primary care
Dependent variable	GFR (CKD-EPI)	GFR (CKD-EPI)
One-way ANOVA		
Overall	<i>p</i> < 0.001	<i>p</i> < 0.001
Gene	<i>p</i> = 0.046	<i>p</i> = 0.020
Covariates		
BP status	<i>p</i> = 0.054	all with hypertension
Age	<i>p</i> < 0.001	<i>p</i> < 0.001
Drug	<i>p</i> = 0.056	<i>p</i> = 0.382
Sex	<i>p</i> = 0.221	<i>p</i> = 0.890
GFR, ml/min/1.73 m ²		
KCNMB1: E/E	74.8 ± 0.4 (n = 971)	66.9 ± 0.9 (n = 479)
KCNMB1: E/K+K/K	77.0 ± 1.0 (n = 181)	73.1 ± 2.4 (n = 68)

Values in italics indicate significance.

p = 0.019); by 10 years, terminal event-free renal survival had declined to only ~41% in 65Lys carriers, as compared to ~59% in Glu/Glu homozygotes.

Genetic Admixture. The possibility that GFR slope differed as a polygenic function of degree of global genetic admixture was approached by comparison of genotype frequencies at widely dispersed markers, since admixture is a genome-wide phenomenon [26]. 126 bi-allelic markers were genotyped in subjects with the chronic GFR slope phenotype. GAMOVA (see Methods) [25] indicated that individuals with similar predictor variables (GFR slope) are not genetically closer to each other than expected by chance (*p* = 0.4516). Therefore, the allele frequency differences that we found at *KCNMB1* cannot be attributed to differential admixture between the higher and lower GFR decline rate groups.

Discussion

Overview: GFR and Ion Channels

GFR, a direct indicator of renal function in CKD, is a heritable trait [1, 2], though the responsible genes remain largely undetermined. Paradoxically, hyperfiltration (elevated GFR in ‘remnant’ nephrons) may accelerate pro-

Fig. 2. *KCNMB1* Glu65Lys effect on GFR in a primary care (VA) hypertension population. GFR was estimated by the MDRD algorithm. The gene effect on GFR was determined by one-way ANOVA. Numbers in parentheses (n) indicate the n for that observation.

gression of CKD. GFR is influenced by the pressure differential between afferent and efferent arterioles [27–29], as well as factors that regulate glomerular capillary surface area [30, 31], such as mesangial cells. Glomerular mesangial cells, responding much like smooth muscle cells to vasoactive hormones [32–34], affect GFR by changing capillary surface area as well as redirecting capillary flow through mesangial loops.

Regulation of contraction by excitable vascular smooth myocytes and mesangial cells is dependent on voltage-gated Na⁺ (and hence Ca²⁺) influx. In response to contractile stimulation, cation channels depolarize the plasma membrane and thereby activate voltage-gated calcium channels, inducing contraction [35]. Depolarization then sets in motion the reciprocal process of re-polarization, via K⁺ efflux.

Physiological Role of KCNMB1

A crucial element of this re-polarization ‘feedback’ response involves activation/opening of the heteromeric plasma membrane ‘BK’ K⁺ channel, a large-conductance, Ca²⁺-responsive channel widely expressed on vascular myocytes. Re-polarization of the plasma membrane then terminates voltage-gated Ca²⁺ influx. The heteromeric BK channel has two subunits: a pore-forming/K⁺-trans-

Fig. 3. *KCNMB1* Glu65Lys effect on GFR decline in non-diabetic CKD (NIDDK AASK). **a** Effect on chronic GFR slope in the genotyped cohort. GFR was determined by [¹²⁵I]-iothalamate clearance. Chronic GFR slope refers to changes after 3 months on randomized study drug. The significance of the gene effect was determined with one-way ANOVA. **b** Progression to renal failure:

survival analysis. The composite endpoint for Kaplan-Meier analysis was either ESRD requiring dialysis, or doubling of serum creatinine. Cox regression $p = 0.019$; covariates tested included gender, years of hypertension, SBP, DBP, baseline GFR, uric acid, baseline urine protein/creatinine, BP treatment goal, and drug group.

locating α -subunit (typically the α_1 -subunit KCNMA1), complexed with a membrane-spanning regulatory β -subunit (typically the β_1 -subunit KCNMB1). The KCNMB1 subunit, expressed in smooth muscle and mesangial cells, increases Ca^{2+} sensitivity of the BK channel [10, 11], increasing the efficiency of the negative-feedback mechanism, hence determining contraction as well as basal tone [10, 11, 36–38].

Although there are several genetic variants distributed across exon-3, ~11-kbp *KCNMB1* locus on chromosome 5q34 [14], one variant has clearly documented functional consequences for K^+ ion fluxes [15]: amino acid substitution Glu65Lys (G352A) of exon-3, within the extracellular loop domain of the regulatory/ β -subunit. Glu65Lys has been associated with hypertension, in that the 65Lys variant seems to protect against development of hypertension [15]. Functional analyses of *KCNMB1* 65Lys channel currents reveal a gain-of-function mutant, with enhanced outward K^+ fluxes through the α -subunit and increased sensitivity to cytoplasmic Ca^{2+} [15]; a more efficient BK- β_1 channel would be predicted to yield an enhanced feedback mechanism, leading to a reduced Ca^{2+} entry via voltage-dependent Ca^{2+}

channels, resulting in vascular smooth muscle and mesangial cell relaxation. Thus the BK channel plays a role much like an endogenous calcium channel blocker, and in this sense the gain-of-function 65Lys allele achieves more effective calcium entry blockade than the wild-type Glu65 allele (fig. 4).

Effects of Ca^{2+} Channels and *KCNMB1* Genetic Variation on GFR

Calcium channel antagonist effects to increase GFR have been reported in both animal models and human subjects [39]. In the AASK trial, GFR acutely increased during the first 3 months after initiation of the amlodipine limb, but subsequently displayed enhanced decline [40]. By contrast, angiotensin-converting enzyme inhibitors appeared to be more effective than β -blockers or dihydropyridine calcium channel blockers in slowing GFR decline, though there was no additional benefit to slowing progression with more intensive BP lowering. Since *KCNMB1* variant 65Lys acts to reduce voltage-gated Ca^{2+} influx [15], its actions resemble in some respects those of a calcium channel antagonist. Thus, an increase in GFR, with more rapid GFR decline in the face of hypertension,

Fig. 4. *KCNMB1* variant Glu65Lys: hypothesis for the effect on GFR. The gain-of-function *KCNMB1* 65Lys variant permits greater K^+ efflux through *KCNMA1*, and thus more efficiently impedes Ca^{2+} entry through voltage operated Ca^{2+} channels (VOCC). Diminished cytosolic Ca^{2+} results in relaxation of mesangial or vascular smooth muscle cells, thereby increasing GFR.

may not be unexpected in subjects carrying the 65Lys allele.

Here we found initially that 65Lys carriers had higher eGFR in two independent primary care populations (Kaiser and VA), each including patients with hypertension. Then, in a population with progressive hypertensive renal disease (NIDDK AASK), we found that the 65Lys allele accelerated long-term renal decline (GFR slope).

Previous studies suggested that the action of calcium channel antagonists to increase GFR might be dependent upon elevated basal renal vascular tone: the drugs acutely elevate GFR in patients with hypertension [40], while in animals the increase in GFR is most apparent during states of increased basal renal vascular tone [39]. Here we found that one population sample equally composed of normotensives and hypertensives (Kaiser) exhibited the eGFR effect, as did an exclusively hypertensive sample (VA) with pharmacological control of BP (average SBP/DBP $\sim 140/\sim 76$ mm Hg, in $\sim 90\%$ of treated individuals). Thus, the 65Lys variant may act in even in early stages of nephropathy, without substantial BP elevation.

In progressive renal disease (fig. 3), Glu65Lys influenced not only GFR decline slope, but also timing of arrival of renal failure (defined as arrival of ESRD requiring dialysis, or doubling of serum creatinine): since glo-

merular hyperfiltration is a risk factor for accelerated renal decline [41], the coordinate effects of 65Lys on GFR (fig. 1, 2) and progressive renal disease (fig. 3) are mechanistically consistent. Glu65Lys did not predict baseline GFR in AASK, perhaps a reflection of the rather narrow GFR entry range (20–65 ml/min/1.73 m²) specified by the AASK enrollment criteria [19], or the multiple vasoactive medications consumed by the AASK subjects even at initial enrollment.

The Literature in Context: Genes and GFR

Here we used a classical, hypothesis-testing experimental approach, proceeding from candidate gene to complex trait, by taking advantage of previous knowledge of vascular and sympathetic physiology and pathology. By contrast, hypothesis-free approaches, such as GWA studies present the theoretical advantage of discovering previously unsuspected contributory loci. Indeed, meta-analyses of four population-based cohorts in the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortium recently discovered novel, replicable effects of at least 3 genetic loci (*UMOD*, *SHROOM3*, *GATM-SPATA5L1*) on eGFR and CKD. However, these loci account for only $\sim 0.7\%$ of population GFR variance. Since twin and family studies indicate that up to $\sim 75\text{--}80\%$ of BP variance is heritable [1], a substantial discrepancy arises, which has been re-

ferred to as the ‘missing heritability problem’ of GWA studies [42], a problem arising for most (if not all) complex traits, including not only GFR but also BP, height and body mass index [43]. What accounts for this discrepancy, or the ‘underperformance’ of GWA for complex traits? A number of explanations have been proposed, including contributions of rare variants [44] (whose effects are not readily detected by the high-minor-allele-frequency SNPs on GWA chips), incomplete linkage disequilibrium between marker and disease loci, the likelihood that individual SNP effects may be too small to pass stringent significance testing (as low as $p < 5 \times 10^{-8}$ for genome-wide multiple tests) [45], gene-by-gene interactions (epistasis), or gene-by-environment interactions. Of note, even here we estimate that *KCNMB1* variant Glu65Lys accounts for only ~0.2–0.9% of GFR trait variance in the three groups studied (table 2). Newer strategies, perhaps encompassing extensive resequencing across physiological pathway loci [43] may finally yield a comprehensive view of the relative roles of heredity and environment in shaping the very complex renal traits of GFR and CKD.

Conclusions and Perspectives

Here we found that *KCNMB1* common variant Glu65Lys influences GFR across multiple populations of different clinical characteristics and biogeographic ancestries. The specificity and reproducibility of such effects were documented by replication, alternative statistical approaches, including multivariable tests (e.g., multiple

linear regression), exact (permutation) tests, interaction effects, and admixture analyses. Finally, the practical significance of the GFR findings was confirmed by prediction of development of renal failure in subjects with progressive renal disease (fig. 3).

Figure 4 illustrates a hypothetical schema integrating our observations in light of the electrophysiology of the BK channel, to yield a framework for future mechanistic studies of the role of *KCNMB1* genetic variation in control of renal function. Since the minor allele frequency of Glu65Lys is substantial in worldwide populations (table 1), ranging from ~5 to 10%, our observations might be of importance to large numbers of individuals at risk of nephropathy; in this respect, it will be of high interest to extend our observations into populations such as Asians and Hispanics, where 65Lys allele frequencies of ~9–10% would predict that upwards of ~18–20% of the population might be susceptible to the hyperfiltration effects we describe.

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