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High frequency of variants of candidate genes in black Africans with low renin-resistant hypertension

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BRIEF COMMUNICATION

High Frequency of Variants of Candidate Genes in Black Africans with Low Renin-Resistant Hypertension

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OBJECTIVES
Black subjects tend to retain salt and water, be more sensitive to aldosterone, and have suppression of plasma renin activity. Variants of the renal sodium channel (ENaC, *SCNN1B*) account for approximately 6% of resistant hypertension (RHT) in Blacks; other candidate genes may be important.

METHODS
Six candidate genes associated with low renin-resistant hypertension were sequenced in Black Africans from clinics in Kenya and South Africa. *CYP11B2* was sequenced if the aldosterone level was high (primary aldosteronism phenotype); *SCNN1B*, *NEDD4L*, *GRK4*, *UMOD*, and *NPPA* genes were sequenced if the aldosterone level was low (Liddle phenotype).

RESULTS
There were 14 nonsynonymous variants (NSVs) of *CYP11B2*: 3 previously described and associated with alterations in aldosterone synthase production (R87G, V386A, and G435S). Out of 14, 9 variants were found in all 9 patients sequenced. There were 4 NSV of *GRK4* (R65L, A116T, A142V, V486A): at least one was found in all 9 patients; 3 were previously described and associated with hypertension. There were 3 NSV of *SCNN1B* (R206Q, G442V, and R563Q); 2 previously described and 1 associated with hypertension. *NPPA* was found to have 1 NSV (V32M), not previously described and *NEDD4L* did not have any variants. *UMOD* had 3 NSV: D25G, L180V, and T585I.

CONCLUSIONS
A phenotypic approach to investigating the genetic architecture of RHT uncovered a surprisingly high yield of variants in candidate genes. These preliminary findings suggest that this novel approach may assist in understanding the genetic architecture of RHT in Blacks and explain their two fold risk of stroke.

Keywords: blood pressure; candidate genes; *CYP11B2*; *GRK4*; hypertension; *NPPA*; *NEDD4L*; *SCNN1B*; *UMOD*.

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Low renin hypertension (LRH) is more common in Black Africans and African Americans,[1] and may account for the observation that in the US Black patients have twice the risk of stroke,[2] and are less likely to have their blood pressure (BP) controlled, despite greater awareness of their hypertension, greater likelihood of being treated, and greater likelihood of being treated more intensively.[3]

LRH can be divided into 2 distinct phenotypes—low renin and elevated aldosterone (primary aldosteronism) and low renin and low aldosterone (Liddle type syndrome). For example, in South Africa a variant of the beta *ENaC* (*SCNN1B*) (R563Q) causes low renin/low aldosterone resistant hypertension (RHT) in 6% of Blacks in urban South Africa and responds to treatment with amiloride, a specific antagonist of the ENaC.[4]

Variants of *GRK4* have also been implicated in LRH/salt-sensitive hypertension; the R65L and A142V alleles were found in most patients tested in a South African study.[5]

Primary aldosteronism due to bilateral adrenal hyperplasia is more common in African Americans and variants of aldosterone synthase (*CYP11B2*) causing familial primary aldosteronism[6,7] may be more prevalent in the African populations.

Other genes implicated in LRH include those encoding atrial natriuretic peptide (*NPPA*), *NEDD4* ligand (which influences the activity of the *ENaC*), and uromodulin (*UMOD*).

Our aim was to search for novel variants of genes associated with salt and water retention in Black African patients with low renin RHT.

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METHODS

Patients

The genetic analysis was part of a larger study that enrolled patients from three sites in sub-Saharan Africa: Nigeria, Kenya, and South Africa. That study aimed to determine if managing RHT according to physiological parameters compared to usual guideline based treatment improved BP control.[8] The number of cases selected for sequencing was determined by funding available. Cases were selected for genotyping were based on inspection of the baseline plasma renin activity (PRA) and plasma aldosterone levels, selecting the most extreme 9 cases for each phenotype: low renin/high aldosterone (primary aldosteronism phenotype) and low renin/low aldosterone (Liddle phenotype).

Measurement of PRA and plasma aldosterone

Blood was drawn at the randomization visit in a fasting condition, while seated for approximately 10 minutes (after BPs were measured). PRA and plasma aldosterone were measured using kits from Diagnostics Biochem (London, Canada). The PRA ELISA kit determined the concentration of angiotensin I in EDTA-plasma using a very specific anti-angiotensin I antibody, a biotinylated angiotensin I tracer and other reagents. PRA was then calculated from the difference in concentration of angiotensin I in samples that were incubated either at 4 °C or 37 °C at slightly acidic pH for approximately 2 hours. Aldosterone concentration was determined directly in the same samples from the DBC ELISA, which also relies on a highly specific antialdosterone antibody. Both kits present good precision with coefficients of variation intra- and inter-assay smaller than 9% (PRA kit) and 13% (aldosterone kit).

DNA collection and analysis

Blood for DNA was collected at 2 of the sites: Kenya and South Africa. DNA was extracted from whole blood at the University of Cape Town. The DNA was sent to the London Regional Genomics Centre at the Robarts Research Institute in London, Canada, for Sanger sequencing. Only patients with low aldosterone/high aldosterone (primary aldosteronism phenotype) and low renin/low aldosterone (Liddle phenotype) were selected for analysis. The *SCNN1B*, *NPPA*, *NEDD4L*, *GRK4*, and *UMOD* genes were sequenced in patients with the Liddle phenotype and *CYP11B2* was sequenced in those with the primary aldosteronism phenotype.

Genomic DNA analysis

PCR amplifications were performed using primers covering the coding regions and intron-exon boundaries of the *SCNN1B*, *NPPA*, *NEDD4L*, *GRK4*, *UMOD*, and *CYP11B2* genes (primers are described in Supplementary Material). PCR amplifications were carried out in 30 μl mixtures containing 32 pmol of each primer, 0.2 mM of each dATP, dCTP, dGTP, and dTTP, 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.0 units of Taq

Table 1. Baseline characteristics of the cases chosen for sequencing

	Age	Sex	PRA	Aldo	Aldo/renin ratio	Baseline systolic	Baseline diastolic
Primary aldosteronism phenotype: sequencing of *Cyp11B2*							
Kenya 1	48	M	2.7	421	156	189	115
2	42	F	1.5	379	253	191	132
3	75	M	1.2	402	335	212	102
4	51	M	0.3	306	1020	179	92
South Africa 1	60	M	0.3	179	597	160	90
2	62	M	1.5	309	206	146	88
3	58	F	0.5	381	762	201	134
4	52	M	0.5	147	294	184	125
5	50	M	0.9	760	844	180	130
Liddle phenotype: sequencing of *SCNN1B*, *NEDD4L*, *GRK4*, *UMOD*, and *NPPA*							
Kenya 1	69	M	4.5	68	15	150	100
2	17	F	9	61	7	156	110
South Africa 1	45	F	7.2	51	7	182	10
2	45	M	1.2	129	108	184	134
3	73	F	0.7	73	104	150	59
4	32	M	2.3	140	61	172	120
5	60	M	1.1	36	33	151	98
6	32	M	3	116	39	157	107
7	36	M	0.4	98.2	246	152	85

Age, years; Sex, M = male, F = female. Abbreviations: Aldo, plasma aldosterone, pg/ml; PRA, plasma renin activity, ng/ml/hr.

platinum DNA polymerase (Invitrogen). Thirty cycles were performed, consisting of denaturation at 95 °C, annealing at 60 °C, and extension at 72 °C for 30 seconds each, followed by a final extension for 7 minutes at 72 °C and cooling to 4 °C. PCR products were purified with CIP/ExoI (New England Biolabs, Pickering, ON, Canada) and sequenced on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster city, CA). DNA sequences were analyzed using Seq Scape v2.6 (Applied Biosystems).

RESULTS

There were 105 patients enrolled in the study, of which 94 completed the study. DNA was collected from 48 patients: 19 from South Africa and 29 from Kenya (see Supplementary Material). Nine patients with low renin and low aldosterone (Liddle phenotype) and 9 patients with low renin and high aldosterone (primary aldosteronism phenotype) were analyzed; the number was dictated by availability of funds. Table 1 shows the baseline characteristics of the patients whose DNA was selected for sequencing.

Table 2 shows the nonsynonymous variants (NSVs) detected by sequencing 9 patients with low renin and low aldosterone and 9 patients with low renin and high aldosterone. There were no NSV detected in the *NEDD4L* gene. However, there were numerous variants detected that did not result in an amino acid change (Supplementary Material).

Table 2. Nonsynonymous variants

Number	Chr:Start	Reference	Altered	Gene	Exon	Amino acid change	HGMD report	Number of patients
CYP11B2: aldosterone synthase								
1	8:143999168	G	A	*CYP11B2* (c.89G>A)	1	R30Q	No	2
2	8:143998626	A	G	*CYP11B2* (c.244A>G)	2	N82D	No	9
3	8:143998614	C	G	*CYP11B2* (c.256C>G)	2	P86A	No	9
4	8:143998611	C	G	*CYP11B2* (c.259C>G)	2	R87G	Altered steroid production	9
5	8:143998545	T	C	*CYP11B2* (c.325T>C)	2	C109R	No	9
6	8:143998544	G	A	*CYP11B2* (c.326G>A)	2	C109Y	No	9
7	8:143998535	T	G	*CYP11B2* (c.335T>G)	2	I112S	No	9
8	8:143996616	T	A	*CYP11B2* (c.441T>A)	4	D147E	No	9
9	8:143996601	G	C	*CYP11B2* (c.456G>C)	4	K152N	No	9
10	8:143996177	T	C	*CYP11B2* (c.743T>C)	5	I248T	No	9
11	8:143994806	T	C	*CYP11B2* (c.1016T>C)	7	I339T	No	6
13	8:143994266	T	C	*CYP11B2* (c.1157T>C)	8	V386A	Corticosterone methyl oxidase deficiency	1
14	8:143994041	G	A	*CYP11B2* (c.1303G>A)	9	G435S	Corticosterone methyl oxidase deficiency	1
NPPA: natriuretic peptide A								
1	1:11907648	G	A	*NPPA* (c.94G>A)	1	V32M	Associated with lower DBP	3
SCNN1B: beta subunit of the epithelial sodium channel								
1	16:23366651	G	A	*SCNN1B* (c.617G>A)	4	R206Q	No	2
2	16:23388540	G	T	*SCNN1B* (c.1325G>T)	9	G442V	Lower aldosterone excretion	1
3	16:23391887	G	A	*SCNN1B* (c.1688G>A)	13	R563Q	Hypertension	1
UMOD: uromodulin								
1	16:20361986	A	G	*UMOD* (c.74A>G)	2	D25G	No	2
2	16:20360085	C	G	*UMOD* (c.538C>G)	3	L180V	No	2
3	16:20348036	C	T	*UMOD* (c.1754C>T)	9	T585I	No	1
GRK4: G protein coupled receptor kinase 4								
1	4:2990499	G	T	*GRK4* (c.194G>T)	3	R65L	Altered activity	3
2	4:3005964	G	A	*GRK4* (c.346G>A)	5	A116T	No	1
3	4:3006043	C	T	*GRK4* (c.425C>T)	5	A142V	Altered activity	8
4	4:3039150	T	C	*GRK4* (c.1457T>C)	14	V486A	Associated with hypertension	9

In the *CYP11B2* gene, there were 14 NSV detected. Three of these variants have previously been described, one of which was present in all 9 sequenced patients. In 8 of the 11 remaining previously undocumented variants, all 9 patients were found to have the variant.

There was one NSV found in the *NPPA* gene. This variant was detected in 3 patients. *SCNN1B* was found to have 3 NSV; 2 of them previously described (G442V and R563Q) and each found in 1 patient, respectively. The previously undocumented variant (R206Q) was found in 2 patients.

The *UMOD* gene was found to have 3 variants, none previously described. Sequencing of the *GRK4* gene revealed 4 variants, of which 3 have previously been described. One of these variants was found in all patients sequenced and another in 8 of the 9 patients sequenced.

The patients that were sequenced originated from a variety of genetic backgrounds. Table 3 shows the origins of the patients and the nucleotide variations that were detected per individual. There was no difference detected in this small number of patients screened, despite different origins.

DISCUSSION

We report a high frequency of genetic variants associated with amino acid mutations in 6 candidate genes implicated in sodium and water retention in Black patients with low renin-resistant hypertension. The selection of which genes to screen was based on aldosterone levels. There was high variability in the genes that were selected, in keeping with the genetic diversity that has previously been documented in Africa.[9]

The beta subunit of the epithelial sodium channel (*SCNN1B*) had 3 variants (R206Q, G442V, and R563Q). The R563Q variant has been previously linked to hypertension in South Africa.[4] The G442V variant of the ENaC was previously described in Black people in London[10] but was not associated with hypertension, only lower aldosterone secretion suggesting increased ENaC activity.

Sequencing of the G protein coupled receptor 4 revealed 4 variants with amino acid changes. One of the variants (A116T) has not been previously reported. However, the *GRK4* R65L, *GRK4* A142V, and *GRK4* A486V are 94.4% predictive of salt sensitive hypertension, and the number of *GRK4* variants is inversely related to salt excretion. The R65L and A142V *GRK4* variants have been linked to BP response to antihypertensive drugs and salt restriction, and ability to excrete sodium.[5,11] Crucially, *GRK4* variants in this study were homozygous for the alternate allele (Table 2) and were described in all patients with low renin/low aldosterone despite different geographic locations and genetic backgrounds. Rayner *et al.*[4] reported that in Black subjects these variants were present in 96% for A142V and 95% for R65L. These preliminary results suggest that *GRK4* may possibly be the main driver of low renin/low aldosterone hypertension in Africa. This hypothesis should be investigated in African American patients with RHT.

Table 3. Patient variants

PT NO.	Tribe	Country												
			NPPA	*SCNN1B*	*SCNN1B*	*SCNN1B*	*UMOD*	*UMOD*	*UMOD*	*GRK4*	*GRK4*	*GRK4*	*GRK4*	
LRLA			V32M	R206Q	G442V	R563Q	D25G	L180V	T585I	R65L	A116T	A142V	V486A	
4002	Colored	SA	GA									TT	TC	
4021	Colored	SA										CT	TC	
4024	Colored	SA	GA										CC	
4051	Xhosa	SA							CT	TT		TT	CC	
4053	Xhosa	SA		GA		GA		CG				CT	CC	
4059	Xhosa	SA		GA								TT	CC	
4065	MOZ	SA	GA				AG					CT	CC	
3019	Kikuyu	Kenya						CG		GT	GA	TT	CC	
3031	Kikuyu	Kenya			GT		AG			GT		TT	CC	
LRHA, *CYP11B2*			R30Q	N82D	P86A	R87G	C109R	C109Y	I112S	D147E	K152N	I248T	I339T	V386A	G435S
4005	Colored	SA		AG	CG	CG	TC	GA	TG	TA	GC	TC	TC		
4023	Colored	SA		AG	CG	CG	TC	GA	TG	TA	GC	TC			GA
4031	Colored	SA	GA	AG	CG	CG	TC	GA	TG	TA	GC	TC	CC		
4044	Xhosa	SA	GA	AG	CG	CG	TC	GA	TG	TA	GC	TC	TC		
4048	Colored	SA		AG	CG	CG	TC	GA	TG	TA	GC	TC			
3004	Kalenjin	Kenya		AG	CG	CG	TC	GA	TG	TA	GC	TC		TC	
3005	Kikuyu	Kenya		AG	CG	CG	TC	GA	TG	TA	GC	TC	TC		
3034	Kikuyu	Kenya		AG	CG	CG	TC	GA	TG	TA	GC	TC	CC		
3035	Kalenjin	Kenya		AG	CG	CG	TC	GA	TG	TA	GC	TC	CC		

The *NPPA* variant has been previously reported to be associated with a lower diastolic BP. The patients in this study all had hypertension. None of the *UMOD* variants was previously described. The variants in this paper were not compared, so further studies to look for association with renin, aldosterone, and BP are required.

The only gene sequenced for the patients with the primary aldosteronism phenotype was the gene encoding aldosterone synthase: *CYP11B2*. Multiple amino acid alterations were detected, most of them in the heterozygous form. There were 2 that were found to be homozygous for the variant allele, but neither of these variants has previously been described. Of the 3 variants that have previously been described, all were found to affect steroid production: R87G, V386A, and G435S have been associated with a decreased aldosterone production.[12–14] However, in this study, the patients with these genotypes had higher aldosterone levels. It is interesting that most of the variants were found in all of the patients, with 1 variant allele.

The most important limitation of this preliminary study was the small numbers of patients studied. However, despite heredity contributing 40–50% to pathogenesis of essential hypertension,[15] genome-wide association studies have only identified 1% of the genetic contribution.[16] This novel approach of enriching the sample by using a phenotypic approach to African patients with low renin-resistant hypertension has revealed a surprising number of previously described and novel variants in candidate genes associated with salt and water retention. It informs a basis for conducting a larger and more powerful study to link these variants with low renin-resistant hypertension in Blacks.

In conclusion, this small preliminary study of variants of candidate genes in African patients from diverse backgrounds with low renin-resistant hypertension reveals a surprising number of novel variants and variants previously linked toLRH. Further study is indicated to determine the significance of these findings and the possibility of translating this research into a more physiological approach to treatment of hypertension.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *American Journal of Hypertension* online.

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DISCLOSURES

Dr Jones has performed contract research in the past two years with Eli Lilly, Otsuka, Bayer, and ZS Pharma. She has received lecture honoraria from Bonitas Medical Aid. Dr Rayner has received grants from the National Research Foundation and Medical Research Council of South Africa. In the past 2 years, he has received lecture honoraria/consulting fees from Servier, Novartis, Merck, Boehringer-Ingelheim, and Cipla and has performed contract research with Otsuka, Novartis, Eli Lilly, Takeda, Boehringer-Ingelheim, and Merck. He is a member of the Editorial Boards of Cardiovascular Journal of South Africa, Nephron Clinical Practice, and Austen Hypertension. Dr Spence has in the past 2 years received lecture honoraria/consulting fees from Bayer and Bristol Myers Squibb, and has performed contract research with Pfizer, Bayer, Bristol Myers Squibb, Acasti Pharma, POM Wonderful, CVRx, and Gore. He is an officer and shareholder of Vascularis Inc., a company seeking to market software for vascular risk reclassification based on measurement of carotid plaque burden. He receives royalties on books from Vanderbilt University Press and McGraw-Hill Medical publishers. Other authors declared no conflict of interest.

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