ORIGINAL RESEARCH

ARMC5 Variants and Risk of Hypertension in Blacks: MH-GRID Study

Mihail Zilbermint, MD;* Amadou Gaye, PhD;* Annabel Berthon, PhD; Fady Hannah-Shmouni, MD; Fabio R. Faucz, PhD; Maya B. Lodish, MD, MHSc; Adam R. Davis, PhD; Gary H. Gibbons, MD; Constantine A. Stratakis, MD, D(Med)Sc

***Background*—**We recently found that *ARMC5* variants may be associated with primary aldosteronism in blacks. We investigated a cohort from the MH-GRID (Minority Health Genomics and Translational Research Bio-Repository Database) and tested the association between *ARMC5* variants and blood pressure in blacks.

***Methods and Results*—**Whole exome sequencing data of 1377 blacks were analyzed. Target single-variant and gene-based association analyses of hypertension were performed for *ARMC5*, and replicated in a subset of 3015 individuals of African descent from the UK Biobank cohort. Sixteen rare variants were significantly associated with hypertension (P=0.0402) in the gene-based (optimized sequenced kernel association test) analysis; the 16 and one other, *rs116201073*, together, showed a strong association (P=0.0003) with blood pressure in this data set. The presence of the *rs116201073* variant was associated with lower blood pressure. We then used human embryonic kidney 293 and adrenocortical H295R cells transfected with an *ARMC5* construct containing *rs116201073* (c.*920T>C). The latter was common in both the discovery (MH-GRID) and replication (UK Biobank) data and reached statistical significance (P=0.044 [odds ratio, 0.7] and P=0.007 [odds ratio, 0.76], respectively). The allele carrying *rs116201073* increased levels of *ARMC5* mRNA, consistent with its protective effect in the epidemiological data.

Conclusions*—ARMC5* shows an association with hypertension in blacks when rare variants within the gene are considered. We also identified a protective variant of the *ARMC5* gene with an effect on *ARMC5* expression confirmed in vitro. These results extend our previous report of *ARMC5*'s possible involvement in the determination of blood pressure in blacks. **(*J Am Heart Assoc*. 2019;8:e012508. DOI: 10.1161/JAHA.119.012508.)**

Key Words: adrenocortical adenoma • *ARMC5* • black • Conn syndrome • genetics • hypertension • primary aldosteronism

Hypertension is one of the preventable risk factors for cardiovascular disease and death. It is estimated that by the year 2030, over 23 million Americans will die from cardiovascular disease.[1] According to the Centers for Disease Control and Prevention, up to 32.5% of Americans older than 20 years have hypertension,[2] with varying rates across various ethnicities. Blacks have a disproportionately increased prevalence, earlier age of onset, and greater morbidity related to hypertension. The National Health and Nutrition Examination Survey found that 42.1% of non-Hispanic black individuals have hypertension.[3] The predisposition of hypertension in blacks has been linked to retention of salt and water, either by excess aldosterone secretion, or to excess sensitivity to aldosterone, and genetic variants that may result in overactivity of the epithelial sodium channel.[4] Nevertheless, increased risk of hypertension in blacks is likely related to complex interactions between genetic, behavioral, and social-environmental determinants that are yet to be determined.

From the Section on Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD (M.Z., A.B., F.H.-S., F.R.F., M.B.L., C.A.S.); Division of Endocrinology, Diabetes, and Metabolism, Johns Hopkins University School of Medicine, Baltimore, MD (M.Z.); Johns Hopkins Community Physicians at Suburban Hospital, Bethesda, MD (M.Z.); Johns Hopkins University Carey Business School, Baltimore, MD (M.Z.); Genomics of Metabolic, Cardiovascular and Inflammatory Disease Branch, Cardiovascular Section, National Human Genome Research Institute, Bethesda, MD (A.G., G.H.G.); Technological Research and Innovation, Uniformed Services University, Bethesda, MD (A.R.D.); National Heart, Lung, and Blood Institute, Bethesda, MD (G.H.G.).

Accompanying Tables S1 through S3 and Figures S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.012508

*Dr Zilbermint and Dr Gaye contributed equally to this work and are co-first authors.

Correspondence to: Mihail Zilbermint, MD, Section on Endocrinology and Genetics, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, 10 Center Drive, Building 10, NIH Clinical Research Center, Room 1-3330, MSC 1103, Bethesda, MD 20892. E-mail: mihail.zilbermint@nih.gov

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Clinical Perspective

What Is New?

- Germline variants in *ARMC5* may in part be responsible for regulation of blood pressure in blacks through their in adrenocortical function.

What Are the Clinical Implications?

- Recognition of hyperaldosteronism and/or its genetics among blacks may lead to earlier and more effective treatments that prevent cardiovascular and renal consequences of hypertension in this population.

Hyperaldosteronism is associated with insulin resistance,[5] diabetes mellitus,[6,7] metabolic syndrome,[7,8] and cardiovascular inflammation and fibrosis,[9] suggesting that aldosterone plays an important role in the development of cardiovascular disease. In young adult blacks, hyperaldosteronism has been linked to insulin resistance that is independent of age, sex, and blood pressure (BP).[10] Primary aldosteronism (PA) is the most common cause of endocrine hypertension and leads to significant morbidity and mortality across all ethnicities. PA is characterized by an autonomous secretion of aldosterone that is independent of renin and sodium status, usually attributable to bilateral adrenocortical hyperplasia. Blacks are more likely to have PA because of bilateral adrenocortical hyperplasia,[4,11–17] although one report suggests a similar prevalence in whites and blacks.[18] Several genetic defects have been identified in PA, although their link to the increased ethnic predisposition to hypertension has not been fully studied or understood.

Our group recently found an association between *ARMC5* gene variants, predicted to be damaging, in patients with PA of black descent.[19] The *ARMC5* gene is a tumor suppressor implicated in cortisol and/or aldosterone-producing primary macronodular adrenal hyperplasia, a rare form of endogenous hypercortisolemia. We identified 12 germline *ARMC5* genetic alterations in 20 unrelated and 2 related individuals (39.3%), in which all affected patients carrying a variant predicted to be damaging were black. This study provided the first evidence of a germline genetic alteration in association with PA specifically for the black population. This genetic association could in part explain the increased predisposition of blacks to low-renin hypertension. Recognition of genetic causes of low-renin hypertension and/or PA and its appropriate treatment may lead to a significant reduction of morbidity and mortality from cardiovascular disease in black individiduals.

To date, over 1000 genetic variants contribute to hypertension, explaining in aggregate ≈6% of the trait variance.[20–28] However, none of these studies demonstrated an association with *ARMC5*. Moreover, investigations of genetic and transcriptome alterations in black patients with hypertension is limited.[29–31] Several genetic variants have been described as associated with hypertension[32,33] and compromised arterial elasticity[34–36] in blacks.[4] Several studies failed to discover any relationship.[37,38] Population-specific genetic variants, variation in allele frequency, and small statistical power were among reasons why some of the genetic loci associated with hypertension lacked the replicability.

Given the paucity of proven genetic drivers of hypertension in this population at risk, we sought to investigate *ARMC5*'s involvement in the regulation of BP among participants in the MH-GRID (Minority Health Genomics and Translational Research Bio-Repository Database) study.[39]

Methods

The data that support the findings of this study are available from the corresponding author upon request.

MH-GRID Data

The MH-GRID project is a study of hypertension with data collected across 8 sites in the United States. The data included in this analysis consist of genotype, from whole exome sequencing, and phenotype information of self-identified black men or women aged 30 to 55 years. Cases are individuals taking ≥2 antihypertensive drugs on a stable regimen (≥6 months) including a diuretic, and controls are individuals with optimal BP (≤120/80 mm Hg) without antihypertension medication and with normal kidney function (estimated glomerular filtration rate >90 mL/min). Patients with kidney disease, diabetes mellitus, heart failure, HIV, and liver disease were excluded. More details of inclusion and exclusion criteria for the MH-GRID are available in Table S1.

UK Biobank Data

The data included in this analysis for the purpose of replication is from the UK Biobank, a large prospective study of 502 628 participants recruited between 2006 and 2010.[40] The subset used in the replication analysis consists of 3015 participants identified as African. Participants provided their medical history, medication information, and lifestyle/behavior factors. BP was measured as the mean of 2 sitting systolic and diastolic BP measurements, taken at baseline using the Omron HEM-7015IT digital BP monitor (Omron Healthcare). For the purpose of replication, participants with any form of cancer were excluded. Cases were defined as individuals with BP ≥140/90 mm Hg regardless of medication status, and controls were defined as individuals with optimal BP (≤120/80 mm Hg) without BP medication. The genetic data are from the June 2017 release. Details of the design of the arrays and

quality control have been described elsewhere.[40] The participants were genotyped on the UK Biobank Axiom array, which has 805 426 markers. For this analysis, the genotypes were imputed, via the Michigan Imputation Server,[41] using the "freeze5b" release of the Trans-Omics for Precision Medicine (TOPMed) whole-genome sequencing data. TOPMed is an initiative sponsored by the National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH) and one of its goals is to achieve ancestral and ethnic diversity and as such it is currently composed of about 60% of participants with substantial non-European ancestry. That diversity was the rationale for the choice of TOPMed as the imputation platform for this analysis.

GENE-FORECAST Data

Plasma renin activity (PRA) data were not available for the MH-GRID or UK Biobank data sets included in this analysis. Therefore, to investigate the relationship between a common *ARMC5* variant and PRA, a smaller data set of 299 samples was used from the GENE-FORECAST (Genomics, Environmental Factors and the Social Determinants of Cardiovascular Disease in African Americans Study). This is a research platform that establishes a strategic, multiomics systems biology approach amenable to the deep, multidimensional characterization of minority health and disease in blacks (ClinicalTrials.gov identifier: NCT02055209). GENE-FORECAST is an ongoing study designed to create a cohort established on a community-based sampling frame of US-born, black men and women (aged 21–65 years) to be recruited from the metropolitan Washington, DC, area. GENE-FORECAST samples were genotyped on a customized Illumina Infinium Multi-Ethnic Global-8 array platform (Illumina, Inc).

Ethics

The study was approved by the institutional review boards of Morehouse School of Medicine, Kaiser Permanente, Grady Health System Research Oversight Committee, and the NIH (ClinicalTrials.gov identifier: NCT02290392). The institutional review boards of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (until 2010) and National Institute of Diabetes and Digestive and Kidney Diseases (2010–present) at NIH approved the research for other protocols (ClinicalTrials.gov identifier: NCT00005927 and NCT00001595). All research participants signed informed consent.

Genetic Association Analyses

Two analyses were performed using the *ARMC5* locus information from genome-built GRCh37 1: chromosome=16, start position=3146941, and end position=31478484. Single-variant analysis of common variants (minor allele frequency [MAF] ≥0.05) within *ARMC5* was conducted in PLINK 1.9.[42,43] Gene-based analyses combining common, low frequency (MAF ≥0.01 and <0.05) and rare variants (MAF <0.01) within *ARMC5* was performed using the optimized sequence kernel association test (SKAT-O),[44] a method recommended when the genetic architecture of a locus of interest is not known.[45] SKAT-O "collapses" variants into 1 single nucleotide polymorphism set, which is then assigned a single score used to predict trait values. Quality controls for the whole exome data are summarized in Figure S1. We used 1377 patients and 44 variants within *ARMC5* (3 common, 4 low frequency, and 37 rare variants) for analysis. All of the association analyses were performed with the genetic variants treated as an additive and the linear model adjusted for potential confounders.

Statistical Analysis

Previous work has shown that known heritable traits (eg, body mass index) can reduce power when included as covariates in regression models.[46–48] Therefore, rather than adjusting indiscriminately for all of the covariates associated with hypertension (Table 1), we used the method of Pirinen et al[48] to identify the model that maximizes power (ie, the model that provides the lowest standard error and *P* value). Our

Table 1. Baseline Characteristics of Patients From the MH-GRID Study

Characteristics	Cases (n=623)	Controls (n=754)	*P* Value
Age, y	48.25±6.06	43.35±7.23	1.17×10^{-40}
Sex			
Women, %	57.11	67.18	1.10×10^{-4}
Men, %	42.89	32.82	
Current smoker (no/yes)	451/165	449/302	2.59×10^{-7}
BMI, kg/m^2	33.92±7.5	28.8±7.46	1.25×10^{-34}
SBP	140±16	109±7	$<2.2\times10^{-16}$
DBP	89±10	70±7	$<2.2\times10^{-16}$
HDL, mg/dL	53.28±15.18	55.42±16.55	1.45×10^{-2}
LDL, mg/dL	120.01±34.57	112.32±34.19	5.81×10^{-5}
Triglycerides, mg/dL	106.95±57.04	87.01±52.24	8.10×10^{-11}

BMI indicates body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MH-GRID, Minority Health Genomics and Translational Research Bio-Repository Database; SBP, systolic blood pressure.

preliminary investigations showed that the optimal model is the one adjusted for age, sex, high-density lipoprotein, low-density lipoprotein, smoking, and the relevant principal components (PCs) of the PC analyses (PCA) performed to investigate admixture.

For MH-GRID, the PCA results show that PC1 separates the 2 main continental ancestries (West African and European) of blacks (Figure S2). Thus, PC1 was added to the model to adjust for global ancestry.

We chose the "African" subset that had MAFs similar to those observed in MH-GRID for the replicated loci. This was done to avoid population stratification, which decreases statistical power. For UK Biobank, the PCA results show, since the analysis was restricted to the African subset, PC2 and PC3, which account for admixture in the African populations were added to the model to adjust for global ancestry (Figure S3).

Molecular Analysis

The DNA sequence of *ARMC5-203* isoform (NM_024742) was cloned in pUCminusMCS plasmid (Blue Heron). The rs116201073 (c.*920T>C) DNA change was introduced by targeted mutagenesis following the manufacturer's instructions (200555-12, Agilent Technologies UK Ltd).

Human embryonic kidney 293 (HEK293) cells were grown in Dulbecco's modified Eagle medium (DMEM) (11995, Gibco) enriched with 10% fetal bovine serum (FBS) (900, Gemini Bio-Products Inc), GlutaMAX (35050, Gibco), and Anti (15240, Gibco), whereas human adrenocarcinoma (H295R) cells were maintained in DMEM-F12 (11320, Gibco) containing 10% FBS (900, Gemini Bio-Products Inc), GlutaMAX (35050, Gibco), Anti (15240, Gibco), and Insulin-Transferrin-Selenium (41400, Thermo Fisher Scientific). A total of 300 000 HEK293 or 400 000 H295R cells were seeded in a well of 6-well plate and were transfected the next day with 1 μg of empty, wild-type or mutant plasmid using Lipofectamine LTX (15338100, Thermo Fisher Scientific) or Effectene (301425, Qiagen), respectively. Where indicated, cells were treated with 10 ng of cycloheximide (C4859, Sigma-Aldrich) or DMSO (34869, Sigma-Aldrich) for the control for 1, 2, or 3 hours before collection. Cells were harvested in Trizol (15596018, Ambion, Inc) 48 hours after transfection for RNA extraction following the manufacturer's protocol. Five hundred thousand nanograms of RNA were then reverse transcribed (11753-050, Thermo Fisher Scientific) as indicated in the manufacturer's instructions. One microliter of a one-twentieth dilution of cDNA was amplified by quantitative polymerase chain reaction conducted with SybrGreen (4364344, Thermo Fisher Scientific). The primers used to analyze *ARMC5*, *ARMC5-203* expression were previously described.[49]

Results

Demographics

The baseline characteristics of the 1377 individuals who passed quality controls are reported in Table 1. The hypertensive group was older and contained more men and had a larger body mass index. The proportion of smokers was higher in the control group and lipid profiles were better (lower low-density lipoprotein and triglycerides and higher high-density lipoprotein) in the control group. The strong association between BP pressure (systolic BP and diastolic BP) and hypertension reported in Table 1 is attributable to the design of MH-GRID, which focused on the tails of hypertension distribution (controls with optimal BP versus cases taking ≥2 BP medications).

Single-Variant Analysis

In the discovery analysis (MH-GRID data), *ARMC5* variant *rs116201073* reached nominal significance (P=0.044; odds ratio, 0.7), suggesting a protective effect for this variant (Table 2). For the replication analysis (GENE-FORECAST data), the variant *rs116201073* was imputed with high confidence (R^2=0.96) and its MAF (0.077) was similar to what was observed in MH-GRID. For the results presented in Table 2, the association, in UK Biobank, was adjusted for age, sex, smoking, alcohol, and PC2 and PC3. The other variables we adjusted for in MH-GRID were not available in UK Biobank.

Gene-Based Analysis

Discovery (MH-GRID)

The gene-based analysis was adjusted for the same covariates as in the single-variant analysis and conducted by combining all 37 rare variants and then applying conditional analysis in SKAT-O to sift out noise variants[50] and identify the variants that truly contribute to the effect (ie, those that decrease the P value of the association). That process identified 16 rare variants that together are associated with hypertension (P=0.0011). SKAT-O of a set that consists of those 16 variants and the common variant identified in the single-variant analysis was more strongly associated with hypertension (Table 3). Subsequent SKAT-O analyses considering low-frequency variants alone or in combination with the rare variants were not conclusive. The SKAT-O results for MH-GRID are summarized in Table S2.

All of the 16 rare variants associated with hypertension in the SKAT-O and outlined in Table 4 have the same effect as the common variant *rs116201073* and this explains the stronger association for the set of 17 (16 rare+1 common). Figure 1 reports the number of mutant alleles across the 16 rare variants by cases and control. Across the 1377

Table 2. Single-Variant Analysis Results and Details of the SNPs

	MH-GRID Study		UK Biobank	
Variant	*rs116201073*	*rs11863886*	*rs11150624*	*rs116201073*
Position on chromosome 16	31477442	31477460	31476458	31477442
Alleles (minor/major)	Cytosine/thymine	Adenine/guanine	Thymine/cytosine	Cytosine/thymine
MAF	0.071	0.182	0.096	0.077
OR	0.7	1.21	1.08	0.76
SD	0.18	0.11	0.14	0.1
P value	0.044	0.089	0.580	0.0068
Frequency in cases, %	5.50	18.70	10.10	7.61
Frequency in controls, %	8.20	16.90	9.50	8.85
Functional information	Synonymous	Synonymous	Missense	Synonymous

MAF indicates minor allele frequency; MH-GRID, Minority Health Genomics and Translational Research Bio-Repository Database; OR, odds ratio; SNP, single nucleotide polymorphism.

individuals, only 17 were heterozygous and 1 was homozygous for the mutant allele and all are controls.

Table 4 lists the 17 variants. Seven of the 16 rare variants have been previously reported while the remainder, along with 4 of 7 variants known as rare and nonsynonymous, are novel from the MH-GRID exome data. Overall, 6 variants, including the common single nucleotide polymorphism, exhibit evidence of selective constraint as computed by 2 mammalian conservation algorithms, the Genomic Evolutionary Rate Profiling[51] and SiPhy,[52] as reported in the HaploReg v4.1 database.[53]

Replication (UK Biobank)

Because of the known challenge of imputing rare variants, only 4 of the 16 rare could be imputed and 2 of those were monomorphic in the UK Biobank data. Therefore, SKAT-O replication was attempted with 2 rare variants imputed with respective R^2 values of 0.92 (*rs367810854*. MAF=0.0008 in UK Biobank) and 0.67 (*rs141923065*, MAF=0.0012 in UK Biobank). The results of the gene-based analysis with the 3 variants, adjusted for age, sex, smoking, and alcohol, was significant in UK Biobank (P=0.0083). The frequency of the replicated variants in various populations is reported in Table S3.

Table 3. SKAT-O Gene-Based Analysis Results

Variant	*P* Value	No. of Variants Collapsed
Rare variants	0.0011	16
Rare variants+*rs116201073*	0.0003	17

SKAT-O indicates optimized sequence kernel association test.

The common variant *rs116201073* seems to be specific to Africans, where it is present only in Africans or African-admixed populations included in the 1000 Genomes Project (Table 4). In the TOPMed data available from the BRAVO portal (University of Michigan), specific allele frequency are not available for some minority populations and the same info as in the 1000 Genomes Project. In the Genome Aggregation Database (gnomAD), the frequencies reported are 0.075 for African, 0.002 for Latino, 0.002 for "other," and essentially 0 (<0.00001) for the other populations.

As for the rare variants included in the replication analysis, in the 1000 Genomes Project: *rs141923065* is observed only in African-Caribbean in Barbados, African American from the Southwest (ASW), and Han Chinese. Intriguingly, *rs367810854* is observed only in South Asian populations (gnomAD provides similar information with 0.0499 for the category "South Asian" and <0.0001 or other continental populations). Additional details are available in Table S3.

ARMC5 Variant *rs116201073* and PRA

First, we evaluated the relationship between hypertension and PRA across the 299 samples (115 with hypertension versus 184 controls). The results showed lower PRA in the hypertensive group (mean=1.40 ng/mL per hour in patients with hypertension versus 2.04 ng/mL per hour in controls), but the difference was not statistically significant (t statistic=1.50, P=0.13 [95% CI, −0.20 to 1.47]). Then we estimated the association between the variant and PRA dichotomized using a cutoff of 0.65 ng/mL per hour[54] to have, respectively, 82 and 134 patients in the low and high renin groups across the 216 samples for which genotype data were available in GENE-FORECAST. The variant was less frequent in the low renin group (6% versus 7% in the rest of the sample); however,

Table 4. List of the Set of 17 Variants Significantly Associated With Hypertension and MAF in the MH-GRID Study

Variant	POS (GRCh37)	MAF	Functional Information (dbSNP)	Under Selection (GERP/SiPhy)
16:31473335	31473335	0.0004		
16:31473823	31473823	0.0004		
rs141923065	31474091	0.0004	Missense (glutamine ⇒ arginine)	Yes
16:31474095	31474095	0.0007		
rs202103062	31476361	0.0004	Missense (glycine ⇒ cysteine)	No
rs370836071	31477234	0.0004	Missense (threonine ⇒ methionine)	
16:31477236	31477236	0.0004		
rs116201073	31477442	0.0711	Synonymous	Yes
16:31477452	31477452	0.0004		
16:31477486	31477486	0.0004		
16:31477569	31477569	0.0004		
16:31477574	31477574	0.0004		
rs181967284	31477834	0.0004	Missense (arginine ⇒ glutamine)	Yes
rs367810854	31477859	0.0004	Synonymous	
rs372567714	31477945	0.0004	Missense (glutamic acid ⇒ valine)	
16:31478013	31478013	0.0004		
rs61734240	31478192	0.0004	Synonymous	Yes

MAF indicates minor allele frequency; MH-GRID, Minority Health Genomics and Translational Research Bio-Repository Database; POS, position; GRCh37, Genome Reference Consortium Human Build 37; dbSNP, The Single Nucleotide Polymorphism Database; GERP, Genomic Evolutionary Rate Profiling; SiPhy, SIte-specific PHYlogenetic analysis.

the association was not statistically significant (odds ratio, 0.87; $P>0.05$).

Effect of *ARMC5* Variant, *rs116201073*

The *rs116201073* variant is a synonymous variant in most of the *ARMC5* isoforms except in the NM_024742 isoform (referred to as *ARMC5*-203 in the *Ensembl* database) in which it is located in the 3′-untranslated transcribed region (UTR). To determine its potential effect on *ARMC5*'s expression, we transfected wild-type and mutant *ARMC5*-203 plasmids in HEK293 cells and analyzed *ARMC5* presence by real-time quantitative polymerase chain reaction, 24 and 48 hours after transfection. Whereas at 24 hours, no difference was observed between the 2 groups, at 48 hours, there was a significant increase in *ARMC5* mRNA accumulation when *ARMC5* carried the variant allele (Figure 2).

Similar results were obtained using either primers targeting all *ARMC5* isoforms (Figure 2A) or specifically the *ARMC5-203* isoform that was overexpressed (Figure 2B). The treatment of the transfected cells with a translation inhibitor, cycloheximide, for 2 or 3 hours before collection led to a normalization of the ratio of *ARMC5-203* mutant and wild-type mRNA demonstrating that the elevation of mutant *ARMC5* mRNA was the result of a decrease of its translation rate. A similar elevation of *ARMC5-203* mRNA was found at 48 and 72 hours after transfection in an adrenocortical cell line H295R, but this increase was not significant (Figure S4).

Discussion

Analysis of our data identified one common variant (*rs116201073*) located in the 3′UTR end of the *ARMC5* gene that was associated with decreased risk of hypertension (odds ratio, 0.7) in a sample set of 1377 blacks from the MH-GRID study. That single-variant association was replicated with a smaller *P* value in a UK Biobank sample set of 3015 African participants. Gene-based SKAT-O analysis, in MH-GRID, also revealed a set of 16 rare variants associated with hypertension in blacks with the same protective effect as the common variant *rs116201073*. Together, these 16 rare and 1 common variant (a set of 17 variants) were significantly associated with BP in a subsequent gene-based test. The gene-based results were also replicated in the UK Biobank data with the rare variants that could be imputed. These results confirm our previous report of *ARMC5*'s possible involvement in regulating BP in blacks, possibly as a result of its role in determining the presence of bilateral adrenocortical hyperplasia and/or hyperaldosteronism.[19]

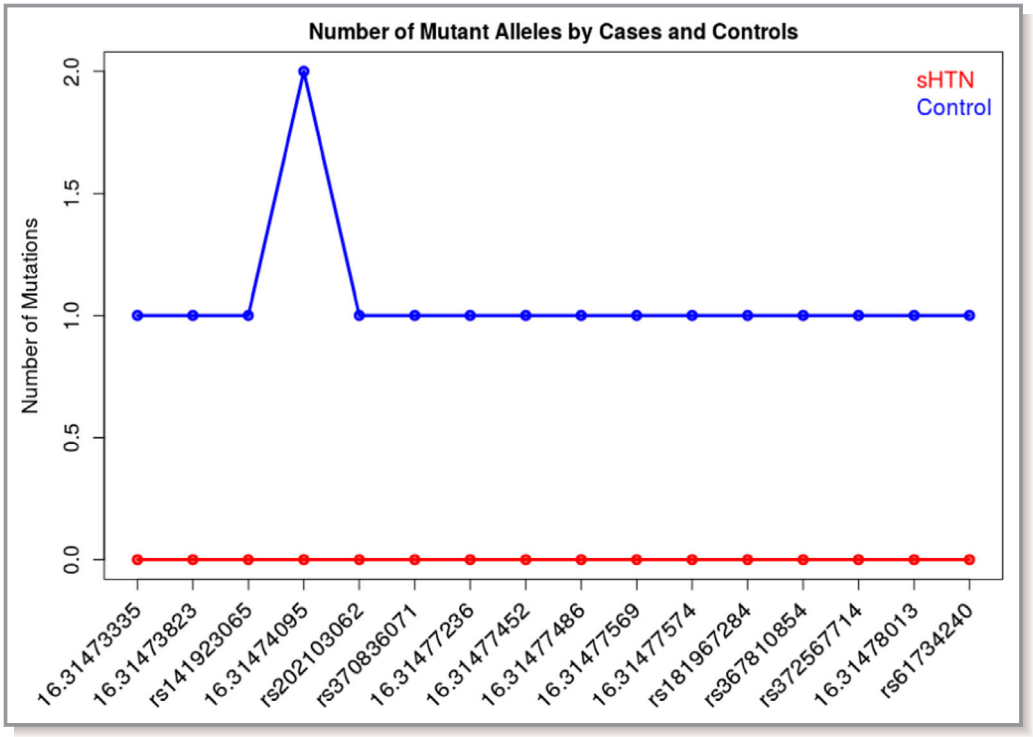

Figure 1. Number of mutant alleles by cases and controls across the Minority Health Genomics and Translational Research Bio-Repository Database. sHTN indicates severe hypertension.

The *ARMC5* gene is a putative tumor suppressor that is located on chromosome 16p11.2 and belongs to the family of armadillo-repeat–containing proteins.[55,56] In humans, *ARMC5* consists of 8 exons and has an unknown function,[19,49] although recent evidence suggests that it plays a critical role for fetal development and immune responses through interactions with proteins from different pathways.[57,58] Four *ARMC5* isoforms exist, with a different pattern of expression, although all 4 are expressed in the adrenal glands.[55] The *ARMC5* gene has been recently implicated in endogenous hypercortisolemia due to primary macronodular adrenal hyperplasia,[59–64] which is characterized by multiple nodules (>1 cm) in the adrenal cortex and hypercortisolemia.[19] Biallelic inactivation of *ARMC5* (germline and somatic) is required for the development of adrenocortical hyperplasia, which is consistent with the 2-hit hypothesis of tumorigenesis.[59] Most disease-causing variants in *ARMC5* are frameshift and/or nonsense, and lead to loss of function of the gene.[62] Overexpression of *ARMC5* in adrenocortical carcinoma cell line H295R leads to increased cell death,[59] while silencing of the gene in nonmutated primary macronodular adrenal hyperplasia cell cultures leads to a decrease of apoptosis.[65]

Genetic variants in *ARMC5* have rarely been implicated in PA. The largest study to date examined 56 patients with PA and found 12 different germline variants in *ARMC5* (6 predicted to be damaging by in silico analysis) in 20 unrelated and 2 related individuals (39%).[19] These variants were exclusively found in black individuals and silencing of *ARMC5* in H295R cells decreased *CYP11B2* expression.[19]

A recent study in a different cohort of patients with PA reported 18 *ARMC5* variants (5 rare with an allele frequency <1%) and 2 new variants that were not predicted to be damaging.[66] Variants in *ARMC5* are difficult to identify as pathogenic because *ARMC5*'s function remains unclear; however, some missense variants fail to induce apoptosis after transfection in a human adrenocortical cancer cell line H295R.[59] Although the link between *ARMC5* and PA is yet to be explained, our data support a potential link between *ARMC5* variants and hypertension in people of African descent.

PRA and aldosterone are often used as a screening test for PA. We studied possible associations between PRA and hypertension in patients from the GENE-FORECAST study. Although these results were not significant, perhaps because of small sample size, the directions of the relationships were consistent with the seemingly protective effect of the variant (*rs116201073*) reported in the genetic associations' analyses of MH-GRID and UK Biobank data sets.

Interestingly, the *rs116201073* variant seems to be specific to the African population with the C allele present only in African and African admixed populations included in the 1000 Genomes Project. Given the significantly higher prevalence of the variant in the control we can hypothesize that either the

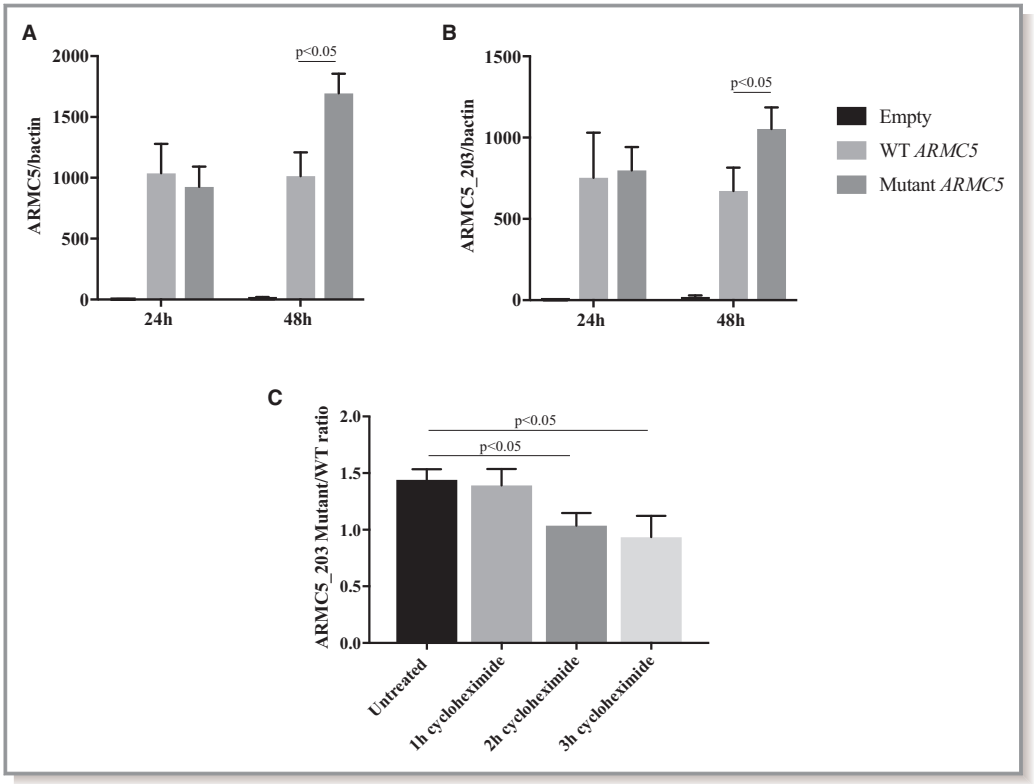

Figure 2. Comparison of wild-type (WT) and mutant *ARMC5* (*rs116201073*) expression in human embryonic kidney 293 (HEK293) cell line. **A** and **B**, Real-time quantitative polymerase chain reaction (RTqPCR) using primers recognizing all *ARMC5* isoforms (**A**) or only the *ARMC5*-203 isoform (**B**) on HEK293 cells transfected with WT or mutant (variant-carrying) *ARMC5*-203 plasmid for 24 and 48 hours. **C**, The ratio of the mutant and the WT *ARMC5*-203 expression detected by RTqPCR on HEK293 cells untreated or treated 1, 2, or 3 hours with a translation blocker, cycloheximide. The graph represents the means of at least 3 independent experiments±standard error of the mean (SEM).

minor C allele has a direct protective effect against hypertension in this population predisposed to low-renin hypertension or this variant is a genetic marker of a protector factor. This variant predicted to be benign is synonymous in 3 *ARMC5* isoforms: *ARMC5-201*, *ARMC5-202*, and *ARMC5-205*. In the *ARMC5-203* isoform, which is ubiquitously expressed[49] and even overexpressed in our hypertensive cohort compared with controls, the *rs116201073* variant is located in the 3′-UTR. The 3′-UTR region is essential for the regulation of mRNA stability, expression, and localization.[67] Indeed, our in vitro experiments in HEK293 cells demonstrate that the *ARMC5-203* carrying the variant mRNA is accumulated compared with the wild-type mRNA and this is the result, at least in part, of a reduction in its translation rate.

ARMC5-203 function has not yet been studied but it is noteworthy that it is the only protein isoform that has the Armadillo domain but not the BTB (BR-C, ttk, and bab)/POZ (Pox virus and Zinc finger) domain. This suggests a specific and nonredundant role between this isoform and the 3 other *ARMC5* isoforms.[49] Altogether, these data suggest that the *ARMC5-203* protein would promote hypertension, as the variant decreasing its protein translation is protective against hypertension.

Predisposition to low-renin hypertension in blacks has been broadly studied. Part of this predisposition is attributed to the activation of the renin-angiotensin-aldosterone system by its promotion of sodium retention.[4,68] It could be explained by genetic variants that predispose to inappropriate secretion of aldosterone, or genetic variants that affect the epithelial sodium channel (eg, Liddle syndrome phenotype), ultimately resulting in water preservation.[4,69] The following genetic variants were found to predispose to PA and/or inappropriate secretion of aldosterone: *CYP11B2*, *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D*, and *ARMC5*.[19,70–72] The Liddle syndrome phenotype could be caused by *GRK*, *NEDD4L*, *CYP4A11*, *NPPA*, *UMOD*, and perhaps other, yet to be discovered genetic variants.[4,70,73]

Why do people of African origin have more hypertension than foreign-born Africans in the United States? It is possible, that natural selection for salt and water retention created a survival advantage for people transported across the Atlantic Ocean in hot conditions. This phenomenon is known as the African Diaspora hypothesis.[4,74–76]

Study Limitations

Our study has limitations and should be interpreted with caution. First, the MH-GRID hypertensive case definition was based on hypertension diagnosis by a clinician and the participant being prescribed ≥2 antihypertensive medications for at least 6 months before enrollment into the study. The available UK Biobank data set did not have information about the number of antihypertensive medications that the study participants were taking, as the cases were defined as those with hypertension diagnosed by a clinician regardless of medication status and/or those with average BP ≥140/90 mm Hg across several measures at the visit;. Second, a "healthy volunteer" effect was demonstrated in the UK Biobank study,[77] thus generalization of the present study is likely limited. Third, we do not have information about the duration of antihypertensive therapy, and it is possible that the duration of medication use may be considered to be a proxy for disease severity. Fourth, we have limited data on the biochemical phenotype of the participants: the association between the common genetic variant and low renin could not be reliably established because of the small sample. Fifth, we do not know whether patients had the Liddle syndrome phenotype (low renin and low aldosterone) or PA (low renin and high aldosterone). Finally, we did not consider other variants/loci of interest for an additive effect on hypertension.

Conclusions

We identified one common variant (*rs116201073*) of the *ARMC5* gene that was associated with a decreased risk of hypertension in blacks and a set of 16 rare variants associated with hypertension. These results extend our previous report of germline *ARMC5* variants that may be linked to hypertension in blacks. Although not conclusive, the evaluation of the main variant with respect to PRA may suggest a link to low-renin hypertension. Further genetic and molecular studies are needed to confirm and complement these findings.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1. *ARMC5* variants and risk of hypertension in African Americans: Minority Health-GRID study. Minority Health Genomics and Translational Research Bio-Repository Database (MH-GRID) study: inclusion and exclusion criteria.

Inclusion Criteria
• Self-identified African-Americans males or females ages 30-55 years. • Cases: Severe-Controlled Hypertension (SCH): SBP ≤ 140 and/or DBP ≤ 90 mmHg on a stable regimen (≥6 months) with ≥ 2 anti-hypertensive drugs (must include a diuretic). • Cases: Severe-Resistant Hypertension (SRH): SBP > 140 and/or DBP > 90 mmHg on a stable regimen (≥3 months) of ≥ 3 drugs (must include a diuretic). • Controls: Individuals with optimal blood pressure: ≤ 120/80 mmHg and normal kidney function (eGFR > 90 ml/min).
Exclusion Criteria
• Failure to meet the inclusion criteria. • Primary chronic kidney disease or proteinuria unrelated to hypertension. • Secondary forms of hypertension*. • Chronic diseases that may secondarily compromise renal function such as diabetes, chronic congestive heart failure, HIV or liver disease. • Patients with recent hospitalizations (< 3 months). • Unable to give informed consent. • Pregnant or lactating women.

SBP: Systolic blood pressure, DBP: Diastolic Blood Pressure, eGFR: estimated glomerular filtration rate.*no aldosterone or plasma renin activity data was available

Table S2. Gene-based analysis results, in MH-GRID.

SNP	P-Value	Number of variants in SNP set
Rare variants	0.0011	16
Low frequency variants	0.1656	4
Rare + Low frequency variants	0.0070	20
Rare variants + rs116201073	0.0003	17
Low frequency variants + rs116201073	0.1090	5
Rare + Low + rs116201073	0.0057	21

MH-GRID: Minority Health Genomics and Translational Research Bio-Repository Database; SNP: Single nucleotide polymorphisms.

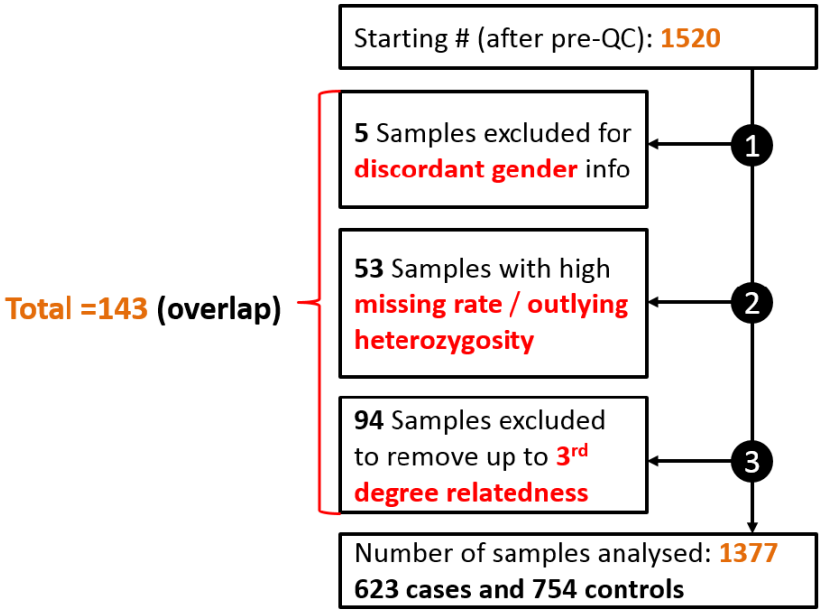
Table S3. Minor Allele Frequency of rs116201073, rs141923065 and rs367810854 in the 1000 Genomes Project populations.

Population	Population	rs116201073	rs141923065	rs367810854
African	ACB	0.0469	0.0012	0
	ASW	0.0984	0.0052	0
	YRI	0.1019	0	0
	ESN	0.0758	0	0
	LWK	0.101	0	0
	GWD	0.1018	0	0
	MSL	0.1	0	0
Admix American	MXL	0	0	0
	PUR	0.0144	0	0
	CLM	0	0	0
	PEL	0.0059	0	0
South Asian	GIH	0	0	0.0631
	PJL	0	0	0.0938
	BEB	0	0	0.0581
	STU	0	0	0.0735
	ITU	0	0	0.0784
European	CEU	0	0	0
	TSI	0	0	0
	FIN	0	0	0
	GBR	0	0	0
	IBS	0	0	0
East Asian	JPT	0	0	0
	KHV	0	0	0
	CHB	0	0.0097	0
	CDX	0	0	0
	CHS	0	0	0

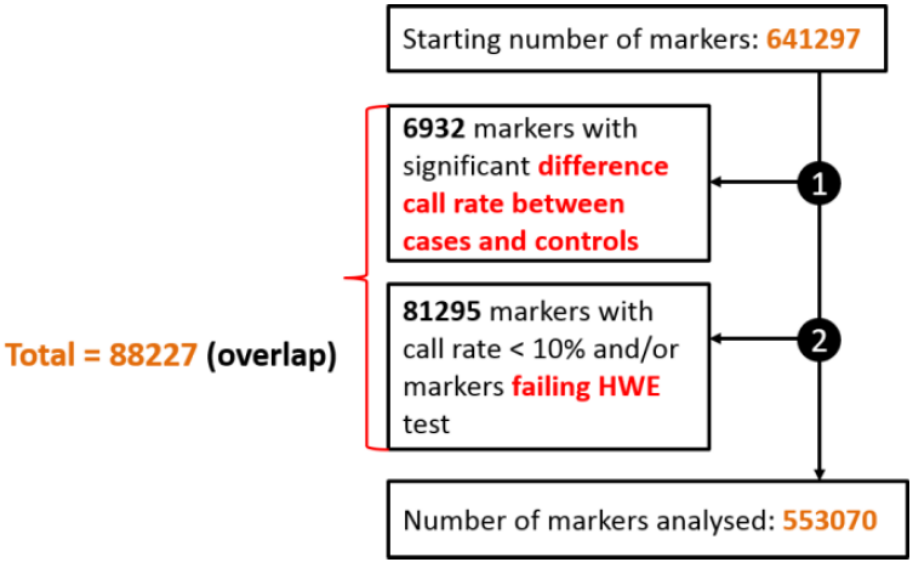
ASW: African ancestry in SW USA; ACB: African Caribbean in Barbados; BEB: Bengali in Bangladesh; GBR: British from England and Scotland; CDX: Chinese Dai in Xishuangbanna, China; CLM: Colombian in Medellín, Colombia; ESN: Esan in Nigeria; FIN: Finnish in Finland; GWD: Gambian in Western Division – Mandinka; GIH: Gujarati Indians in Houston, Texas, United States; CHB: Han Chinese in Beijing, China; CHS: Han Chinese South, China; IBS: Iberian populations in Spain; ITU: Indian Telugu in the U.K.; JPT: Japanese in Tokyo, Japan; KHV: Kinh in Ho Chi Minh City, Vietnam; LWK: Luhya in Webuye, Kenya; MS: Mende in Sierra Leone; MXL: Mexican Ancestry in Los Angeles CA United States; PEL: Peruvian in Lima, Peru; PUR: Puerto Rican in Puerto Rico; PJL: Punjabi in Lahore, Pakistan; STU: Sri Lankan Tamil in the UK; TSI: Toscani in Italy; YRI: Yoruba in Ibadan, Nigeria; CEU: Utah residents with Northern and Western European ancestry from the CEPH collection.

Figure S1. Summary of Quality Controls (QC) for MH-GRID Exome-Wide Sequencing Data.

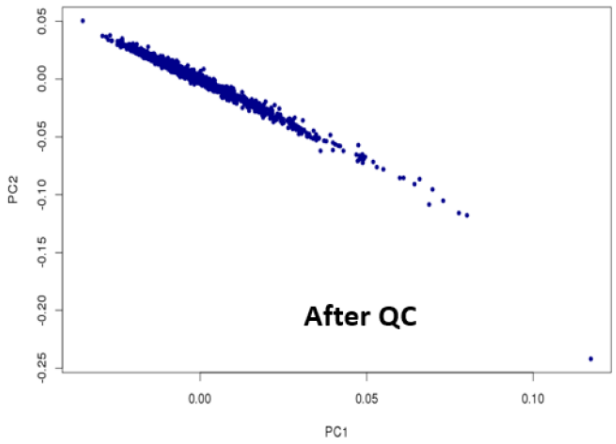
A. Sample QC



B. Markers QC

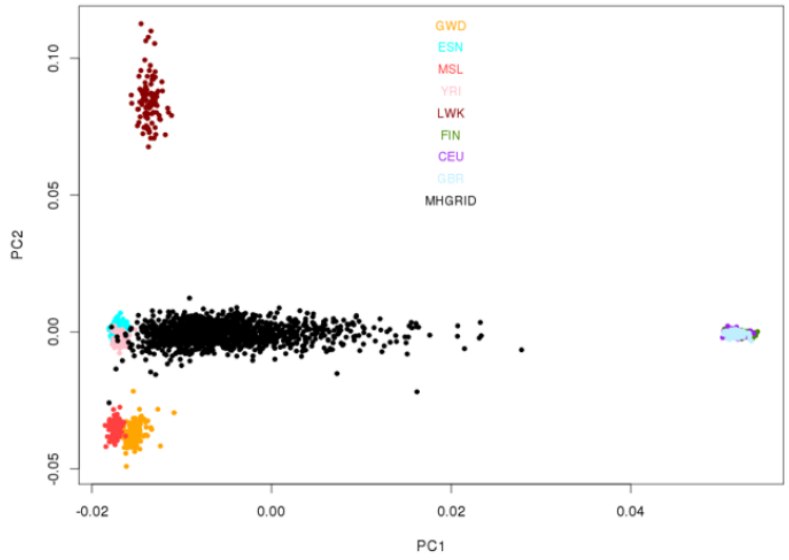


C. Principal component analysis graph after all QC show homogeneous sample set



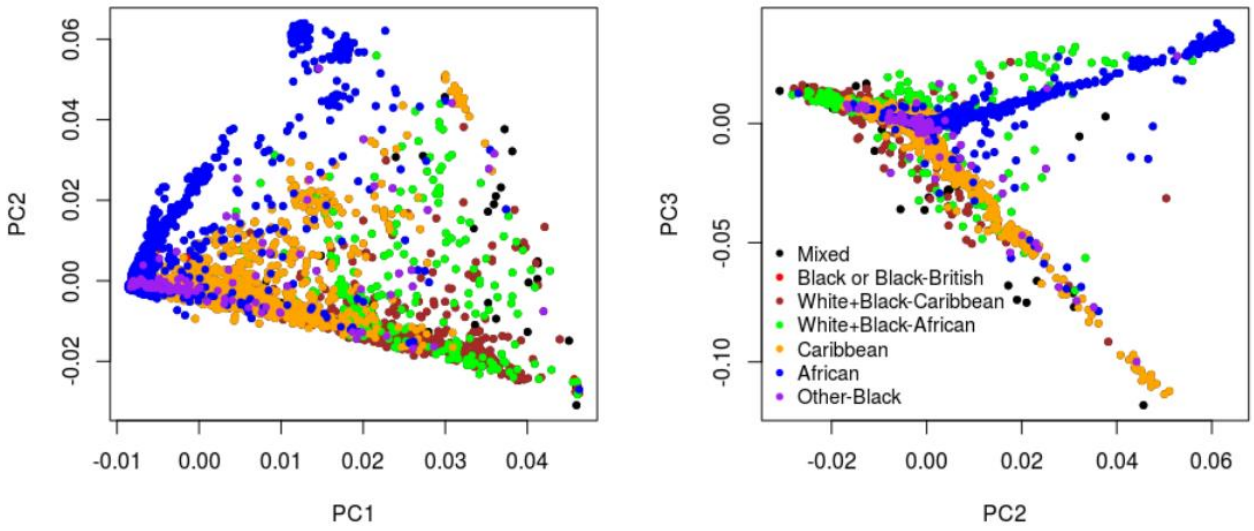
A. After excluding samples failing quality control filters 1377 samples remained for analysis. **B.** 44 variants between the start and end position of the *ARMC5* gene are among the 553070 variants that passed quality control. The Ti/Tv ratio after quality control is 3.31 indicating good quality data with regards to sequencing errors. **C.** There was no evidence of batch effect after quality control as shown by the homogenous cluster where the smear shape just reflects admixture from West-African to European ancestry shown in.

Figure S2. Principal component analyses of MH-GRID data with 1000 Genomes Project samples.



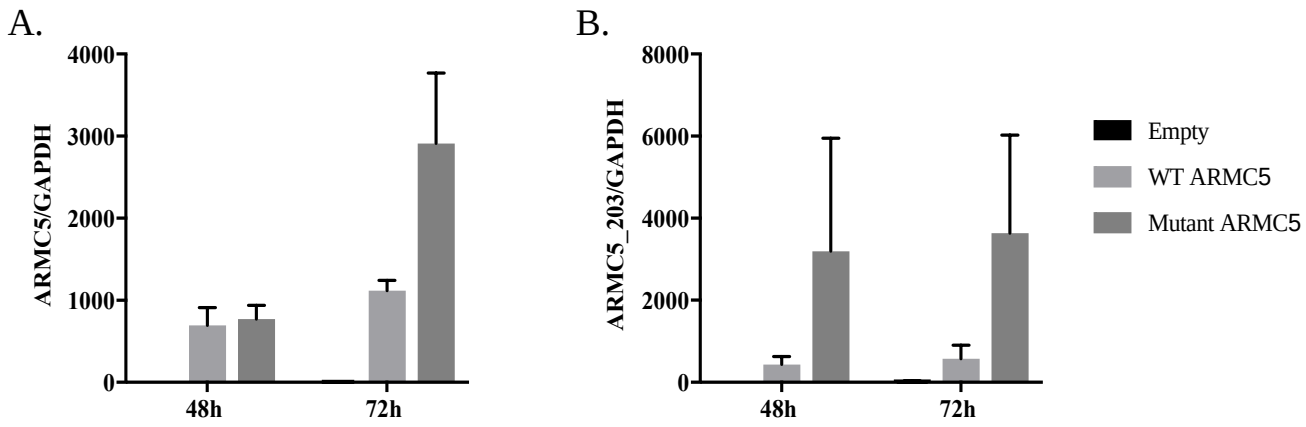
MH-GRID: Minority Health Genomics and Translational Research Bio-Repository Database; Optimal model is adjusted for age, sex, HDL, LDL, smoking and the first principal component (PC1) of the principal component analysis carried out to investigate admixture. PC1 separates the 2 continental ancestries relevant for this analysis. The graph represents a PCA plot of 1377 MH-GRID samples with eight 1000 Genome populations: 5 African, GWD (Gambian in Western Division), ESN (Esan in Nigeria), MSL (Mende in Sierra Leone), YRI (Yoruba in Ibadan, Nigeria) and LWK (Luhya in Webuye, Kenya) and 3 European, FIN (Finnish in Finland), CEU (Utah Residents with Northern and Western European Ancestry) and GBR(British from England and Scotland).

Figure S3. Graphs of principal component analyses (PCA) of UK Biobank genotype data.



PCA analyses of UK Biobank genotype data to determine principal components (PC)s to consider adjusting for ancestry. In the below figure the plots of PC1 vs. PC2 and PC2 vs. PC3 PC2 and PC3 are the relevant PC for the analysis restricted to the African populations (in blue). Therefore, PC2 and PC3 were added to the model described in the manuscript.

Figure S4. Comparison of wild type (WT) and mutant ARMC5 (rs116201073) expression in HEK293 cell line.



Expression of wild type (WT) and mutant *ARMC5* variant (rs116201073) after transfection in the adrenocortical cell line, H295R. The *ARMC5* expression is analyzed by RTqPCR using primers targeting all *ARMC5* isoforms (A) or only the transfected 203 isoforms (B). The graph represents the means of at least 2 independent experiments ± SEM.