Prevalence of the Apolipoprotein E Arg145Cys Dyslipidemia At-Risk Polymorphism in African-Derived Populations

Maen D. Abou Ziki, BA[a,b], Yael Strulovici-Barel, MS[a], Neil R. Hackett, PhD[a], Juan L. Rodriguez-Flores, PhD[a], Jason G. Mezey, PhD[a,c], Jacqueline Salit, MS[a], Sharon Radisch, MS[a], Charleen Hollmann, PhD[a], Lotfi Chouchane, MD[b], Joel Malek, PhD[b], Mahmoud A. Zirie, MD[d], Amin Jayyuosi, MD[d], Antonio M. Gotto, Jr., MD, DPhil[e], and Ronald G. Crystal, MD[a,e,*]

Apolipoprotein E, a protein component of blood lipid particles, plays an important role in lipid transport. Different mutations in the apolipoprotein E gene have been associated with various clinical phenotypes. In an initiated study of Qataris, we observed that 17% of the African-derived genetic subgroup were heterozygotes for a rare Arg145Cys (R145C) variant that functions as a dominant trait with incomplete penetrance associated with type III hyperlipoproteinemia. On the basis of this observation, we hypothesized that the R145C polymorphism might be common in African-derived populations. The prevalence of the R145C variant was assessed worldwide in the "1000 Genomes Project" and in 1,012 whites and 1,226 African-Americans in New York, New York. The 1000 Genomes Project data demonstrated that the R145C polymorphism is rare in non−African-derived populations but present in 5% to 12% of Sub-Saharan African-derived populations. The R145C polymorphism was also rare in New York whites (1 of 1,012, 0.1%); however, strikingly, 53 of the 1,226 New York African-Americans (4.3%) were R145C heterozygotes. The lipid profiles of the Qatari and New York R145C heterozygotes were compared with those of controls. The Qatari R145C subjects had higher triglyceride levels than the Qatari controls ($p < 0.007$) and the New York African-American R145C subjects had an average of 52% greater fasting triglyceride levels than the New York African-American controls ($p < 0.002$). From these observations, likely millions of people worldwide derived from Sub-Saharan Africans are apolipoprotein E R145C. In conclusion, although larger epidemiologic studies are necessary to determine the long-term consequences of this polymorphism, the available evidence suggests it is a common cause of a mild triglyceride dyslipidemia. © 2013 Elsevier Inc. All rights reserved. (Am J Cardiol 2013;■:■−■)

Apolipoprotein E (ApoE), a major component of blood chylomicrons, very-low-density lipoprotein, and high-density lipoprotein (HDL) particles,[1−4] has several alleles that differ by point mutations that are associated with a range of clinical phenotypes (Supplemental Table 1). As part of a study to assess the genetic variations of medical importance among Qataris, a population that evolved at the migration crossroads of Africa and characterized by a high prevalence of type 2 diabetes mellitus, obesity, and cardiovascular disease,[5,6] we observed a high frequency of a point mutation in amino acid 145 of ApoE, in which arginine is substituted by cysteine (R145C). Since its discovery in 1982, the mutation has been described in 32 subjects (Supplemental Table 2) as inherited in a dominant fashion with incomplete penetrance[7,8] and hypothesized to be a rare cause of type III hyperlipoproteinemia. The Qatari population is composed of 3 distinct genetic subpopulations, including Arab, Persian, and African (Q3).[5] Recognizing that all the R145C variants were in Qataris of African genetic ancestry, we hypothesized that the R145C polymorphism might be far more common in those of African descent than previously recognized. To assess this, we evaluated the genomes represented by the "1000 Genomes Project" and of 1,012 whites and 1,226 African-Americans in the New York City Metropolitan Area. The prevalence of the mutation in the study population suggests that ApoE R145C might put large numbers of Africans and African descendants worldwide at risk of a mild triglyceride dyslipidemia.

Methods

Under protocols approved by the Weill Cornell New York and Weill Cornell-Qatar institutional review boards and the Hamad Medical Corporation Ethics Committee

[a]Department of Genetic Medicine, Weill Cornell Medical College, New York, New York; [b]Department of Genetic Medicine, Weill Cornell Medical College-Qatar, Doha, Qatar; [c]Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, New York; [d]Department of Medicine, Hamad Medical Corporation, Doha, Qatar; and [e]Department of Medicine, Weill Cornell Medical College, New York, New York. Manuscript received August 2, 2013; revised manuscript received and accepted September 27, 2013.

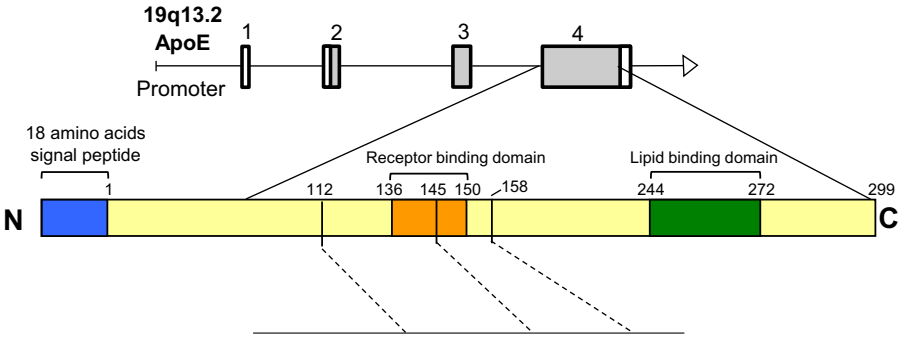
These studies were supported, in part, by Weill Cornell Medical College-Qatar and the Qatar Foundation, Doha, Qatar; and National Institutes of Health grant UL1-RR024996. Dr. Rodriguez-Flores was supported, in part, by National Institutes of Health grant T32-HL094284.

Maen Abou Ziki and Yael Strulovici-Barel contributed equally to this study.

See page 7 for disclosure information.

*Corresponding author: Tel: (646) 962-4363; fax: (646) 962-0220.
E-mail address: geneticmedicine@med.cornell.edu (R.G. Crystal).

0002-9149/13/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.amjcard.2013.09.021



	Amino acid		
ApoE allele	**112**	**145**	**158**
E2	Cys	Arg	Cys
E3	Cys	Arg	Arg
E4	Arg	Arg	Arg
E3 R145C	Cys	**Cys**	Arg
E4 R145C	Arg	**Cys**	Arg

Figure 1. ApoE gene, common alleles, and the Arg145Cys (R145C) variant. ApoE is a 3.1-kb gene composed of 4 exons located on the long arm of chromosome 19. The gene encodes a 317-amino acid precursor protein; after the signal peptide is removed, the mature 299-amino acid ApoE protein is secreted. The *white areas* represent 5′ and 3′ noncoding regions; *shaded areas* represent coding regions; and *vertical lines* in exons 2 and 4 indicate the start and stop sites, respectively. More than 95% of ApoE variations worldwide result from 2 single nucleotide polymorphisms in exon 4, resulting in amino acid substitutions at positions 112 and 158, dictating the common ApoE2, ApoE3, and ApoE4 alleles. A C→T transition at single nucleotide polymorphism rs769455, corresponding to amino acid 145 within the positively charged receptor-binding domain, gives rise to alleles ApoE3R145C and ApoE4R145C, depending on whether the R145C variant is on the E3 or E4 allele.

(Doha, Qatar), all participants in Qatar and New York signed informed consent documents for participation in the present study. Three populations were assessed: Qataris (n = 228; subgrouped into 3 distinct genetic subpopulations: Arabian, n = 123; Persian, n = 82; and African [Q3], n = 23), worldwide subjects from the 1000 Genomes Project (n = 1,094), and whites (n = 1,012) and African-Americans (n = 1,226) in the New York metropolitan area (Supplemental Methods).

ApoE variants were identified in the Qatari and New York African-American populations using polymerase chain reaction (Supplemental Table 3) and sequencing, when necessary. The ApoE variants in the 1000 Genomes Project data were identified from the single nucleotide polymorphisms and, when available, sequences (Supplemental Methods).

The clinical phenotypes of the Qatari Q3 R145C were identified from a review of medical records, including 4 to 14 fasting lipid profiles within 7 to 9 years (i.e., triglycerides, cholesterol, low-density lipoprotein [LDL] cholesterol, HDL cholesterol; Supplemental Table 4). The Q3 R145C subjects were compared with 15 Q3 controls for whom lipid levels were available (1 to 10 fasting lipid profiles within 1 to 9 years).

Of the 53 New York African-American R145C, 23 had fasting routine lipid values available from the medical records (73% with 1 measurement and 27% with 2 to 47 measurements within 2 to 12 years). Of the African-American controls, 72 randomly chosen subjects had fasting lipid studies retrieved from their medical records (71% had 1 study and 29% had 2 to 36 studies within 1 to 12 years). Attempts were made to interview all 53 New York African-American R145C subjects. Of these 53, 16 (30%) returned for additional studies. Of these 16, 5 also had previous values available from their medical records. The fasting plasma from these 16 R145C subjects was assessed for lipoprotein subclass profiling, hemoglobin A1C, high-sensitivity C-reactive protein, lipoprotein (a), ApoA1, ApoB, thyroid-stimulating hormone, and glucose using proton nuclear magnetic resonance spectroscopy (Liposceince, Raleigh, North Carolina), and for LDL electrophoresis and subfractionation (Berkeley HeartLab, Alameda, Calif).

The fasting lipid studies of the R145C subjects and controls were compared using 2 methods: (1) using all mean values from each subject, with a single-factor analysis of variance (ANOVA) and (2) weighting the mean value for each subject by the variance in mean response, with a nested ANOVA (Supplemental Methods).

Results

ApoE2, ApoE3, and ApoE4 represent >95% of ApoE alleles worldwide.[1,2] In most populations, ApoE3 is the most common, followed by ApoE4 and then ApoE2[4,9] (Figure 1). Of the common ApoE alleles in the overall Qatari population, ApoE3 dominated, with ApoE4 more common than ApoE2 (Table 1). However, in the context of the Qatari genetic subpopulations,[5] the proportion of the common ApoE alleles differed ($p < 10^{-3}$, chi-square), with the greatest percentage of the ApoE3 allele in the Persian group (93%), the greatest percentage of the ApoE2 allele in the Q3 (African) group (11%), and the greatest percentage of the ApoE4 allele also in the Q3 group (15%).

Of the 456 Qatari ApoE alleles assessed, 4 (0.9%) had the R145C polymorphism (Table 1). All were on the ApoE3 background, all were heterozygotes, and all were in the Q3

Table 1
ApoE genotypes and alleles in Qatari population

ApoE Genotypes and Alleles*	All Qataris	Q1 (Arab)	Q2 (Persian)	Q3 (African)
Genotype				
Total	228 (100)	123 (100)	82 (100)	23 (100)
E2/E2	1 (0.4)	1 (0.8)	0 (0)	0 (0)
E3/E3	169 (74.1)	91 (74.0)	70 (85.3)	8 (34.8)
E4/E4	1 (0.4)	1 (0.8)	0 (0)	0 (0)
E2/E3	13 (5.7)	3 (2.4)	6 (7.3)	4 (17.4)
E2/E4	0 (0)	0 (0)	0 (0)	0 (0)
E3/E4	38 (16.7)	26 (21.1)	6 (7.3)	6 (26.1)
E3/E5†	1 (0.4)	1 (0.8)	0 (0)	0 (0)
E2R150H/E2‡	1 (0.4)	0 (0)	0 (0)	1 (4.3)
E3R145C/E3§	3 (1.3)	0 (0)	0 (0)	3 (13.0)
E3R145C/E4‖	1 (0.4)	0 (0)	0 (0)	1 (4.3)
All R145C heterozygotes	4 (1.8)	0 (0)	0 (0)	4 (17.4)
Alleles				
Total	456 (100)	246 (100)	164 (100)	46 (100)
E2	16 (3.5)	5 (2.0)	6 (3.7)	5 (10.9)
E3	393 (86.2)	212 (86.2)	152 (92.7)	29 (63.0)
E4	41 (9.0)	28 (11.4)	6 (3.7)	7 (15.2)
E5	1 (0.2)	1 (0.4)	0 (0)	0 (0)
E2R150H	1 (0.2)	0 (0)	0 (0)	1 (2.2)
E3R145C	4 (0.9)	0 (0)	0 (0)	4 (8.7)
All R145C heterozygotes	4 (0.9)	0 (0)	0 (0)	4 (8.7)

Data are presented as n (%) of the population.

* The DNA for all Qataris was first assessed using TaqMan polymerase chain reaction at single nucleotide polymorphisms rs429358 (amino acid 112), rs769455 (amino acid 145), and rs7412 (amino acid 158). This was followed by sequencing of all ApoE exons and flanking introns. If heterozygosity was present at 2 single nucleotide polymorphisms in the same subject, cloning and complete sequencing was performed to unambiguously identify the genotype.

† The E5 allele (E212K on the E3 background) has been previously described[10]; this subject had no dyslipidemia.

‡ The E2R150H (R150H substitution on the E2 background) allele was novel to the present study; this subject had no dyslipidemia.

§ See Supplemental Table 4 for demographics and values for each subject of the 3 E3R145C/E3 heterozygotes (age 52.7 ± 2.8, 1 man and 2 women, body mass index 35.3 ± 1.6 kg/m^2).

‖ For the E3R145C/E4 heterozygote, 1 subject was a 40-year-old woman, with a body mass index of 48.6 kg/m^2; see Supplemental Table 4 for additional details.

(African) subpopulation, representing 17.4% of all Q3 subjects. All 4 subjects had type 2 diabetes, all were obese, 1 had cataracts, 2 had cardiovascular disease, and 1 had kidney disease (Supplemental Table 4). None had thyroid disease or xanthomas; 3 had hypertriglyceridemia (mean level 193 to 465 mg/dl), 2 of the 4 had hypercholesterolemia, 1 had low HDL cholesterol, and 3 of the 4 had elevated LDL cholesterol. Of the 15 Q3 Qatari controls, 4 (27%) had type 2 diabetes compared with 100% of the Qatari R145C subjects ($p < 0.04$). The incidence of xanthomas, cataracts, hypertension, and thyroid disease was similar in the control group and R145C group ($p > 0.1$, for all comparisons). The Qatari R145C triglyceride levels were significantly greater than those in the Qatari controls ($p < 0.006$, single-factor ANOVA, $p < 0.007$ nested ANOVA). The cholesterol, HDL, and LDL levels were not significantly different between the 2 groups ($p > 0.2$, all comparisons, single-factor or nested ANOVA; Figure 2).

Assessment of the 1000 Genomes Project populations revealed that 12 of the 97 (12%) Luhya subjects in the Webuye, Kenya population and 7 of the 88 (8%) Yoruban subjects in Ibadan, Nigeria were R145C (Table 2). In addition, 3 of 61 (5%) of African ancestry in the Southwest United States population were R145C. The R145C polymorphism was not observed in the British, Finnish, Northern and Western European ancestry, Iberian populations in Spain, Toscani in Italy, Han Chinese in Beijing, China, Han Chinese South, Japanese in Tokyo, Japan, or Colombian in Medellin, Colombia, populations and was only rarely present in the Puerto Rican and Mexican populations (1.5% to 1.8%).

Consistent with our hypothesis, 53 of 1,226 (4.3%) New York African-Americans were R145C heterozygotes (Table 3). Most were on the ApoE3 allele (ApoE3>> ApoE4). One subject with mixed African-American-Hispanic ancestry (0.1% of the population) had the E4E13 K.R145C allele, novel to the present study. Of the 1,012 whites, none had the R145C polymorphism on the common ApoE2, ApoE3, or ApoE4 alleles. One white subject had the ApoE3E13 K.R145 C allele ($ApoE4_{Philadelphia}$, associated with type III hyperlipidemia with incomplete dominance[10]; Supplemental Tables 1 and 2).

Of the 53 African-American R145C subjects, no difference was found compared with the 72 African-American controls in age, gender, body mass index, human immunodeficiency virus (HIV) status, or the proportion of those with type 2 diabetes, thyroid disease, or cataracts ($p > 0.4$, for all comparisons). The African-American R145C subjects had 52% greater triglyceride levels than the controls ($p < 0.001$, single-factor ANOVA; $p < 0.002$, nested ANOVA; Figure 2; Supplemental Table 5). The triglyceride levels remained significantly greater in the R145C group when considering HIV status as a covariate ($p < 0.007$ single factor ANOVA, $p < 0.003$ nested ANOVA). The cholesterol and HDL cholesterol levels of the R145C and control groups were not significantly different ($p > 0.1$, both comparisons, single-factor or nested ANOVA, with and without considering HIV status). The LDL cholesterol levels were borderline lower in the R145C subjects ($p < 0.03$, single-factor ANOVA; $p > 0.06$, nested ANOVA; $p < 0.03$, single-factor ANOVA, and $p > 0.1$, nested ANOVA when considering HIV status). Of the subgroup of African-American R145C subjects with average triglyceride levels greater than the normal range (>150 mg/dl), the average lipid profile included triglycerides 276 ± 98 mg/dl, cholesterol 198 ± 29 mg/dl, LDL cholesterol 96 ± 25 mg/dl, and HDL cholesterol 43 ± 15 mg/dl.

Of the 53 African-American R145C subjects, 16 were available for additional fasting lipid analysis (Supplemental Table 6). The most striking observation was an increase in the levels of small very-low-density lipoprotein particle levels and HDL particle size (31% and 38% of the population studied, respectively). To a variable degree, some of the R145C subjects had an increase in the levels of LDL particles and HDL particles. LDL gel electrophoresis identified 31% of R145C subjects with an LDL IIIa + b peak in the intermediate or high cardiovascular risk range. Finally, 25% of the R145C subjects had elevated levels of LDL

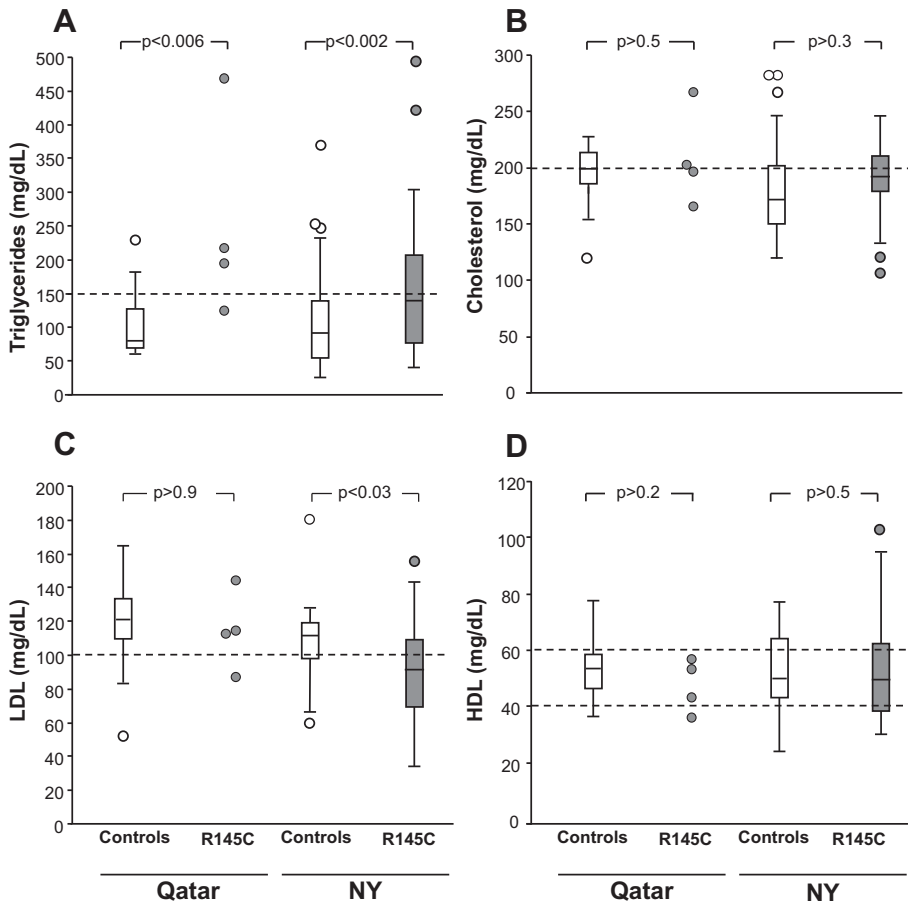


Figure 2. Fasting lipid studies of ApoE R145C compared with controls in Qatar and New York. *(A)* Triglycerides, *(B)* cholesterol, *(C)* LDL cholesterol, and *(D)* HDL cholesterol. All Qataris were of the Q3 (African) genetic subgroup. The Qatari R145C subjects are represented as individual data points; the Qatari Q3 controls and New York African-American R145C subjects and controls are represented as box and whisker plots, with the median and 4 quartiles indicated. Outliers indicate greater and less than the average ± 2 SDs for each group. For both the Qatari and New York data, if >1 value was available, the average value for each subject was used. p Values were calculated using single-factor ANOVA. *Dashed lines* indicate the high and low normal levels. *(A,B)* Qatari controls, n = 15; Qatari R145C, n = 4; New York African-American controls, n = 72; and New York African-American R145C subjects, n = 30; *(C,D)* Qatari controls, n = 14; Qatari R145C subjects, n = 4; New York African-American controls, n = 24; and New York African-American R145C subjects, n = 26.

particles, 50% had elevated lipoprotein (a) levels, 44% had elevated ApoA1 levels, and 31% had high-sensitivity C-reactive protein levels in the high-risk range.

Discussion

Discovered in the early 1970s as a constituent of very-low-density lipoprotein, ApoE is a polymorphic protein also found in chylomicrons, HDL, and remnant particles of chylomicrons and very-low-density lipoprotein.[1–3,11] ApoE plays an important role in the overall maintenance of cholesterol homeostasis by stabilization of these particles in the circulation and enhancing uptake by the liver and other tissues.[1–3,12] ApoE also participates in cholesterol efflux from macrophages,[13,14] prevents platelet aggregation,[15] and inhibits endothelial cell and T-lymphocyte proliferation.[15–17] The ApoE knockout mouse has been used as an in vivo model for atherosclerosis.[18] The LDL receptor binds to ApoE3 with high affinity; this is markedly lower with ApoE2, which has only 2% the binding affinity of ApoE3 and ApoE4.[19] Only 1% to 10% of ApoE2/ApoE2 homozygotes will develop type III hyperlipoproteinemia.[3,8,20]

In addition to the common ApoE2, ApoE3, and ApoE4 alleles, 38 rare ApoE variants have been identified, associated with a range of clinical phenotypes, including dyslipidemias and the risk of cardiovascular disease[3] (Supplemental Table 1). Of these rare variants, R145C has been reported in 32 cases in the past 3 decades, all in subjects treated in lipid-related clinics or their families (Supplemental Table 2), and has been described as a dominant mutation with incomplete penetrance in association with familial type III hyperlipidemia.[7,8] The R145C variant reduces the positive charge of this domain,[8,21] because it has 40% of the binding affinity of ApoE3 to the LDL receptor.[8,21] This discrepancy in disease manifestation might be explained by considering the role of other ApoE receptors in the clearance of remnant particles from the circulation, including the LDL-like receptor and heparin sulfate proteoglycans.[3,8,12] Although the R145C variant has better binding affinity to the LDL receptor than ApoE2, it binds much more poorly to heparin sulfate

Table 2
ApoE genotypes and alleles in subjects from 1000 Genomes Database*

Variable	Europe†					Asia‡			America§				Africa‖		All 1000 Genome Project Subjects
	CEU	FIN	GBR	IBS	TSI	CHB	CHS	JPT	ASW	CLM	MXL	PUR	LWK	YRI	
Genotype															
Total	87 (100)	93 (100)	89 (100)	14 (100)	98 (100)	97 (100)	100 (100)	89 (100)	61 (100)	60 (100)	66 (100)	55 (100)	97 (100)	88 (100)	1,094 (100)
E2/E2	0	0	1 (1.1)	0	0	0	0	0	1 (1.6)	1 (1.7)	0	0	1 (1.0)	0	4 (0.4)
E2/E3	10 (11.5)	13 (14.0)	10 (11.2)	0	8 (8.2)	18 (18.6)	16 (16.0)	8 (9.0)	10 (16.4)	6 (10.0)	8 (12.1)	7 (12.7)	8 (8.2)	15 (17.0)	137 (12.4)
E2/E4	1 (1.1)	0	2 (2.2)	1 (7.1)	1 (1.0)	2 (2.1)	1 (1.0)	0	4 (6.6)	1 (1.7)	0	0	6 (6.2)	9 (10.2)	28 (2.7)
E3/E3	51 (58.6)	50 (53.8)	54 (60.7)	9 (64.3)	72 (73.5)	57 (58.8)	66 (66.0)	67 (75.3)	28 (45.9)	35 (58.3)	46 (69.7)	36 (65.5)	27 (27.8)	35 (39.8)	633 (57.8)
E3/E4	24 (27.6)	27 (29.0)	19 (21.3)	4 (28.6)	17 (17.3)	20 (20.6)	17 (17.0)	12 (13.5)	13 (21.3)	16 (26.7)	10 (15.2)	11 (20.0)	31 (32.0)	21 (23.9)	242 (22.2)
E4/E4	1 (1.1)	3 (3.2)	3 (3.4)	0	0	0	0	2 (2.2)	2 (3.3)	1 (1.7)	0	0	5 (5.2)	1 (1.1)	18 (1.6)
E3/E3R145C	0	0	0	0	0	0	0	0	3 (4.9)	0	1 (1.5)	1 (1.8)	2 (2.1)	4 (4.5)	11 (1.0)
E4/E3R145C	0	0	0	0	0	0	0	0	0	0	0	0	4 (4.1)	2 (2.3)	6 (0.5)
E3/E4R145C	0	0	0	0	0	0	0	0	0	0	0	0	2 (2.1)	0	2 (0.2)
E3/E4R158C	0	0	0	0	0	0	0	0	0	0	1 (1.5)	0	2 (2.1)	0	3 (0.3)
E3R145C/E4R145C	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.0)	0	1 (0.1)
E4/E4R145C	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.0)	1 (1.1)	2 (0.2)
E4/E4R145CR158C	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.0)	0	1 (0.1)
E4/E4R158C	0	0	0	0	0	0	0	0	0	0	0	0	5 (5.2)	0	5 (0.5)
E4R145C/E4R158C	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.0)	0	1 (0.1)
Total R145C genotypes	0	0	0	0	0	0	0	0	3 (4.9)	0	1 (1.5)	1 (1.8)	12 (12.4)	7 (8.0)	24 (2.2)
Alleles															
Total	174 (100)	186 (100)	178 (100)	28 (100)	196 (100)	194 (100)	200 (100)	178 (100)	122 (100)	120 (100)	132 (100)	110 (100)	194 (100)	176 (100)	2,188 (100)
E2	11 (6.3)	13 (7.0)	14 (7.9)	1 (3.6)	9 (4.6)	20 (10.3)	17 (8.5)	8 (4.5)	16 (13.1)	9 (7.5)	8 (6.1)	7 (6.4)	16 (8.2)	24 (13.6)	173 (7.9)
E3	136 (78.2)	140 (75.3)	137 (77.0)	22 (78.6)	169 (86.2)	152 (78.4)	165 (82.5)	154 (86.5)	82 (67.2)	92 (76.7)	112 (84.8)	91 (82.7)	99 (51.0)	110 (62.5)	1,661 (75.9)
E4	27 (15.5)	33 (17.7)	27 (15.2)	5 (17.9)	18 (9.2)	22 (11.3)	18 (9.0)	16 (9.0)	21 (17.2)	19 (15.8)	10 (7.6)	11 (10.0)	58 (29.9)	35 (19.9)	320 (14.6)
E3R145C	0	0	0	0	0	0	0	0	3 (2.5)	0	1 (0.8)	1 (0.9)	7 (3.6)	6 (3.4)	18 (0.8)
E4R145C	0	0	0	0	0	0	0	0	0	0	0	0	5 (2.6)	1 (0.6)	6 (0.3)
E4R145C.R158C¶	0	0	0	0	0	0	0	0	0	0	0	0	1 (0.5)	0	1 (0.05)
E4R158C#	0	0	0	0	0	0	0	0	0	0	1 (0.8)	0	8 (4.1)	0	9 (0.4)
Total R145 alleles	0	0	0	0	0	0	0	0	3 (2.5)	0	1 (0.8)	1 (0.9)	13 (6.7)	7 (4.0)	25 (1.1)

Data are presented as n (%) of the population.

* Haplotype frequency observed in 1,094 subjects. Phased haplotypes for 3 ApoE single nucleotide polymorphisms on chromosome 19 were extracted from the May 2011 release of the 1000 Genomes Project data (available at: *ftp://ftptrace.ncbi.nih.gov/1000genomes/ftp/release/20101123/interim_phase1_release/ALL.chr19.phase1.projectConsensus.genotypes.vcf.gz*).

† European populations included subjects of Northern and Western European ancestry (CEU), Finnish from Finland (FIN), British from England and Scotland (GBR), Iberian populations in Spain (IBS), and Toscani in Italy (TSI).

‡ Asian populations included Han Chinese in Beijing, China (CHB), Han Chinese South (CHS), and Japanese in Tokyo, Japan (JPT).

§ American populations included subjects with African ancestry in the Southwest United States (ASW), Colombian in Medellin, Colombia (CLM), Mexican ancestry in Los Angeles, California (MXL), and Puerto Rican in Puerto Rico (PUR).

‖ African Populations included Luhya in Webuye, Kenya (LWK) and Yoruba in Ibadan, Nigeria (YRI).

¶ An allele novel to the present study with 2 mutations on the E4 background, R145C and R158C; thus, E4R145C.R158C.

An allele novel to the present study with 2 mutations compared with E3:C112R, which results in the E4 background and then the R158C mutation; thus, E4R158C.

Table 3
Prevalence of the apolipoprotein (Apo)E Arg145Cys polymorphism among New York City Metropolitan Area white and African-American populations

Genotype*	Population With R145C Allele†	
	White (n = 1,012)	African-American (n = 1,226)
E2/E3R145C	0	5 (0.4)
E3/E3R145C	0	39 (3.2)
E4/E3R145C	0	8 (0.7)
E3/E4E13K.R145C‡	0	1 (0.1)§
E4/E3E13K.R145C‖	1 (0.1)	0
All R145C heterozygotes	1 (0.1)	53 (4.3)

* TaqMan was used to identify the R145C allele; all R145C were then sequenced. In the setting of a double mutation, the samples were cloned and sequenced to determine the "cis" or "trans" relation.

† Subjects with self-reported ethnicities from the New York City Metropolitan Area.

‡ An allele novel to the present study with 2 mutations in the amino acid sequence resulting in substitution of lysine (K) for glutamate (E) at residue 13 and cysteine for arginine at residue 145 (R145C).

§ This subject was of African-American-Hispanic mixed ancestry.

‖ $ApoE_{Philadelphia}$ a double mutant of E13K and R145C on an ApoE3 background; this allele has been previously described.[29,30]

proteoglycans, compromising the LDL-like receptor internalization pathway and decreasing the efficiency of the ApoE interaction with the LDL receptor.[8,21]

In a study investigating the genetic variations of the Qatari genome, the assessment of 228 Qataris identified that 4 of 23 (17%) with a Q3 (Sub-Saharan African) genetic background were heterozygotes for the R145C polymorphism on the ApoE3 background. In contrast, Qataris from an Arabian or Persian genetic subgroup did not have this ApoE variation. This led to the hypothesis that the ApoE R145C variant might be prevalent in African-derived populations and, if so, could be a risk factor for dyslipidemia far more common than would be expected from the rare reports of this variant in the published data. This was verified by assessment of the May 2011 release of the 1000 Genomes Project data, demonstrating that the R145C variant is rare to nonexistent in populations that are not African or descended from African populations but common (5% to 12%) among African-derived populations.

This observation was further confirmed in subjects randomly recruited in the New York Metropolitan area for a study on smoking-related lung health. The data demonstrated that, although rare (0.1%) in the white population, 4.3% of African-Americans in the New York Metropolitan region were heterozygous for the R145C allele. On the average, the New York African-American R145C subject had fasting dyslipidemia, with fasting triglyceride levels 52% greater than those of matched New York African-American controls. Consistent with the dominant, incomplete penetrance of this at-risk allele, variable proportions of the African-American R145C subjects also had elevated levels of very-low-density lipoprotein, LDL, and HDL particles; elevated levels of ApoA1, lipoprotein (a), and high-sensitivity C-reactive protein at the high risk ranges; and an elevated LDL gel electrophoresis IIIa + b peak in the intermediate and high cardiovascular risk range. The high rate of R145C among Q3 Qataris compared with that of the 1000 Genomes Project African-descendant populations or the New York African-American subjects might have resulted from the Q3 disassortative mating pattern that exceeds that of other African-descendant groups and leads to a greater frequency of heterozygosity.[5,22]

From the available data, it is clear that the R145C variant carries an increased risk of dyslipidemia; however, the extent of that risk is not clear. All previous descriptions of the R145C allele have been in subjects identified in lipid clinics or their family members (Supplemental Table 2; see also Supplemental Methods for ApoE Nomenclature). No studies have been published of the prevalence of the R145C variant among different populations. There appears to be no increased risk of R145C homozygosity compared with heterozygosity; most cases have been associated with elevated levels of triglycerides and cholesterol and low levels of HDL cholesterol and, in some, xanthomas and/or cardiovascular disease (Supplemental Table 2). The overall consensus has been that the R145C variant conveys increased risk inherited in a dominant fashion with incomplete penetrance,[7] likely dependent on environmental factors.

Of the Qatari R145C subjects, the triglyceride levels were significantly greater than those of the control group; however, no significant differences were found in the cholesterol, HDL cholesterol, or LDL cholesterol levels between the 2 groups. All 4 were obese and had diabetes, 2 had cardiovascular disease, 3 had hypertension, and 1 had cataracts (Supplemental Table 4).

In New York, the identification of the R145C allele among African-Americans was also an unbiased assessment of the risk associated with the R145C allele, because all subjects with the R145C allele were obtained from a random sample of subjects in the New York Metropolitan Area, recruited to a study regarding lung health. On average, the African-American R145C subjects had 52% significantly greater triglyceride levels compared with the African-American controls but no differences in the cholesterol or HDL cholesterol levels and a borderline decrease in LDL cholesterol. A more detailed assessment of a subgroup of the African-American R145C subjects demonstrated that 31% of the population studied had higher levels of small very-low-density lipoprotein particles than the normal range (Supplemental Table 6). Elevated levels of small very-low-density lipoprotein particles have been associated with an increased risk of coronary artery disease and peripheral arterial disease.[23]

It appears, therefore, that the R145C allele is an at-risk allele for mild dyslipidemia, with a subgroup of the R145C heterozygotes developing a more severe dyslipidemia in accordance with other genetic variations, age, diet, body mass index, diabetes status, thyroid hormone levels, and medications.[3,24] Although HIV infection can be associated with dyslipidemia,[25,26] no difference was found in the lipid levels between the HIV-positive and HIV-negative R145C African-Americans, and ANOVA analysis demonstrated that HIV status could not explain the elevated triglyceride levels in the R145C heterozygotes.

The overall risk of dyslipidemia conveyed by the R145C allele appears to be mild, and additional epidemiologic

studies are needed to confirm the observations described in the present study. However, remembering that there are approximately 39 million African-Americans in the United States[27] and approximately 836 million Sub-Saharan Africans, and large numbers of people derived from this population, worldwide,[28] the observations from the present study suggest 1.7 million African-Americans or 36 million Sub-Saharan Africans worldwide could have the R145C allele, likely contributing to the global risk of dyslipidemias and the associated risk of cardiovascular disease.

Acknowledgment: We thank Alya A. Al-Shakaki, BS, and Katrina Bandong, BA, for help with this study; DN McCarthy and N Mohamed for help in preparing this report; and Mohammad Fathy Saoud, President of Qatar Foundation, and Her Highness Sheikha Moza Bint Nasser, Chair of Qatar Foundation, for their continued encouragement and support.

Disclosures

The authors have no conflicts of interest to disclose.

Supplementary Data

Supplementary data related to this article can be found, in online version, at http://dx.doi.org/10.1016/j.amjcard.2013.09.021.

1. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 1988;8:1−21.
2. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. *Am J Epidemiol* 2002;155:487−495.
3. Mahley RW, Rall SC Jr. Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Bases of Inherited Disease. 7th ed. New York: McGraw-Hill, 1995:1953−1980.
4. Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000;1:507−537.
5. Hunter-Zinck H, Musharoff S, Salit J, Al-Ali KA, Chouchane L, Gohar A, Matthews R, Butler MW, Fuller J, Hackett NR, Crystal RG, Clark AG. Population genetic structure of the people of Qatar. *Am J Hum Genet* 2010;87:17−25.
6. Bener A, Zirie M, Janahi IM, Al-Hamaq AO, Musallam M, Wareham NJ. Prevalence of diagnosed and undiagnosed diabetes mellitus and its risk factors in a population-based study of Qatar. *Diabetes Res Clin Pract* 2009;84:99−106.
7. de Villiers WJ, van der Westhuyzen DR, Coetzee GA, Henderson HE, Marais AD. The apolipoprotein E2 (Arg145Cys) mutation causes autosomal dominant type III hyperlipoproteinemia with incomplete penetrance. *Arterioscler Thromb Vasc Biol* 1997;17:865−872.
8. Ji ZS, Fazio S, Mahley RW. Variable heparan sulfate proteoglycan binding of apolipoprotein E variants may modulate the expression of type III hyperlipoproteinemia. *J Biol Chem* 1994;269:13421−13428.
9. Gerdes LU, Gerdes C, Hansen PS, Klausen IC, Faergeman O, Dyerberg J. The apolipoprotein E polymorphism in Greenland Inuit in its global perspective. *Hum Genet* 1996;98:546−550.
10. Feussner G, Scharnagl H, Scherbaum C, Acar J, Dobmeyer J, Lohrmann J, Wieland H, Marz W. Apolipoprotein E5 (Glu212–>Lys): increased binding to cell surface proteoglycans but decreased uptake and lysosomal degradation in cultured fibroblasts. *J Lipid Res* 1996;37:1632−1645.
11. Shore VG, Shore B. Heterogeneity of human plasma very low density lipoproteins: separation of species differing in protein components. *Biochemistry* 1973;12:502−507.
12. Mahley RW. Heparan sulfate proteoglycan/low density lipoprotein receptor-related protein pathway involved in type III hyperlipoproteinemia and Alzheimer's disease. *Isr J Med Sci* 1996;32:414−429.
13. Cullen P, Cignarella A, Brennhausen B, Mohr S, Assmann G, von EA. Phenotype-dependent differences in apolipoprotein E metabolism and in cholesterol homeostasis in human monocyte-derived macrophages. *J Clin Invest* 1998;101:1670−1677.
14. Smith JD, Miyata M, Ginsberg M, Grigaux C, Shmookler E, Plump AS. Cyclic AMP induces apolipoprotein E binding activity and promotes cholesterol efflux from a macrophage cell line to apolipoprotein acceptors. *J Biol Chem* 1996;271:30647−30655.
15. Riddell DR, Graham A, Owen JS. Apolipoprotein E inhibits platelet aggregation through the L-arginine:nitric oxide pathway: implications for vascular disease. *J Biol Chem* 1997;272:89−95.
16. Mistry MJ, Clay MA, Kelly ME, Steiner MA, Harmony JA. Apolipoprotein E restricts interleukin-dependent T lymphocyte proliferation at the G1A/G1B boundary. *Cell Immunol* 1995;160:14−23.
17. Vogel T, Guo NH, Guy R, Drezlich N, Krutzsch HC, Blake DA, Panet A, Roberts DD. Apolipoprotein E: a potent inhibitor of endothelial and tumor cell proliferation. *J Cell Biochem* 1994;54:299−308.
18. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb* 1994;14:133−140.
19. Weisgraber KH, Innerarity TL, Mahley RW. Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cysteine-arginine interchange at a single site. *J Biol Chem* 1982;257:2518−2521.
20. Rall SC Jr, Mahley RW. The role of apolipoprotein E genetic variants in lipoprotein disorders. *J Intern Med* 1992;231:653−659.
21. Rall SC Jr, Weisgraber KH, Innerarity TL, Mahley RW. Structural basis for receptor binding heterogeneity of apolipoprotein E from type III hyperlipoproteinemic subjects. *Proc Natl Acad Sci U S A* 1982;79:4696−4700.
22. Raven P, Johnson G, Mason KA, Losos JB, Singer SS. Genes within populations. In: Biology. 9th ed. New York: McGraw Hill, 2010:401−402.
23. Rizzo M, Berneis K. Lipid triad or atherogenic lipoprotein phenotype: a role in cardiovascular prevention? *J Atheroscler Thromb* 2005;12:237−239.
24. Morganroth J, Levy RI, Fredrickson DS. The biochemical, clinical, and genetic features of type III hyperlipoproteinemia. *Ann Intern Med* 1975;82:158−174.
25. Grunfeld C, Kotler DP, Hamadeh R, Tierney A, Wang J, Pierson RN. Hypertriglyceridemia in the acquired immunodeficiency syndrome. *Am J Med* 1989;86:27−31.
26. Periard D, Telenti A, Sudre P, Cheseaux JJ, Halfon P, Reymond MJ, Marcovina SM, Glauser MP, Nicod P, Darioli R, Mooser V. Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors: the Swiss HIV Cohort Study. *Circulation* 1999;100:700−705.
27. Overview of race and Hispanic origin: 2010. 2010 Census Briefs 2011. Available at: *http://www.census.gov/prod/cen2010/briefs/c2010br-02.pdf*. Accessed on September 1, 2013.
28. 2009 World population data sheet. Population Reference Bureau 2011;1−19. Available at: *http://www.prb.org/pdf09/09wpds_eng.pdf*. Accessed on September 1, 2013.
29. Lohse P, Rader DJ, Brewer HB Jr. Heterozygosity for apolipoprotein E-4Philadelphia(Glu13−–Lys, Arg145−–Cys) is associated with incomplete dominance of type III hyperlipoproteinemia. *J Biol Chem* 1992;267:13642−13646.
30. Lohse P, Mann WA, Stein EA, Brewer HB Jr. Apolipoprotein E-4Philadelphia (Glu13−–Lys, Arg145−–Cys): homozygosity for two rare point mutations in the apolipoprotein E gene combined with severe type III hyperlipoproteinemia. *J Biol Chem* 1991;266:10479−10484.