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Prevalence of the ApoE Arg145Cys Dyslipidemia At-risk Polymorphism in African-derived Populations

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Abstract

Apolipoprotein E (ApoE), a protein component of blood lipid particles, plays an important role in lipid transport. Different mutations in the ApoE 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. Based on this observation, we hypothesized that the R145C polymorphism may be common in African-derived populations. The prevalence of the R145C variant was assessed worldwide in the 1000 Genomes Project (1000G) and in 1012 Caucasians and 1226 African-Americans in New York City. The 1000G data demonstrated that the R145C polymorphism is rare in non-African derived populations, but present in 5–12% of sub-Saharan African-derived populations. The R145C polymorphism was also rare in New York City Caucasians (1/1012, 0.1%), but strikingly, 53 (4.3%) of 1226 New York City African-Americans were R145C heterozygotes. Lipid profiles of the Qatari and New York R145C were compared to controls. Qatari R145C had higher triglyceride levels compared to Qatari controls ($p < 0.007$) and NY African-Americans R145C had an average of 52% higher fasting triglyceride levels compared to NY African-American controls ($p < 0.002$). Based on these observations, there are likely millions of people worldwide derived from Sub-Saharan Africans that are ApoE R145C. In conclusion, while 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.

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Keywords

Apolipoprotein E; polymorphism; lipids; dyslipidemia; ethnicity

Introduction

Apolipoprotein E (Apo E), a major component of blood chylomicrons, very low density lipoprotein (VLDL) and high density lipoprotein (HDL) particles¹⁻⁴, 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 genetic variations of medical importance among Qataris, a population that evolved at the migration crossroads of Africa and is characterized by a high prevalence of type 2 diabetes, obesity and cardiovascular disease^{5,6}, we observed a high frequency of a point mutation in amino acid 145 of ApoE, where arginine is substituted with cysteine (R145C). Since its discovery in 1982, the mutation has been described in 32 individuals (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 comprised of 3 distinct genetic subpopulations, including Arab (Q1), Persian (Q2) and African (Q3)⁵. Recognizing that all of the R145C variants were in Qataris of African genetic ancestry, we hypothesized that the R145C polymorphism might be far more common in individuals of African descent than previously recognized. To assess this, we evaluated the genomes represented by the 1000 Genomes Project (1000G) and of 1012 Caucasians and 1226 African-Americans in the New York City metropolitan area. The prevalence of the mutation in the studies population suggests that the ApoE R145C may put large numbers of Africans and African descendants worldwide at risk for 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, Doha, Qatar, all individuals in Qatar and New York signed informed consent documents for participation in the study. Three populations were assessed: Qataris (n=228; subgrouped into 3 distinct genetic sub-populations: Q1, Arabian, n=123, Q2, Persian, n=82; Q3, African, n=23), 1000G worldwide subjects (n=1094), and Caucasians (n=1012) and African-Americans (n=1226) in the New York metropolitan area (Supplemental Methods).

ApoE variants were identified in the Qatari, and New York African-American populations using PCR (Supplemental Table 3) and sequencing when necessary. The ApoE variants in the 1000G data were identified from the SNPs and, when available, sequences (Supplemental Methods).

The clinical phenotypes of Qatari Q3 R145C were identified from review of medical records, including 4 to 14 fasting lipid profiles over 7 to 9 yr (triglycerides, cholesterol, low density lipoprotein (LDL)-cholesterol, HDL-cholesterol; Supplemental Table 4). The Q3 R145C were compared to 15 Q3 controls for which lipid levels were available (1 to 10 fasting lipid profiles over 1 to 9 yr).

Of the 53 NY African-American R145C, 23 had fasting routine lipid values available from medical records (73% with 1 measurement; 27% with 2 to 47 measurements over 2 to 12 yr). Of the African-American controls, 72 randomly chosen individuals had fasting lipid studies retrieved from medical records (71% had 1 study, 29% 2 to 36 studies over 1 to 12 yr). Attempts were made to contact all of the New York City African-American R145C. Of these 53, 16 (30%) returned for additional studies; 5/16 also had prior values available from

medical records. Fasting plasma from these 16 R145C individuals was assessed for lipoprotein subclass profiling by proton NMR spectroscopy, hemoglobin A1C (HbA1C), high sensitivity C-reactive protein (hs-CRP), lipoprotein (a) [Lp(a)], ApoA1, ApoB, thyroid stimulating hormone (TSH) and glucose (Liposceince, Raleigh, NC) and for LDL electrophoresis and subfractionation (Berkeley Heart Labs, Alameda, CA).

Fasting lipid studies of R145C individuals vs controls were compared in 2 ways: (1) using all mean values from each individual, with a single factor ANOVA; and (2) weighting the mean value for each individual by the variance in mean response, with a nested ANOVA (Supplemental Methods).

Results

ApoE2, E3 and E4, represent >95% of ApoE alleles worldwide^{1,2}, and in most populations, ApoE3 is the most common, followed by E4 and then E2^{4,9} (Figure 1). Of the common ApoE alleles in the overall Qatari population, E3 dominated, with E4 being more common than E2 (Table 1). However, in the context of the Qatari genetic subpopulations⁵, the proportion of the common ApoE alleles were different ($p < 10^{-3}$, chi squared), with the highest % E3 allele in the Q2 group (93%), highest % E2 allele in the Q3 group (11%) and the highest % E4 allele also in the Q3 group (15%).

Among the 456 Qatari ApoE alleles assessed, 4 (0.9%) had the R145C polymorphism (Table 1). All were on the E3 background, all were heterozygotes and all were on the Q3 (African) subpopulation, representing 17.4% of all Q3 individuals. All 4 had type 2 diabetes, all were obese, 1 had cataracts, 2 had cardiovascular disease, and another had kidney disease (Supplemental Table 4). None had thyroid disease or xanthomas. Three had hypertriglyceridemia (mean levels 193–465 mg/dl), 2 of 4 had hypercholesterolemia, 1 a low HDL-cholesterol and 3 of 4 elevated LDL-cholesterol. Of the 15 Q3 Qatari controls, 4 (27%) had type 2 diabetes, compared to 100% of the Qatari R145C ($p < 0.04$). The incidence of xanthomas, cataracts, hypertension or thyroid disease was similar in the control group and in the R145C group ($p > 0.1$, all comparisons). The Qatari R145C triglyceride levels were significantly higher than the Qatari controls ($p < 0.006$ single factor ANOVA, $p < 0.007$ nested ANOVA). Cholesterol, HDL and LDL levels were not significantly different between the groups ($p > 0.2$, all comparisons, single factor or nested ANOVA, Figure 2).

Assessment of the 1000G populations revealed that, of 97 Luhya subjects in the Webuye, Kenya population, 12 (12%) were R145C, as were 7 of the 88 (8%) Yoruban subjects in Ibadan, Nigeria (Table 2). In addition, 3 of 61 (5%) of African ancestry in the Southwest US population were R145C. The R145C polymorphism was not observed in the GBR, FIN, CEU, IBS, TSI, CHB, CHS, JPT, or CLM populations, and was only rarely present in the Puerto Rican (PUR) and Mexican (MXL) populations (1.5–1.8%).

Consistent with our hypothesis, 53 of 1226 (4%) African-Americans were R145C heterozygotes (Table 3). Most were on the E3 allele ($E3 \gg E4$). One (0.08% of the population, a subject with mixed African-American-Hispanic ancestry) had the E4E13K.R145C allele, novel to this study. Of the 1012 Caucasians, none had the R145C polymorphism on the common E2, E3 or E4 alleles. One had the ApoE3E13K.R145C allele (ApoE4^{Philadelphia}, associated with type III hyperlipidemia with incomplete dominance¹⁰, see Supplemental Tables 1, 2).

Of the 53 African-American R145C, there was no difference compared to the 72 African-American controls in age, gender, BMI, HIV1 status, or the proportion of individuals with type 2 diabetes, thyroid disease or cataracts ($p > 0.4$, all comparisons). The African-American

R145C had 52% higher triglyceride levels than the controls ($p<0.001$ single factor ANOVA, $p<0.002$ nested ANOVA; Figure 2, Supplemental Table 5). Triglyceride levels remained significantly higher in the R145C group when considering HIV1 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 HIV1 status). The LDL-cholesterol levels were borderline lower in R145C ($p<0.03$, single factor ANOVA, $p>0.06$ nested ANOVA; $p<0.03$ single factor ANOVA, $p>0.1$ nested ANOVA when considering HIV1 status). Of the subgroup of African-American R145C 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.

Sixteen of the 53 African-American R145C were available for additional fasting lipid analysis (Supplemental Table 6). The most striking observations were an increase in levels of small VLDL particle levels and HDL particle size (31% and 38% of the population studied, respectively). To a variable degree, some of the R145C had increases in the levels of LDL particles and HDL particles. LDL gel electrophoresis identified 31% of R145C with a LDL IIIa+b peak in the intermediate or high cardiovascular risk range. Finally, 25% of the R145C had elevated levels of IDL particles, 50% had elevated Lp(a) levels, 44% had elevated ApoA1 levels, and 31% had hs-CRP levels in the high risk ranges.

Discussion

Discovered in the early 1970s as a constituent of VLDL, ApoE is a polymorphic protein also found in chylomicrons, HDL and remnant particles of chylomicrons and VLDL^{1-3;11}. ApoE plays an important role in 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 aggregation¹⁵, and inhibits endothelial cell and T-lymphocyte proliferation¹⁵⁻¹⁷. The ApoE knockout mouse is used as an *in vivo* model for atherosclerosis¹⁸. The LDL receptor binds to ApoE3 with high affinity; this is markedly lower with ApoE2 which has only 2% the binding affinity of E3 and E4¹⁹. Interestingly, only 1 to 10% of E2/E2 homozygotes develop type III hyperlipoproteinemia^{3;8;20}.

In addition to the common E2, E3 and E4 alleles, 38 rare ApoE variants have been identified, associated with a range of clinical phenotypes, including dyslipidemias and risk for cardiovascular disease³ (See Supplemental Table 1). Among these rare variants, R145C has been reported in 32 cases in the past 3 decades, all in individuals treated in lipid-related clinics or their families (see Supplemental Table 2), and 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} as it has 40% the binding affinity of E3 to the LDL receptor^{8;21}. This discrepancy in disease manifestation may be explained by considering the role of other ApoE receptors in the clearance of remnant particles from the circulation, including the LDL-like receptor (LRP) and heparin sulfate proteoglycans (HSPG)^{3;8;12}. Although the R145C variant has better binding affinity to the LDL receptor than ApoE2, it binds much more poorly to HSPG, compromising the LRP internalization pathway and decreasing the efficiency of ApoE interaction with the LDL receptor^{8;21}.

In a study to investigate genetic variations of the Qatari genome, 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 (Q1) or Persian (Q2) genetic subgroup did not have this ApoE variation. This led to the hypothesis that the ApoE R145C variant may be prevalent in African-derived populations, and if so, may be a risk factor for dyslipidemia far more common than would be expected from the rare reports of this variant in the literature. This was verified by assessment of the May 2011 release of 1000 Genomes Project data, demonstrating that the R145C variant is rare to non-existent in populations that are not African or African-descendants, but common (5 to 12%) among African-derived populations.

This observation was further confirmed in individuals randomly recruited in the New York Metropolitan area for a study on smoking-related lung health. The data demonstrated that, while rare (0.1%) in the Caucasian population, 4% of African-Americans in the New York Metropolitan region are heterozygous for the R145C allele. On the average, the New York African-American R145C had fasting dyslipidemia, with fasting triglyceride levels 52% above those of matched New York African-American controls. Consistent with dominant, incomplete penetrance of this at-risk allele, variable proportions of the African-Americans R145C also had elevated levels of VLDL, LDL and HDL particles, elevated levels of ApoA1, Lp(a) and hs-CRP 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 in comparison to that of the 1000G African-descendant populations or the NY African-American individuals might be as a result of the Q3 disassortative mating pattern that exceeds that of other African-descendant groups that leads to a higher frequency of heterozygosity^{5:22}.

From the available data, it is clear that the R145C variant carries an increased risk for dyslipidemia, but the extent of that risk is not clear. All prior descriptions of the R145C allele have been in individuals identified in lipid clinics or their family members (Supplemental Table 2; see Supplemental Methods for ApoE nomenclature). There are no studies of the prevalence of the R145C variant among different populations. There appears to be no increased risk of R145C homozygosity compared to heterozygosity; most cases have been associated with elevated levels of triglycerides and cholesterol, low levels of HDL-cholesterol, and, in some, xanthomas and/or cardiovascular disease (See Supplemental Table 2). The overall consensus is that the R145C variant conveys increased risk inherited in a dominant fashion with incomplete penetrance⁷, likely dependent on environmental factors.

Of the Qatari R145C, the triglyceride levels were significantly higher than those of the control group, but there were no significant differences in the cholesterol, HDL-cholesterol or LDL-cholesterol levels between the groups. All were obese and had diabetes, 2/4 had cardiovascular disease, 3/4 had hypertension and 1 had cataracts.

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, as all individuals were obtained from a random sample of individuals in the New York metropolitan area, recruited to a study regarding lung health. On the average, the African-American R145C had 52% significantly higher triglyceride levels compared to African-American controls, but no differences in cholesterol levels or HDL-cholesterol and a borderline decrease in LDL-cholesterol. More detailed assessment of a subgroup of the African-American R145C demonstrated that 31% of the population studied had levels of small VLDL particles above the normal range (see Supplemental Table 6). Elevated levels of small VLDL particles are associated with an increased risk of coronary artery disease and peripheral arterial disease²³.

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 based on other genetic variations, age, diet, BMI, diabetes status, thyroid hormone levels and

medications^{3;24}. Although HIV infection can be associated with dyslipidemia^{25;26}, there was no difference in the lipid levels between the HIV positive and negative R145C African-Americans, and ANOVA analysis demonstrated that HIV status could not explain the elevated triglyceride levels among R145C heterozygotes.

While the overall risk for dyslipidemia conveyed by the R145C allele appears to be mild, and there is a need for further epidemiologic studies to confirm the observations described in this study, when put in the context that there are approximately 39 million African-Americans in the US²⁷, and approximately 836 million Sub-Saharan Africans, and large numbers of peoples derived from this population, worldwide²⁸, the observations in the present study suggest there could be 1.7 million African-Americans or 36 million Sub-Saharan Africans worldwide with the R145C allele likely contributing to the global risk for dyslipidemias and the associated risk for cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Apolipoprotein E gene, common alleles and the Arg145Cys (R145C) variant. ApoE is a 3.1 kb gene comprised 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' non-coding regions; shaded areas represent coding regions; the vertical lines in exons 2 and 4 are the start and stop sites, respectively. Greater than 95% of ApoE variations worldwide result from 2 SNPs in exon 4, resulting in amino acid substitutions at positions 112 and 158, dictating the common E2, E3 and E4 alleles. A C→T transition at SNP 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.

Figure 2.

Fasting lipid studies of ApoE R145C compared to controls in Qatar and New York. **A.** Triglycerides; **B.** Cholesterol; **C.** Low density lipoprotein (LDL)-cholesterol; and **D.** High density lipoprotein (HDL)-cholesterol. All Qataris were of the Q3 (African) genetic subgroup. The Qatari R145C are presented as individual data points for each subject; the Qatari Q3 controls and New York African-American R145C and controls are presented as box and whisker plots with the median and 4 quartiles indicated. Outliers are greater/lesser than the average ± 2 standard deviations for each group. For both the Qatari and New York data, if more than 1 value was available, the average value for each individual was used. The p values were calculated by a single factor ANOVA. The dashed lines indicate the high and low normal levels. For panels **A**, **B**, Qatari controls n=15, Qatari R145C n=4, NY African-American controls n=72, NY African-American R145C n=30; for panels **C**, **D**, Qatari

controls n=14, Qatari R145C n=4, NY African-American controls n=24, NY African-American R145C n=26.

Table 1**ApoE Genotypes and Alleles in the Qatari Population**

ApoE genotypes and alleles¹	All Qataris²	Q1 (Arab)²	Q2 (Persian)²	Q3 (African)²
Genotypes				
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 ³	1 (0.4)	1 (0.8)	0 (0)	0 (0)
E2R150H/E2 ⁴	1 (0.4)	0 (0)	0 (0)	1 (4.3)
E3R145C/E3 ⁵	3 (1.3)	0 (0)	0 (0)	3 (13.0)
E3R145C/E4 ⁶	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)

¹The DNA for all Qataris was first assessed by TaqMan PCR at SNPs rs429358 [amino acid (aa) 112], rs769455 (aa 145) and rs7412 (aa 158). This was followed by sequencing of all ApoE exons and flanking introns. If there was heterozygosity at two SNPs in the same individual, cloning and complete sequencing was carried out to unambiguously identify the genotype.

²Data is presented as "n (%)" of the population.

³The E5 allele (E212K on the E3 background) has been previously described ¹⁰; this individual had no dyslipidemia.

⁴The E2R150H (R150H substitution on the E2 background) allele is novel to this study; this individual had no dyslipidemia.

⁵See Supplemental Table 2 for demographics and values for each individual of the n=3 E3R145C/E3 heterozygotes (age 52.7 ± 2.8, 1 male, 2 female, BMI 35.3 ± 1.6).

⁶For the E3R145C/E4 heterozygote, the n=1 individual was 40 yr old, female, BMI 48.6; see Supplemental Table 4 for further details.

ApoE Genotypes and Alleles in Subjects from the 1000Genomes Database^{1,2}

Table 2

Genotype	Europe (n, %) ³					Asia (n, %) ⁴			America (n, %) ⁵				Africa (n, %) ⁶		
	CEU	FIN	GBR	IBS	TSI	CHB	CHS	JPT	ASW	CLM	MXL	PUR	LWK	YRI	All 1000Gs
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)	1094 (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/E4R145CRI58C	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)	2188 (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)	1661 (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 ⁷	0	0	0	0	0	0	0	0	0	0	0	0	1 (0.5)	0	1 (0.05)

Genotype	Europe (n, %) ³					Asia (n, %) ⁴			America (n, %) ⁵			Africa (n, %) ⁶		
	CEU	FIN	GBR	IBS	TSI	CHB	CHS	JPT	ASW	CLM	MXL	PUR	LWK	YRI
E4R158C ⁸	0	0	0	0	0	0	0	0	0	0	1 (0.8)	0	8 (4.1)	0
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)

¹ Haplotype frequency observed in 1094 individuals. Phased haplotypes for three APOE SNPs on chromosome 19 were extracted from the May 2011 release of 1000 Genomes Project data [ftp://ftptrace.ncbi.nih.gov/1000genomes/ftp/release/20101123/interim_phase1_release/ALL.chr19.phase1.projectConsensus.genotypes.vcf.gz]

² Genotypes and alleles are presented as “n (%)” of the population

³ European populations included subjects from Northern and Western European Ancestry (CEU), Finnish from Finland (FIN), British from England and Scotland (GBR), Iberian populations in Spain (IBS) and Toscani in Italia (TSI).

⁴ Asian populations included Han Chinese in Beijing, China (CHB), Han Chinese South (CHS) and Japanese in Tokyo, Japan (JPT).

⁵ American populations included subjects with African Ancestry in Southwest US (ASW), Colombian in Medellin, Colombia (CLM), Mexican Ancestry in Los Angeles, CA (MXL) and Puerto Rican in Puerto Rico (PUR).

⁶ African Populations included Luhya in Webuye, Kenya (LWK) and Yoruba in Ibadan, Nigeria (YRI).

⁷ An allele novel to this study with two mutations on the E4 background: R145C and the R158C, thus E4R145C:R158C.

⁸ An allele novel to this study with two mutations compared to E3: C112R which results in the E4 background and then the R158C mutation, thus E4R158C.

Table 3

Prevalence of the ApoE Arg145Cys Polymorphism Among New York City Metropolitan Area Caucasian and African-American Populations

Genotypes ²	Population (n, % with the R145C allele) ¹	
	Caucasian (n=1012)	African-American (n=1226)
E2/E3R145C	0	5 (0.4%)
E3/E3R145C	0	39 (3.2%)
E4/E3R145C	0	8 (0.7%)
E3/E4E13K.R145C ³	0	1 (0.08%) ⁴
E4/E3E13K.R145C ⁵	1 (0.1%)	0
All R145C heterozygotes	1 (0.1%)	53 (4.3%)

¹ Subjects with self-reported ethnicities from the New York City Metropolitan Area.

² TaqMan to identify the R145C allele; all R145C were then sequenced. In the setting of a double mutation, samples were cloned and sequenced to determine the *cis* or *trans* relationship.

³ A novel allele to this study with two mutations in the amino acid sequence resulting in the substitution of lysine (K) for glutamate (E) at residue 13 and in the substitution of cysteine for arginine at residue 145 (R145C).

⁴ This subject is of African-American Hispanic mixed ancestry.

⁵ ApoE^{Philadelphia} a double mutant of E13K and R145C on an E3 background; this allele has been previously described^{29;30}.