Brief Communication

*High Frequency of the Apolipoprotein E *4 Allele in African Pygmies and Most of the African Populations in Sub-Saharan Africa*

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Abstract Apolipoprotein E genotypes (alleles **2, *3,* and **4*) have been determined in 70 Aka Pygmies and 470 unrelated African sub-Saharan subjects. Allele frequencies for Pygmies are 5.7% for *APOE*2,* 53.6% for *APOE*3,* and 40.7% for *APOE*4,* and the global proportions for sub-Saharan subjects are 11.6% for *APOE*2,* 70.6% for *APOE*3,* and 17.8% for *APOE*4.* The frequencies in some ethnic groups are statistically different from the overall mean in the Afar and the Isa, the Ewe (Togo), the Malinke (Guinea), and the Mossi; three ethnic groups have a higher allele frequency of *APOE*4* (Fon, 29.4%; Zairians, 33.3%; Tutsi, 38.5%). The *APOE*4* allele is considered the ancestral form because of its high frequency in African Pygmies and other aboriginal populations.

Apolipoprotein E (apoE) plays an important role in plasma lipoprotein metabolism and in local lipid homeostasis (Malhey 1988). Three common genetic forms of the *APOE* gene exist in human populations (Utermann et al. 1977), encoded by three alleles (**2, *3,* and **4*) at the *APOE* locus on chromosome 19q13.2. These forms differ by single amino acid substitutions at one of two positions of the residue protein: *APOE*3,* which is the most frequent isoform, has cysteine at position 112 and arginine at position 158; *APOE*2* has cysteine at position 158; and *APOE*4* has arginine at position 112. These changes cause significant differences in the structure and physical properties of the protein and are responsible for the association of apoE protein variants with cholesterol metabolism and risk of atherosclerotic vascular disease.

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Table 1. Frequencies of *APOE* Genotypes (%) and *APOE* Alleles in Pygmies (n = 70) from the Central African Republic and in Other Africans (n = 470) from West, Central, and East Africa

Genotype or Allele	*Pygmies*	*Africans*
Genotype		
*APOE*2/*2*	0	2.3
*APOE*2/*3*	7.2	14.3
*APOE*3/*3*	27.1	52.1
*APOE*2/*4*	4.3	4.3
*APOE*3/*4*	45.7	22.8
*APOE*4/*4*	15.7	4.3
Allele		
*APOE*2*	5.7	11.6
*APOE*3*	53.6	70.6
*APOE*4*	40.7	17.8

Hallman et al. (1991) determined *APOE* allele frequencies in various ethnic groups. We have now extended these studies to Aka Pygmies from Central Africa and to various populations of West, Central, and East Africa. The Pygmy sample consists of 70 Pygmies from the Central African Republic, and the non-Pygmy sample consists of 470 unrelated African subjects from various countries of sub-Saharan Africa (mainly Benin, Burkina Faso, Cameroon, Chad, Congo, Gabon, Ivory Coast, Mali, Niger, Senegal, Togo, and Zaire). The 470 non-Pygmy subjects are young adults, mainly males, for whom we have obtained information on their parents' ethnic background.

Genomic DNA was prepared from white blood cells by phenol extraction. The Pygmy DNA sample (Lucotte et al. 1989, 1990) and the African non-Pygmy DNA sample (Lucotte et al. 1994) have already been described and studied for anthropological reasons.

APOE genotypes were determined using polymerase chain reaction and digestion with *Hha*I, using a method (Lagarde et al. 1995) modified from that described by Hixson and Vernier (1990). Allele frequencies of the ethnic groups, in the non-Pygmy DNA sample, where the number of studied subjects was sufficient, were compared by means of a chi-square test. Comparisons were made between each subgroup and the whole population.

Frequencies of *APOE* genotypes and alleles are shown in Table 1. The frequency of *APOE*4* (0.407) reported here for Pygmies is the highest reported from any human ethnic group (Sandholzer et al. 1995): 16% of the Pygmies are *APOE*4/*4* homozygotes, and 50% are heterozygotes (either *APOE*3/*4* or *APOE*2/*4*); altogether, 66% carry at least one *APOE*4* allele. High values of *APOE*4* frequencies have also been reported for Bushmen (Sandholzer et al. 1995) and for New Guineans (Kamboh et al. 1990). *APOE*4* allele frequencies are also high in Australian Aborigines (Kamboh

Table 2. Frequencies of *APOE* Allles (%) in the Various Non-Pygmy Ethnic Groups Studied[a]

Ethnic Group (Country)	*Number of Alleles*	*Allele Frequency*			
		*APOE*2*	*APOE*3*	*APOE*4*	*p*
Afars and Issas	34	2.9	85.3	11.8	<0.20
Arabs (Mauritania)	20	7.5	82.5	10.0	NS
Bambara (Mali)	16	6.2	71.9	21.9	NS
Bamileke (Cameroon)	18	11.1	66.7	22.2	NS
Djerna (Niger)	16	6.2	81.2	12.5	NS
Ewe (Togo)	19	31.6	47.4	21.0	<0.02
Fang (Gabon)	25	12.0	68.0	20.0	NS
Fon (Benin)	17	0.0	70.6	29.4	<0.20
Haoussa (Niger)	37	2.7	78.4	18.9	NS
Hutu (Rwanda)	21	9.5	66.7	23.8	NS
Malinke (Guinea)	30	23.3	60.0	16.7	<0.20
Merina (Madagascar)	22	22.7	59.1	18.2	NS
Mossi (Burkina Faso)	20	37.5	50.0	12.5	0.01
Wolof (Senegal)	33	3.0	93.9	3.0	<0.5
Peul (Senegal)	45	7.8	67.8	24.4	NS
Songhai (Mali)	17	20.6	73.5	5.9	NS
Chadians	22	4.5	68.2	27.3	NS
Toucouleur (Senegal)	17	11.8	70.6	17.6	NS
Tutsi (Burundi)	13	0.0	61.5	38.5	<0.10
Zairians	24	4.2	62.5	33.3	<0.10

NS, nonsignificant.

a. In some ethnic groups allele frequencies are statistically different from the whole group of sub-Saharan non-Pygmy Africans considered.

et al. 1991). Table 1 also shows that the *APOE*4* frequency is high (17.8%) in our total non-Pygmy sample, as it is in other African populations (Nigerians, Sudanese, and African Americans) (Sepehrnia et al. 1989; Hallman et al. 1991; Kamboh et al. 1990, 1991; Eichner et al. 1989; Maestre et al. 1995).

Table 2 shows *APOE* allele frequencies in different ethnic groups included in our non-Pygmy sample from sub-Saharan Africa. In most of the subjects the allele frequencies are statistically similar to those (Table 1) of the whole group of 470 subjects, but for some ethnic groups allele frequencies are statistically different from the overall sample. Several ethnic groups from Benin to East Africa have a higher *APOE*4* allele frequency: the Fon (29.4%, $p < 0.20$), Zairians (33.3%, $p < 0.10$), and the Tutsi (38.5%, $p < 0.10$). *APOE*2* allele frequencies are significantly higher in groups such as the Mossi (37.5%, $p < 0.01$), the Ewe (31.6%, $p < 0.02$), and the Malinke (located between Guinea and Togo) (23.3%, $p < 0.2$). On the contrary, frequencies are lower (<3%) or absent in groups such as the Fon, the Haoussa, and the Tutsi. Both the *APOE*2* and *APOE*4* alleles have high values in the

Mossi and the Ewe, who consequently have a low frequency of the *APOE*3* allele (50% and 47.4%, respectively). In some West African ethnic groups only the *APOE*3* allele is expressed, for example, in the Wolof (93.9%, $p < 0.05$).

No subjects in this study showed *Hha*I restriction fragments that were different from those corresponding to the three classical alleles at the *APOE* locus. From Table 2 it is clear that some ethnic groups are genetic isolates, probably because of limitations imposed by local customs regarding interethnic marriages.

The frequencies of the *APOE* alleles in sub-Saharan African populations reported in this study and found in the literature are shown in Figure 1. The highest frequency of *APOE*4* was observed in Aka Pygmies (40.7%). A value of 37% was obtained for another aboriginal African population: the Khoi San (Bushmen), published previously by Sandholzer et al. (1995). Several nonaboriginal African populations also have high frequencies of the *APOE*4* allele: the Tutsi from Burundi (38.5%) (without the *APOE*2* allele), Zairians (33.3%), and the Fon from Benin (29.4%; also without the *APOE*2* allele). The highest *APOE*4* frequencies obtained for the Northern Hemisphere are for Finns (22.7%) and Inuits from Greenland (22.9%) (Ehnholm et al. 1986; De Knijff et al. 1992).

There is some disagreement in the literature concerning the identity of the ancestral allele at the *APOE* locus. Because *APOE*3* is the most prevalent allele in most human populations, it has been considered the original allele state (Malhey 1988). At the protein level both *APOE*2* and *APOE*4* can be derived from the *APOE*3* sequence by single mutational events, whereas two successive mutations would be needed to derive *APOE*2* from *APOE*4*. However, analysis of the *APOE* protein of apes by electrophoresis gave signals at a similar position to the human *APOE*2*, suggesting (Zannis et al. 1985) that *APOE*2* may be the ancestral allele (this analysis depends on a protein's molecular weight and charge and is not a reliable indicator for sequence comparisons between species).

At the genomic level, for both positions 112 and 158, there is a change of the nucleotide sequence from CGC to TGC, resulting in a change from arginine to cysteine in the protein; because the likely direction of mutation is CpG → TpG, Larsen et al. (1993) suggested that the *APOE*4* allele is the ancestral one. In favor of this, the sequenced *APOE* genes of baboons (Hixson et al. 1988) and cynomolgus monkeys (Marotti et al. 1989) are of the *APOE*4* type. Restriction isotyping of the DNA of chimpanzees, according to Hixson and Vernier (1990), shows an *Hha*I fragment of ≈72 base pairs, characteristic of the *APOE*4* allele (Gearing et al. 1994). Chimpanzees and other primate species are all homozygous for CGC at positions homologous to codon 112 (Hanlon and Rubinsztein 1995).

All these lines of genomic evidence suggest that *APOE*4* is the ancestral allele and that the *APOE*3* and *APOE*2* alleles arose after the chimpan-

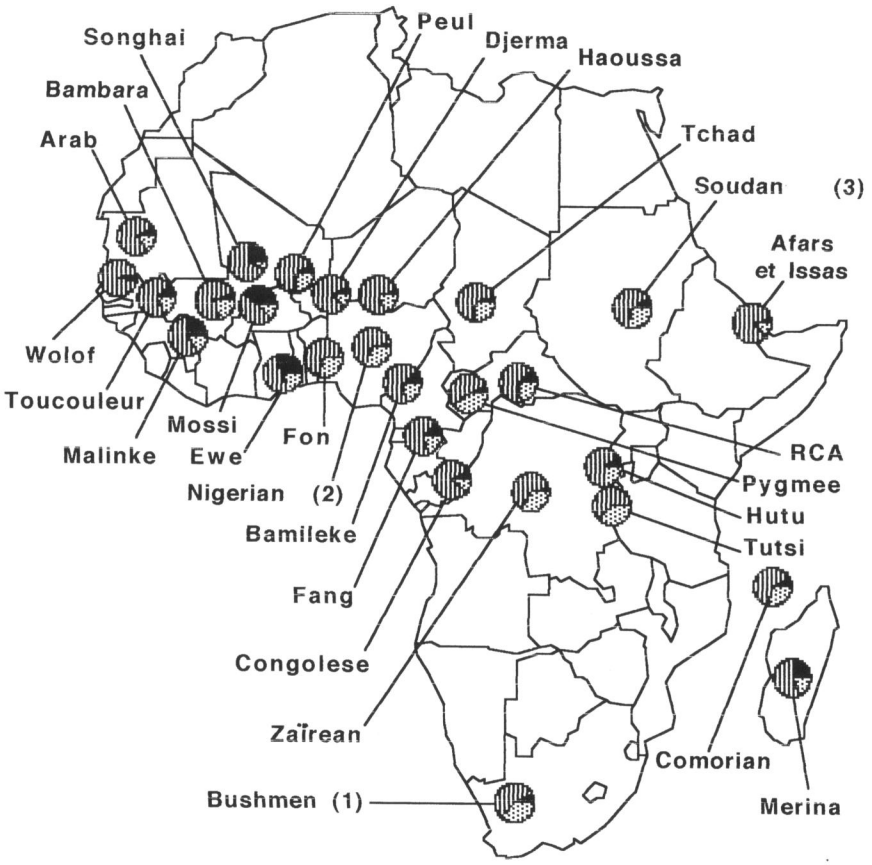

Figure 1. *APOE* allele frequencies in the various African populations studied. Other African populations represented have high frequencies of the *APOE*4* allele, for example, (1) Bushmen, with a 37% frequency (Sandholzer et al. 1995), (2) the Nigerian population (Sepehrnia et al. 1989), and (3) the population of Khartoum, Sudan (Hallman et al. 1991), but for these last two populations the original references do not give the ethnic origin. In the pie charts, solid sections refer to the *APOE*2* allele, vertically ruled sections to the *APOE*3* allele, and stippled sections to the *APOE*4* allele.

zee and human lineages split. The high *APOE*4* allele frequencies reported here in "old" aboriginal populations, such as African Pygmies, and previously published for the Khoi San Bushmen (Sandholzer et al. 1995), New Guineans (Kamboh et al. 1990), Australian Aborigines (Kamboh et al. 1991), and Inuits (De Knijff et al. 1992) are also compatible with the hypothesis that the *APOE*4* allele and not the *APOE*3* allele is the ancestral form. High *APOE*4* frequencies reported here for most of the African ethnic groups

studied also support the hypothesis that the *APOE*4* allele is the ancestral form.

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