REFERENCES

Allele Frequencies of Apolipoprotein E Gene Polymorphisms in the Protein Coding Region and Promoter Region (-491A/T) *in a Healthy Norwegian Population*

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Abstract This study examines the distribution of apolipoprotein E (*APOE*) alleles in a population of healthy male and female Norwegians (n = 798) below the age of 40. The *-491A/T* polymorphism of the promoter region of the *APOE* gene was also examined. A seminested polymerase chain reaction was applied in the genotyping. The results showed that the *E3* allele had the highest frequency (0.744), followed by *E4* (0.198) and *E2* (0.058). The *APOE* frequencies found in this study differ significantly from those obtained in earlier Norwegian *APOE* phenotypings. The allele frequencies in the -491 site of the promoter region were 0.845 for the *A* allele and 0.155 for the *T* allele. The genotype frequency was highest for *AA* (0.707), followed by *AT* (0.277) and *TT* (0.016). Moreover, the *A* allele was in linkage disequilibrium to *E4*.

Apolipoprotein E is a 299-amino-acid protein coded by a polymorphic gene (*APOE*) on chromosome 19q (Lucotte et al. 1997; Rosseneu and Labeur 1995). The polymorphism influences the risk for cardiovascular disease and Alzheimer's disease. Three common isoforms are coded by the alleles *E2*, *E3,* and *E4* (Lucotte et al. 1997; Mastana et al. 1998; Hixson and Vernier 1990). Other single nucleotide polymorphisms are to be found in the *APOE* locus (Nickerson et al. 2000). Compared to the most prevalent isoform E3 (Rubinsztein 1995), E4 has arginine instead of cysteine at position 112, and E2 has cysteine instead of arginine at position 158 (Mastana et al. 1998; Hixson and Vernier 1990). The frequencies of the *APOE* genotypes are highly variable among different populations (Mastana et al. 1998; Siest et al. 1995). The frequency of the *E4* allele ranges from 5% (Taiwan and Sardinia) to 40% (Pygmies), and the frequency of the *E3* allele is in the range of 49% (Papua New Guinea) to 90% (Sardinia) (Corbo and Scacchi 1999; Siest et al. 1995). Interestingly, there is a clear north-to-south decreasing gradient of the *E4* allele in Europe (Lucotte et al. 1997), with frequen-

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cies above 20% in Finland and Sweden and below 8% in Italy (Table 1). The opposite is true for the *E3* allele, with frequencies about 90% in Sardinia and Greece and below 70% in Sweden. These gradients coincide with the well-known falling gradient in the prevalence of coronary artery disease from north to south, but no similar gradient in the prevalence of Alzheimer's disease has been found.

The previously reported *APOE* allele frequencies from Norwegian populations deviate quite markedly from those reported in other Nordic countries. In one study the frequencies of *E2*, *E3,* and *E4* alleles were 0.083, 0.760, and 0.157 (Pedersen and Berg 1989), and another study found frequencies of 0.090, 0.795, and 0.115 (Pedersen and Berg 1990). However, a third study with a small sample found frequencies of 0.035, 0.757, and 0.208 (Hagve 1993).

The *-491A/T* polymorphism of the promoter region of the *APOE* gene was initially detected by Artiga et al. (1998) and Bullido et al. (1998). Studies have shown that for some populations (e.g., Spain, Australia, Finland, Italy, and France) the *AA* genotype is associated with Alzheimer's disease, while for other populations (e.g., China and Japan) no such association has been found (Bullido and Valdivieso 2000). Linkage disequilibrium between the *APOE* allele *E4* and the promoter allele *A*, as well as between the *APOE* allele *E2* and the promoter allele *T,* has been described (Bullido and Valdivieso 2000).

For future research on the influence of various *APOE* alleles on disease it is important to have the best estimate for the allele frequencies in the studied population. Achieving such an estimate was our motivation for examining the allele frequencies in a large number of healthy Norwegian individuals.

Materials and Methods

Peripheral blood samples were drawn from 798 healthy white Norwegian blood donors recruited at Ullevål University Hospital in Oslo. The donors were below the age of 40 (45% were females) and were all recruited from the counties Oslo or Akershus. Samples were stored in EDTA or citrate. Genomic DNA was extracted from the samples using QIAamp Blood Kit (Qiagen GmbH, 40724 Hilden, Germany). DNA was amplified by polymerase chain reaction (PCR) in a thermal cycler, GeneAmp 2400 (PE-Biosystems, Foster City, CA 94404, USA). The method used was a seminested PCR described elsewhere (Ghebremedhin et al. 1998). The amplification resulted in a 188-base-pair PCR product, which was subjected to digestion by a restriction enzyme (*Hha*I) to distinguish between the different alleles of *APOE* (Ghebremedhin et al. 1998). The restriction fragments were separated by polyacrylamide gel (15%) electrophoresis at 200 V for 5.5 hours.

The *-491A/T* alleles were determined from the same DNA extracts with a nested PCR, followed by digestion by the restriction enzyme *Dra*I and polyacrylamide gel (8%) electrophoresis at 200 V for 4 hours (Bullido et al. 1998). All gels were stained with ethidium bromide and examined in UV light at 312 nm.

Statistical comparisons of allele frequencies were based on chi-square tests. When testing the association between *APOE* and *-491A/T*, we merged *E2/E2* and *E2/E3*, *E3/E4* and *E4/E4*, and excluded *E2/E4* to obtain sufficient cells counts in the chi-square test.

Results

This study examined the *APOE* allele and genotype frequencies in a healthy blood donor group ($n = 798$) of Norwegian persons under the age of 40 years. Allele *E3* had the highest frequency, 0.744, followed by *E4* (0.198) and *E2* (0.058) (Table 1). The highest genotype frequency was *E3/E3* at 0.555, followed by *E3/E4* (0.292), *E2/E3* (0.085), *E4/E4* (0.040), *E2/E4* (0.024), and *E2/E2* (0.004) (for absolute values see Table 2). Allele distributions were similar for males and females.

Table 1. The Allele Frequencies for *E2, E3,* and *E4* in Our Study Compared with Earlier Norwegian Studies and Studies from Other Countries in Europe

Country	*E2*	*E3*	*E4*	*N*
Norway (our results)	0.058	0.744	0.198	798
Norway (Pedersen and Berg 1989)	0.083	0.760	0.157	159
Norway (Pedersen and Berg 1990)	0.090	0.795	0.115	239
Norway (Hagve 1993)	0.035	0.757	0.208	60
Finland (Corbo et al. 1995)	0.044	0.748	0.208	2245
Sweden (Eggertsen et al. 1993)	0.078	0.719	0.203	279
Denmark (Gerdes et al. 1992)	0.085	0.741	0.174	466
Germany (Corbo et al. 1995)	0.077	0.778	0.145	2031
France (Corbo et al. 1995)	0.108	0.771	0.121	1228
Italy (Corbo et al. 1995)	0.060	0.849	0.091	2000
Sardinia (Corbo et al. 1995)	0.050	0.898	0.052	280

Table 2. The Simultaneous Distribution in Absolute Numbers of *APOE* Alleles and Alleles in the -491 Site in the Promoter Region[a]

Allele	*AA*	*AT*	*TT*	*Sum APOE*
E4/E4	30	1	0	31 (+1)
E3/E4	179	51	2	232 (+1)
E3/E3	314	123	5	442 (+1)
E2/E4	11	8	0	19
E2/E3	27	36	5	68
E2/E2	1	1	1	3
Sum *-491A/T*	562	220	13	795 (+3)

a. Three of the blood donors were genotyped for *APOE*, but not for the promoter polymorphism.

Concerning the *-491A/T* polymorphism, the *A* allele had the highest frequency, 0.845, while the *T* allele had a frequency of 0.155. The highest genotype frequency was *AA* at 0.707, followed by *AT* at 0.277 and *TT* at 0.016. The results showed a highly significant linkage disequilibrium ($p < 0.0001$) between the *E4* allele and the *A* allele in the *-491A/T* polymorphism, and a corresponding linkage disequilibrium between *E2* and *T*. The genotypic frequencies showed no systematic deviation from Hardy-Weinberg expectation, neither for the *E2, E3, E4* system nor for the *-491A/T* polymorphism.

A comparison of *APOE* allele frequencies from our study and those from the two earlier, larger Norwegian studies shows a highly significant difference ($p < 0.001$), mainly caused by the difference in *E4* frequency between our study (*E4* frequency: 0.198) and the larger of the earlier studies (*E4* frequency: 0.115).

Discussion

We found that the Norwegian *APOE* allele frequencies (*E2*: 0.058, *E3*: 0.774, *E4*: 0.198) are similar to those reported from other Nordic countries (Table 1). Our results also correspond well with those reported in a small ($n = 60$) Norwegian study from 1993 (Hagve 1993), but they contrast with the lower *E4* allele frequencies and higher *E2* allele frequencies reported earlier (Pedersen and Berg 1989; 1990). The *E4* frequencies were 0.157 and 0.115 in these two studies, but both studies were relatively small (159 and 239 participants, respectively), which makes the standard deviation of the estimates fairly high. Moreover, a bias may have been introduced, since *E4* carriers may be less fertile than persons with the genotype *E3/E3* (Gerdes et al. 1996), and the subjects in the study had been selected in the direction of high fertility (Pedersen and Berg 1989). Finally, there may also be methodological explanations, since Pedersen and Berg used two-dimensional polyacrylamide electrophoresis to phenotype *APOE*, while we used PCR to genotype the individuals.

Isoelectric focusing or two-dimensional gel electrophoresis (Børresen and Berg 1981) were used for a phenotypic approach to determine the frequencies of the *APOE* alleles in the other studies mentioned above (Pedersen and Berg 1989; 1990). Studies of the isoelectric focusing/immunoblotting method report that it is the most reliable for scoring the E2 phenotype, whereas there will be some uncertainty in the identification of other phenotypes (Hagve 1993). Similar uncertainty connected to the method of two-dimensional gel electrophoresis can contribute to the discrepancy between our results and previous results by Pedersen and Berg (1989; 1990).

The results of the genotyping for the *-491A/T* polymorphism (*A* frequency = 0.845) are similar to those found in studies on a French population and on a US white population (*A* frequency = 0.82 for both) (Bullido et al. 1998; Lambert et al. 1998), while different allele distributions were reported for a Japanese population (*A* frequency = 0.95; Toji et al. 1999). We found a clear linkage disequilibrium between the *APOE* allele *E4* and the promoter allele *A*, and also a linkage dis-

equilibrium between *E2* and *T*, findings that correspond with those from other population studies (Bullido and Valdivieso 2000).

In conclusion, Norwegian allele frequencies for the apolipoprotein E polymorphism obtained from nearly 800 healthy young individuals appear to be similar to those of other Nordic countries and fit well into a European north-south gradient. This study is the first to give the allele distribution of the *-491A/T* polymorphism in a Norwegian population.

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Literature Cited

Artiga, M.J., M.J. Bullido, I. Sastre et al. 1998. Allelic polymorphisms in the transcriptional regulatory region of apolipoprotein E gene. *FEBS Lett.* 421:105–108.

Bullido, M.J., M.J. Artiga, M. Recuero et al. 1998. A polymorphism in the regulatory region of APOE associated with risk for Alzheimer's dementia. *Nat. Genet.* 18:69–71.

Bullido, M.J., and F. Valdivieso. 2000. Apolipoprotein E gene promoter polymorphisms in Alzheimer's disease. *Microsc. Res. Tech.* 50:261–267.

Børresen, A.L., and K. Berg. 1981. The *APOE* polymorphism studied by two-dimensional, high-resolution gel electrophoresis of serum. *Clin. Gen.* 20:438–448.

Corbo, R.M., and R. Scacchi. 1999. Apolipoprotein E (*APOE*) allele distribution in the world. Is *APOE4* a 'thrifty' allele? *Ann. Hum. Genet.* 63:301–310.

Corbo, R.M., R. Scacchi, L. Mureddu et al. 1995. Apolipoprotein E polymorphism in Italy investigated in native plasma by a simple polyacrylamide gel isoelectric focusing technique. Comparison with frequency data of other European populations. *Ann. Hum. Genet.* 59:197–209.

Eggertsen, G., R. Tegelman, S. Ericsson et al. 1993. Apolipoprotein E polymorphism in a healthy Swedish population: Variation of allele frequency with age and relation to serum lipid concentrations. *Clin. Chem.* 39:2125–2129.

Gerdes, L.U., C. Gerdes, P.S. Hansen et al. 1996. Are men carrying the apolipoprotein epsilon 4- or epsilon 2 allele less fertile than epsilon 3 epsilon 3 genotypes? *Hum. Genet.* 98:239–242.

Gerdes, L.U., I.C. Klausen, I. Shim et al. 1992. Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study population around the world. *Genet. Epidemiol.* 9:155–167.

Ghebremedhin, E., H. Braak, E. Braak et al. 1998. Improved method facilitates reliable *APOE* genotyping of genomic DNA extracted from formaldehyde-fixed pathology specimens. *Neurosci. Methods* 79:229–231.

Hagve, T.A. 1993. The usefulness of apolipoprotein E phenotyping in the diagnosis of type III hyperlipoproteinemia. *Tidsskr. Nor. Lægeforen.* 113:853–856.

Hixson, J.E., and D.T. Vernier. 1990. Restriction isotyping of human apolipoprotein E by gene amplification with *Hha*I. *J. Lipid. Res.* 31:545–548.

Lambert, J.C., C. Berr, F. Pasquier et al. 1998. Pronounced impact of Th1/E47cs mutation compared with -491 AT mutation on neural APOE gene expression and risk of developing Alzheimer's disease. *Hum. Mol. Genet.* Sep;7(9):1511–1516.

Lucotte, G., F. Loirat, and S. Hazout. 1997. Pattern of gradient of Apolipoprotein E allele 4 frequencies in Western Europe. *Hum. Biol.* 69:253–262.

Mastana, S.S., R. Calderon, J. Pena et al. 1998. Anthropology of the apolipoprotein E (apo E) gene: Low frequency of apo *E4* allele in Basques and in tribal (Baiga) populations of India. *Ann. of Hum. Biol.* 25:137–143.

Nickerson, D.A., S.L. Taylor, S.M. Fullerton et al. 2000. Sequence diversity and large-scale typing of SNPs in the human apolipoprotein E gene. *Genome Res.* 10:1532–1545.

Pedersen, J.C., and K. Berg. 1989. Interaction between low density lipoprotein receptor (LDLR) and apolipoprotein E (*APOE*) alleles contributes to normal variation in lipid level. *Clin. Gen.* 35:331–335.

Pedersen, J.C., and K. Berg. 1990. Gene-gene interaction between the low density lipoprotein receptor and apolipoprotein E loci affects lipid levels. *Clin. Gen.* 38:287–294.

Rosseneu, M., and C. Labeur. 1995. Physiological significance of apolipoprotein mutants. *Faseb. J.* 9:768–776.

Rubinsztein, D.C. 1995. Apolipoprotein E: A review of its roles in lipoprotein metabolism, neuronal growth and repair and as a risk factor for Alzheimer's disease. *Psychol. Med.* 25:223–229.

Siest, G., T. Pillot, A. Régis-Bailly et al. 1995. Apolipoprotein E: An important gene and protein to follow in laboratory medicine. *Clin. Chem.* 41:1068–1086.

Toji, H., H. Maruyama, K. Sasaki et al. 1999. Apolipoprotein E promoter polymorphism and sporadic Alzheimer's disease in a Japanese population. *Neurosci. Lett.* 259:56–58.