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RAPID COMMUNICATION

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Are men carrying the apolipoprotein ε4- or ε2 allele less fertile than ε3ε3 genotypes?

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Abstract The ε3 allele in the human gene coding for apolipoprotein E (apoE) is the most common worldwide, but ε4 is probably the ancestral allele. Since apoE is involved in many important biological processes, selection forces could have favoured ε3. We hypothesized that apoE genotypes may affect reproductive efficiency, and we therefore compared the distributions of 40-year-old married men with known genotypes by the numbers of their biological children. The distributions were statistically significantly different (P = 0.0026). On average, men with the ε3ε3 genotype (n = 212) had 1.93 children, men with the ε3ε4 or ε4ε4 genotype (n = 105) had 1.50, and men with the ε3ε2 or ε2ε2 genotypes (n = 53) had 1.66 children. Of the men in the three groups, 6%, 26% and 19%, respectively, reported being childless. These findings are unlikely to be due to gross error in the reported prevalence of childlessness, differences in socioeconomic status or other likely sources of bias. They are compatible with higher fertility in men with the ε3ε3 genotype than in those with the other common apoE genotypes.

Introduction

The gene coding for apolipoprotein E (apoE) on chromosome 19q13.2 is polymorphic, and three alleles, ε2, ε3 and ε4, are common in most human populations (Davignon et al. 1988; Hallman et al. 1991; Gerdes et al. 1992; Corbo et al. 1995). Epsilon 3 is the most common, ranging in frequency from 50–55% in Papua New Guineans (Kamboh et al. 1990) and African Bushmen (Sandholzer et al. 1995) to 85–90% in Mayans of Mexico (Kamboh et al. 1991), Chinese (Hallman et al. 1991; Kao et al. 1995), Japanese (Gerdes et al. 1992), Spaniards (Fiol et al. 1991), and Turks (Mahley et al. 1995b). It is therefore usually considered to be the ancestral (wild-type) allele.

A recent study of non-human primates suggests, however, that ε4 is the ancestral allele, and that ε3 may have resulted from a $[\mathbf{C}GC \rightarrow \mathbf{T}CG]_{112}$ mutation (causing an Arg→Cys substitution in the gene product), and ε2 from an additional $[\mathbf{C}GC \rightarrow \mathbf{T}CG]_{158}$ mutation (Hanlon and Rubinsztein 1995). If this is true, the high frequency of ε3 is due to random genetic drift, migration and/or natural selection (Cavalli-Sforza et al. 1994). Selection may have been played a role, since apoE is involved in many biological processes in which the structural and functional characteristics of the genetic isoforms (Weisgraber 1994) could affect survival to reproductive age. For example, apoE is involved in lipid transport between and within tissues, absorption of dietary fat, immunological preparedness and neuronal function (Mahley 1988; Keltikangas-Järvinen et al. 1993; Mahley et al. 1995a). Since apoE may also be involved in gonadal function, implantation of the embryo in the uterus, and transplacental transport of fat (Rindler et al. 1991; Schneider and Nimpf 1993; Olson et al. 1995), we hypothesized that the apoE genotype could also affect reproductive efficiency. As a first step in examining this possibility, we compared the numbers of biological children of 40-year-old married men with known apoE genotypes.

Materials and methods

Five hundred and eighty-five men, randomly selected from the national register of all men born in 1948 and residing in the city of Aarhus, were invited to participate in a study of the prevalence of cardiovascular risk factors in the Spring of 1989 (The RisiKalk Project). The study was approved by the Ethics Committee of Aarhus County. Four hundred and seventy-seven men (82%) even-

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tually participated, 466 of whom were of Danish or north European ancestry. ApoE genotypes were determined as previously described (Hansen et al. 1994). Demographic variables and information on vital status, year of birth and death, and diseases in all first-degree biological relatives were obtained from questionnaires. For the purpose of the present study we considered only the 379 of the 466 men (81.3%) who were married or had a common-law wife. Wives and living children were not examined, and inquiries were not made as to the wives' histories of abortions or miscarriages. Stillborn children may not always have been reported, and some men may not have reported illegitimate children. Conversely, some men may have reported children that they had not fathered. Men who reported being childless were not asked whether childlessness was voluntary.

The distributions of men with 0, 1, 2, or ≥3 children among ε3ε3 genotypes, ε3ε4 or ε4ε4 genotypes (ε4 carriers), and ε3ε2 or ε2ε2 genotypes (ε2 carriers), respectively, were compared using a likelihood ratio χ^2 with 6 degrees of freedom.

Results and discussion

Figure 1 shows the distributions of men with 0, 1, 2, or ≥ 3 children among ε3ε3 genotypes, ε4 carriers, and ε2 carriers, respectively. The differences are statistically significant (P = 0.0026). Crude numbers are shown in Table 1. Only trivial differences are encountered when the nine ε4ε2 genotypes are included as either ε4 or ε2 carriers. The most marked difference is between ε3ε3 genotypes and ε4 carriers, who had an average of 1.93 and 1.50 children, respectively, whereas ε2 carriers had 1.66. At the age of 40, about 6% of the married men with genotype ε3ε3, 26% of the ε4 carriers and 19% of the ε2 carriers were childless. These figures correspond to risk odds ratios (with 95% confidence intervals) for being childless of 5.3 (2.6–10.8) in ε4 carriers and 3.6 (1.5–8.7) in ε2 carriers, relative to ε3ε3 genotypes.

The low P value for our data under the null hypothesis suggests that our findings are not due to chance. However, they could be due to one or more sources of bias. First, the numbers of biological children reported may be inaccurate. However, errors should either be non-differential and quite extreme (Dosemeci et al. 1990), or differential among apoE genotypes, to have this effect. We are confident in particular that childlessness is not overreported in our study. According to the Danish Fertility Database (Statistics Denmark, Copenhagen), about 19% of 40-year-old Danish men in 1989 were childless, a figure closely similar to the 20% in all 466 participants in our study, and 14% in the 379 married men. Also, important differential errors as regards childlessness are unlikely, since the proportions of childless men who reported having children in their homes (i.e. adopted children, or biological children of their wives) did not differ between apoE genotypes: 6/13 = 46% of ε3ε3 genotypes, 11/27 = 41% of ε4 carriers, and 4/10 = 40% of ε2 carriers.

Secondly, confounding by differences in socioeconomic status across apoE genotype groups, possibly generated by differences in, e.g., temperament or mental activity (Keltikangas-Järvinen et al. 1993) is unlikely, because we obtained essentially the same results in three strata defined by total numbers of years spent in school by the men, or in three strata defined by the number of rooms in their homes.

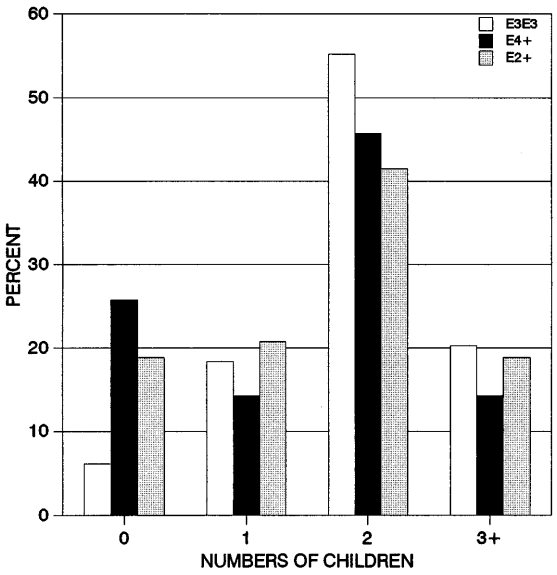

Fig. 1 Distributions of men with apolipoprotein E genotype ε3ε3 (n = 212), genotypes ε3ε4 or ε4ε4 (ε4+; n = 105) and genotypes ε3ε2 or ε2ε2 (ε2+; n = 53) by the numbers of their biological children

Table 1 Distributions of 40-year-old married or cohabiting Danish men by the numbers of biological children and apolipoprotein E genotype

Genotype	Numbers of children							Total
	0	1	2	3	4	5	9	
ε3ε3	13	39	117	32	9	2	0	212
ε3ε4	24	13	41	13	2	0	0	93
ε4ε4	3	2	7	0	0	0	0	12
ε3ε2	10	10	20	5	3	0	0	48
ε2ε2	0	1	2	2	0	0	0	5
ε4ε2	1	3	4	0	0	0	1	9
Total	51	68	191	52	14	2	1	379

Thirdly, we speculated whether ε4 and ε2 carriers, again due for instance to differences in temperaments, were more likely to have postponed having (more) children until their forties. In that case, a very extreme distribution of apoE genotypes in men having children in their forties would be expected, if 40-year-old ε4 and ε2 carriers had not yet caught up with ε3ε3 genotypes in reproductive powers. Men with children, moreover, did not differ in mean age at time of first child, second child etc.

Fourthly, a higher prevalence of premature cardiovascular diseases among parents or siblings of ε4 carriers may have caused more of these men (or their wives) to decide not to have children (Davignon et al. 1988; Gerdes 1994). Although a family history of disease before age 55 years was more frequently reported by ε4 carriers (about 11%) than by ε3ε3 genotypes (about 7%), there were no differences in the average number of children (or in the

percentages of childless men) in either ε4 carriers or in ε3ε3 genotypes with or without a positive family history.

Finally, we speculated whether the different numbers of children could be due to a "rebound effect" in the attitude towards having (more or many) children, generated by the men's experience in their childhood, i.e. that ε4 and ε2 carriers were more often from larger sibships than ε3ε3 genotypes – a possibility that would actually undermine our hypothesis. This was not the case, however. In fact, ε4 and ε2 carriers had an average of 2.06 and 1.92 siblings, whereas ε3ε3 genotypes had 2.35. These differences were not statistically significant.

In conclusion, the present study indicates that 40-year-old male ε4 and ε2 carriers have fewer children than men with the ε3ε3 genotype, and in particular that they are more often childless. Further research is warranted, however, since we cannot exclude a play of chance, or unambiguously ascribe the findings as due to differences in fertility. Subfertility due to, e.g., oligoasthenoteratozoospermia is relatively common and aggregates in families (Lilford et al. 1994), and we are beginning a study of infertile married couples as the next step to examine the possible role of apoE polymorphism in this context.

The evolutionary aspects of our findings are intriguing. If ε4 is indeed the ancestral allele (Hanlon and Rubinsztein 1995), a relatively higher fertility in ε3 homozygotes could certainly contribute to an explanation of the high frequency of this allele in the human lineage. For example, if we let the ratio of average numbers of children observed in this study correspond to relative fitness coefficients in ε4 homo- and heterozygotes of about 0.75, then a simple selection model predicts that the frequency of ε3 in a population would raise from 1–2% to 100% in 100–300 generations (i.e. 2500–7500 years). We do not believe, however, that such figures can either weaken or strengthen the credibility of our findings, considering the oversimplifications implied in the calculations (Vogel and Motulsky 1986). We find it unreasonable, in particular, to assume a higher fertility in ε3ε3 genotypes to have had an invariable effect on net maternity functions in populations everywhere, and at all times. It could be, for instance, that the relative advantage of this genotype is mostly a characteristic of older reproductive individuals, so that the impact on the sojourn of allele frequencies in a population depends on, for example, mean life expectancy and mating structure. ApoE genotypes may also have independent and different effects on probability of survival to reproductive age conditional on the environment. Hence, the cumulative net maternity function (fitness) associated with a particular apoE genotype can be highly variable among populations, and our findings do not imply, for instance, that ε4 should have been extinct in modern humans.

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References

Cavalli-Sforza LL, Menozzi P, Piazza A (1994) The history and geography of human genes. Princeton University Press, Princeton, New Jersey

Corbo RM, Scacchi R, Mureddu L, Mulas G, Alfano G (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

Davignon J, Gregg RE, Sing CF (1988) Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8: 1-21

Dosemeci M, Wacholder S, Lubin JH (1990) Does nondifferential misclassification of exposure always bias a true effect toward the null value ? Am J Epidemiol 132: 746-748

Fiol C, Argimón JM, Hurtado I, Machuca I, Pinto X, Castiñeiras MJ, Gracia V, Llarguès y J, Jiménez E (1991) Estudio poblacional de la distribución del fenotipo de la apolipoproteína E. Clin Invest Arteriosclerosis 3: 130-134

Gerdes LU (1994) Apolipoprotein E genotypes and cardiovascular disease: a quantitative overview of 41 studies. Genet Epidemiol 11: 294 (Abstract)

Gerdes LU, Klausen IC, Sihm I, Faergeman O (1992) Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the World. Genet Epidemiol 9: 155-167

Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Csazar A, Utermann G (1991) The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 49: 338-349

Hanlon CS, Rubinsztein DC (1995) Arginine residues at codons 112 and 158 in the apolipoprotein E gene correspond to the ancestral state in humans. Atherosclerosis 112: 85-90

Hansen PS, Gerdes LU, Klausen IC, Gregersen N, Faergeman O (1994) Genotyping compared with protein phenotyping of the common apolipoprotein E polymorphism. Clin Chim Acta 224: 131-137

Kamboh MI, Bhatia KK, Ferrell RE (1990) Genetic studies of human apolipoproteins: XII. Population genetics of apolipoproteins in Papua New Guinea. Am J Hum Biol 2: 17-23

Kamboh MI, Weiss KM, Ferrell RE (1991) Genetic studies of human apolipoproteins. XVI. APOE polymorphism and cholesterol levels in the Mayans of the Yucatan Peninsula, Mexico. Clin Genet 39: 26-32

Kao JT, Tsai KS, Chang CJ, Huang PC (1995) The effects of apolipoprotein E polymorphism on the distribution of lipids and lipoproteins in the Chinese population. Atherosclerosis 114: 55-59

Keltikangas-Järvinen L, Räikkönen K, Lehtimäki T (1993) Dependence between apolipoprotein E phenotype and temperament in children, adolescents, and young adults. Psychosom Med 55: 155-163

Lilford R, Jones AM, Bishop DT, Thornton J, Mueller R (1994) Case-control study of whether subfertility in men is familial. BMJ 309: 570-573

Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240: 622-630

Mahley RW, Nathan BP, Bellosta S, Pitas RE (1995a) Apolipoprotein E: impact of cytoskeletal stability in neurons and the relationship to Alzheimer's disease. Curr Opin Lipidol 6: 86-91

Mahley RW, Palaoglu KE, Atak Z, Dawson-Pepin J, Langlois AM, Cheung V, Onat H, Fulks P, Mahley LL, Vakar F, Özbayrakci O, Winkler W (1995b) Turkish Heart Study: lipids, lipoproteins, and apolipoproteins. J Lipid Res 36: 839-859

Olson LM, Zhou X, Schreiber JR (1995) Cell-specific localization of apolipoprotein E messenger ribonucleic acid in the testis and epididymis of the rat. Biol Reprod 52: 1003-1011

Rindler MJ, Traber MG, Esterman AL, Bersinger NA, Dancis J (1991) Synthesis and secretion of apolipoprotein E by human placenta and choriocarcinoma cell lines. Placenta 12: 615-624

Sandholzer C, Delport R, Vermaak H, Utermann G (1995) High frequency of the apo epsilon 4 allele in Khoi San from South Africa. Hum Genet 95: 46-48

Schneider WJ, Nimpf J (1993) Lipoprotein receptors: old relatives and new arrivals. Curr Opin Lipidol 4: 205-209

Vogel F, Motulsky AG (1986) Human genetics. Problems and approaches, 2nd edn. Springer, Berlin Heidelberg New York

Weisgraber KH (1994) Apolipoprotein E: structure-function relationships. Adv Prot Chem 45: 249-302