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Apolipoprotein E polymorphism and fertility: a study in pre-industrial populations

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Human apolipoprotein E is the most important supplier of the cholesterol precursor for steroid hormone production in steroidogenic tissues and therefore could play a role in the regulation of steroid hormone function and influence human reproduction. This hypothesis has been confirmed by studies describing a differential fertility associated with common apolipoprotein (APOE) genotypes in two European populations. In the present investigation the impact of APOE genetic variation on fertility was studied in two Ecuadorian populations, African-Ecuadorians (57 women) and Cayapa Indians (27 women). In addition some biodemographic variables concerning women's fertility were investigated (124 African-Ecuadorian women; 40 Cayapa women) to better understand the APOE–fertility relationships in these pre-industrial populations. General fertility rates in both populations were very high (6.5 and 6.2 for the African-Ecuadorians and for the Cayapa respectively). When considering only women near the end of reproductive life (≥40 years), a more marked difference was observed between the two groups (9.1 versus 7.7, $P = 0.09$). In both communities, the highest number of children was found to be associated with the e*4/e*3 genotype; the e*4/e*3 genotype frequency (0.50) in the African-Ecuadorian women with 9–17 children was about three times that of the women with 0–8 children (0.14) ($P = 0.02$). The present findings are at variance with those observed in European populations, where e*3/e*3 was the genotype associated with the highest reproductive efficiency. A possible explanation for this inconsistency could be due to the different functional properties associated with the e*3 and e*4 alleles and to genotype interactions with environmental factors including reproductive strategies.

Key words: African-Ecuadorians/APOE gene polymophism/Cayapa Indians/fertility

Introduction

Apolipoprotein E (APOE = gene, apoE = protein) plays a central role in plasma lipoprotein metabolism and in lipid transport within tissues; it is also involved in cholesterol absorption from the intestine (Kesaniemi *et al.*, 1987; Mahley and Rall, 2000). APOE shows a genetic polymorphism determined by three common alleles, e*2, e*3, e*4, that can be revealed both at the protein and the DNA level (Utermann *et al.*, 1977; Wenham *et al.*, 1991). ApoE is the most important supplier of the cholesterol precursor for steroid hormone production in steroidogenic tissues (Polacek *et al.*, 1992; Swarnakar *et al.*, 1998; Mahley and Rall, 2000; Zofkova *et al.*, 2002). This suggests that apoE could play a role in the regulation of steroid hormone function and, therefore, influence human reproduction at various stages such as age at puberty or menopause, or fertility (Finch and Sapolky *et al.*, 1999). This hypothesis has been confirmed by studies reporting an effect of APOE common polymorphism on serum levels of testosterone and dehydroepiandrosterone in postmenopausal women (Zofkova *et al.*, 2002) and by other studies describing a differential fertility associated with common APOE genotypes in two European populations (Gerdes *et al.*, 1996; Corbo *et al.*, 2004). The latter studies shared the finding that homozygotes for e*3 (112 cys,158 arg), the most common allele, showed a higher fertility (number of children) than did e*2 (112 cys,158 cys) or e*4 (112 arg,158 arg) carriers. It was suggested (Corbo *et al.*, 2004) that the different reproductive efficiency of the APOE genotypes could reflect an effect on steroidogenesis of the different total cholesterol levels associated with the APOE genotypes. Actually the products of the three alleles (isoforms) have different functional properties which affect plasma lipid levels: compared with e*3, the e*4 allele is associated with higher levels of plasma total and LDL cholesterol, and apolipoprotein B, and lower apoE, whereas e*2 is associated with lower levels of total and LDL cholesterol, and apolipoprotein B, and higher apoE (Mahley and Rall, 2000). The finding of an increased fertility in e*3/e*3 homozygotes is consistent with the hypothesis that the E3 isoform properties are the most advantageous for humans, the e*3 allele being the most frequent in human populations (Corbo and Scacchi, 1999).

Because the relationships between the APOE polymorphism and lipid metabolism are influenced by environmental factors, the impact of APOE genetic variation on fertility could also vary according to the ethnicity and/or the lifestyle of different populations. To test this hypothesis, we extended the analysis of the relationship between APOE and fertility to two samples from the African-Ecuadorian and Native American (the Cayapa Indians) communities living in Esmeraldas province, northwestern Ecuador. In addition several relevant biodemographic variables concerning women's fertility and newborn viability were investigated to better understand the APOE/fertility relationships in these populations.

The samples for which records on reproductive habits could be collected are quite small, especially for the Cayapa Indians.

Nonetheless the Cayapa sample represents ~15‰ of all the Cayapa women living today. The two samples are collected from pre-industrial populations which represent the only ones nowadays in which factors affecting 'natural' fertility can be still studied. In developed countries, the homogenization of reproductive patterns at very low levels makes it quite difficult to identify the genetic and environmental components of fertility.

Materials and methods

Subjects

The samples analysed in this paper are part of a larger sampling, randomly collected, constituted of healthy individuals of both sexes, apparently unrelated, belonging to all age classes. Data on fertility and newborn viability were collected through interviews with 164 female subjects (124 African-Ecuadorians and 40 Cayapa Indians) living along the Cayapas river and its tributaries in the province of Esmeraldas, northwestern Ecuador. The African-Ecuadorian community is a rural settlement derived mostly from slaves who escaped from Colombia and phenotypically resemble West Africans. The family structure is mainly polygamic and patrilineal. A more detailed description of the community's living conditions and genetic and demographic structures is given in a previous exhaustive paper (Martinez-Labarga *et al.*, 1999). The Cayapa Indians, also known as the Chachi, are of Andean origin (De Boer, 1987). The total population numbers ~3600 individuals (Carrasco, 1988); at present, they represent more than half the population living in the Cayapas area. The family structure is nuclear; although they live near the African-origin communities, no intermarriage seems to occur between them (Rickards *et al.*, 1994, 1999; Scacchi *et al.*, 1994; De Stefano *et al.*, 2000).

Blood samples could only be collected from part of the total sample ($n = 87$). Venous blood was drawn in acid citrate dextrose (ACD) from all subjects after overnight fasting. Donors were asked to supply their name, birthplace, language and ethnicity for three ascending generations to allow us to determine the extent of recent admixture. Informed consent was obtained from all subjects.

DNA analysis

High-molecular weight DNA was extracted from whole blood using the salting out method described elsewhere (Miller *et al.*, 1988). The APOE genotypes were detected by restriction fragment length polymorphism (RFLP) analysis described elsewhere (Wenham *et al.*, 1991). The sequence including the two polymorphic sites was amplified by PCR in a Perkin–Elmer thermal cycler apparatus. The PCR products were digested with *Cfo*I and the fragments were electrophoresed on agarose gel according to standard techniques.

Statistical and demographic analysis

Allele frequencies were determined by the gene counting method, and the agreement of the genotype distribution with Hardy–Weinberg expectations was calculated by a χ^2-test. Both samples were in Hardy–Weinberg equilibrium. Standard statistical tests were performed to compare fertility differences between the two populations. Comparisons between the mean number of children observed in the different APOE genotypes were performed by parametric (ANOVA) and non-parametric tests (Kruskall–Wallis). Estimates of statistical power ($1 - \beta$) were calculated according to Cohen (1988), i.e. we calculated the probability of rejecting the null hypothesis, when the null is false. In this analysis the null hypothesis is that the APOE genotypes are associated with the same number of children. Prior to the analyses, the number of children values were adjusted for mother's age by linear regression analysis.

For the demographic variables, perinatal mortality includes stillbirth rate plus deaths in the first week of life. Infant mortality refers to babies that died in the first year of life per 1000 livebirths. Data on abortion were quite scanty and scattered and so were considered unreliable for the purposes of this study.

Results

Demographic variables concerning the women's fertility and newborn viability of the populations are reported in Table I.

Table I. Demography of the study populations

	Afro-Ecuadorians	Cayapa Indians
Age	38.6 ± 15.7 (124)	39.1 ± 12.3 (40)
Median (range)	35.5 (15–82)	38.0 (19–70)
Age at menarche	13.9 ± 1.9 (63)	14.2 ± 1.3 (10)
Age at first birth	18.2 ± 4.3 (95)	17.7 ± 2.5 (18)
Birth interval (years)	2.1 ± 0.8 (38)	2.2 ± 0.6 (10)
Pregnancies/woman	7.4 ± 4.9 (124)	6.5 ± 2.8 (40)
Pregnancies/woman (≥40years)[a]	10.6 ± 4.2 (53)	7.7 ± 2.8 (19)
Livebirths/woman	6.5 ± 4.2 (124)	6.2 ± 2.9 (40)
Livebirths/woman (≥40years)	9.1 ± 4.2 (53)	7.7 ± 2.8 (19)
Livebirths	800	246
Stillbirths	13	–
Stillbirth rate (‰)	16.0	–
Deaths in 1st week	37	–
Perinatal mortality (‰)	61.5	–
Deaths in 1st year	66	–
Infant mortality (‰)	82.5	–

Values are mean ± SD (numbers of subjects).
[a]Statistically significant difference: $P = 0.004$.

The mean age at menarche was 13.9 ± 1.9 years for the African-Ecuadorians and 14.2 ± 1.3 years for the Cayapa, which fell within the ranges reported for African or South-American populations (Thomas *et al.*, 2001). First pregnancy age and birth interval were quite low in both samples. In the African-Ecuadorians and in the smaller sample of the Cayapa Indians, the mean number of pregnancies per woman and the mean number of liveborn children per woman were calculated from the total sample of mothers. To obtain information on fertility at the end of reproductive life, both parameters were calculated also for mothers aged ≥40 years. The decision to set the threshold age at 40 years was based on the result of a preliminary analysis on fertility in different maternal ages, which showed that the mean number of children plateaued out after age 40 years. The only statistically significant difference between the two communities was the mean number of pregnancies per woman in mothers aged ≥40 years ($P = 0.004$). The comparison between livebirths per woman aged ≥40 years was approaching significance but was not actually significant ($P = 0.09$). In the African-Ecuadorian population, the stillbirth rate is 16.0‰, the perinatal mortality (stillbirth plus mortality within the first week) is 61.5‰ and the mortality within the first year of life is 82.5‰. The small size of the Cayapa sample ($n = 40$) did not produce reliable results on early mortality.

APOE allele frequencies in the African-Ecuadorian sample were e*2 = 0.158 ± 0.034, e*3 = 0.553 ± 0.046, e*4 = 0.289 ± 0.042. They fell within the range of allele frequencies reported for South-Saharan Africans (Corbo and Scacchi, 1999). Table II lists the mean number of liveborn children by mother's APOE genotype. Because the number of children strongly depends on the mother's age (Table I), both crude values and values adjusted for mother's age are listed (Table II). In the crude and the adjusted values, the highest number of children was found in e*4/e*3 heterozygotes, though the difference in the mean number of children among the APOE genotypes was not significant. When the number of pregnancies was considered, the same result was observed. The power calculation of these analyses is low ($1 - \beta < 0.80$) and the probability of making a type II error is high ($\beta > 0.20$). In fact a significant result was achieved with a different approach: the women were divided into two groups according to the number of children they had, using as a threshold value the median (8.1) of the distribution of the number of children (adjusted for mother's age); the APOE genotype and the allele frequencies were then calculated for each group (Table III). In the group of women with 9–17 children, the e*4/e*3 genotype frequency was about three times that observed in the group with

Table II. Number of liveborn children by apolipoprotein E genotype in African-Ecuadorians

Genotype	Women	Pregnancies	Pregnancies (adjusted for mother's age)	Children	Children (adjusted for mother's age)
e*3/e*3	17	7.0 ± 5.0	7.7 ± 3.4	6.1 ± 4.7	6.7 ± 3.4
e*3/e*2	13	8.6 ± 6.4	8.7 ± 5.3	7.7 ± 5.3	7.7 ± 4.5
e*4/e*4	6	6.8 ± 4.5	7.1 ± 4.7	6.7 ± 4.3	6.9 ± 4.4
e*4/e*3	16	10.8 ± 5.0	9.9 ± 4.3	9.6 ± 3.9	8.9 ± 3.4
e*4/e*2	5	5.8 ± 4.0	7.7 ± 0.9	5.6 ± 3.8	7.3 ± 0.6
n	57	8.3 ± 5.2	8.5 ± 4.2	7.5 ± 4.6	7.6 ± 3.7
P		0.19	0.42	0.19	0.31

Values are mean ± SD.

Table III. Apolipoprotein E genotype and allele frequency (±SE) distribution in African-Ecuadorian women with a different number of children

Genotypes and alleles	0–8 children	9–17 children
e*3/e*3	14 (0.40)	3 (0.14)
e*3/e*2	8 (0.23)	5 (0.23)
e*4/e*4	3 (0.09)	3 (0.14)
e*4/e*3	5 (0.14)	11 (0.50)
e*4/e*2	5 (0.14)	–
n	35	22
P^a	0.015	
e*2	0.19 ± 0.05	0.11 ± 0.05
e*3	0.59 ± 0.06	0.50 ± 0.08
e*4	0.23 ± 0.05	0.39 ± 0.07
P	0.17	

[a]e*4/e*3 was pooled with e*4/e*4 for χ^2 calculation, e*4/e*2 was excluded.

0–8 children, while the e*3/e*3 frequency dropped from 40 to 14% (heterogeneity test: $\chi^2 = 8.4$, df 2, $P = 0.015$).

The allele frequencies in the Cayapa (e*3 = 0.679 ± 0.062, e*4 = 0.321 ± 0.062) were similar to those reported in a larger sample (e*3 = 0.720 ± 0.033, e*4 = 0.280 ± 0.033) (Scacchi *et al.*, 1997). The absence of e*2 allele is a feature typical of Native Americans (Scacchi *et al.*, 1997). Table IV lists the mean number of pregnancies and liveborn children by mother's APOE genotype in the Cayapa. In mothers carrying the e*4/e*3 genotype, a tendency toward having more children was observed, although no significant difference was found between the groups. Also in this case the statistical power is very low ($1 - \beta \ll 0.80$), probably because of the sample size. The sample size was too small to subdivide it according to the number of children.

Discussion

ApoE is recognized as an important molecule in several metabolic functions such as lipid transport or neuronal repair/protection and it is also involved in related pathologies of advanced age, such as cardiovascular disease (CVD) and Alzheimer's disease (AD) (Mahley and Rall, 2000). There are indications that apoE may play a role in reproductive functions as well. The present study investigated the relationships between APOE polymorphism and reproductive efficiency in two pre-industrial populations of African origin and in Native Americans. The findings were compared with those previously obtained from European populations to detect possible differences in heterogeneous environmental conditions.

The African-Ecuadorian and the Cayapa communities offer a good example for exploring natural fertility in pre-industrial populations. Since at the time of data collection the subjects were not using any contraception methods, the number of children reported here can be considered an actual measure of biological fertility. In both populations, the general fertility rates (6.5 and 6.2 for African-Ecuadorians and for the Cayapa Indians respectively) agree closely with the value reported for the Esmeraldas province (6.1) which, in turn, has one of the highest fertility levels among the provinces of Ecuador (Pozo Avalos, 1997). When considering only women near the end of reproductive life (≥40 years), a marked difference was observed between the two groups (9.1 versus 7.7, Table I). The reason for the higher reproduction levels observed in the African-Ecuadorian group might be partly explained by the hypothesis that multiplicity of sexual unions has a positive effect on fertility (Chen *et al.*, 1977). This observation is in agreement with the different sexual behaviours of the two communities, i.e. unstable sexual unions in the African-Ecuadorians versus stable matings in the Cayapa (De Stefano, 1994). The mean maternal age at first birth is lower than that reported for the overall Ecuadorian population (20.7 years) (On line Population Reports, 1994) and the birth interval is near the lower limits for an optimum spacing between births (27–38 months). These results could explain the high fertility and the high infant mortality rate found among African-Ecuadorians, 82.5‰. This value is higher than the index of infant mortality observed in the overall Ecuadorian population, 53‰, for the period of the sample collection (Anonymous, 1991). On the whole, the biodemographic indices reflect the poor socioeconomic conditions of these communities that have a subsistence economy based on fishing, hunting and agriculture for domestic consumption (Rickards *et al.*, 1994; Martinez-Labarga *et al.*, 1999; De Stefano *et al.*, 2000).

In the African-Ecuadorians, the largest sample, the relationship between APOE genotypes and number of liveborn children seems to indicate that the highest fertility is associated with the e*4/e*3 genotype because women carrying it tend to have the highest number of children, and because the e*4/e*3 genotype is the most frequent (50%) among women with 9–17 children. Unfortunately, the sample size was too small to make a fertility scale for the other genotypes, which seem to be loosely associated with a similar number of children. In the sample of the Cayapa, only a tendency in the same direction was seen, but substantially the three genotypes show similar reproductive efficiency. The present findings are at variance with those observed in European populations (Gerdes *et al.*, 1996; Corbo *et al.*, 2004), where e*3/e*3 was the genotype found to be associated with the highest reproductive efficiency (1.9 children in Denmark, 3.9 in Italy) and e*4 and e*2 carrying genotypes with reduced fertility. A possible explanation for this inconsistency could be due to genotype–environment interactions. Most pre-industrial populations have lower plasma cholesterol levels than those observed in western countries. In these populations individuals carrying e*3 and the hypercholesterolaemic e*4 allele would be favoured because this allele could help in rebalancing cholesterol levels which would otherwise be too low (Corbo and Scacchi, 1999) and enhancing steroidogenesis, could play a favourable role in fertility. e*4/e*4 homozygotes would not be associated with increased

Table IV. Number of children and pregnancies by apolipoprotein E genotype in the Cayapa Indians

Genotype	Subjects	Pregnancies	Pregnancies (adjusted for mother's age)	Children	Children (adjusted for mother's age)
e*3/e*3	13	6.3 ± 3.4	6.1 ± 2.4	6.3 ± 3.4	6.1 ± 2.4
e*4/e*4	4	6.3 ± 4.8	5.0 ± 4.8	6.2 ± 4.8	4.9 ± 4.8
e*4/e*3	10	6.6 ± 2.2	7.3 ± 2.2	6.3 ± 2.1	7.1 ± 1.9
n	27	6.4 ± 3.2	6.4 ± 2.8	6.3 ± 3.2	6.3 ± 2.7
P		0.97	0.37	0.99	0.37

Values are mean ± SD.

reproductive efficiency as they lack the useful properties of the E3 isoform. The observation that the e*3/e*2 genotype is also associated with somewhat higher fertility compared with e*3/e*3 is in contrast with the result obtained in the Italian sample, where the lowest fertility was found in subjects carrying e*2, the allele generally associated with lower total cholesterol levels (Corbo *et al.*, 2004). However, the diffence between e*3/e*3 and e*3/e*2 genotypes does not have the same statistical relevance as that between e*3/e*3 and e*4/e*3 genotypes.

The present data indicate that the differences in functional properties among apoE isoforms may be relevant not only in biological processes of advanced age (cardiovascular diseases and neurodegenerative disorders) but also during reproductive age. In this case too, their effects seem to be modulated by environmental factors, including not only dietary habits, but also reproductive strategies (number of children, birth interval, stable/unstable matings, age at first birth, etc.). It should be noted that some factors (number of children, birth interval) are those reproductive characteristics that present family planning programmes try to act on. The implication is that the genetic component of fertility will diminish with time and that the fertility scale is likely to be tipped in favour of the environmental component. As has occurred in industrialized countries, so too in pre-industrialized populations like the African-Ecuadorians and the Cayapa Indians 'cultural' fertility may eventually substitute 'natural' fertility, thus changing the pre-existing genotype–environment relationships.

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References

Anonymous (1991) Sociodemographic indicators of the Andean Pact Countries. Profamilia 7,15–16.

Carrasco E (1988) El pueblo Chachi: el jeengume avanza. Abya-Yala ediciones. Quito, pp 11–31.

Chen KH, Wishik SM and Scrimshaw S (1977) Effects of unstable sexual unions on fertility in Guayaquil, Ecuador. Fam Plann Resume 1,85–88.

Cohen J (1988) 2nd edn. Statistical Power Analysis for Behavioural Sciences, Academic Press, New York.

Corbo RM and Scacchi R (1999) Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele? Ann Hum Genet 63,301–310.

Corbo RM, Scacchi R and Cresta M (2004) Differential reproductive efficiency associated with common apolipoprotein E alleles in postreproductive aged subjects. Fertil Steril 81,104–107.

De Boer WR (1987) Returning to Pueblo Viejo: a review of Chachi (Cayapa) origin, 11th South American Indian Conference, Bennington, Vermont.

De Stefano GF (1994) Biodiversity and Selection in man: an example of biodiversity maintenance by non-adaptive factors. In Hauser G, Little M, and Roberts D (eds), Man, Culture and Biodiversity: Understanding. Biology International Special Issue no.32. Denver, pp 63–71.

De Stefano GF, Martínez-Labarga C, Casalotti R and Rickards O (2000) Allelic genealogy in the Ecuadorian Cayapa Indians and other Native American Populations. In Bhasin MK and Bhasin V (eds), Man Environment Relationship. Human Ecology Special Issue no.9. Kamla-Raj Enterprises, Delhi, India, pp. 267–277.

Finch CE and Sapolsky RM (1999) The evolution of Alzheimer disease the reproductive schedule and apoE isoforms. Neurobiol Aging 20,407–428.

Gerdes LU, Gerdes C, Hansen PS, Klausen IC and Faergeman O (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.

Kesaniemi YA, Ehnholm C and Miettinen TA (1987) Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. J Clin Invest 80,578–581.

Mahley RW and Rall SC (2000) Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 1,507–537.

Martinez-Labarga C, Rickards O, Scacchi R, Corbo RM, Biondi G, Pena JA, Varas de Vieira C, Guevara AE, Santurino MS and dc Stcfano GF (1999) Genetic population structure of two African-Ecuadorian communities of Esmeraldas. Am J Phys Anthropol 109, 159–74.

Miller SA, Dykes DD and Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16,1215.

On line Population Reports, Series M-12 (1994) Opportunities for women through reproductive choice, table 2, vol XXII/1. http://www.infoforhealth.com/pr/online.shtml

Polacek D, Beckmann MW and Schreiber JR (1992) Rat ovarian apolipoprotein E: localization and gonadotropic control of messenger RNA. Biol Reprod 46,65–72.

Pozo Avalos, A (1997) Fertility in Loja, Bolivar, Esmeraldas, Azuay and El Oro. Series: Provincial reports from the Demographic and Maternal-Child Health Survey, 1994 (ENDEMAIN-94). Correo Poblac Salud 5 24-29.

Rickards O, Tartaglia M, Martínez-Labarga C and De Stefano GF (1994) Genetic characterization of the Cayapa Indians of Ecuador and their genetic relationships to other Native American populations. Hum Biol 66,299–322.

Rickards O, Martínez-Labarga C, Lum JK, De Stefano GF and Cann RL (1999) Mitochondrial DNA history of the Cayapa Amerinds of Ecuador: detection of additional founding lineages for the native American populations. Am J Hum Genet 65,519–530.

Scacchi R, Corbo RM, Rickards O and De Stefano GF (1994) The Cayapa Indians of Ecuador: a population study of seven protein genetic polymorphisms. Ann Hum Biol 21,67–77.

Scacchi R, Corbo RM, Rickards O, Mantuano E, Guevara A and De Stefano GF (1997) Apolipoprotein B and E genetic polymorphisms in the Cayapa Indians of Ecuador. Hum Biol 69,375–382.

Swarnakar S, Reyland ME, Deng J, Azhar S and Williams DL (1998) Selective uptake of low density lipoprotein-cholesteryl ester is enhanced by inducible apolipoprotein E expression in cultured mouse adrenocortical cells. J Biol Chem 273,12140–12147.

Thomas F, Renaud F, Benefice E, de Meeus T and Guegan JF (2001) International variability of ages at menarche and menopause: patterns and main determinants. Hum Biol 73,271–290.

Utermann G, Hees M and Steinmetz A (1977) Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man. Nature 269, 604–607.

Wenham PR, Price WH and Blandell G (1991) Apolipoprotein E genotyping by one-stage PCR. Lancet 337,1158–1159.

Zofkova I, Zajickova K, Hill M and Horinek A (2002) Apolipoprotein E gene determines serum testosterone and dehydroepiandrosterone levels in postmenopausal women. Eur J Endocrinol, 147 503–506.

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