Biogerontology (2008) 9:317–323
DOI 10.1007/s10522-008-9143-9

RESEARCH ARTICLE

# Study on a possible effect of four longevity candidate genes (ACE, PON1, PPAR-$\gamma$, and APOE) on human fertility

**Rosa Maria Corbo · Laura Ulizzi · Laura Piombo · Renato Scacchi**

Received: 26 February 2008 / Accepted: 14 April 2008 / Published online: 26 April 2008


**Abstract** The present study investigated for a possible effect on fertility of four longevity candidate genes (ACE, PON1, PPAR-$\gamma$, APOE) in order to determine whether they have a pleiotropic action at different life ages. The study population was 151 healthy unrelated subjects. Only PPAR-$\gamma$ and APOE showed an effect on fertility. The PPAR-$\gamma$ Pro/Ala genotype, which had showed an association with longevity only in men, was found associated only in men with having produced more children ($6.1 \pm 3.3$) than the Pro/Pro genotype ($3.3 \pm 1.9$; $P = 0.001$). APOE*2 allele, which has been consistently associated with longevity, was confirmed to be associated with the lowest fertility ($P = 0.03$). The logistic regression analysis indicated that APOE and PPAR-$\gamma$ polymorphisms may be considered independent determinants of reproductive efficiency. These data suggest that the APOE*2 allele follows the model of antagonist pleiotropy, while the PPAR-$\gamma$ Pro/Ala genotype seems to exert beneficial effects both early in life and in advanced age in a gender-specific way.

**Keywords** Human fertility · Longevity · Antagonist pleiotropy · APOE polymorphism · PPAR-$\gamma$ polymorphism

R. M. Corbo (✉) · L. Ulizzi
Department of Genetics and Molecular Biology, University "La Sapienza", P. A. Moro 5, 00185 Rome, Italy
e-mail: rosamaria.corbo@uniroma1.it

L. Piombo
Department of Animal and Human Biology, University "La Sapienza", P. A. Moro 5, 00185 Rome, Italy

R. M. Corbo · R. Scacchi
CNR Institute of Molecular Biology and Pathology, P. A. Moro 5, 00185 Rome, Italy

## Introduction

The increasing number of older persons in modern society has prompted extensive study into the complexity of the human life-span (longevity) phenotype and its environmental and genetic determinants. A related problem is that, like other organisms, human beings may possess an antagonist longevity-fertility trade-off mechanism. Insights into this intriguing question have come from studies on *D. melanogaster* and *C. elegans* showing that an organism's resources are employed to either maintain the organism itself (somatic maintenance) or ensure successful reproduction (Partridge et al. 2005; Leroi et al. 2005). Since natural selection tends to promote an individual's reproductive ability over its longevity, it is plausible that an antagonistic relationship exists between these two functions as they compete for the same resources.

In the search for a similar longevity-fertility antagonist pattern in humans, the cost of reproduction can be successfully studied in populations that have

not yet undergone a demographic transition, i.e., the process in which a population's fertility and mortality rates both decrease over time, eventually reaching the low levels now seen in Western countries. Research on the longevity-fertility trade-off mechanism has nearly always been done using a historic-demographic approach on nineteenth century European populations (Westendorp and Kirkwood 1998; Korpelainen 2000). Despite certain discrepancies (Le Bourg 2007), these studies appear to confirm the existence of a longevity-fertility trade-off.

Data on pleiotropic genes with an antagonistic effect on fertility and longevity are scarce. A candidate gene could be the interleukin 10 gene (IL-10) which was found to be associated with differential fecundity in women and with longevity (Westendorp et al. 2001; Lio et al. 2004). Another promising candidate, the Apolipoprotein E (APOE) gene, has been associated with susceptibility to degenerative diseases and with differential survival as well. APOE has three common alleles, APOE*2, APOE*3, APOE*4. APOE*4 is a recognized risk factor for Alzheimer's disease (AD) and, to a lesser extent, for coronary heart disease (CHD), whereas APOE*2 seems to protect against both illnesses and has been consistently associated with longer lifespan (Gerdes et al. 2000; Mahley and Rall 2000; Christensen et al. 2006). Recently the APOE*2 allele was found to be associated also with reduced fertility in a sample of the Italian population (Corbo et al. 2004).

The present investigation sought in the same population sample a possible effect on fertility of three genes (ACE, PON1 and PPAR-$\gamma$) previously found associated with longevity in order to determine whether they may exert their action at a different life age. The Italian sample was quite suitable for a fertility study as it comprised subjects born before 1930, who lived their reproductive life in a society where the demographic transition had just started (Ulizzi et al. 1979) and where a differential reproductive efficiency can still be found. The APOE gene was then included in a logistic regression analysis to evaluate the overall effect of the genes found to affect the fertility of the study population.

ACE (angiotensin 1-converting enzyme) hydrolyses angiotensin I to angiotensin II, a potent vasopressor. The ACE gene has an insertion (I)/deletion (D) polymorphism thought to be involved in susceptibility of various pathological conditions (cardiovascular disease, AD, etc.) and to influence longevity as well (Sayed-Tabatabaei et al. 2006). After the initial finding of elevated frequencies of the ACE*D allele in a cohort of French centenarians (Schachter et al. 1994) later studies provided conflicting evidence for a link between the allele and longevity (Panza et al. 2003; Sayed-Tabatabaei et al. 2006). PON1 (Paroxonase) is an arylesterase associated with high density lipoproteins (HDLs) in plasma. It not only helps the body detoxify organophosphate insecticides such as parathion but it may also confer protection against CHD by destroying the proinflammatory oxidized lipids present in oxidized low density lipoproteins (LDLs) (Mackness et al. 1998). While PON1 polymorphisms have been linked with the risk of CHD (Mackness et al. 1998; Christiansen et al. 2004), a small survival advantage has recently been discovered for centenarians carrying the PON1*R allele (Rea et al. 2004), and it even seems to confer significant protection against the onset of sporadic AD (Scacchi et al. 2003). The human peroxisome proliferator-activated receptor gamma (PPAR-$\gamma$2), the most studied isoform of the nuclear receptor superfamily, has a key role in regulating lipid and glucose homeostasis, in adipocyte differentiation and in fatty acid storage. The PPAR-$\gamma$ Pro12Ala polymorphism has been widely investigated in relation to various disorders, including type 2 diabetes, insulin sensitivity, obesity, cardiovascular disease, and cancer (Altshuler et al. 2000; Mori et al. 2001; Meirhaeghe and Amouyel 2004). A male gender-specific association of the Pro/Ala heterozygous genotype with longevity has been recently observed (Barbieri et al. 2004) and explained as probably due to the effect of PPAR-$\gamma$ Pro12Ala polymorphism on BMI and in general on body composition.

## Materials and methods

The Italian sample (151 healthy unrelated subjects in post-reproductive age) was recruited for a multidisciplinary study (LONCILE study) on the anthropological and biological characteristics of the elderly population of the Cilento area in the district of Salerno, southern Italy (Cresta and Gregorio 2001). All subjects were consecutively enrolled without selection criteria. Fertility rates were derived from

responses to a questionnaire item asking about the number of children the subjects reported they had had. The protocol for the collection of biological material for the scientific studies was approved by the institutional ethics committees. Informed consent was obtained from all subjects.

Venous blood was drawn in ethylenediaminetetraacetic acid (EDTA) from all subjects after overnight fasting. High molecular weight DNA was extracted from whole blood according to the procedure described by Miller et al. (1988). The PON1 Gln192 → Arg polymorphism was investigated by Polymerase Chain Reaction (PCR) according to the technique of Serrato and Marian (1995). The PCR product, digested overnight by *Bsp*PI (isoschizomer of *Alw*I), was examined on agarose gel and visualized under ethidium bromide. The insertion-deletion polymorphism of the ACE gene was analyzed according to the method of Tiret et al. (1993). The PPAR-$\gamma$ Pro12Ala polymorphism was investigated by PCR using the primers reported by Mori et al. (2001). The PCR product, digested overnight by *Hha*I, was examined on agarose gel and visualized under ethidium bromide.

Allele frequencies were determined by the gene counting method; agreement of genotype distribution with Hardy–Weinberg expectations was verified by an exact test. Comparisons among the mean number of children associated with different genotypes were performed by parametric (ANOVA) and non-parametric tests (Kruskal–Wallis). Logistic regression was performed to estimate the relationships between fertility (dependent variable) and ACE, PON1, PPAR-$\gamma$, and APOE genotypes (explanatory variables).

**Table 1** Study sample characteristics

| | Total ($n = 151$) | Men ($n = 73$) | Women ($n = 78$) |
|---|---|---|---|
| Mean age (yr) | 82.2 ± 4.8 | 83.9 ± 4.5 | 82 ± 5.8 |
| No. of children | 542 | 268 | 274 |
| Mean n° of children | 3.6 ± 2.3 | 3.7 ± 2.3 | 3.5 ± 2.4 |
| Children sex ratio (males/females) | 1.02 | 0.9 | 1.1 |
| Childless subjects (%) | 6 | 3 | 9 |

**Table 2** Mean number of children stratified by ACE, PON1, PPAR-$\gamma$ and APOE genotypes

| Gene | Genotype | Number | No. of children |
|---|---|---|---|
| ACE | I I | 19 (0.13) | 3.2 ± 2.0 |
| | ID | 71 (0.47) | 3.6 ± 2.1 |
| | DD | 61 (0.40) | 3.7 ± 2.7 |
| | $P = 0.70$ | | |
| PON 1 | Q/Q | 74 (0.49) | 3.6 ± 2.3 |
| | Q/R | 71 (0.47) | 3.7 ± 2.5 |
| | R/R | 6 (0.04) | 2.8 ± 1.2 |
| | $P = 0.69$ | | |
| PPAR-$\gamma$ | Pro/Pro | 131 (0.87) | 3.4 ± 2.1 |
| | Pro/Ala | 19 (0.13) | 5.1 ± 3.4 |
| | $P = 0.047$ | | |
| APOE | E*2/E*2 | 2 (0.013) | 1.5 ± 0.7 |
| | E*3/E*3 | 115 (0.177) | 3.8 ± 2.5 |
| | E*2/E*3 | 14 (0.09) | 2.6 ± 1.6 |
| | E*3/E*4 | 19 (0.13) | 3.7 ± 1.6 |
| | $P = 0.03$ | | |

Plus-minus values are means ± SD

Allele frequencies: ACE*D = 0.639 ± 0.03; PON1*R = 0.275 ± 0.03; PPAR-$\gamma$*Ala = 0.063 ± 0.01; APOE*2 = 0.06 ± 0.01; APOE*3 = 0.877 ± 0.02

## Results

The characteristics of the study sample are reported in Table 1. The subjects were in post-reproductive age and had completed fertility. The mean number of children was 3.6 ± 2.3, range 0–13, median 3.

Table 2 reports the mean number of children produced by ACE, PON1, PPAR-$\gamma$, and APOE genotypes. Genotype frequencies fitted with those expected according to Hardy–Weinberg equilibrium. APOE*2 carrying genotypes were associated with the lowest number of children. These data, previously reported by Corbo et al. (2004) are given here for completeness. Besides to the APOE polymorhism, only the PPAR-$\gamma$ polymorphism appeared to significantly influence fertility, since the Pro/Ala heterozygous genotypes was associated with the highest number of children (5.1 ± 3.4). Subjects carrying PON1 RR genotype, the genotype associated with a survival advantage, showed a trend to lower fertility, but the difference in the mean number of children associated with the PON1 genotypes was not significant. The ACE polymorphism had no impact on the number of children produced. As the

association of PPAR-$\gamma$ Pro12Ala with longevity had been observed only in the men (Barbieri et al. 2004), the fertility data were stratified by gender and the effect of PPAR-$\gamma$ on fertility resulted gender-specific only for the men ($P < 0.001$) (Table 3).

To better understand the overall role of these genes in fertility and to study the effect on fertility of each genotype adjusted for the other variables, the data were tested by logistic regression analysis. Because the number of childless subjects in the sample was very low ($n = 9$; 6%) and the results indicated that both the APOE and PPAR-$\gamma$ genes may influence the number of children produced rather than to have children or not, the dependent variable in the logistic regression was having produced a number of children higher than the median (or not). The median value was chosen as cut point to transform the continuous variable "number of children" in a dichotomous variable as its value seemed a more representative measure of the average children number than the mean which was influenced by the presence of few subjects ($n = 4$) with a very high number of children (11 and 13). The explanatory variables were APOE and PPAR-$\gamma$ genotypes and gender (Table 4). The analysis indicated that the APOE and PPAR-$\gamma$ polymorphisms may both be independent determinants of reproductive efficiency. The APOE*2 carriers were significantly less likely to produce a number of children higher than the median (adjusted Odds Ratio [OR] = 0.20, 95% Confidence Interval [CI] 0.05–0.79; $P = 0.026$). Consistent with ANOVA results, the PPAR-$\gamma$ Pro/Ala genotype was found to be significantly associated with producing a number of children higher than the median only when its interaction with male gender (interaction term adjusted OR = 19.13, 95% CI 1.42–257.5; $P = 0.026$) was taken into account, with an estimated OR = 10.9 for men carrying the Pro/Ala genotype versus women not carrying it.

The genetic polymorphisms examined did not show any effect on children sex ratio.

**Table 3** Mean number of children stratified by gender and PPAR-$\gamma$ genotypes

| | Genotype | Number | No. of children |
|---|---|---|---|
| Men | Pro/Pro | 62 | 3.3 ± 1.9 |
| | Pro/Ala | 10 | 6.1 ± 3.3 |
| | $P = 0.001$ | | |
| Women | Pro/Pro | 69 | 3.5 ± 2.2 |
| | Pro/Ala | 9 | 3.9 ± 3.4 |
| | $P = 0.61$ | | |

Plus-minus values are means ± SD

**Table 4** Estimates of the effects of APOE and PPAR-$\gamma$ genotypes on the probability of having a number of children higher than the median (median = 3)

| Variables | OR | 95% CI | *P*-value |
|---|---|---|---|
| Gender | 0.57 | 0.28–1.16 | 0.57 |
| APOE*2 carrying genotypes | 0.20 | 0.05–0.83 | 0.026 |
| PPAR-$\gamma$ Pro/Ala | 1 | 0.23–4.29 | 1 |
| PPAR-$\gamma$ Pro/Ala *x* gender | 19.1 | 1.42–257.53 | 0.026 |

OR, odds ratio; CI, confidence interval

## Discussion

This study investigated the effect on fertility of genes previously found associated with longevity in order to verify whether they act as pleiotropic genes and follow or not the model of antagonistic pleiotropy. In this study sample the fertility rate (3.6 ± 2.3) was an intermediate value with respect to the range of 4.4–2.4 reported for cohorts of women born in the same geographic area and during the same period (1910–1930) (Istituto Centrale di Statistica 1974), i.e., in a period when family planning began to be accepted (Ulizzi et al. 1979). However, the mean number of children produced and the range are such as to be still useful to detect a differential reproductive efficiency associated with different genotypes. The proportion of childless subjects is also comparable with the demographic data (8.1–7.1). The allele frequencies were similar to those previously reported for the not aged population of Southern Italy (ACE*D = 0.61, Corbo and Scacchi 2001; PON1*R = 0.264, Rea et al. 2004; APOE*3 = 0.869, APOE*2 = 0.055, Corbo et al. 1995; PPAR-$\gamma$*Ala = 0.05–0.07, Barbieri et al. 2004; Scacchi et al. 2007). Since the study subjects are elderly, but not extremely old [mean age = 82 years; mean life expectancy = 7 years for women and 6 years for men (ISTAT 2008)], it is not surprising that the effect of longevity genes, i.e., different allele frequencies between not aged and elderly subjects, is not apparent in this sample, although it has been observed in other Italian samples

(Barbieri et al. 2004; Rea et al. 2004; Seripa et al. 2006).

Of these four longevity candidate genes, only PPAR-$\gamma$ and APOE appeared to have a significant effect on fertility as well and to influence the level of reproductive efficiency. In the analysis of the PPAR-$\gamma$ gene, the Pro/Ala genotype was associated only in men with the highest fertility, showing that the male Pro/Ala carriers had a number of children about two-fold higher than those of the Pro/Pro homozygotes. PPAR-$\gamma$, a nuclear receptor mainly expressed in adipose tissue, is also present at lower levels in many other tissues. Expression of the PPAR-$\gamma$ receptor has recently been demonstrated in male and female reproductive tissues (Froment et al. 2006). In males it has been detected in Sertoli cells and spermatozoa (Aquila et al. 2006; Froment et al. 2006), where its activation may influence sperm physiology (Aquila et al. 2006). This receptor has a recognized role in regulating lipid and glucose homeostasis and it may act as a link between energy metabolism and reproduction (Froment et al. 2006). The PPAR-$\gamma$ Ala allele, which has been found to be associated with improved insulin sensitivity (Deeb et al. 1998), could exert a beneficial effect on glucose metabolism and energy balance. Of note is that in women with the polycystic ovary syndrome increased insulin sensitivity may improve ovulatory function and fertility (Aquila et al. 2006). This suggests that the PPAR-$\gamma$ Ala carrying genotypes would improve male fertility with a similar mechanism, although the results should be confirmed in a larger sample. Unfortunately the reduced frequency of PPAR-$\gamma$ Ala allele ($\sim$0.07) and Pro/Ala genotype ($\sim$0.13) in the Italian population represents a limitation of the study.

The observation that the PPAR-$\gamma$ Pro/Ala genotype was found to be associated with longevity only in males (Barbieri et al. 2004) suggests that the PPAR-$\gamma$ gene, or better its Ala allele, may have a positive, rather than an antagonistic, pleiotropic effect across different life ages, but in a gender specific way. In other words, the same genotype would confer males carrying the Ala allele a reproductive advantage early in life and would predispose them to longevity, perhaps through the protective role this allele exerts against diabetes, CHD and other age-related illnesses. This finding does not dismantle the hypothesis of a negative relationship between fertility and longevity, because the physiological cost of reproduction purported to exist for women could not be expected for men as confirmed by studies that frequently found no trade-off mechanism in males (Korpelainen 2000; Doblhammer and Oeppen 2003). Moreover present observations on PPAR-$\gamma$ seem to support the hypothesis (Barbieri et al. 2004) that gender, interacting with genetic factors, may influence substantially human life-span determination, and that men and women can reach longevity by means of gender specific strategies.

In our logistic regression model, the APOE*2 carrying genotypes were included as an explanatory variable because APOE*2 was previously found associated with the lowest fertility in the population sample. The negative effect on higher fertility of APOE*2 was still present after adjustment for PPAR-$\gamma$ genotype, and no gender interaction was observed. The role of APOE as a candidate gene for life-span determination has been consistently confirmed by several studies showing different APOE allele frequencies across age groups including centenarians (Gerdes et al. 2000; Zubenko et al. 2002; Christensen et al. 2006). APOE*2 allele frequencies tend to increase with increasing age, indicating that the allele favours a longer life-span probably through its protective action against susceptibility to common illnesses such as cardiovascular disease and AD (Gerdes et al. 2000).

The association of the APOE*2 allele with lower fertility and a longer life-span makes it a unique example of an allele with an antagonistic pleiotropic effect on fertility and longevity. A possible explanation for these findings could come from the lower total cholesterol levels that are generally associated with the APOE*2 allele (Mahley and Rall 2000). This property could have an unfavourable effect on steroidogenesis in APOE*2 carriers and, as a consequence, on their fertility in reproductive age. But it could turn out to be advantageous in advanced age, protecting such carriers from hypercholesterolemia and atherosclerosis. Moreover, mounting evidence that past fertility can be a risk factor for AD in women and influence its onset age (Corbo et al. 2007) offers a further explanation of a protective role of APOE*2 against the disease through its lowering effect on reproductive efficiency, thus predisposing the individual to a longer life.

An additional observation is that the most frequent APOE*3 allele, associated with the highest fertility, do

not show significantly reduced frequencies in the very old subjects, whereas APOE*4, associated with a lower fertility than APOE*3 (Gerdes et al. 1996), is associated with a shorter life-span, given its recognized role of risk allele for AD and cardiovascular disease (Gerdes et al. 2000). In brief, APOE represents an example of the various kinds of pleiotropic actions a polymorphic gene can exert across different life ages.

This study was a first attempt at unravelling the complex longevity-fertility relationship at the gene level. By comparing candidate genes (APOE and PPAR-$\gamma$) and several alleles at a single locus (APOE alleles), the study shows that some longevity candidate genes can influence reproductive function and that they can exert a pleiotropic action in all sorts of different ways.

**Acknowledgements** We are grateful to K.A. Britsch for reviewing the manuscript. Financial support from University 'La Sapienza'.

## References

Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J et al (2000) The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 26:76–80

Aquila S, Bonofiglio D, Gentile M, Middea E, Gabriele S, Belmonte M, Catalano S, Pellegrino M, Andò S (2006) Peroxisome proliferator-activated receptor (PPAR) gamma is expressed by human spermatozoa: its potential role on the sperm physiology. J Cell Physiol 209:977–986

Barbieri M, Bonafè M, Rizzo MR, Ragno E, Olivieri F, Marchegiani F, Franceschi C, Paolisso G (2004) Gender specific association of genetic variation in peroxisome proliferator-activated receptor (PPAR) gamma-2 with longevity. Exp Gerontol 3:1095–1100

Christensen K, Johnson TE, Vaupel JW (2006) The quest for genetic determinants of human longevity: challenges and insights. Nat Rev Genet 7:436–448

Christiansen L, Bathum L, Frederiksen H, Christensen K (2004) Paraoxonase 1 polymorphisms and survival. Eur J Hum Genet 12:843–847

Corbo RM, Scacchi R (2001) Distribuzione di alcuni fattori genetici di rischio per la malattia coronaria in un campione di ultraottuagenari del Cilento. Confronto con gli anziani di altre popolazioni europee. Risultati dello studio LONCILE. Riv Antrop 79(suppl): 49–58

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 tecnique comparison with frequency data of other European populations. Ann Hum Genet 59:197–209

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

Corbo RM, Gambina G, Ulizzi L, Monini P, Broggio E, Rosano A, Scacchi R (2007) Combined effect of apolipoprotein e genotype and past fertility on age at onset of Alzheimer's disease in women. Dement Geriatr Cogn Disord 24:82–85

Cresta M, Gregorio G (2001) Il disegno dello studio LONCILE. Riv Antrop 79:11–18

Deeb SS, Fajas L, Nemoto M, Pihlajamäki J, Mykkänen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20:284–287

Doblhammer G, Oeppen J (2003) Reproduction and longevity among the British peerage: the effect of frailty and health selection. Proc Biol Sci 270:1541–1547

Froment P, Gizard F, Defever D, Staels B, Dupont J, Monget P (2006) Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition. J Endocrinol 189:199–209

Gerdes LU, Gerdes C, Hansen PS, Klausen IC, 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

Gerdes LU, Jeune B, Ranberg KA, Nybo H, Vaupel JW (2000) Estimation of apolipoprotein E genotype-specific relative mortality risks from the distribution of genotypes in centenarians and middle-aged men: apolipoprotein E gene is a "frailty gene", not a "longevity gene". Genet Epidemiol 19:202–210

ISTAT (2008) http://demo.istat.it/unitav/index.html

Istituto Centrale di Statistica (1974) Indagine sulla fecondita' della donna. Note Relazioni 50:96–97

Korpelainen H (2000) Fitness, reproduction and longevity among European aristocratic and rural Finnish families in the 1700s and 1800s. Proc R Soc Lond B Biol Sci 267:1765–1770

Le Bourg E (2007) Does reproduction decrease longevity in human beings? Ageing Res Rev 6:141–149

Leroi AM, Bartke A, De Benedictis G, Franceschi C, Gartner A, Gonos ES, Fedei ME, Kivisild T, Lee S, Kartaf-Ozer N, Schumacher M, Sikora E, Slagboom E, Tatar M, Yashin AI, Vijg J, Zwaan B (2005) What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? Mech Ageing Dev 126:421–429

Lio D, Candore G, Crivello A, Scola L, Colonna-Romano G, Cavallone L, Hoffmann E, Caruso M, Licastro F, Caldarera CM, Branzi A, Franceschi C, Caruso C (2004) Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: genetic background of male centenarians is protective against coronary heart disease. J Med Genet 41:790–794

Mackness B, Durrington PN, Mackness MI (1998) Human serum paraoxonase. Gen Pharmacol 31:329–336

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

Meirhaeghe A, Amouyel P (2004) Impact of genetic variation of PPARgamma in humans. Mol Genet Metab 83:93–102

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

Mori H, Ikegami H, Kawaguchi Y, Seino S, Yokoi N, Takeda J, Inoue I, Seino Y, Yasuda K, Hanafusa T, Yamagata K, Awata T, Kadowaki T, Hara K, Yamada N, Gotoda T, Iwasaki N, Iwamoto Y, Sanke T, Nanjo K, Oka Y, Matsutani A, Maeda E, Kasuga M (2001) The Pro12/Ala substitution in PPARgamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. Diabetes 50:891–894

Panza F, Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Kehoe PG, Capurso A (2003) Angiotensin I converting enzyme (ACE) gene polymorphism in centenarians: different allele frequencies between the North and South of Europe. Exp Gerontol 38:1015–1020

Partridge L, Gems D, Withers DJ (2005) Sex and death: what is the connection? Cell 120:461–472

Rea IM, McKeown PP, McMaster D, Young IS, Patterson C, Savage MJ, Belton C, Marchegiani F, Olivieri F, Bonafe M, Franceschi C (2004) Paraoxonase polymorphisms PON1 192 and 55 and longevity in Italian centenarians and Irish nonagenarians. A pooled analysis. Exp Gerontol 39:629–635

Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JC (2006) ACE polymorphisms. Circ Res 98:1123–1133

Scacchi R, Gambina G, Martini MC, Broggio E, Vilardo T, Corbo RM (2003) Different pattern of association of paraoxonase Gln192→Arg polymorphism with sporadic late-onset Alzheimer's disease and coronary artery disease. Neurosci Lett 339:17–20

Scacchi R, Pinto A, Rickards O, Pacella A, De Stefano GF, Cannella C, Corbo RM (2007) An analysis of peroxisome proliferator-activated receptor gamma (PPAR-gamma 2) Pro12Ala polymorphism distribution and prevalence of type 2 diabetes mellitus (T2DM) in world populations in relation to dietary habits. Nutr Metab Cardiovasc Dis 17:632–641

Schachter F, Faure-Delanef L, Guenot F, Rouger H, Froguel P, Lesueur-Ginot L, Cohen D (1994) Genetic associations with human longevity at the APOE and ACE loci. Nat Genet 6:29–32

Seripa D, Franceschi M, Matera MG, Panza F, Kehoe PG, Gravina C, Orsitto G, Solfrizzi V, Di Minno G, Dallapiccola B, Pilotto A (2006) Sex differences in the association of apolipoprotein E and angiotensin-converting enzyme gene polymorphisms with healthy aging and longevity: a population-based study from Southern Italy. J Gerontol A Biol Sci Med Sci 61:918–923

Serrato M, Marian AJ (1995) A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. J Clin Invest 96:3005–3008

Tiret L, Kee F, Poirier O, Nicaud V, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Amouyel P (1993) Deletion polymorphism in angiotensin-converting enzyme gene associated with parental history of myocardial infarction. Lancet 341:991–992

Ulizzi L, San Martini A, Terrenato L (1979) Changes of selection opportunities with a changing environment: regional heterogeneity in Italy. Ann Hum Genet 43: 137–141

Westendorp RG, Kirkwood TB (1998) Human longevity at the cost of reproductive success. Nature 396:743–746

Westendorp RG, van Dunne FM, Kirkwood TB, Helmerhorst FM, Huizinga TW (2001) Optimizing human fertility and survival. Nat Med 7:873

Zubenko GS, Stiffler JS, Hughes HB III, Fatigati MJ, Zubenko WN (2002) Genome survey for loci that influence successful aging: sample characterization, method validation, and initial results for the Y chromosome. Am J Geriatr Psychiatry 10:619–630