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# Estrogen receptor 1 gene polymorphisms and decreased risk of obesity in women

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## Abstract

Estrogen receptor alpha gene (ESR1) polymorphisms have been associated with several diseases, but whether they are associated with obesity is uncertain. To elucidate the role of genetic variation in the ESR1 gene with body mass index (BMI), 543 Caucasian women (median age 63 years) from the Women's Health Study were examined. Most were postmenopausal (99.3%). The relationships between rs2234693 and rs9340799 genotypes and their associated haplotypes with obesity (BMI $\geq$ $30kg/m^2$) and overweight (BMI$\geq 25kg/m^2$) were evaluated. Among women with the rs2234693 TT genotype, 18.3% were obese, while only 8.2% of those with the CC genotype only were obese (p=0.04). In a logistic regression model assuming additive inheritance, rs2234693 was associated with decreased odds of obesity (BMI $\geq$ 30) (crude odds ratio [OR] = 0.63, 95%CI= 0.44-0.90, *P*=0.01). For rs9340799, only an inverse trend was observed for BMI (*P*=0.08). Haplotypes that included the variant C allele were associated with a reduced risk of obesity (crude OR=0.65, 95% CI=0.44-0.94, *P*= 0.02 for C-G). The rs2234693 C allele of ESR1 and its associated genotypes and haplotypes were inversely and consistently associated with obesity. One or more copies of the C allele were associated with decreased risk of obesity in white post-menopausal women.

### Keywords

estrogen receptor α; genetic polymorphism; obesity; body mass index; women

## 1. Introduction

Obesity is a complex disease, which can be influenced by multiple genetic and environmental factors(1). Recently the Human Obesity Gene Map listed the estrogen receptor alpha gene (ESR1) as one of 127 possible candidate genes associated with obesity(2). ESR1 resides on chromosome 6q25 and is comprised of 8 exons and 7 introns with a total size of 140 kilobases (3).

ER-α expression has been related both to menopausal status and adiposity in women (4,5). Polymorphisms of ESR1 have been also associated with variations in BMI and waist circumference (6-11)(8)(9)(10)(11). In a Japanese study, the rs9340799 polymorphism, particularly the GG genotype, was associated with decreased whole-body and abdominal fat in older women individuals (7). In the Framingham Heart Study (FHS), three ESR1 polymorphisms (rs2234693, rs9340799 and rs1801132) were associated with measures of adiposity in men, but not women (9). A number of other small studies have found diverse results for the relationship between body mass index and ESR1 polymorphisms (6-10).

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However, relatively few studies of genetic variation of ESR1 and obesity in postmenopausal women have been conducted (10)(10). The purpose of this study was to examine the relationship of two polymorphisms in ESR1 (rs2234693 and rs9340799) with risk of overweight or obesity in women.

## 2. Materials and Methods

### 2.1. Subjects

Study participants were enrolled in the Women's Health Study (WHS), a recently completed, randomized, double-blinded, placebo-controlled trial of low-dose aspirin and vitamin E initiated in 1992 among 39876 female, predominantly Caucasian, U.S. health professionals, 45- 89 years of age at study entry (12,13). Before randomization, 28,524 participants provided an EDTA-anticoagulant blood sample that was stored for genetic analysis. All participants were free of prior MI, stroke or any serious illness that might preclude participation at study entry (12). Women enrolled in the WHS completed a baseline questionnaire, which included questions on demographics, health characteristics/behaviors (height, weight, alcohol use, diet, smoking status, physical activity, hormone therapy use), menopausal status, past medical history of hypertension, diabetes mellitus and elevated cholesterol. For those women aged 60 years or older, self-reported of permanent cessation of menstrual periods due to natural menopause, complete oophorectomy, radiation, or chemotherapy was considered postmenopausal status. Yearly follow-up self-report questionnaires were used to update information(12).

Data on ESR1 genetic variation were available for 543 Caucasian WHS participants who had previously been selected as control subjects for a nested case-control study of genetic variation of ESR1 and CVD. Of these, 539 (99.3%) were post-menopausal, with an additional 4 women who had biologically uncertain menopause. The study protocol was approved by the Brigham and Women's Hospital Institutional Review Board for Human Subjects Research.

### 2.2. Exposure Variables

Baseline BMI [self-reported weight in kilograms divided by the square of self-reported height in meters $(kg/m^2)$] was analyzed as a continuous and categorical variable, according to World Health Organization criteria as follows: $< 25 \, kg/m^2$ (normal), 25 to$<30.0 \, kg/m^2$ (overweight) and $\geq 30 \, kg/m^2$ (obesity) (14). Self-reported weight were highly correlated (r=0.96) in a similar population female health professionals(15).

### 2.3. Genotype Determination

Two single -nucleotide polymorphisms in the ESR1 gene (rs2234693 and rs9340799) were chosen based on prior associations for cardiovascular disease in the literature (8,16). Both of these polymorphisms are in the first intron of ESR1 gene, 397 and 351 base pairs upstream of exon 2. The rs2234693 polymorphism is characterized by a T→C transition (also known as *c. 454-497T>C*) that obliterates the *Pvu*II restriction site. The T allele has previously been called the *p* allele, while the C allele has been called the *P* allele, denoting the absence of the *Pvu*II restriction site. The rs9340799 polymorphism marks an A→G transition (also known as *c. 454-351A>G*). Those with the G allele have an absent *Xba*I site which has previously been called *X* in the literature, with the A allele denoted by *x*. This polymorphism has previously also been called *IVS1-354A/G*.

Genotype determination was performed using ABI fluorescence-based allelic discrimination method (Applied Biosystems, Foster City, USA). Each 10 mL amplification reaction volume contained 1X Taqman Universal Master Mix (Applied Biosystems, Foster City, USA) and 10

ng of template DNA. Amplification reactions were carried out in duplicates on ABI 7900HT Sequence Detection System according to the manufacture's specifications.

To confirm genotype assignment, two independents observers carried out scoring. Discordant results (1% of all scoring) were resolved by a joint reading, and where necessary, repeat genotyping.

### 2.4. Statistical Analysis

The baseline characteristics were examined according to BMI categories based on WHO criteria (14). Based on nonparametric distributions, and Kruskal Wallis- was used to test medians across categories-- add citation), and Chi-square test was used for categorical variables. Genotype and allele frequencies were calculated and Hardy-Weinberg equilibrium was tested using $\chi^2$ -analysis. The association between ESR1 genotypes and adiposity was examined using BMI, as a continuous variable as well as according to WHO BMI categories (14). General linear regression models were used to determine whether BMI varied according to ESR1genotypes. In addition, the relationships between genotypes and adiposity were examined by logistic regression using dichotomous outcomes, obesity (BMI $< 30kg/m^2$ vs $\geq$ $30kg/m^2$) and overweight (BMI $< 25kg/m^2$ vs $\geq 25kg/m^2$). Additional adjustment for age at randomization, age at menopause, smoking status (never, past and current), physical activity (rarely/never, <1/week, 1-3/week and $\geq$ 4/week) and hormone therapy use (yes/no), was also performed. All analyses were conducted assuming an additive mode of inheritance. Possible associations between several covariates [alcohol and red wine consumption; total intakes of carbohydrate, protein, saturated, monounsaturated and polyunsaturated fat (all of them adjusted by energy); educational level; marital status and income] were evaluated, and considered in the regression model. Potential interactions between hormone therapy use (HT) and ESR1 genotypes were tested using a formal interaction term (genotype * HT) as well as in analyses stratified by HT. Pairwise linkage disequilibrum (LD) was examined as described by Devlin and Risch (17). Haplotype frequencies were estimated from genotype data using the PHASE v2.1 algorithm (18,19). For each odds ratio, we calculated 95% confidence intervals (CIs). A two-tailed p-value of 0.05 was considered to represent a statistically significant result. All statistical analyses were conducted with the use of SAS software (version 9.1; SAS institute, Cary, NC).

## 3. Results

Baseline characteristics of the 543 women are shown in Table 1. The median age was 63 years, and most (99.3%) were postmenopausal. The prevalence of BMI $\geq 25$ kg/m$^2$ was 46%.

The allele frequencies for rs2234693 were 54% for T and 46% for C alleles, while for rs9340799 the frequencies were 66% for A and 34% for G alleles. Median BMI did not differ by genotype for either polymorphism. (Data not shown) There was borderline significance (p=0.09) between BMI categories (normal, overweight and obese) and rs2234693 genotypes. We found no evidence for any significant association with BMI categories and rs9340799 genotypes. (Data not shown)

Of those with the TT genotype, 18.3% were obese, compared with 13.5% of those with TC and only 8.2% of those with CC genotypes. (Table 1) When obese women were compared with non-obese women ($< 30kg/m^2$), the inverse association between BMI and rs2234693 genotype was more evident (p=0.04). (Data not shown) No significant associations between BMI and ERS1 genotypes were observed in general linear regression models. (Data not shown)

In logistic regression analyses, the rs2234693 polymorphism was inversely associated with obesity; the age-adjusted OR was 0.63 (95% 95%CI=0.44-0.90) for BMI $> 30$ kg/m$^2$, compared

to BMI <30 kg/m$^2$. Additional adjustment slightly strengthened the association for obesity. (Table 2) For rs9340799 there was only a trend in the same direction, comparing obese to nonobese women (p= 0.08). In stratified analyses, we explored potential effect modifications by hormone use. The rs2234693 polymorphism was associated with decreased risk of obesity among women who did not use HT (p=0.001). (Data not shown) There was no significant association for obesity among hormone therapy users, but very few hormone users were obese (n=16). The interaction terms between hormone use and ESR1 genotypes were not significant (p for interaction term=0.13 and 0.41 for rs2234693 and rs9340799, respectively).

The polymorphisms tested were in LD (normalized Lewontin's *D'* = 0.96). Of four possible haplotypes, one haplotype (T-G) had a frequency of <5%. The most common haplotype, T-A, had a frequency of 52%, and the C-A and C-G haplotypes had frequencies of 13% and 33%, respectively. In logistic regression analyses, adjusting for the same covariates used in the genotype models was observed a decreased risk of obesity among women with the C-G haplotype (OR=0.65, 95%CI=0.44-0.94, p= 0.02) (Table 3). Further, adjustment resulted in only minor changes in risk estimates.

## 4. Discussion

In these women, one or more copies of the variant C allele of rs2234693 and its associated haplotypes were associated with decreased risk of prevalent obesity. Although there has been considerable controversy about the relationship between ESR1 polymorphisms and BMI, our findings are similar to several other reports (7,8,10). The FHS investigated the association between the ESR1 polymorphisms and BMI, as well as the relationship with waist circumference in 1763 unrelated men and women (mean age 56 years) (9). In this population, men homozygous for the rs2234693 C allele had lower waist circumference (99.3 cm), than TT homozygotes (99.8cm) and heterozygotes (100.6 cm) (P> 0.004). Similar results were also observed for the *rs9340799* polymorphism. Although no association was observed for women, this may have been due to a smaller sample size. Women in the FHS were also somewhat younger than women in our sample and not all were postmenopausal (9). A Brazilian case-control study of 295 subjects (mean age 44 years), investigated the association of ESR1 polymorphisms with premature coronary artery disease and some cardiovascular risk factors (8). In this younger sample, the rs9340799 polymorphism, but not rs2234693, was associated with lower BMI (8). Okura et al also found a similar inverse association with adiposity parameters and the rs9340799 polymorphism in 2238 middle-aged and elderly Japanese population (7) Older women (60-79 years) homozygous for the variant (GG) had a lower whole-body fat mass and waist circumference compared to those homozygous for wild type (7). No difference was found for rs2234693, with the exception of a slightly lower mean BMI (22.0 ±0.3kg/m$^2$) among older men (60-79y) with the CC genotype (TT>CC, *P*=0.03) (7). Although some previous studies included post-menopausal women in their analyses, few have studied the association between ESR1 and adiposity only in this population (10). Deng et al examined the association of the ESR1 rs2234693 genotypes and BMI in a small sample of 108 healthy post-menopausal Caucasian women (10). Women with TC and CC genotypes had, respectively, 4.8% and 11.4% higher mean BMI than those with the TT genotype (10). In comparison, women with the CC genotype in our study had a lower prevalence of obesity (8.2%) compared to those with TT and TC (18.3% and 13.5%, respectively) (*P*=0.04). A similar, but nonsignificant trend was also observed for the rs9340799 genotype, which is not unexpected given the tight linkage disequilibrium between these two polymorphisms.

ER-α expression has been related both to menopausal status and obesity (5). Meza-Munoz et al found lower levels of ER-α and progesterone receptors in adipose tissue of post-menopausal than pre menopausal women. Non obese menopausal women had lower levels of ER-α expression in adipose tissue (*P*< .03) than postmenopausal obese women (5). In post-

menopausal women, the primary source of estrogens is adrenal steroids converted to bioactive estrogens in fat tissue(20). It has been proposed of the ER-α may determine tissue sensitivity to estrogens(21). This study has several strengths and limitations. It is one of the largest reported studies of ERS1 polymorphisms and obesity in postmenopausal women. Of note, our analyses were restricted to Caucasian participants, and thus cannot necessarily be applied to other racial or ethnic groups.

While we have observed an inverse association between these polymorphisms and obesity in women, we have no direct evidence of causative relationship for these particular polymorphisms. We had limited power to detect interactions or perform subgroups analyses. However, the current results do replicate findings of some prior groups.

In conclusion, we found a significant inverse association between the ESR1 rs2234693 polymorphism and prevalent obesity in women. Carriers of the rs2234693-C minor allele had a 38% lower prevalence of obesity. The mechanisms by which the ESR1 gene might be related to obesity deserve further exploration.

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## References

1. Comuzzie AG, Williams JT, Martin LJ, Blangero J. Searching for genes underlying normal variation in human adiposity. J Mol Med 2001;79:57–70. [PubMed: 11327104]

2. Rankinen T, Zuberi A, Chagnon YC, et al. The human obesity gene map: the 2005 update. Obesity (Silver Spring) 2006;14:529–644. [PubMed: 16741264]

3. Ponglikitmongkol M, Green S, Chambon P. Genomic organization of the human oestrogen receptor gene. Embo J 1988;7:3385–3388. [PubMed: 3145193]

4. Reimann M, Vafeiadou K, Hall WL, et al. Evidence for associations between common polymorphisms of estrogen receptor beta gene with homocysteine and nitric oxide. Climacteric 2006;9:215–223. [PubMed: 16766435]

5. Meza-Munoz DE, Fajardo ME, Perez-Luque EL, Malacara JM. Factors associated with estrogen receptors-alpha (ER-alpha) and -beta (ER-beta) and progesterone receptor abundance in obese and non obese pre- and post-menopausal women. Steroids 2006;71:498–503. [PubMed: 16566954]

6. Jian WX, Yang YJ, Long JR, et al. Estrogen receptor alpha gene relationship with peak bone mass and body mass index in Chinese nuclear families. J Hum Genet 2005;50:477–482. [PubMed: 16160748]

7. Okura T, Koda M, Ando F, Niino N, Ohta S, Shimokata H. Association of polymorphisms in the estrogen receptor alpha gene with body fat distribution. Int J Obes Relat Metab Disord 2003;27:1020–1027. [PubMed: 12917706]

8. Mansur Ade P, Nogueira CC, Strunz CM, Aldrighi JM, Ramires JA. Genetic polymorphisms of estrogen receptors in patients with premature coronary artery disease. Arch Med Res 2005;36:511–517. [PubMed: 16099331]

9. Fox CS, Yang Q, Cupples LA, et al. Sex-specific association between estrogen receptor-alpha gene variation and measures of adiposity: the Framingham Heart Study. J Clin Endocrinol Metab 2005;90:6257–6262. [PubMed: 16144952]

10. Deng HW, Li J, Li JL, et al. Association of estrogen receptor-alpha genotypes with body mass index in normal healthy postmenopausal Caucasian women. J Clin Endocrinol Metab 2000;85:2748–2751. [PubMed: 10946876]

11. Speer G, Cseh K, Winkler G, et al. Vitamin D and estrogen receptor gene polymorphisms in type 2 diabetes mellitus and in android type obesity. Eur J Endocrinol 2001;144:385–389. [PubMed: 11275948]

12. Rexrode KM, Lee IM, Cook NR, Hennekens CH, Buring JE. Baseline characteristics of participants in the Women's Health Study. J Womens Health Gend Based Med 2000;9:19–27. [PubMed: 10718501]

13. Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med 2005;352:1293–1304. [PubMed: 15753114]

14. WHO. Physical status: the use and interpretation of anthropometry. WHO; Geneva: 1995. Report No.: 584

15. Willett WC, Stampfer MJ, bain C, Lipnick R, Speizer FE, Rosner B, et al. Cigarrete smoking, relative weight, and menopause. J Epidemiol 1983;117:651–658.

16. Schuit SC, Oei HH, Witteman JC, et al. Estrogen receptor alpha gene polymorphisms and risk of myocardial infarction. Jama 2004;291:2969–2977. [PubMed: 15213208]

17. Devlin B, Risch N. A comparison of linkage disequilibrium measures for finescale mapping. Genomics 1995;29:311–322. [PubMed: 8666377]

18. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162–1169. [PubMed: 14574645]

19. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–989. [PubMed: 11254454]

20. Malacara JM, Fajardo ME, Nava LE. Gonadotropins at menopause: the influence of obesity, insulin resistance, and estrogens. Steroids 2001;66:559–567. [PubMed: 11322964]

21. Malacara JM, Perez-Luque EL, Martinez-Garza S, Sanchez-Marin FJ. The relationship of estrogen receptor-alpha polymorphism with symptoms and other characteristics in post-menopausal women. Maturitas 2004;49:163–169. [PubMed: 15474761]

**Table 1**

Baseline characteristics according to body mass index in 543 apparently healthy Caucasian women in the Women's Health Study

| | Body mass index (kg/m$^2$) | | | |
|---|---|---|---|---|
| **Characteristics** | **Normal (<25)** | **Overweight (25-<30)** | **Obese (≥30)** | **P** |
| Age [*] (IQR) | 63(57-68) | 64(59-69) | 61(57-65) | 0.03 |
| Age at menopause [*] (IQR) | 49 (45-52) | 49 (44-52) | 49 (45-52) | 0.75 |
| Total fat [**] (IQR) | 57.5 (50.0-64.9) | 58.6 (50.1-65.3) | 59.9 (53.3-69.7) | 0.12 |
| Saturated fat [**] (IQR) | 19.4 (16.1-22.7) | 19.6 (17.0-22.7) | 20.6 (18.3-24.8) | 0.06 |
| Hormone use (%) | | | | |
| Never | 32.1 | 31.3 | 46.0 | |
| Past | 19.5 | 27.8 | 28.4 | |
| Current | 48.5 | 40.9 | 25.7 | 0.004 |
| Smoking status (%) | | | | |
| Never | 41.3 | 44.3 | 48.7 | |
| Past | 35.8 | 38.6 | 36.5 | |
| Current | 22.9 | 17.1 | 14.9 | 0.041 |
| Alcohol (%) | | | | |
| Rarely/never | 43.3 | 46.0 | 62.2 | |
| 1-3 drinks/month | 13.7 | 17.1 | 10.8 | |
| 1-6 drinks/week | 30.4 | 23.9 | 23.0 | |
| ≥ 1 drink/day | 12.6 | 13.1 | 4.1 | 0.05 |
| Exercise (%) | | | | |
| Rarely/never | 35.5 | 35.8 | 54.1 | |
| < 1/week | 19.1 | 18.2 | 23.0 | |
| 1-3/week | 28.7 | 36.9 | 17.6 | |
| ≥ 4/week | 16.7 | 9.1 | 5.4 | 0.001 |
| Total cholesterol ≥ 240 mg/dl | 50.0 | 36.1 | 13.9 | 0.28 |
| Hypertension % [†] | 39.8 | 39.8 | 20.5 | < 0.0001 |
| Diabetes % | 17.7 | 47.1 | 35.3 | 0.003 |
| Genotype distribution | | | | |
| rs2234693 [‡] | | | | |
| TT | 54.4 | 27.2 | 18.3 | |
| TC | 52.5 | 34.0 | 13.5 | |
| CC | 54.9 | 36.9 | 8.2 | 0.10 |
| rs 9340799 | | | | |
| AA | 52.5 | 31.5 | 16.0 | |
| AG | 55.1 | 32.7 | 12.2 | |
| GG | 54.4 | 35.4 | 10.1 | 0.65 |

[*] IQR is interquartile range (25 th to 75 th percentile).

[**] All macronutrients (mg/day) were adjusted for total energy intake.

[†]Hypertension defined as physician diagnosis of hypertension or reported BP of >140 mmHg systolic or >90 mmHg diastolic blood pressure. *P* values were obtained from Kruskal-Wallis (nonparametric) for continuous variables and Chi-square for categorical variables. Exact *P*-values were considered for genotype association.

[‡]rs 2234693 genotype distribution was in Hardy-Weinberg equilibrium.

**Table 2**

Odds ratios for obesity and overweight in 543 apparently healthy Caucasian women in the Women's Health Study, according to ESR1 genotypes ( additive model)

| | Odds Ratio[**] (OR, 95%CI, $P$) | | Adjusted Odds Ratio [†] (OR, 95%CI, $P$) | |
|---|---|---|---|---|
| Overweight [*] | | $P$ | | $P$ |
| rs2234693 | | | | |
| Normal | Referent (1.0) | | Referent (1.0) | |
| Overweight | 0.99 (0.79-1.26) | 0.97 | 0.96 (0.75-1.24) | 0.75 |
| rs 9340799 | | | | |
| Normal | Referent (1.0) | | Referent (1.0) | |
| Overweight | 0.94 (0.74-1.19) | 0.62 | 0.89(0.69-1.15) | 0.39 |
| Obese [*] | | $P$ | | $P$ |
| rs2234693 | | | | |
| Normal | Referent (1.0) | | Referent (1.0) | |
| Obese | 0.63(0.44-0.90) | 0.01 | 0.57(0.39-0.84) | 0.005 |
| rs 9340799 | | | | |
| Normal | Referent (1.0) | | Referent (1.0) | |
| Obese | 0.73 (0.51-1.04) | 0.08 | 0.69(0.46-1.04) | 0.07 |

[*] Overweight women (25.0-> 30.0kg/m$^2$) were compared to women with normal BMI (< 25.0kg/m$^2$). Obese women (≥ 30.0kg/m$^2$) were compared to women with BMI < 30.0kg/m$^2$.

[**] Adjustment by age.

[†] Multivariate adjustment by age at randomization, age at menopause, hormones use, exercise, educational level, alcohol consumption, smoking status, total fat adjusted for energy intake. OR, odds ratio; CI, confidence interval.

**Table 3**

Odds ratios for obesity and overweight in 543 apparently healthy Caucasian women in the Women's Health Study, according to ESR1 haplotype (additive model)

| | Odds Ratio[**] (OR, 95%CI, *P*) | | Adjusted Odds Ratio[†] (OR, 95%CI, *P*) | |
|---|---|---|---|---|
| Overweight [*] | | *P* | | *P* |
| T-A | Referent (1.0) | | Referent (1.0) | |
| T-G | 0.69 (0.16-2.95) | 0.62 | 0.99 (0.22-4.51) | 0.99 |
| C-A | 1.09 (0.76-1.58) | 0.64 | 1.14 (0.76-1.72) | 0.53 |
| C-G | 0.96 (0.75-1.23) | 0.75 | 0.91 (0.69-1.20) | 0.49 |
| Obese [*] | | *P* | | *P* |
| T-A | Referent (1.0) | | Referent (1.0) | |
| T-G | 0.72 (0.09-6.01) | 0.76 | 1.35 (0.14-13.30) | 0.80 |
| C-A | 0.58(0.32-1.06) | 0.08 | 0.49 (0.24-0.99) | 0.04 |
| C-G | 0.65(0.44-0.94) | 0.02 | 0.60 (0.39-0.92) | 0.02 |

[*] Overweight women (25.0-> 30.0kg/m$^2$) were compared to women with normal BMI (< 25.0kg/m$^2$). Obese women (≥ 30.0kg/m$^2$) were compared to women with BMI < 30.0kg/m$^2$.

[**] Adjustment by age.

[†] Multivariate adjustment as in table 2. OR, odds ratio; CI, confidence interval.