0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(1):303–309

doi: 10.1210/jc.2003-031095

Height in Pre- and Postmenopausal Women Is Influenced by Estrogen Receptor α Gene Polymorphisms

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The estrogen receptor α gene (*ESR1*) is known to be involved in metabolic pathways influencing growth. We have performed two population-based association studies using three common polymorphisms within this candidate gene to determine whether these are associated with variation in adult stature. In 607 women, aged 55–80 yr, from the Rotterdam Study, the *ESR1 Pvu*II-*Xba*I haplotype 1 (px) and the L allele of the TA repeat polymorphism (<18 TA repeats) were significantly associated with an allele dose-dependent decrease in height. The per allele copy of *ESR1 PvuII-XbaI* haplotype 1 height was 0.9 cm shorter (*P* trend = 0.02) and 1.0 cm/allele copy of the TA repeat L allele (*P* trend = 0.003). These results were independent of age, age at menarche and menopause, and lumbar spine bone mineral density and remained significant after participants with vertebral fractures were excluded. In 483 men from the Rotterdam Study we found no association with height. In 1500 pre- and perimenopausal women from the Eindhoven Study a similar association was observed; women were 0.5 cm shorter per allele copy of the *ESR1* haplotype 1 (*P* for trend = 0.03). In conclusion, we demonstrate a role for genetic variations in the estrogen receptor α gene in determining adult stature in women. (*J Clin Endocrinol Metab* 89: 303–309, 2004)

ADULT STATURE HAS been a topic of genetic research since the beginning of the 20th century. Early studies considered the racial differences in stature proof of heritability (1). Later, studies of twins and families quantified this heritability, which is generally believed to be over 80% (2–4). Today, adult stature is commonly recognized as a complex trait that is regulated by multiple genetic and environmental factors.

The importance of genetic research of height lies not only in unraveling the physiological processes involved in growth. In clinical practice, short stature is frequently treated in pediatric endocrine departments. In addition, aspects of skeletal size have been implicated in the risk of many diverse diseases, including osteoporotic fractures (5), cancer (6), and cardiovascular disease (7). These associations emphasize the heterogeneity of factors that determine stature; that is variations in genes affecting skeletal size may also determine the risk for certain diseases through direct or indirect pathways (pleiotropy). Thus, unraveling the genetic origins of stature will not only give important information about the physiology of growth, but may also provide new insights into the mechanisms of diseases such as osteoporosis, cancer, and cardiovascular disease.

To identify the individual genetic factors underlying differences in height, several approaches can be pursued, including genome searches by linkage analysis, followed by candidate gene studies by association analysis. Recently, the first four genome-wide linkage studies of stature were published (8–11), leading to the identification of several potential regions of linkage. The region on chromosome 6 (6q24–25) is of special interest not only because the results were independently replicated (8, 11), but also because it is centered on a gene that is known to be involved in metabolic pathways influencing growth, the estrogen receptor α gene (*ESR1*). The estrogen-resistant male described in 1994 (12) illustrates the importance of the *ESR1* gene in bone development and growth. A disruptive mutation in the *ESR1* gene, producing a nonfunctional estrogen receptor in this man, led to absence of the pubertal growth spurt, delayed bone maturation, unfused epiphyses, and continued growth into adulthood. Given the striking effects of complete estrogen resistance on stature, it is conceivable that subtler and more frequent variations (polymorphisms) in the *ESR1* gene may affect skeletal size in healthy individuals.

The aim of this study was to determine whether polymorphisms in the *ESR1* gene are associated with variation in height in an adult population. We addressed this question in two Dutch population-based studies.

Abbreviations: BMI, Body mass index; BMD, bone mineral density; DEXA, dual energy x-ray absorptiometry; LRH, long-range haplotype; RFLP, restriction fragment length polymorphism; VNTR, variable number of tandem repeat.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Subjects and Methods

The Rotterdam Study

Study population. The Rotterdam Study is a population-based, prospective cohort study of men and women, aged 55 yr and over. The rationale and design have been described previously (13). All 10,275 inhabitants, aged 55 yr or older, of Ommoord, a district of Rotterdam, The Neth-

erlands, were invited to participate. Baseline examinations, including a home interview and an extensive physical examination, took place between 1990 and 1993. The overall response rate was 77% for the home interview, and 6,451 participants (71%) were also able to visit the research center, where anthropometrics were measured, and blood samples were taken.

Subjects. For the present study we included independently living subjects, aged 55–80 yr and of Northern European decent, who were initially part of a large epidemiological study of bone mineral density. Baseline measurements of bone mineral density were available for 5931 independently living subjects from the initial 7983 participants of the Rotterdam Study, 1453 of these were excluded on the basis of age (>80 yr), use of a walking aid, known diabetes mellitus, or use of diuretic, estrogen, thyroid hormone, or cytostatistic drug therapy. From the 4478 remaining individuals, we studied a random sample of 2042 subjects. Information on vertebral fractures was available for 1184 participants of the study. Subjects with 1 or more prevalent vertebral fractures at baseline (n = 94) were excluded. In the remaining sample of 1090 subjects (607 women and 483 men), the following polymorphisms were determined: the *Pvu*II and *Xba*I restriction fragment length polymorphism (RFLP) haplotype and the TA repeat variable number of tandem repeat (VNTR) polymorphism in the *ESR1* gene.

Clinical examination. During the home interview, female participants were asked to recall their age at menarche and menopause, and responses were validated as described previously (14). At the research center, height and weight were measured in standing position in indoor clothing without shoes. All height measurements were attained by a research assistant using a standard wall-mounted statiometer. Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared. At baseline, the average bone mineral density (BMD; expressed in grams per square centimeter) was measured over L2–L4 of the lumbar spine by dual energy x-ray absorptiometry (DEXA; Lunar DPX-L densitometer, Lunar Corp., Madison, WI) as described previously (14). Spine radiographs were assessed for the presence of vertebral fractures at baseline by the McCloskey-Kanis method (15, 16). Vertebral body area (square centimeters) was measured over the second through fourth lumbar vertebra by postero-anterior scanning using DEXA (Lunar DPX-L, Lunar Corp.).

The Eindhoven Perimenopausal Osteoporosis Study

Study population. The Eindhoven Study is a population-based cohort study of women born between 1941 and 1947, living in the city of Eindhoven, The Netherlands. Rationale and design have been described previously (17). Of the eligible 8503 women, 6700 (79%) participated. Baseline examinations, including an interview by a trained research assistant and an extensive physical examination, took place between 1994 and 1995. All participants gave their written informed consent, and two medical ethical committees approved the study.

Subjects. For the present study we included a random sample of 1500 pre- and perimenopausal women of Northern European decent. Pre- and perimenopausal status was defined as last menses less than 1 yr ago. This cohort was initially part of a large epidemiological study of cholesterol in which subjects were excluded according to the following criteria: use of hormone replacement therapy, oral contraceptives, and cholesterol-lowering therapy.

Clinical examination. During the interview participants were asked about age at menarche and menopause. Height and weight were measured in indoor clothing without shoes at either of two research centers: Diagnostic Center Eindhoven and St. Joseph Hospital in Veldhoven, a suburb of Eindhoven. All height measurements were made by a research assistant using a standard wall-mounted statiometer. BMI was computed as weight in kilograms divided by height in meters squared.

Genotyping

Genomic DNA was isolated from peripheral leukocytes by standard procedures. The *Pvu*II (C to T substitution) and *Xba*I (G to A substitution) RFLPs are located in intron 1 of the *ESR1* gene, 397 and 351 bp, respectively, upstream of exon 2. To increase genetic resolution, we constructed haplotypes in this area of the *ESR1* gene (18). As the two RFLPs are separated by only 46 bp, we identified haplotypes by a direct molecular haplotyping method. A 346-bp PCR fragment was generated by a forward primer (estrogen receptor forward, 5′-GATATCCAGGGT TATGTGGCA-3′) and a reverse primer (estrogen receptor reverse, 5′-AGGTGTTGCCTATTATATTAACCTTGA-3′) in a reaction mixture of 10 μl containing 20 ng genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.2 mM deoxy-NTP, 2 pM of each primer, and 0.2 U SuperTaq polymerase (HT Biotechnology Ltd., Cambridge, UK). The reactions were performed in 384-well format in a thermocycler (MJ-tetrad) with a cycling protocol of 94, 60, and 72 C for 45 sec each for 30 cycles. Ten microliters of PCR product were digested by addition of 5 μl digestion mixture containing 5 U *Pvu*II, 7 U *Xba*I restriction enzyme (MBI Fermentas, Hannover, Germany), and 1.5 μl ReactBuffer 2 (Life Technologies, Gaithersburg, MD) and incubating for 90 min at 37 C. The digestion products were analyzed by electrophoresis in a 3% agarose gel in 0.5× TBE (1× TBE = 89 mM Tris, 89 mM boric acid, and 2 mM Na_2EDTA) for 80 min at 125 V. Separation patterns were documented with a digital camera (DC120, Eastman Kodak Co., Rochester, NY) under UV illumination (302 nm).

The alleles were defined as haplotypes such as Px, with capitals denoting the absence and lowercase letters denoting the presence of the restriction sites for the *Pvu*II (P/p) and *Xba*I (X/x) enzymes on each of the alleles. The haplotype alleles were coded as haplotype numbers 1–4 in order of decreasing frequency in the population (1 = px, 2 = PX, 3 = Px, and 4 = pX). Genotypes are analyzed as combinations of two alleles.

The $(TA)_n$ VNTR polymorphism is located 1 kb upstream of the first exon at −1118 bp from the transcription start site and −1351 bp from the translation start site. A 160- to 194-bp PCR fragment containing the TA repeat VNTR was generated using a 6-carboxy fluorescein (FAM)-labeled forward primer (5′-GACGCATGATATACTTCACC-3′) and reverse primer (5′-GCAGAATCAAATATCCAGATG-3′) in a reaction mixture of 10 μl containing 10 ng genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.2 mM deoxy-NTP, 5 pM of each primer, and 0.2 U SuperTaq polymerase (HT Biotechnology Ltd., Cambridge, UK). The reactions were performed in 384-well format in a thermocycler (MJ-tetrad) with a cycling protocol of 94, 59, and 72 C for 30 sec each for 28 cycles. The labeled PCR products were analyzed on an ABI 3100 automated capillary DNA sequencer using Genescan software (PE Applied Biosystems, PerkinElmer, Capelle a/d IJssel, The Netherlands). The allele length was determined using internal size standards.

Statistical analysis

One-way ANOVA and Pearson's χ^2 test were used to compare age, gender, and age at menarche in our study population to those in the entire Rotterdam Study cohort. To account for the possible confounding effects of age and gender, all other baseline characteristics were compared by adjusted analysis of covariance.

To analyze the relationship between the *ESR1* genotypes, height, and potential confounders, we stratified subjects by allele copy number (0, 1, or 2) for the *Pvu*II-*Xba*I haplotype in the *ESR1* gene. In view of the large number of individual genotypes, the *ESR1* TA repeat alleles were grouped according to the number of TA repeats; group H included alleles with a high number of TA repeats (TA ≥18) and group L included alleles with a low number of TA repeats (TA <18). Individuals were then genotyped and analyzed as LL, LH. or HH. The cut off value of 18 TA repeats is based on previous findings indicating that 18 or more TA repeats are associated with osteoporosis in our population (18).

We allowed for three possible models to explain the differences between genotype groups, *i.e.* an allele dose effect, a dominant effect, or a recessive effect. Allele dose was defined as the number of copies of a certain allele in the genotype. In case of a consistent trend, reflected as an allele dose effect, we performed a (multiple) linear regression analysis to quantify the association. In case of a dominant or recessive effect of the test allele, ANOVA and analysis of covariance tests were performed. For dominant effects we compared test allele carriers *vs.* noncarriers, whereas for recessive effects, subjects homozygous for the test allele were compared with heterozygous carriers and noncarriers. First, the crude differences between genotype groups were calculated stratified by gender. Subsequently, the analysis was adjusted for the potential confounding effects of age and age at menarche in women.

We searched for possible intergenic interactions within the *ESR1* gene. We used genotype data for each of the polymorphisms to infer frequency of the long-range haplotype (LRH) alleles within each gene using the PHASE program (19).

All statistical analyses were performed using SPSS version 11.0.1 (SPSS, Inc., Chicago, IL).

Results

The baseline characteristics of the subjects selected from the Rotterdam Study differ from the total cohort. More men have been included in our study (44% *vs.* 39%), and the participants of our study were, on the average, 5.2 yr younger compared with the entire Rotterdam Study population. There were no significant differences in age at menarche or menopause. After adjustment for age, women in our study population weighed less (68.1 *vs.* 69.4 kg; $P < 0.01$) and had a significantly lower BMI (26.2 *vs.* 26.7 kg/m^2; $P < 0.01$) compared with the entire Rotterdam Study cohort. In both men and women, after adjustment for age, there no were significant differences in height compared with the entire Rotterdam Study cohort.

To study the effects of the *ESR1 Pvu*II-*Xba*I haplotypes on height in women before menopause, we analyzed a random set of 1500 pre- and perimenopausal women from the Eindhoven Perimenopausal Osteoporosis Study. The participants selected for our study were, on the average, 0.8 yr younger then the entire Eindhoven Study cohort. There was no significant difference in age at menarche and after adjustment for age, and there were no significant differences in any of the anthropometric parameters between our study population and the total cohort.

Table 1 shows the location, number of subjects analyzed, and allele frequencies for all polymorphisms. All genotypes and haplotypes were in Hardy-Weinberg equilibrium, and frequencies were similar to those in other studies of Caucasian subjects (20–22).

In the 607 female subjects remaining after exclusion of participants with prevalent vertebral fractures at baseline (n = 94), an association was seen between height and *ESR1 Pvu*II-*Xba*I haplotypes. In women, a significant allele dose effect was observed for the *ESR1* haplotype 1 (the most frequent allele), corresponding to a 0.9-cm decrease in height per allele copy (*P* for trend = 0.02; Table 2), extreme genotypes varied 1.8 cm. We observed a trend for association of *ESR1* haplotype 1 with age at menopause (*P* for trend = 0.06), in that homozygous carriers of the *ESR1* haplotype 1 had a 1.1-yr later age at menopause. However, age at menarche was not associated with *ESR1* haplotype 1. Lumbar spine BMD and lumbar vertebral area were both significantly associated with *ESR1* haplotype 1 (*P* for trend = 0.05 and 0.01, respectively). A significant variation in weight was also observed; however investigation of the relationship with BMI revealed this association to be driven by height differences. Adjustment for age, age at menarche, age at menopause, and lumbar spine BMD did not significantly change the observed association between *ESR1* haplotype 1 and height. In women, the *ESR1* haplotype 2 showed a similar, but opposite, allele dose effect on height corresponding to a 0.7-cm increase in height per haplotype 2 allele copy (*P* for trend = 0.06); extreme genotypes differed 1.6 cm (results not shown). No association with height was observed for the *ESR1* haplotype 3 (results not shown).

When these same analyses were performed on the 483 male subjects (mean ± SD, age, 65.2 ± 6.6), no relationship with height was seen; noncarriers of haplotype 1 were 176.5 cm, heterozygous carriers were 175.7 cm, and homozygous carriers were 176.0 cm (*P* for trend = 0.6; Table 2). We also analyzed *ESR1* haplotype 1-dependent height differences in men in age categories above and below the median age of 65 yr. However, in both age categories no association was observed; the *P* value for trend was 0.4 in the youngest age category and 0.7 in the oldest age category.

We analyzed the association between the *ESR1* TA repeat VNTR polymorphism, located in the promoter region and height in the Rotterdam Study. Nineteen different alleles were identified. The bimodal distribution pattern of the TA repeat alleles in our population (Fig. 1) showed two peaks at 13–15 and 21–23 TA repeats. A low frequency was seen for the intermediate 16–20 TA repeats, and the extremes of the spectrum (9–12 and 24–33 TA repeats).

In women, the L allele (<18 TA repeats) of the TA repeat VNTR polymorphism showed a similar significant association with decreased height as the *ESR1* haplotype 1. A 1.1-cm decrease in height per allele copy of the L allele (*P* for trend < 0.01), independent of age, age at menarche, and age at menopause was observed (results not shown); extreme genotypes differed 2.2 cm in mean height. Apparent lumbar vertebral area was also significantly associated with the L allele; extreme genotypes differed by 0.4 cm^2 (*P* for trend = 0.02). In men, as for the *Pvu*II-*Xba*I haplotypes, no association with height or apparent lumbar vertebral area was seen.

We observed strong linkage disequilibrium between the polymorphic sites at the 5′ region of the *ESR1* gene. To

TABLE 1. Polymorphisms analyzed in this study

Polymorphism	Alleles	No. of subjects analyzed	Allele frequency (%)
The Rotterdam Study			
*Pvu*II RFLP (intron 1-397 C/T)/	Haplotype 1-px-T-A	1090	1176/2180 (54)
*Xba*I RFLP (intron 1-351 G/A)	Haplotype 2-PX-C-G	1090	754/2180 (35)
	Haplotype 3-Px-C-A	1090	250/2180 (11)
	Haplotype 4-pX-T-G	1090	–/2180 (0)
$(TA)_n$ (-1351 from translation start site)	L allele (<18 repeats)	1090	1173/2180 (54)
The Eindhoven Study			
*Pvu*II RFLP (intron 1-397 C/T)/	Haplotype 1-px-T-A	1500	1620/3000 (54)
*Xba*I RFLP (intron 1-351 G/A)	Haplotype 2-PX-C-G	1500	1077/3000 (36)
	Haplotype 3-Px-C-A	1500	303/3000 (10)
	Haplotype 4-pX-T-G	1500	–/3000 (0)

TABLE 2. Characteristics of 607 women and 483 men by *ESR1 Pvu*II-*Xba*I haplotype 1, The Rotterdam Study

No. of allele copies *ESR1* haplotype 1	Women				Men			
	0	1	2	*P* value	0	1	2	*P* value
No. (%)	142 (23.4)	294 (48.4)	171 (28.2)	0.5[a]	95 (19.7)	236 (48.9)	152 (31.5)	0.8[a]
Age ± SE (yr)	65.8 ± 0.5	65.2 ± 0.4	66.0 ± 0.5	0.4[b]	65.0 ± 0.7	65.3 ± 0.4	65.2 ± 0.5	0.9[b]
Age at menarche ± SE (yr)	13.8 ± 0.1	13.7 ± 0.1	13.6 ± 0.1	0.5[c]				
Age at menopause ± SE (yr)	48.0 ± 0.4	48.9 ± 0.3	49.1 ± 0.4	0.06[c]				
Weight ± SE (kg)	70.1 ± 0.8	68.9 ± 0.6	67.6 ± 0.8	0.03[c]	77.6 ± 1.0	79.6 ± 0.7	79.8 ± 0.8	0.1[c]
BMI ± SE (kg/m^2)	26.3 ± 0.3	26.1 ± 0.2	26.0 ± 0.3	0.5[c]	24.9 ± 0.3	25.8 ± 0.2	25.8 ± 0.2	0.04[c]
Lumbar spine BMD (g/cm^2)	1.034 ± 0.014	1.032 ± 0.010	0.998 ± 0.013	0.05[c]	1.158 ± 0.019	1.172 ± 0.012	1.140 ± 0.015	0.3[c]
Height ± SE (cm)	163.4 ± 0.6	162.6 ± 0.4	161.6 ± 0.5	0.02[c]	176.5 ± 0.6	175.7 ± 0.4	176.0 ± 0.5	0.6[c]
Height ± SE (cm)[d]	163.4 ± 0.5	162.5 ± 0.4	161.6 ± 0.5	0.02[c]	176.5 ± 0.6	175.7 ± 0.4	176.0 ± 0.5	0.6[c]
Height ± SE (cm)[e]	163.3 ± 0.5	162.5 ± 0.4	161.8 ± 0.5	0.04[c]	176.5 ± 0.6	175.6 ± 0.4	176.0 ± 0.5	0.7[c]
Lumbar apparent vertebral area (cm^2)	14.5 ± 0.1	14.3 ± 0.1	14.1 ± 0.1	0.01[c]	17.4 ± 0.2	17.5 ± 0.1	17.6 ± 0.1	0.4[c]

[a] *P* value for Hardy-Weinberg equilibrium.
[b] ANOVA.
[c] Linear regression.
[d] Adjusted for age (and age at menarche and menopause in women).
[e] Adjusted for age and lumbar spine BMD (and age at menarche and menopause in women).

determine which of the polymorphic sites is driving the association with height and vertebral bone area, we constructed LRH using the PHASE program (19). Table 3 shows frequencies of each of the six observed haplotypes. Due to the strong linkage disequilibrium between the *Pvu*II-*Xba*I haplotype and the TA repeat VNTR, we observed two frequent LRH alleles (alleles A and E) among the six possible haplotypes. Table 3 also shows the difference in mean height between carriers and noncarriers of each particular LRH allele. The greatest effect on height was observed for LRH allele A, which is the combination of a low number of TA repeats and *Pvu*II-*Xba*I haplotype 1.

To study the effects of the *ESR1 Pvu*II-*Xba*I haplotype 1 on height in women before menopause, we analyzed a random set of 1500 pre- and perimenopausal women from the Eindhoven Perimenopausal Osteoporosis Study. The *ESR1* haplotypes were also associated with variation in height in these pre- and perimenopausal women. A significant allele dose effect was observed for *ESR1* haplotype 1, corresponding to a 0.5-cm decrease in height per allele copy (*P* for trend = 0.03; Table 4).

Discussion

The first genome-wide linkage studies of stature (8–11) identified a region of potential linkage to adult stature on chromosome 6 (6q24–25). This region is centered on a gene that is known to be involved in metabolic pathways influencing growth, *ESR1*. After these findings, three polymorphic sites in the estrogen receptor α gene were investigated for associations with height. We found these common polymorphisms in the 5′ region of the *ESR1* gene to be significantly associated with height in postmenopausal women as well as pre- and perimenopausal women. Differences in height between extreme genotypes ranging from 1.0–2.2 cm were observed depending upon the group of women studied and the polymorphism analyzed.

In the Rotterdam Study, a population-based study of adults aged 55 yr and older, the *ESR1 Pvu*II-*Xba*I haplotype 1 (−397 T and −351 A) and the L allele of the TA repeat VNTR polymorphism (<18 TA repeats) were significantly associated with a decrease in height per allele copy (0.9 and 1.0 cm/allele copy, respectively). Homozygous carriers of *Pvu*II-*Xba*I haplotype 1 were 1.8 cm shorter than subjects who did not carry this haplotype; homozygous carriers of the TA repeat L allele were 2.2 cm shorter than noncarriers. Furthermore, these polymorphisms were associated with a smaller apparent lumbar vertebral area (0.4 cm^2). These associations were not found in the male participants of our study.

We considered that the postmenopausal drop in estrogen levels may be instrumental in the association we found for the *ESR1* gene with height. This would indicate that it is not attained adult height that is influenced by *ESR1* gene polymorphisms, but the age-related decrease in stature. To address this hypothesis we excluded participants with radiologically confirmed vertebral fractures at baseline and adjusted for age at menopause, and we found that each did not change our results. However, vertebral fractures that do not meet the McCloskey-Kanis criteria; that is, vertebral deformities with a reduction in vertebral height that do not meet the cut-off level may still be

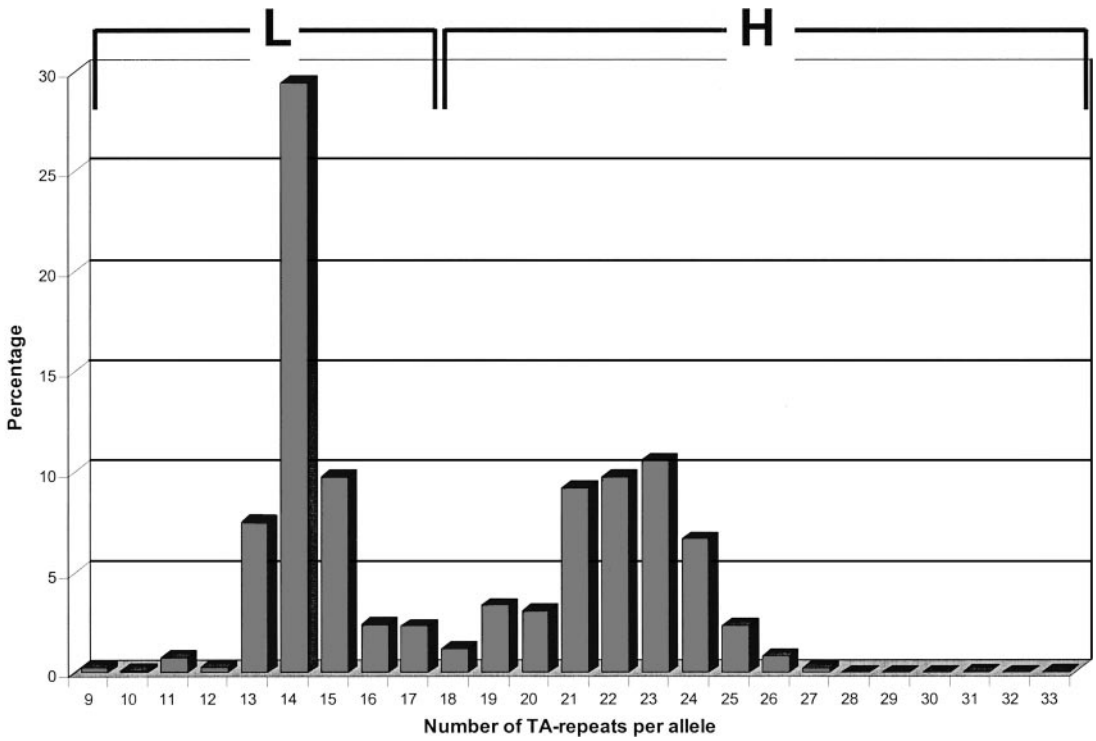

FIG. 1. Frequency distribution of the *ESR1* dinucleotide (TA) repeat polymorphism in 1090 subjects (2180 chromosomes). Group H includes alleles with a high number of TA repeats (TA ≥18), and group L includes alleles with a low number of TA repeats (TA <18).

TABLE 3. Association of the 6 *ESR1 PvuII-XbaI*-TA repeat LRH alleles in women (n = 607), The Rotterdam Study

LRH allele	Haplotype alleles		No. of carriers	Δ height (cm)
	TA repeat	*Pvu*II-*Xba*I haplotype		
A	L	1	441	−1.30
B	L	2	30	−1.28
C	L	3	28	+0.90
D	H	1	54	+0.75
E	H	2	339	+0.77
F	H	3	101	+1.10

L, Low number of TA repeats (<18); H, high number of TA repeats (≥18); Δ height, mean height of allele carriers minus mean height of noncarriers.

present in the Rotterdam population. We have tried to address this by adjusting for lumbar spine BMD. Women most susceptible to these less severe vertebral fractures will, on the average, have a lower lumbar spine BMD. Furthermore, as has been reported previously, *ESR1* haplotype 1 is associated with lumbar spine BMD (18). Therefore, by adjusting our analysis for lumbar spine BMD, we have adjusted for a surrogate for vertebral fractures and have shown that these fractures most likely do not play an essential role in the observed association. Furthermore, we analyzed the association between the *ESR1 Pvu*II-*Xba*I haplotypes and height in 1500 pre- and perimenopausal women from the Eindhoven Study, a population-based cohort of women aged 46–57 yr. In this study the *Pvu*II-*Xba*I haplotype 1 was also significantly associated with decreased height, where haplotype 1 was associated with a 0.5-cm shorter stature per allele copy. Thus, in pre- and perimenopausal women, in whom vertebral fractures are not likely to be an important confounder, the results were similar to the association found in postmenopausal women. Given these findings we expect that confounding by less severe vertebral fractures that do not meet the McCloskey-Kanis cut-off level will not have influenced our results. Furthermore, we hypothesize that *ESR1* polymorphisms influence attained height earlier in life, rather than after menopause. However, the effect does seem to be greater in postmenopausal women than in pre- and perimenopausal women (1.8 *vs.* 1.0 cm). Yet, we cannot be sure whether this is a significant difference or falls within the 95% confidence intervals for both populations.

As seen in other populations, in the Rotterdam Study strong linkage disequilibrium exists between the intron 1 *Pvu*II and *Xba*I polymorphic sites and the TA repeat VNTR located approximately 21 kb upstream of these polymorphisms (18, 21, 23). It is still unclear which of these polymorphisms is driving the association we observed with stature. It is difficult to distinguish the different effects of the two polymorphic sites by association analyses because of the strong linkage between them. However, the association studies with the LRHs suggest that both sites are contributing, because the LRH defined by carrier status for both the *Pvu*II-*Xba*I as well as the TA repeat VNTR risk alleles (LRH A) shows the strongest effect on stature, especially when we compare LRH alleles A and B *vs.* C and D and E *vs.* F. However, larger studies are necessary to prove this hypothesis.

In this study we observed that the genetic associations between *ESR1* polymorphisms and height were found only in women. This is unexpected, because the only known clinical example of the consequences of a nonfunctional estrogen receptor α on height was observed in a male. Two explanations arise. First, the physiology of pubertal growth in males differs from that in females in that it begins and ends later

TABLE 4. Characteristics of women by *ESR1 PvuII-XbaI* haplotype 1 (n = 1500), The Eindhoven Study

	Copy number of *ESR1* haplotype 1 allele			*P* value
	0	1	2	
No. (%)[a]	331 (22.13)	718 (47.9)	451 (30.1)	0.2[a]
Age ± SE (yr)	49.8 ± 0.1	50.1 ± 0.1	50.0 ± 0.1	0.3[b]
Age at menarche ± SE (yr)	14.0 ± 0.4	14.2 ± 0.3	14.2 ± 0.4	0.7[b]
Weight ± SE (kg)[c]	68.8 ± 0.7	69.3 ± 0.4	68.7 ± 0.6	0.8[b]
BMI ± SE (kg/m^2)[c]	25.2 ± 0.3	25.7 ± 0.2	25.5 ± 0.2	0.5[b]
Height ± SE (cm)	165.3 ± 0.4	164.7 ± 0.2	164.3 ± 0.3	0.03[b]
Height ± SE (cm)[d]	165.3 ± 0.4	164.7 ± 0.2	164.3 ± 0.3	0.04[b]

[a] P value for Hardy-Weinberg equilibrium.
[b] Linear regression.
[c] Adjusted for age and age at menarche.
[d] Adjusted for age.

in life and has a higher peak velocity. Furthermore, men are taller than women, more as a result of longer legs rather than longer torsos (24). In addition, there are structural and physiological differences between vertebral and long-shafted bone growth. This raises the possibility that the *ESR1* gene polymorphisms particularly influence mechanisms controlling vertebral bone growth. To investigate this theory we measured average vertebral area from lumbar spine DEXA scans. Indeed, *ESR1* polymorphisms were significantly associated with apparent vertebral body area, which supports the hypothesis that *ESR1* gene polymorphisms influence vertebral bone growth.

Another explanation for the fact that the association with stature was only seen in women relates to the predominant role of androgens in males. Perhaps although in the predominantly estrogenic puberty of females polymorphisms in the *ESR1* gene lead to height differences, in males variances in androgenic response (perhaps through polymorphisms) may override these variances.

The *ESR1 Pvu*II and *Xba*I polymorphisms have been an important area of research in diseases such as osteoporosis, cardiovascular disease, and cancer (21, 25, 26). Until recently, neither of the *Pvu*II or *Xba*I RFLPs was known to have functional consequences. It was thought that the *Pvu*II and *Xba*I polymorphisms acted as a marker through linkage disequilibrium for a truly functional sequence variation elsewhere in the gene. Therefore, to increase genetic resolution, we combined the *Pvu*II and *Xba*I polymorphisms in multiallelic haplotype makers. However, recently Herrington *et al.* have shown that the C allele of the *Pvu*II RFLP produces a functional binding site for the transcription factor B-myb, suggesting that the presence of this allele may result in augmented estrogen receptor α transcription or produce estrogen receptor α isoforms that have different properties than the full-length gene product (27).

The TA repeat VNTR polymorphism is located in the promoter region of the *ESR1* gene. The location of the TA repeat VNTR polymorphism in the promoter region, 700 bp upstream of promoter B and 600 bp downstream of promoter C, indicates it may have functional significance (28). Studies have shown that VNTR polymorphisms in proximity to gene promoters, such as the TA repeat, can have a significant influence on transcriptional regulation (29). It is, therefore, plausible that the number of TA repeats could be important for *ESR1* gene transcription, but molecular biological functional studies are necessary to address this issue.

How do these possibly functional polymorphisms in the *ESR1* gene influence attained height in women? Perhaps early age at menarche leads to premature closure of the epiphyseal growth plates and subsequent shorter stature (30). Stavrou *et al.* (31) have shown that age at menarche is influenced by *ESR1* polymorphisms. However, adjustment for age at menarche did not change the association, making this a less likely mechanism. Therefore, we hypothesize that the *ESR1* polymorphisms lead to genotype-dependent differences in *ESR1* expression and consequently variable estrogen sensitivity directly at the site of linear bone growth, the epiphyseal growth plates. A prerequisite for a direct estrogen action at the level of the growth plate is the presence of *ESR1* on chondrocytes in the growth plate. Indeed, *ESR1* has been localized in all zones of the growth plate, *i.e.* resting, proliferating, and hypertrophic chondrocytes (32).

Two previous studies have shown an association between *ESR1* polymorphisms and adult stature. Lehrer *et al.* (33) showed that a rare synonymous polymorphism in codon 87 in exon 2 of the *ESR1* gene (B variant) was associated with height in American women of multiracial descent. However, given the synonymous nature of this polymorphism, it is unlikely that the B variant allele will lead to a functionally different estrogen receptor α. This B variant polymorphism has previously been shown to be in strong linkage disequilibrium with the *Pvu*II and *Xba*I polymorphisms (34), raising the possibility that it is the *Pvu*II or *Xba*I polymorphism that is driving this observed association. In a study of adolescent boys, Lorentzon *et al.* (35) found an association between shorter stature and the *Pvu*II T allele and the *Xba*I A allele, which corresponds to the *ESR1* haplotype 1. Although this study was performed in young boys, it is in line with our findings in adult women.

Our study has limitations. Within the Rotterdam Study vertebral fracture data were only collected in individuals who survived the follow-up period of approximately 7 yr. This resulted in a healthy responder bias (as illustrated by differences in mean age with the entire Rotterdam Study cohort). However, this bias is not likely to be genotype dependent, because we did not find an influence of the *ESR1* polymorphisms we studied on survival (data not shown). Genetic association studies can be influenced by population heterogeneity. This is especially true for case-control studies in a population of mixed racial origin. However, our study groups are drawn from two population-based cohort studies, and all subjects were Dutch Caucasians of similar social backgrounds. Therefore, our study populations

may be considered ethnically homogeneous and representative of the Dutch population.

In conclusion, in the present population-based association studies of stature, we have confirmed the role of the estrogen receptor α gene in determining adult stature. The effects were present in pre-, peri-, and postmenopausal women, but were not seen in males. However, confirmation of the role of this candidate gene in determining height does not exclude that other genes also located in the region defined by recently published genome scans do not also play a role in between-person variations in height.

Acknowledgments

We are very grateful to the participants of the Rotterdam study and to the DXA and radiograph technicians, L. Buist and H. W. M. Mathot. Furthermore, we acknowledge all of the participating general practitioners and the many field workers in the research center in Ommoord (Rotterdam, The Netherlands).

Received June 26, 2003. Accepted October 2,2003.

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This work was supported by The Netherlands Organization of Scientific Research Institute for Diseases in the Elderly (Grant 014-90-001).

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