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Association of estrogen receptor β (*ESR2*) gene polymorphism with blood pressure

Abstract We investigated the association between a dinucleotide (cytosine-adenine; CA) repeat polymorphism located in the flanking region of the human estrogen receptor β (*ESR2*) gene and systemic blood pressure in 187 healthy postmenopausal Japanese women. The genotype was classified as "A" through "O" according to the number of these repeats from 18 to 32. When we separated the subjects into two groups — bearing at least one "I" allele (26 CA repeats) and those who did not — we found that the former subjects had significantly higher systolic blood pressure than the latter (mean $\pm$ SD, 146.0 ± 25.0 vs 136.6 ± 23.4; $P = 0.032$). These data suggest that genetic variation at the *ESR2* locus may be associated with some determinants of blood pressure, and that there is a possible involvement of this polymorphism in causing hypertension in Japanese women.

Key words *ESR2* · *ESR2* polymorphism · Systemic hypertension · Microsatellite marker · Allelic association

Introduction

Accumulating evidence derived from clinical, epidemiological, and experimental studies suggests that estrogen deficiency plays a major role in the pathogenesis of cardiovascular diseases in postmenopausal women (Akishita et al. 1997; Grady et al. 1992; Mendelsohn and Karas 1999; Sullivan et al. 1995). This is confirmed by the finding that estrogen supplement significantly prevents the diseases (Felson et al. 1993). In general, estrogen action is presumed to be mediated by estrogen receptor (ER), a member of the nuclear receptor superfamily (Evans 1988; Green and Chambon 1988; Mangelsdorf et al. 1995). Both classical ERα and recently identified ESR2 (Kuiper et al. 1996; Ogawa et al. 1998) were differently expressed during the vascular injury response (Iafrati et al. 1997; Lindner et al. 1998), implying their functional roles in the cardiovascular effects.

Hypertension is one of the most important predictors of cardiovascular diseases, which are determined by genetic risk factors as well as environmental factors (Hollenberg, 1996; Pratt and Dzau 1999). The candidate gene approach has revealed that several gene polymorphisms are responsible for the relative risk of hypertension. Therefore, it is necessary to clarify these genetic risk factors for the diagnosis, prevention, and early effective treatment of hypertension. In addition, if the relevant genes were identified, the pathogenesis of these diseases could be explained by variations of these genes or the genes adjacent to these loci. Because the association of the *ER*α genotype with blood pressure was reported (Lehrer et al. 1993), it is a matter of concern whether the *ESR2* genotype is also associated with hypertension.

In the present study, we investigated the association between an *ESR2* gene microsatellite containing a cytosine-adenine (CA) repeat polymorphism (Ogawa et al. 2000; Tsukamoto et al. 1998b) and systolic and diastolic blood pressure in postmenopausal Japanese women.

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Subjects and methods

Subjects

Two hundred and four healthy postmenopausal Japanese women (age 61–91 years; mean age $\pm$ SD, 70.5 ± 6.8 years)

were recruited from among unrelated volunteers living in Nagano prefecture, Japan. DNA samples were obtained from the peripheral blood of subjects as previously described (Emi et al. 1999). All were non-related volunteers and gave their informed consent prior to the study. No participant had medical complications or was undergoing treatment for conditions known to affect blood pressure, such as pituitary disease, hyperthyroidism, hyperparathyroidism, diabetes mellitus, liver disease, or renal disease, and none was receiving anti-hypertensive therapy.

Measurement of blood pressure and biochemical markers

Blood pressure was measured, after at least 5-min supine rest in a quiet room, by sphygmomanometer with appropriate cuff. Serum concentrations of total cholesterol, and triglyceride were also measured.

Determination of microsatellite polymorphism

The CA-repeat microsatellite marker lies within intron 5 of the *ESR2* gene, starting at 3550 bp downstream of exon 5. Polymerase chain reaction (PCR) amplification of the CA-repeat microsatellites was performed in a volume of $10\,\mu l$, containing 20 ng of genomic DNA obtained from peripheral blood, 10 mM of Tris HCl (pH8.4), 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.01% of gelatin, $200\,\mu M$ of each dNTP, and 0.25 units of Taq polymerase, and 2.5 pmol of each set of $[^{32}P]$ end-labeled forward primer and non-labeled reverse primer that we have described previously (Hirayama et al. 1998; Watanabe et al. 1998). Oligonucleotide primers designed to amplify the polymorphic (CA)n repeat at the human *ESR2* locus were described elsewhere (Tsukamoto et al. 1998b). PCR products were electrophoresed for 2 h at 2000 V in 0.3-mm-thick denaturing 6% polyacrylamide gels containing 36% formamide and 8 M urea. Gels were transferred to filter papers, dried at 80°C, and autoradiographed. The size of each allele was determined by comparison with a sequencing ladder of control DNAs (Tsukamoto et al. 1998a).

Statistical analysis

In the statistical analysis of quantitative traits, such as blood pressure, plasma lipoprotein levels, and bone mineral density, several statistical methodologies have been employed; the non-parametric Student-Neumann-Kleuls test is routinely used in population-based studies that examine the correlations of some quantitative traits. The χ^2 test is used to analyze the deviation of genotype frequencies from Hardy-Weinberg expectation in case-control comparisons. Other non-parametric methods, such as the Wilcoxon signed-ranks test, have been used in the analysis of lipoprotein levels in family-based studies.

Because the present study was a population-based genetic analysis in a volunteer general population, we chose to employ the Student-Newman-Kleuls test for the statistical analysis. Thus, comparisons of blood pressure and biochemical markers between the group of individuals possessing one or two alleles of the genotype and the group without that genotype were performed using non-parametric analysis (Student's *t*-test; StatView-J 4.5; Abacus Concept, Tokyo, Japan) as described previously (Tsukamoto et al. 1999). A *P* value less than 0.05 was considered statistically significant.

Results

Fifteen alleles observed in this population were classified as genotypes "A" through "O" according to the number of *ESR2* CA dinucleotide repeats they contained. Correlations between the *ESR2* genotype and systolic blood pressure, diastolic blood pressure, and biochemical parameters were examined in 187 healthy postmenopausal Japanese women from whom all genotypic and phenotypic data were available (aged 61–91 years; mean age 70 years) living in Nagano prefecture (Table 1). Individuals possessing one allele of "I" genotype (26 CA repeats) had significantly higher systolic blood pressure compared with individuals not possessing "I" alleles (Fig. 1A; Table 1). In contrast, comparison of the diastolic blood pressure between those with and without the "I" allele showed no significant difference (Fig. 1B; Table 1).

There were no significant correlations between genotype I and the subject's profiles (age, height, weight, years since menopause), or with their biochemical markers, including serum lipid levels (total cholesterol and triglyceride).

Discussion

The present study is the first to analyze the association between genetic variation in the *ESR2* gene and blood pressure. Our recent study (Ogawa et al. 2000) has revealed that this dinucleotide polymorphism is also associated with high bone mineral density (BMD), suggesting the involvement of the same polymorphism in blood pressure and bone mineral properties. Although the molecular basis of these associations has been unclear, it was speculated that *ESR2* polymorphisms were linked to some other mutation effect of protein quantity and quality, or that they may be linked to the mutation of other regulatory elements that affect the gene expression level through transcriptional regulation. Alternatively, these polymorphisms may be linked with the mutation of another unidentified gene, adjacent to the *ESR2* gene, which affects blood pressure either directly or indirectly.

Studies of cardiovascular phenotypes of *ESR1* knockout mice (ERαKO) were reported, but some of the results seem to be rather complicated to interpret (Gustafsson 1997; Iafrati et al. 1997). In practice, ovariectomy increased the medial area of injured vessels to the same extent in adult

Table 1. Comparison of subjects' baseline data between subjects not possessing "I" allele and those posessing "I" allel

	Genotype		
	I(−)[a]	I(+)[a]	P value
Number of subjects	151	36	—
Age (years)	70.1 (6.3)	72.3 (8.3)	NS
Height (cm)	149.1 (6.9)	147.8 (6.1)	NS
Body weight (kg)	50.3 (8.4)	49.2 (8.0)	NS
Systolic blood pressure (mm Hg)	136.6 (23.4)	146.0 (25.0)	0.032
Diastolic blood pressure (mm Hg)	77.1 (12.6)	79.4 (11.5)	NS
Years since menopause	20.5 (7.11)	21.4 (7.69)	NS
Total cholesterol (mg/dl)	193.6 (33.5)	200.6 (39.9)	NS
Triglyceride (mg/dl)	140.7 (78.6)	133.6 (85.9)	NS
% Fat	32.4 (8.3)	31.8 (9.1)	NS
Body mass index	22.6 (3.3)	22.0 (3.2)	NS

Statistical analysis was done according to non-parametric analysis (Student's *t*-test, StatView-J 4.5). Values are expressed as means (SD in parentheses)

[a] See text for information on genotype classification

Fig. 1A, B. Blood pressure values in the groups of individuals who possessed two alleles of genotype "I" (*2*), one allele of genotype "I" (*1*), and those lacking genotype "I" (*0*). Values are expressed as means ± SE. Numbers of subjects are shown in parentheses. **A** Comparison of systolic blood pressure between the groups with *ESR2* genotype "I". **B** Comparison of diastolic blood pressure between the groups with *ESR2* genotype I
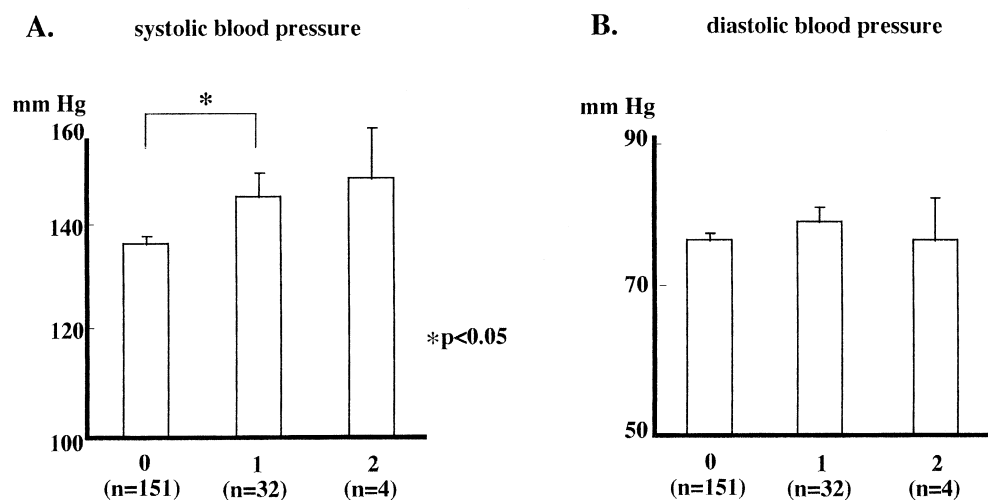
female ERαKO as in WT mice (Iafrati et al. 1997). Moreover, 17 β-estradiol equally inhibited the increase in vascular medial area and the proliferation of vascular smooth muscle cells (SMC) after vascular injury, suggesting the possible compensation of the *ESR1*-mediated cascade by *ESR2*.

Recent studies demonstrated that the vascular walls of *ESR2* knockout mice (βERKO) showed a response to estrogen similar to that in wild-type mice when they were injured (Karas et al. 1999; Krege et al. 1998). Taken together with these knockout studies, it was suggested that either *ESR1* or *ESR2* is sufficient to protect against vascular injury. It is also possible that the vascular protective effect of estrogen is mediated by unidentified ER or by the so-called non-genomic action of estrogen (Couse and Korach 1999; Iafrati et al. 1997; Karas et al. 1999). The effects of estrogen on the vascular tone occur over seconds to minutes, reflecting its rapid effects on vasomotor tone (Mendelsohn and Karas 1994). Some direct effects of estrogen on the vascular wall, such as the modulation of vascular nitric oxide release (Arnal et al. 1996), SMC-

resting potential (Zhang et al. 1994), and calcium channel activity (Mendelsohn and Karas 1994; White et al. 1995), are reported to be involved in the estrogen-mediated vasorelaxation.

In general, we understand that correction for multiple tests was proposed for case-control association studies in which allelic association in normal and case populations is derived from linkage disequilibrium between alleles of certain loci and the affected state (i. e., when disease-bearing chromosomes are descended from one or a few ancestral chromosomes). In allelic association studies, Bonferroni's school considers that this correction would avoid type-1 errors (false-positives). However, other statisticians' schools consider that this correction is too stringent, because when each hypothesis is tested independently, there is no reason to change the significance level according to the number of hypotheses tested in a single study. For instance, in a genome-wide scan for allelic association using 300 markers, only extraordinarily strong associations would be taken as significant when the Bonferroni correction is applied (i.e., *P* < 0.00017, instead of

the ordinary level of $P < 0.05$). In other words, the more loci being analyzed in an association study, the more difficult it would be to obtain significant results under such a hypothesis. However, because our study was small in scale, the possibility of false-positive results can not be ruled out. Therefore, further confirmation in several independent studies would be warranted before a definitive conclusion can be drawn regarding the association between *ESR2* and blood pressure in a variety of different populations.

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