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ORIGINAL ARTICLE

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Coronary artery wall atherosclerosis in relation to the estrogen receptor 1 gene polymorphism: an autopsy study

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Abstract Estrogen receptors (ESR) 1 and 2 are expressed in the normal and atherosclerotic arteries mediating the atheroprotective action of estrogen to artery wall cells. Whether variants of these receptor genes associate with autopsy-verified coronary artery wall atherosclerosis is not known. This study investigated whether variants of the ESR1 gene are associated with autopsy-verified coronary artery wall atherosclerosis and thrombosis. Coronary arteries were taken from 300 white Finnish male autopsy cases aged 33–69 years included in the Helsinki Sudden Death Study. Areas of coronary wall covered with fatty streaks, fibrotic, calcified, and complicated lesions were measured using computer-assisted planimetry and related to ESR1 *Pvu*II genotypes (P/P, P/p, and p/p) determined by PCR. The mean area of complicated lesions of three major coronaries and the presence of coronary thrombosis were significantly associated with the ESR1 genotype in men aged 53 years or older (median age as a cut off point). No such association was found in men aged under 53 years. After adjusting for age and body mass index the men aged 53 years or over with P/p and P/P genotype had areas of complicated lesions on average two- and fivefold larger than subjects with the p/p genotype. The age and body mass index adjusted odds ratios for coronary thrombosis were 6.2 for P/p and 10.6 for P/P compared to men with the p/p genotype. After additional adjustment for diabetes and hypertension the ESR1 genotype persisted as an independent predictor of complicated lesions (P=0.007) and coronary thrombosis. In conclusion, the ESR1 gene is a potential candidate behind the pathogenesis of acute coronary events.

Keywords Estrogen receptor 1 · Coronary artery disease · Complicated lesion · Thrombosis

Abbreviations *ANCOVA:* Analysis of covariance · *ANOVA:* Analysis of variance · *BMI:* Body mass index · *ESR:* Estrogen receptor

Introduction

The atheroprotective action of estrogen is mediated by estrogen receptors (ESR) 1 and 2, which are expressed in atherosclerotic lesions [1]. ESR1 activates specific target genes in vascular smooth muscle [2], inhibits smooth muscle cell migration [3, 4], and accelerates endothelial cell growth in vitro [5] and vivo [6, 7]. In addition, fewer ESR1 are found in premenopausal women with atherosclerotic coronary arteries than in those with normal arteries [8]. Thus it is possible that the effects of estrogen to vascular cells, mediated by ESR1, differ due to the ESR1 variant forms that have different transcriptional effects than the "wild-type" receptor [9, 10]. The ESR1 gene has a common two-allele polymorphism leading to genotypes P/P, P/p, and p/p [11]. We examined the relationship of these ESR1 genotypes to the areas of the different types of atherosclerotic lesions and the presence of thrombosis in the coronary arteries, in an autopsy series of 300 Finnish men included in the Helsinki Sudden Death Study [12].

Methods

Subjects

The Helsinki Sudden Death Study was launched at the beginning of the 1991 to study the lifestyle and genetic factors that predispose to sudden death in middle-aged Finnish men in the Helsinki area [12, 13]. The autopsy series was collected during 1991–1992 at the Department of Forensic Medicine, University of Helsinki. The original study population consisted of a prospective series of 300 white men aged 33–69 years. This autopsy series covered 42% of all deaths of men younger than 65 years of age during those years in the area of Helsinki and surroundings. The cause of death was cardiac disease in 39% (*n*=104), other diseases 40% (*n*=107), and violent death (suicides and accidents) in 21% of cases (*n*=57; Table 1). In addition to the ESR1 genotype, simultaneous autopsy and other risk factor data [i.e., diabetes, hypertension, age, body mass index (BMI)] were available in 126 cases.

Measurement of atherosclerotic lesion area and myocardial infarction

The proximal parts of the right coronary, left anterior descending, and left circumflex coronary arteries were collected for analysis. The definition of atherosclerosis was based on the protocols of two international studies: the International Atherosclerosis Project, Standard Operating Protocol 1962 [14] and the WHO Study Group in Europe [15]. Atherosclerotic changes in the coronary arteries were measured by computer-assisted planimetry, and coronary narrowing were determined from plastic casts as described in detail earlier [12, 13]. The presence of myocardial infarction was confirmed by macroscopic and histological examination of the myocardium [12, 13].

ESR1 genotyping

DNA was extracted from cardiac tissue by the standard phenol-chloform method. A region of the ESR1 gene was amplified using primary and secondary (nested) primers designed from those reported by Yaich and colleagues [11]. After digestion of the PCR product with *Pvu*II endonuclease fragments were detected by standard agarose gel electrophoresis. Uppercase (P, mutated) and lowercase (p, wild type) letters denoted the absence and presence of the restriction sites, respectively.

Statistics

Data analysis was based on linear and logistic regression analysis and on analysis of variance (ANOVA) and covariance (ANCOVA). Nonnormally distributed data were analyzed in square-root form, but the results are displayed as crude data. Since complicated lesions are rare in young persons, we also studied the effect of age, using the median age of 53 years as a cutoff point for dividing the subjects into those aged under 53 years and those aged 53 years or

Table 1 Subjects by ESR1 genotype and age subgroup

	<53 years			≥53 years			All subjects		
	P/P (*n*=24)	P/p (*n*=57)	p/p (*n*=45)	P/P (*n*=25)	P/p (*n*=67)	p/p (*n*=50)	P/P (*n*=49)	P/p (*n*=124)	p/p (*n*=95)
Age (years)[a]	42.4±4.3	43.5±4.7	43.3±4.4	59.5±4.2	60.6±5.2	59.3±4.9	51.1±9.6	52.8±9.9	51.7±9.3
Body mass index[a]	25.0±5.1	24.9±4.8	24.1±4.8	25.1±6.1	25.1±4.9	26.0±4.5	25.1±5.6	25.0±4.8	25.1±4.7
Cause of death: cardiac disease/other[b]	7/17	19/38	9/36	10/15	40/27	19/31	17/32	59/65	28/67
Coronary risk factor[c]									
Smoking (yes/no)	3/9	6/19	0/22	2/8	10/26	9/16	5/17	16/45	9/38
Diabetes (yes/no)	1/7	4/25	1/22	2/10	5/24	6/19	3/17	9/49	7/41
Hypertension (yes/no)	2/6	11/18	4/19	5/7	10/19	7/18	7/13	21/37	11/37

[a] There was no statistically significant differences between ESR1 genotype groups, *P* -values were calculated by using ANOVA

[b] Includes causes of death, due to other diseases, violence, intoxication and unknown reasons (*n*=6)

[c] Simultaneously all risk factors and other data available from 99 cases

over. A similar age categorization has been used previously [12, 13]. The ESR1 genotype and the age groups were used as factors in the two-way ANOVA. One-way ANCOVA was performed also separately for the two age groups using age and BMI as covariates and last significant difference (LSD) test as a post-hoc test.

In linear regression model the mean area of complicated lesions was used as dependent variable. Among explanatory variables in the model we used ESR1 genotype, age, BMI, hypertension (yes/no), smoking (yes/no), and diabetes (yes/no). In the multiple logistic regression analysis thrombosis (yes/no) was used as dependent variable, and ESR1 genotype and age group as independent variables. We also performed logistic regression analysis for separate age groups by using thrombosis (yes/no) as dependent variable, and ESR1 genotype, age, and BMI as explanatory variables.

The power of the study to detect a difference in complicated atherosclerotic lesions between PP and pp genotype subjects was 0.96 and to detect difference in calcified lesions 0.75 with the present number of subjects (within the group aged 53 years or over) and probability for type I error α=0.05. Correction for multiple testing was not performed because of the exploratory approach to a genetically complex disorder in which a phenotype-genotype relationship has not been established [16].

The statistical analysis was performed on a microcomputer using Statistica for Windows 5.1 (StatSoft, Tulsa, Okla., USA) and SPSS 9.0 for Windows 95 software (SPSS, Chicago, Ill., USA). Values in the text are means ±SD if not otherwise stated.

Results

Descriptive data

From 268 genotyped cases (Table 1) autopsy data were missing from 6 cases, and a total of 262 cases thus comprised the final study population used in Table 2. Over all the mean area of complicated atherosclerotic lesions from three major coronary arteries was 1.2%, ranging from 0% to 37.7%. The mean area of complicated lesions was 0.5% (range 0–7.9) in men aged under 53 years and 1.8% (range 0–37.7) in men aged 53 years or older. The frequencies of the P and p alleles were 0.42 and 0.58 for men aged under 53 years and 0.41 and 0.59 for men aged 53 years or over. The distributions of genotypes followed the Hardy-Weinberg equilibrium. The cause of death and coronary risk factors according to ESR1 genotype and age subgroups are shown in Table 1.

ESR1 genotype and different types of atherosclerotic lesions

The mean proportion of the area of the complicated atherosclerotic lesions from three coronary arteries was significantly associated with ESR1 genotype in men aged 53 years or over but not in younger men (ANOVA, *P*=0.003 for age-by-genotype interaction). Among men aged 53 years or over those with the P/p or P/P genotype had an average two- and fivefold larger area of complicated lesions than those with the p/p genotype (*P*=0.001 in ANCOVA for trend, adjusted for age and BMI, Table 2). After adjusting for age, BMI, diabetes, and hypertension the ESR1 genotype was the strongest (*P*=0.007) predictor of complicated lesions (Table 3). After addition

Table 2 Mean proportion of the area of atherosclerotic lesions and narrowing in coronary arteries by ESR1 genotype and age subgroup (*CA* coronary artery)

Lesion type	<53 years				≥53 years				ANCOVA		
	P/P (n=22)	P/p (n=56)	p/p (n=44)	P[a]	P/P (n=24)	P/p (n=66)	p/p (n=50)	P[a]	ESR1	Age	Interaction
Fatty streak	6.10±5.68	4.34±3.94	5.11±4.68	0.219	5.49±3.29	6.51±5.11	6.50±5.47	0.776	0.759	0.042	0.361
Fibrotic	3.05±4.62	3.06±3.87	2.91±4.53	0.844	4.06±3.07	4.04±3.19	3.17±3.49**	0.088	0.163	0.005	0.616
Calcified	0.69±1.24	1.24±2.39	0.95±1.75	0.821	6.66±9.51	2.95±4.59	3.16±3.47***	0.028	0.305	0.000	0.056
Complicated	0.13±0.47	0.86±2.07	0.27±0.94	0.156	4.76±8.41	1.43±2.49	0.89±1.78*	0.001	0.024	0.000	0.003
CA narrowing	31.0±20.9	28.9±22.7	28.5±19.1	0.618	47.2±18.9	47.2±18.4	41.8±20.1	0.200	0.716	0.000	0.385

*P<0.001 P/P vs. p/p and P/p vs. p/p, **P<0.05 P/p vs. p/p, P<0.008 P/P vs. P/p

[a] P values for ESR1 from the ANCOVA performed for two age subgroups separately by using age and BMI as a covariate and LSD post-hoc test

Table 3 The strongest predictors of the area of complicated atherosclerotic lesions in coronary arteries (β standardized beta coefficient)

Explanatory variable	<53 years old[a]		≥53 years old[b]	
	β	P	β	P
ESR1 genotype	0.057	0.630	–0.334	0.007
Body mass index	0.133	0.292	0.177	0.175
Age	0.335	0.008	0.050	0.682
Hypertension (yes vs. no)	0.046	0.705	–0.033	0.799
Diabetes (yes vs. no)	0.272	0.027	0.254	0.040

[a] R^2=0.268, P=0.004
[b] R^2=0.189, P=0.032

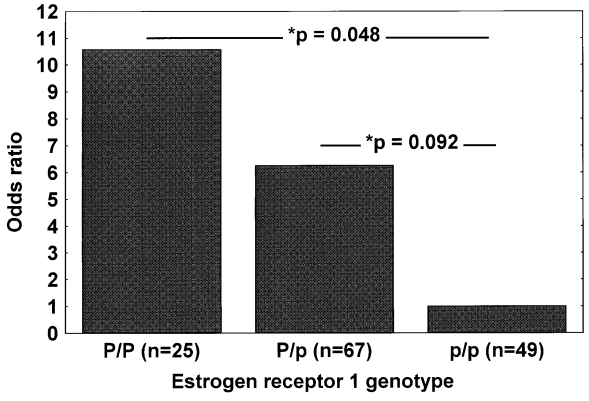

Fig. 1 Odds ratios and *P* values are from logistic regression analysis in men aged 53 years or over using thrombosis (yes/no) as dependent variable, and estrogen receptor 1 *Pvu*II genotype, age, and BMI as explanatory variables (n=141)

of smoking (yes/no) in an otherwise similar model (see Table 3), ESR1 genotype remained a significant (P=0.045) predictor of complicated lesions, although simultaneous smoking and other data were available only for 99 subjects.

The results were parallel when the mean proportion of the area of calcified lesions was used as dependent factor in two-way ANOVA, revealing borderline age by ESR1 genotype interaction (P=0.056). Men aged 53 years or over with P/P either P/p genotypes had an average two-fold larger area of calcified lesions than men with p/p genotype (P=0.028 in ANCOVA for trend, adjusted for age and BMI, Table 2). No such associations were seen in men aged under 53 years (Table 2). For the area of fibrotic lesions the age-by-genotype interaction was not statistically significant (Table 2). ESR1 genotypes had no significant association with fatty streaks or percentages of coronary narrowing (Table 2).

ESR1 genotype and coronary thrombosis

In multiple logistic regression analysis we found a significant age group by ESR1 genotype interaction (P=0.036) for coronary thrombosis. When adjusted for age and BMI the men aged 53 years or over with P/p or P/P genotype had odds ratios on average 6.3-fold (CI 0.74–52.9, P=0.092) and 10.6-fold (CI 1.08–103.5, P=0.043) for coronary thrombosis compared to men with p/p genotype, respectively (Fig. 1). The ESR1 genotype remained as a significant predictor of coronary thrombosis (P=0.043) after additional adjustment with diabetes and hypertension, although simultaneous data were available only for 65 subjects. When confounding factors (age, BMI, smoking, diabetes, hypertension) were added the to the model, the tendencies of the results were the same, although the small number of cases with all data (n=54) weakened the statistical significance.

Discussion

There was an age-dependent association between the ESR1 genotype and the area of complicated coronary lesions and the presence of coronary thrombosis in subjects who suffered sudden death. These results demonstrate at the level of the vessel wall that among men aged 53 years or over the area of complicated lesions in coronary arteries and the presence of coronary thrombosis increase in with allele dose (p/p<p/P<P/P). Further, these findings remained after adjusting for major available coronary risk factors i.e., age, BMI, diabetes, hypertension, and smoking.

ESR1 is expressed in the atherosclerotic plaque [8]. However, no previous studies have investigated the impact of the ESR1 *Pvu*II gene variation regarding the degree of specific atherosclerosis lesion phenotype, measured precisely from the artery wall after autopsy. It is possible that the alterations in ESR1 expression and its function affect the atheroprotective roles of circulating estrogen [1]. It has been speculated that the *Pvu*II polymorphism affects the splicing of ESR1 mRNA, resulting in alteration of protein expression [9, 17], or that the polymorphism is linked to some other polymorphism relevant to protein expression [9]. We believe it most probable that the polymorphism is in linkage disequilibrium with a mutation elsewhere in the ESR1 gene [18], predisposing to the development of advanced atherosclerotic plaques and coronary thrombosis. In fact, we have shown in preliminary studies [18] a strong linkage (P<0.00001) between ESR1 *Pvu*II polymorphism, and one other dinucleotide repeat polymorphism in the ESR1 gene regulatory (upstream) region (unpublished results). Although the mechanisms by which *Pvu*II polymorphism affect ESR1 signaling has not been adequately explained. However, it is strongly associated with the area of advanced atherosclerotic lesions, supporting the hypothesis that at least some variation in ESR1 gene affects the way in which the atheroprotective action of estrogen is mediated to artery wall cells. In men aged 53 years or over both homozygous and heterozygous P-allele carriers had an increased risk of complicated lesions and a tendency to increased prevalence of coronary thrombus

(Fig. 1). These findings suggest that the effect of ESR1 genotype on coronary atherosclerosis is age dependent.

The angiographic study by Matsubara and colleagues [9] found no association between the ESR1 genotype and severity of coronary artery narrowing (*n*=87, aged 38–79 years, 65 men and 22 women). Despite differences in study design our results are concordant with those of this study [9] since we also were unable to find a statistically significant association between coronary narrowing, as determined from a cast rubber model, and ESR1 genotype. In angiography only coronary narrowing can be measured, whereas we used in addition a computer-assisted planimetry to determine the precise area of specific atherosclerotic plaque phenotypes of the coronary arteries, in a substantially larger number of subjects (*n*=262).

In conclusion, our preliminary results show that the ESR1 gene is an interesting candidate behind the pathogenesis of acute coronary events which among elderly men may partly explain differences between individuals in the development of coronary artery disease.

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