EXTENDED REPORT

Oestrogen receptor α gene polymorphisms in systemic lupus erythematosus

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Objective: To analyse associations of two oestrogen receptor α (ORα) gene polymorphisms in 260 patients with SLE from northern Sweden. The two polymorphisms, PvuII T/C and the XbaI A/G, are located in the first intron of the ORα gene.

Methods: All patients fulfilling at least four of the ACR criteria for SLE were consecutively recruited during one year. The SLEDAI score and SLICC damage index were recorded. 670 individuals from the same geographical area served as controls. DNA from the patients and controls was extracted and genotyped using the 5′ nuclease assay with an ABI PRISM 7900HT instrument. The genotype/phenotype relationships were calculated using SPSS.

Results: The unusual PvuII C allele was associated with malar rash and the unusual XbaI G allele with photosensitivity (p = 0.001, OR = 2.53, 95% CI = 1.43 to 4.47 and p = 0.007, OR = 2.12, 95% CI = 1.22 to 3.66, respectively). The common XbaI AA genotype was associated with serositis (p = 0.013, OR = 1.92, 95% CI = 1.15 to 3.22). Based on the SLICC damage index associations of the common TT genotype and AA genotype with cognitive impairment were identified (p = 0.018, OR = 2.47, 95% CI = 1.17 to 5.25 and p = 0.018, OR = 2.75, 95% CI = 1.19 to 6.38 respectively). There was also an association of the XbaI AA genotype with the angina/coronary artery bypass variable (p = 0.042, OR = 2.58, 95% CI = 1.03 to 6.43). Of the variables describing disease severity and duration it was found that carriers of the unusual PvuII C allele showed a later onset of SLE (p = 0.02) and carriers of the unusual XbaI G allele a lower SLICC damage index.

Conclusions: The unusual PvuII C and XbaI G alleles were associated with a milder form of SLE characterised by skin manifestations, later onset, and less organ damage.

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown aetiology predominantly affecting women.[1–4] Both genetic and environmental factors are thought to contribute to disease susceptibility. Among the environmental factors are the female sex hormones, oestrogens, which have proinflammatory properties that might predispose to disease development.[5 6] Oestrogen treatment is known to activate mature peripheral B cells in mice and may increase the production of IgG, including IgG antibodies against double stranded (ds) DNA, in peripheral blood mononuclear cells of SLE patients by enhancing B cell activity and by promoting interleukin 10 (IL10) production in monocytes.[7 8] Higher levels of oestrogen and the oestrogen/androgen ratio have been proposed to explain why women have a greater risk of developing SLE and why the flares appear at certain stages in life. The disease mostly affects women of childbearing age,[9 10] and the risk of disease decreases in post-menopausal women in line with the decline of endogenous oestrogens.[10] Administration of some oestrogen containing oral contraceptives[11–13] and pregnancy[14–18] may cause the disease to flare, and disease activity often fluctuates with the menstrual cycle.[19]

Oestrogens are mainly produced in the ovary and testis, but aromatisation of androgens also generates oestrogen in peripheral tissues and in adrenal glands. The effects of oestrogens are mediated through oestrogen receptors (ORs or ERs). There are two different kinds of oestrogen receptor, ORα (6q25.1) and ORβ (14q22–24), which are homologous to each other. Both are ligand activated transcription factors and have the same structural characteristics and function as nuclear receptors.[20]

Oestrogen receptor α gene polymorphisms have been described as being associated with a various different complex diseases such as myocardial infarction,[21] cognitive impairment,[22] breast cancer,[23] Alzheimer's disease,[24] osteoporosis,[25] rheumatoid arthritis,[26] and lupus nephritis.[27] In all of these reports the polymorphisms of the ORα gene are designated PvuII and XbaI. However, the nomenclature has been inconsistent and quite confusing. Most results are derived using the restriction fragment length polymorphism (RFLP) method whereby PvuII and XbaI denote the restriction enzymes for two specific sites in intron 1 of the ORα gene. PvuII C and XbaI G equate to the absence of restriction site (= P1 = P and = X1 = X, respectively). Other names for PvuII T/C are: ESR1 c.454-397 T→C, IVS1-401 T/C, IVS1-397 T/C, ESR1 nt 938 T→C (GeneBank: AF326912), and rs2234693. The XbaI A/G polymorphism has also been referred to as ESR1 c.454-351 A→G, IVS1-354 A/G, IVS1-351 A/G, and rs9340799.[21–29]

Associations of the PvuII T/C and XbaI A/G polymorphisms have been reported in two studies on Asian patients with SLE.[27 30] The patients included in our cross sectional study were collected from the four most northern counties of Sweden. The population of northern Sweden is relatively homogeneous, making it well suited for genetic association studies. All individuals were diagnosed with SLE and their clinical phenotypes evaluated in relation to the polymorphisms of the ORα gene.

Abbreviations: ACR, American College of Rheumatology; SLE, systemic lupus erythematosus; SLEDAI, SLE activity index; SLICC, Systemic Lupus International Collaborating Clinics; SNP, single nucleotide polymorphism

Table 1 Baseline characteristics of 220 female and 40 male patients diagnosed with systemic lupus erythematosus

Variable	Total (n = 260)	Women (n = 220)	Men (n = 40)
Age (years) (mean (SD))	51.4 (15.2)	51.2 (14.4)	52.5 (18.9)
Age of onset (years) (mean (SD))	39.0 (15.7)	38.4 (15.0)	42.4 (19.1)
ACR criteria‡			
1. Malar rash	149 (57.5%)	132 (60.3%)*	17 (42.5%)
2. Discoid rash	75 (28.8%)	63 (28.6%)	12 (30.0%)
3. Photosensitivity	172 (66.2%)	152 (69.1%)*	20 (50.0%)
4. Oral ulcers	48 (18.7%)	41 (18.9%)	7 (17.5%)
5. Arthritis	195 (75.3%)	171 (78.1%)*	24 (60.0%)
6. Serositis	107 (41.3%)	86 (39.3%)	21 (52.5%)
7. Renal disorder	85 (32.8%)	64 (29.2%)	21 (52.5%)**
8. Neurological disorder	36 (14.0%)	32 (14.7%)	4 (10.0%)
9. Haematological disorder	138 (53.1%)	117 (53.2%)	21 (52.5%)
10. Immunological disorder	188 (72.3%)	155 (70.5%)	33 (82.5%)
11. Antinuclear antibody	254 (97.7%)	216 (98%)	38 (95%)
Number of ACR criteria (mean (SD))	5.5 (1.4)	5.6 (1.3)	5.4 (1.5)
SLICC§ (mean (SD))	2.4 (2.5)	2.3 (2.4)	2.9 (2.9)
Cognitive impairment	33 (12.8%)	28 (12.8%)	5 (12.5%)
Angina or coronary artery bypass	27 (10.4%)	20 (9.1%)	7 (17.5%)
Peripheral vascular involvement	37 (14.4%)	27 (12.3%)	10 (25.0%)*
Diabetes	13 (5.0%)	8 (3.6%)	5 (12.5%)*
SLEDAI¶ (mean (SD))	3.4 (4.4)	3.2 (4.1)	4.5 (5.5)
Haematuria	26 (10.0%)	19 (8.7%)	7 (17.5%)
Proteinuria	25 (9.7%)	17 (7.8%)	8 (20.0%)*
CNS involvement	9 (3.5%)	5 (2)	4 (10)*†

Values are n (%) unless stated otherwise. Of the SLICC and SLEDAI groups only variables significantly different between sexes or by presumptions from published reports are shown.
*$p<0.05$, **$p<0.01$, †Fisher's exact test.
‡American College of Rheumatology criteria.[31, 32]
§SLICC, Systemic Lupus International Collaborating Clinics.[34]
¶SLEDAI, SLE disease activity index.[33]

METHODS

Subjects

All patients with possible SLE were collected from departments of rheumatology (n = 4), internal medicine (n = 12), dermatology (n = 3), all primary health care centres (n = 140), and a private practitioner, from the four most northern counties of Sweden. The patients had to fulfil at least four of the 1982 American College of Rheumatology (ACR) classification criteria[31] and the updated criteria[32] for SLE to be included in the study. Five rheumatologists assessed the patients to determine their disease activity with the SLE activity index (SLEDAI)[33] and the organ damage with the Systemic Lupus International Collaborating Clinics/ACR (SLICC) index.[34] In all, 279 patients fulfilling the ACR criteria for SLE, of whom 260 (220 women (84.6%) and 40 men (15.4%)) were willing to participate in the study. Demographic and significant clinical data on the patients are presented in table 1.

Table 2 Primers and probes for the 5′ nuclease assay

PvuII	
fw primer	5′-CTGTGTTGTCCATCAGTTCATCTG-3′
rev primer	5′-ACTCAGGGTCTCTGGGAAACAG-3′
T allele probe	5′-VIC-CCAGCTGTTTTATG-MGB*-Q†-3′
C allele probe	5′-6FAM-CCAGCCGTTTTAT-MGB*-Q†-3′
XbaI	
fw primer	5′-GTCCATCAGTTCATCTGAGTTCCAA-3′
rev primer	5′-AGAACCATTAGAGACCAATGCTCATC-3′
A allele probe	5′-VIC-AGTGTGGTCTAGAGTTG-MGB*-Q†3′
G allele probe	5′-6FAM-TGTGGTCTGGAGTTG-MGB*-Q†-3′

*Minor groove binder.
†Quencher.

The controls were geographically matched and randomly collected from the northern Sweden part of the World Health Organisation (WHO) study for monitoring of trends and determinants in cardiovascular disease (MONICA). The control cohort was population based, stratified for age and sex, and no individual was excluded. The controls were only used to compare genotype and allele frequencies. The control cohort comprised 670 individuals, 490 women (73.1%) and 180 men (26.9%). The mean (SD) age of the controls was 59.4 (12.4) years for women and 64.1 (10.2) years for men. The project was approved by the regional ethics committee of the University Hospital in Umeå, Sweden.

Methods

Genomic DNA from the patients was extracted from whole blood (EDTA treated) using the standard phenol/chloroform extraction method (n = 161)[35] and the salting out method (n = 99).[36] DNA from the controls was extracted using the FlexiGene DNA kit supplied by Qiagen (Valencia, California, USA). The PvuII and XbaI polymorphisms were determined using the 5′ nuclease assay.[37] Primers and probes were designed using Assays-by-Design (Applied Biosystems, Foster City, California, USA) (table 2). The probes were labelled at their 5′ ends with FAM™ (the first allele) and VIC™ (the second allele), and the 3′ ends contained quenchers. Primers and probes were mixed with TaqMan® Universal PCR Master Mix, No AmpErase® UNG, and added to 96-well microtitre plates, each well containing 10 ng of air dried DNA. The polymerase chain reactions (PCR) were carried out according to Applied Biosystems instructions, and detection of the different genotypes was done using an ABI PRISM® 7900HT sequence detector system (Applied Biosystems). Data were processed using SDS 2.1 software (Applied Biosystems).

Table 3 Genotype frequencies of the patients and controls

		Patients (%)			Controls (%)		
		Women (n = 220)	Men (n = 40)	Total (n = 260)	Women (n = 490)	Men (n = 180)	Total (n = 670)
PvuII	CC	16.8	20.0	17.3	18.0	23.3	19.4
	CT	52.7	40.0	50.8	49.4	50.0	49.6
	TT	30.5	40.0	31.9	32.7	26.7	31.0
XbaI	GG	7.7	10.0	8.1	7.8	11.1	8.6
	GA	37.7	27.5	36.2	40.8	44.4	41.9
	AA	54.5	62.5	55.8	51.4	44.4	49.5

The different genotypes were verified by comparison with controls of known genotype.

Statistical methods

Continuous data were analysed using Student's *t* test for independent samples. Categorical data were analysed using the χ^2 test or Fisher's exact test as appropriate. Calculations of the odds ratios (ORs) were done using binary logistic regression, adjusted for sex, with male subjects as reference, and for age at disease onset. The relation between a variable and an allele, with appropriate adjustments, was analysed using the univariate analysis of variance.

The statistical program used for the calculations was SPSS (SPSS for Windows, version 11.5; Chicago, Illinois, USA). All p values refer to two sided tests, with values less than 0.05 considered significant.

Strategy of analysis

The strategy for choosing variables for binary logistic regression analyses was based on conclusions drawn from previous reports on these single nucleotide polymorphisms (SNPs) in SLE and other diseases, and by including variables found to differ significantly between the sexes. The difference between the sexes could be oestrogen dependent.

RESULTS

The distribution of genotypes in both the patients and the controls was in agreement with the Hardy–Weinberg equilibrium, and the genotypes and allele frequencies of the patients did not differ significantly from the controls (table 3). PvuII and XbaI are tightly linked,[27] so a classification of the nine possible combined genotypes was made (table 4). There was no significant difference in the combined genotypes between patients and controls.

By stratifying for the ACR criteria and genotypes of the PvuII and XbaI polymorphisms it was found that malar rash was associated with the PvuII genotypes ($p = 0.022$, $p_c = 0.242$) and that photosensitivity and serositis were associated with the XbaI genotypes ($p = 0.013$, $p_c = 0.143$ and $p = 0.009$, $p_c = 0.099$, respectively) (table 5). The binary logistic regression showed that malar rash was associated with carriage of the PvuII C allele ($p = 0.001$, OR = 2.53 (95% CI, 1.43 to 4.47)) and the XbaI G allele ($p = 0.027$, OR = 1.82 (1.31 to 4.57)) (table 6). By adjusting for sex and age at disease onset there was a slight increase in odds ratios. Photosensitivity was also associated with the XbaI G allele ($p = 0.007$, OR = 2.12 (95% CI, 1.22 to 3.66)) with an approximately similar odds ratio when adjusted for sex and age at onset (table 6). Serositis was associated with the XbaI AA genotype ($p = 0.013$, OR = 1.92 (95% CI, 1.15 to 3.22)) and the XbaI G allele was protective for serositis ($p = 0.011$, OR = 0.51 (0.31 to 0.86)) (table 6). Adjustments for sex and age at onset did not affect the odds ratios.

Using the SLICC damage index, the relation of genotype with cognitive impairment was analysed. The PvuII TT genotype ($p = 0.018$, OR = 2.47 (95% CI, 1.17 to 5.25)), as well as the XbaI AA genotype ($p = 0.018$, OR = 2.75 (1.19 to 6.38)) was found to be significantly associated with cognitive impairment, unrelated to the adjustments (table 7).

As there are previous reports on PvuII and XbaI associations with coronary artery disease[38 39] and cardiovascular disease,[21 40 41] the predicted values for cardiovascular manifestations were analysed. There was an association for individuals carrying the XbaI AA genotype ($p = 0.042$, OR = 2.58 (95% CI, 1.03 to 6.43)) with the SLICC damage index for angina/coronary artery bypass (table 7). Adjustments did not affect the odds ratios.

Comparison was made of the variables characterising the severity of the disease (number of ACR criteria, SLICC index, and SLEDAI) and age at disease onset in relation to the different genotypes. Carriers of the unusual PvuII C allele showed a later onset of SLE ($p = 0.02$) and carriers of the unusual XbaI G allele indicated a lower value for SLICC index ($p = 0.028$) (table 8). This relation remained after adjustment for disease duration.

There were no significant associations with the ACR criteria for arthritis and renal disorder, or with the SLICC damage index for peripheral vascular involvement and diabetes despite their differences in sexual prevalence.

Table 4 Combined genotype frequencies of the patients and controls

	Patients (%)			Controls (%)		
Genotypes	Women (n = 220)	Men (n = 40)	Total (n = 260)	Women (n = 490)	Men (n = 180)	Total (n = 670)
CCGG	7.7	10.0	8.1	7.6	11.1	8.5
CCGA	6.8	5.0	6.5	8.0	11.1	8.8
CCAA	2.3	5.0	2.7	2.4	1.1	2.1
CTGG	0.0	0.0	0.0	0.2	0.0	0.1
CTGA	30.9	22.5	29.6	32.7	33.3	32.8
CTAA	21.8	17.5	31.2	16.5	16.7	16.6
TTGG	0.0	0.0	0.0	0.0	0.0	0.0
TTGA	0.0	0.0	0.0	0.2	0.0	0.1
TTAA	30.5	40.0	31.9	32.4	26.7	30.9

Table 5 Comparison between the genotypes of the two polymorphisms and the ACR criteria

	PvuII				XbaI			
ACR criteria (1–11)	CC (n = 45)	TC (n = 132)	TT (n = 83)	p Value*	GG (n = 21)	AG (n = 94)	AA (n = 145)	p Value*
1. Malar rash	27 (60.0)	84 (63.6)	38 (45.8)	0.022	14 (66.7)	60 (63.8)	75 (51.7)	0.12
2. Discoid rash	15 (33.3)	42 (31.8)	18 (21.7)	0.21	8 (38.1)	25 (26.6)	42 (29.0)	0.57
3. Photosensitivity	32 (71.1)	89 (67.4)	51 (61.4)	0.49	17 (81.0)	70 (74.5)	85 (58.6)	0.013
4. Oral ulcer	10 (22.2)	24 (18.2)	14 (16.9)	0.75	4 (19.0)	15 (16.0)	29 (20.0)	0.73
5. Arthritis	33 (73.3)	97 (73.5)	65 (78.3)	0.70	12 (57.1)	74 (78.7)	109 (75.2)	0.12
6. Serositis	19 (42.2)	50 (37.9)	36 (45.8)	0.51	10 (47.6)	27 (28.7)	70 (48.3)	0.009
7. Renal disease	16 (35.6)	41 (31.1)	28 (33.7)	0.83	8 (38.1)	29 (30.9)	48 (33.1)	0.81
8. Neurological disease	4 (8.9)	19 (14.4)	13 (15.7)	0.55	1 (4.8)	12 (12.8)	23 (15.9)	0.36
9. Haematological disease	23 (51.1)	72 (51.5)	47 (56.6)	0.73	11 (52.4)	52 (55.3)	75 (51.7)	0.86
10. Immunological disease	34 (75.6)	90 (68.2)	64 (77.1)	0.31	12 (57.1)	66 (70.2)	106 (73.1)	0.32
11. ANA	43 (95.6)	131 (99.2)	80 (96.4)	0.23	20 (95.2)	93 (98.9)	141 (97.2)	0.51

Values are n (%).
*Calculated by χ^2 with two degrees of freedom.

No association analysis was undertaken with osteoporosis as it had been measured inconsistently in the patients.

DISCUSSION

In this cross sectional study of patients diagnosed with SLE, the data collected were related to the ACR criteria, the SLEDAI score, and the SLICC damage index. Data analyses were based on findings of significant differences between the sexes and on significant findings reported by others. The patients originate from a relatively homogeneous population from the four northernmost counties of Sweden, all having the same ethnic background. The controls were derived from the same population. This population is considered to be well suited to genetic association studies as it derives from a founder population that has been relatively isolated throughout history.

When analysing the variables describing the severity of the disease, it was found that individuals carrying the unusual PvuII C allele had a later onset of SLE and those carrying the unusual XbaI G allele had a lower SLICC damage index value, unrelated to disease duration. This could indicate that carriage of these alleles results in a milder form of the disease. However, there are no differences between carriers of the unusual alleles or usual alleles when considering the number of ACR criteria or the sum of disease activity measured by the SLEDAI score. Our finding concerning age at disease onset is consistent with that reported by Lee *et al*,

Table 6 Binary logistic regression analysis of patients with ACR criteria 1, 3, and 6

	Univariate analysis			Multivariate analysis		
Variable	B	p Value	OR (95% CI)	B	p Value	OR (95% CI)
Malar rash						
PvuII carrier of C	0.704	0.009	2.022 (1.191 to 3.433)	0.927	0.001	2.526 (1.427 to 4.473)
Sex (males reference)				−0.564	0.127	0.569 (0.276 to 1.175)
Age at onset				−0.037	0.000	0.964 (0.947 to 0.981)
XbaI carrier of G	0.546	0.034	1.727 (1.043 to 2.858)	0.598	0.027	1.818. (1.070 to 3.088)
Sex (males reference)				−0.605	0.099	0.546 (0.266 to 1.121)
Age at onset				−0.033	0.000	0.967 (0.951 to 0.984)
Photosensitivity						
XbaI carrier of G	0.785	0.004	2.193 (1.279 to 3.760)	0.750	0.007	2.117 (1.224 to 3.661)
Sex (males ref.)				−0.754	0.034	0.470 (0.234 to 0.945)
Age at onset				−0.006	0.471	0.994 (0.977 to 1.011)
Serositis						
XbaI AA	0.664	0.011	1.942 (1.167 to 3.2234)	0.653	0.013	1.922 (1.146 to 3.223)
Sex (males reference)				0.539	0.127	1.714 (0.859 to 3.419)
Age at onset				−0.004	0.656	0.996 (0.980 to 1.013)
XbaI carrier of G	−0.677	0.009	0.508 (0.306 to 0.846)	−0.667	0.011	0.513 (0.306 to 0.860)
Sex (males reference)				0.543	0.124	1.721 (0.862 to 3.435)
Age at onset				−0.003	0.684	0.997 (0.981 to 1.013)

Adjusted for sex and age at disease onset.
CI, confidence interval; OR, odds ratio.

Table 7 Binary logistic regression analysis of patients with systemic lupus erythematosus assessed by SLICC/ACR damage index

	Univariate analysis			Multivariate analysis		
Variables	**B**	**p Value**	**OR (95% CI)**	**B**	**p Value**	**OR (95% CI)**
Cognitive impairment						
PvuII TT	0.940	0.013	2.560 (1.221 to 5.368)	0.906	0.018	2.474 (1.166 to 5.248)
Sex (males reference)				−0.114	0.830	0.893 (0.316 to 2.519)
Age at onset				−0.006	0.632	0.994 (0.970 to 1.019)
XbaI AA	1.024	0.017	2.784 (1.204 to 6.435)	1.012	0.018	2.752 (1.187 to 6.383)
Sex (males reference)				−0.083	0.875	0.920 (0.327 to 2.589)
Age at onset				−0.009	0.485	0.992 (0.968 to 1.016)
Angina or coronary artery bypass						
XbaI AA	0.904	0.049	2.469 (1.005 to 6.062)	0.948	0.042	2.580 (1.034 to 6.434)
Sex (males reference)				0.548	0.270	1.730 (0.653 to 4.579)
Age at onset				0.030	0.024	1.030 (1.004 to 1.057)

Adjusted for sex and age at disease onset.
CI, confidence interval; OR, odds ratio.

who found that SLE patients homozygous for the PvuII C allele were older at disease onset.[30]

Women with SLE more often had skin and joint manifestations than men. The unusual alleles PvuII C and XbaI G were shown to be associated with skin involvement; however, there were no associations of these alleles with arthritis. The C allele of PvuII was related to malar rash with a higher odds ratio than the G allele of XbaI and conversely the G allele of XbaI was in return associated with photosensitivity. As the PvuII C allele and the XbaI G allele are tightly linked, it is impossible to tell which is more highly related to skin involvement. Skin manifestations, such as malar rash and photosensitivity, are generally regarded as signs of a less severe disease in SLE patients. Skin rashes have in some cases been related to the menstrual periods, suggesting a hormonal influence.[42]

The serositis variable from the ACR criteria is associated with the XbaI AA genotype while the XbaI G allele is protective for serositis. From these data, it is not possible to conclude which of the genotypes is of importance, as the significance levels were equal; and in view of the numbers of tests carried out, either one or both of the significance values could be the result of a type II error.

There was a significant difference in central nervous system involvement (from the SLEDAI score) between women and men, whereby men had a higher frequency of CNS involvement. A decline in oestrogen is related to bone loss and possibly to loss of cognitive function. It has been suggested that bone loss and cognitive impairment are concurrent conditions, which could reflect their association with oestrogens.[43 44] Oestrogen receptor polymorphisms have been reported to be involved in cognitive impairment which is a neuropsychiatric criterion of the SLICC damage index. There are also reports indicating associations between the ORα polymorphisms and osteoporosis.[25] We did not undertake any analyses with osteoporosis indices owing to inconsistency in measurements and data collection. Yaffe *et al*, in a study of healthy women aged 65 years or older, found that those elderly women who carried the PvuII T allele had a higher risk of developing cognitive impairment.[22] An association with cognitive function is biologically relevant as oestrogen receptors are located throughout the brain and especially in regions involved with learning and memory.[45] It has also been shown that oestrogen replacement therapy may benefit cognitive function in postmenopausal women.[46] Our results were consistent with those of Yaffe *et al* regarding the T allele[22] when the PvuII TT genotype was found to be significantly associated with cognitive impairment. Cognitive impairment was also found to be associated with the XbaI AA genotype. This is logical because of the strong linkage between the two polymorphisms. The XbaI AA genotype had a slightly higher odds ratio, which may indicate that this polymorphism is the one associated with cognitive impairment while the PvuII TT only shows significance because of the linkage. The data were not further stratified for age and therefore the specific association with older women as reported by Yaffe *et al*[22] could not be identified.

Women with SLE have been reported to have significantly increased rates of myocardial infarction compared with those without SLE.[47 48] The PvuII and the XbaI polymorphisms of the oestrogen receptor gene have been studied extensively in several cardiovascular related diseases. Oestrogen receptor α has been found in smooth muscle cells, endothelial cells, and myocardial cells of the coronary artery wall.[21] Other reports indicate that the XbaI GG genotype and the PvuII TT genotype are associated with coronary artery disease,[38 39] the PvuII CC genotype being associated with cardiovascular disease[40] and with coronary disease[41] and that the PvuII T allele and the XbaI A allele are associated with myocardial infarction.[21] The XbaI AA genotype was associated with angina/coronary artery bypass of the SLICC index in our SLE patients. This can be related to the findings of Schuit *et al*, who reported associations of the TA haplotype (combination of PvuII T and XbaI A) with myocardial infarction and

Table 8 Continuous variables describing the disease and the differences between patients carrying the unusual alleles (PvuII C and XbaI G) versus not carrying these alleles

Type	Age of onset	Number of ACR criteria	SLICC index	SLEDAI
PvuII carrier of C allele/carrier of non-C	40.6 (1.2)*/35.7 (1.7)	5.6 (0.1)/5.5 (0.1)	2.3 (0.2)/2.5 (0.3)	3.5 (0.3)/3.4 (0.6)
XbaI carrier of G allele/carrier of non-G	40.0 (1.4) / 38.2 (1.3)	5.6 (0.1)/5.5 (0.1)	2.0 (0.2)*/2.7 (0.2)	3.2 (0.4)/3.6 (0.4)

Values are mean (SEM).
*$p<0.05$, all analysed by Student's *t* test.
SLEDAI, SLE disease activity index; SLICC, Systemic Lupus International Collaborating Clinics.

ischaemic heart disease in women.[21] There was no association with the other genotypes, which might indicate that the oestrogen receptor involvement in angina/coronary artery bypass is similar to that in myocardial infarction and ischaemic heart disease.

How these polymorphisms alter the function or expression of the receptor is unknown. They are both intronic polymorphisms and it has been reported that the PvuII C allele produces a binding site for the myb family of transcription factors. In presence of B-myb there was a four times greater upregulation of a downstream reporter construct compared with that seen with the T allele.[41 49] Thus the PvuII C allele could lead to an upregulation of the ORα transcription or an alteration of the ORα transcript. The role of the XbaI polymorphism is still unclear except for its strong linkage to the PvuII polymorphism.

The limitations of this study comprise the size of the patient group, which is relatively small owing to the low incidence of SLE, though all patients fulfilling the criteria for SLE from northern Sweden were included. Also, because of the number of statistical tests carried out, some of the results with lower significance values have to be interpreted with care and should be seen as indications rather than truly significant findings until they are confirmed by further studies.

In conclusion, the unusual alleles PvuII C and XbaI G were found in patients with a greater age at disease onset and with a lower SLICC index score, and were also associated with skin involvement. The C allele is highly associated with malar rash and the G allele with photosensitivity. These findings indicate that the unusual alleles of these ORα polymorphisms (PvuII C and XbaI G) are associated with less severe disease manifestations such as skin involvement. The more common alleles PvuII T and XbaI A were found to be associated with the cognitive impairment and angina/coronary artery bypass variables from the SLICC damage index, and also with serositis from the ACR criteria.

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