allele frequencies for patients and controls were used to calculate the OR and the 95% CI using the method of Woolf.[15] Gene–environmental interactions were assessed by both 'case-only' analysis and logistic regression analysis.[16] All statistical analysis were performed using Plink software.[17]

RESULTS AND DISCUSSION

A significant association between the *PADI4* polymorphism and RA susceptibility was observed in the whole set of case–control subjects in the first Japanese set (rs11203367; per allele OR (OR_{allele}) 1.14; 95% CI 1.00 to 1.29; p value for a trend test (p_{trend})=0.045; table 1). In a stratified analysis with sex, the *PADI4* polymorphism was significantly associated only in men (OR_{allele} 1.44; 95% CI 1.14 to 1.81; p_{trend}=0.0022), but not in women (OR_{allele} 1.02; 95% CI 0.82 to 1.25; p_{trend}=0.84). Similarly, when subjects in both cases and controls were stratified for smoking status, the *PADI4* polymorphism had a greater effect in ever-smokers (OR_{allele} 1.35; 95% CI 1.11 to 1.65; p_{trend}=0.0024) compared with never-smokers (OR_{allele} 1.03; 95% CI 0.86 to 1.23; p_{trend}=0.71). Further stratification analysis with sex and smoking status revealed that the *PADI4* polymorphism had the highest risk in the subpopulation of male ever-smokers (OR_{allele} 1.61; 95% CI 1.24 to 2.09; p_{trend}=0.00031). Similar findings were also observed, when only ACPA-positive patients were analysed (supplementary table 3, available online only).

To support these observations, we also analysed other case–control sets in the Japanese population and Dutch population (unstratified controls for smoking status were used in both sets as no information was available). In the second Japanese set, the highest risk in the subpopulation of male ever-smokers was replicated in rs1748033 (OR_{allele} 1.34; 95% CI 1.02 to 1.75; p_{trend}=0.039; table 1). In the Dutch set, the association of the *PADI4* polymorphism (rs1748033) was statistically significant in a dominant model (OR_{dom} 1.32; 95% CI 1.02 to 1.72; p_{dom}=0.03; table 2), but not in a trend test, when evaluated in total (p_{trend}=0.14). When patients were stratified by sex or/and smoking status and compared with control subjects, OR in the dominant model was higher for men (OR 1.36; 95% CI 0.90 to 2.06; p=0.13) than for women and was higher for ever-smokers (OR 1.56; 95% CI 1.06 to 2.31; p=0.02) than for never-smokers. Furthermore, it was highest in male ever-smokers (OR 1.79; 95% CI 0.98 to 3.27; p=0.043).

Table 2 Association of *PADI4* polymorphism and RA stratified with sex and smoking status in a Dutch population*

	Sum		MAF		Genotype frequency test (dominant model)	
	Case	Contol	Case	Contol	OR	p Value
rs11203367						
All	646	385	0.44	0.42	1.19 (0.90 to 1.56)	0.2
Men	218	180	0.47	0.40	1.49 (0.96 to 2.33)	0.063
Women	398	188	0.42	0.43	1.06 (0.73 to 1.56)	0.7
Ever-smoker	174	385	0.43	0.42	1.14 (0.76 to 1.70)	0.5
Never-smoker	178	385	0.45	0.42	1.06 (0.72 to 1.57)	0.7
Male ever-smoker	76	180	0.45	0.40	1.46 (0.78 to 2.71)	0.2
Male never-smoker	40	180	0.53	0.40	1.38 (0.62 to 3.10)	0.4
Female ever-smoker	98	188	0.41	0.43	0.99 (0.58 to 1.72)	0.9
Female never-smoker	138	188	0.43	0.43	0.99 (0.61 to 1.61)	0.9
rs1748033						
All	635	391	0.34	0.30	1.32 (1.02 to 1.72)	0.03
Men	215	183	0.35	0.28	1.36 (0.90 to 2.06)	0.13
Women	389	191	0.32	0.31	1.33 (0.93 to 1.91)	0.11
Ever-smoker	158	391	0.36	0.30	1.56 (1.06 to 2.31)	0.02
Never-smoker	178	391	0.31	0.30	1.06 (0.73 to 1.53)	0.7
Male ever-smoker	70	183	0.38	0.28	1.79 (0.98 to 3.27)	0.043
Male never-smoker	41	183	0.35	0.28	1.35 (0.65 to 2.82)	0.4
Female ever-smoker	88	191	0.34	0.31	1.48 (0.86 to 2.55)	0.13
Female never-smoker	137	191	0.30	0.31	1.03 (0.65 to 1.64)	0.9

*rs11203373 (T/C, T is the minor allele) and rs174803 (T/C, T is the minor allele) were genotyped for the test. Only case subjects were stratified with smoking status.
MAF, minor allele frequency; *PADI4*, peptidyl arginine deiminase 4; RA, rheumatoid arthritis.

These stratified analyses suggested gene–environmental interactions between *PADI4* and sex, and/or between *PADI4* and smoking status. We performed case-only analysis to test these interactions statistically, by comparing the allele frequency of the *PADI4* polymorphism in the stratified subpopulation of patients (the first and second Japanese sets were combined). Allele frequency was significantly higher in men than in women (rs11203367; 0.48 vs 0.42; p_{trend}=0.0016) and in ever-smokers than in never-smokers (rs11203367; 0.47 vs 0.41; p_{trend}=0.00077), suggesting the presence of gene–environmental interactions for *PADI4*. Similar results were obtained for rs1748033. In addition to stratified analyses using the contingency tables, we analysed these gene–environmental interactions using logistic regression models. The first Japanese set was used for analysis because of the availability of smoking status. The *PADI4* polymorphism was associated with RA susceptibility in an additive model, adjusted by sex and smoking status (rs11203367; OR_{add} 1.18; 95% CI 1.01 to 1.38; p_{add}=0.035). When an interaction term between SNP genotype and sex (a product term of genotype×sex) was introduced into the regression model, the logistic coefficient for the term was significant (p=0.029). Similarly, when an interaction term between SNP genotype and smoking status (a product term of genotype×smoking status) was introduced into the model, the coefficient for the term was again significant (p=0.034). We also added the age of subjects into the model, because it could be a confounding factor considering that smoking prevalence has been decreasing in recent decades, especially in Japanese men (OECD Health Data, 2009).[18] The interaction term for SNP and smoking remained significant (p=0.038), whereas the significance level of the interaction term for SNP and sex became marginal (p=0.075).

Finally, we examined the association between the *PADI4* polymorphism and ACPA status in the patients of Japanese sets. The allele frequency of *PADI4* showed a higher trend in ACPA-positive patients compared with ACPA-negative patients (rs11203367; 0.43 vs 0.41; p_{trend}=0.54). When the genotype frequency was compared in a recessive model, the *PADI4* polymorphism was significantly associated with the ACPA status in ever-smokers (rs11203367; OR_{rec} 2.33; 95% CI 1.23 to 4.39; p_{rec}=0.0072; table 3), suggesting that the *PADI4* polymorphism may be involved in the appearance of ACPA in smokers.

Gene–environmental interactions in RA susceptibility have been well described between polymorphisms in *HLA-DRB1* and *PTPN22* genes and smoking habit in populations of European descent.[19 20] Our observations here indicate that the *PADI4* polymorphism is another genetic risk that would interact with smoking in RA susceptibility, although why this interaction is prominent in men remains to be solved. The status of sex hormones may influence the role of *PADI4*, as it is profoundly involved in the onset of RA.[21] Another possible explanation could be gender differences in smoking behaviour, which has also been argued in other smoking-related diseases.[22] Quantitative analysis of smoking history, such as pack-years smoked, may be needed to investigate further for the gender difference.

Smoking prevalence rates differed highly among the populations, and the attribution of smoking to the onset of RA may thus differ among populations. A recent epidemiological survey has shown that smoking prevalences are generally higher in men from Asian countries than in western European countries: Japan, 45.8%; Korea, 46.6%; UK, 25.0%; The Netherlands, 35.0%; Sweden, 13.9%; and USA, 19.1% in 2005.[16] Considering our observation that the *PADI4* polymorphism has the highest risk in male ever-smokers, the attribution of the *PADI4* polymorphism may be relatively high in populations with high smoking prevalences among men, such as Japan and Korea, corresponding to the positive results in association studies for *PADI4* polymorphisms in these countries.[2 4 5]

In conclusion, the *PADI4* polymorphism highly predisposes male smokers to RA, and the genetic heterogeneity observed in the *PADI4* polymorphism between populations of Asian and European countries may be partly explained by differences in smoking prevalences among men.

Table 3 Association of *PADI4* polymorphism and ACPA status in a Japanese population*

	Sum		MAF		Genotype frequency test (recessive model)	
	ACPA+	ACPA−	ACPA+	ACPA−	OR	p Value
rs11203367						
All	1614	401	0.43	0.41	1.25 (0.93 to 1.68)	0.14
Men	295	80	0.46	0.45	1.22 (0.65 to 2.28)	0.52
Women	1319	321	0.42	0.40	1.27 (0.90 to 1.78)	0.17
Ever-smoker	523	116	0.46	0.41	2.33 (1.23 to 4.39)	0.0072
Never-smoker	1091	285	0.41	0.41	0.99 (0.70 to 1.39)	0.96
Male ever-smoker	245	55	0.49	0.44	1.90 (0.85 to 4.25)	0.11
Male never-smoker	50	25	0.34	0.48	0.28 (0.08 to 1.01)	0.045
Female ever-smoker	278	61	0.43	0.39	3.20 (1.11 to 9.22)	0.024
Female never-smoker	1041	260	0.41	0.41	1.09 (0.75 to 1.56)	1
rs1748033						
All	1614	400	0.37	0.37	1.39 (0.98 to 1.98)	0.063
Men	295	80	0.41	0.42	1.40 (0.69 to 2.83)	0.34
Women	1319	320	0.36	0.36	1.41 (0.93 to 2.12)	0.10
Ever-smoker	523	115	0.40	0.38	2.15 (1.08 to 4.28)	0.026
Never-smoker	1091	285	0.35	0.37	1.13 (0.75 to 1.72)	0.54
Male ever-smoker	245	55	0.43	0.41	2.09 (0.84 to 5.16)	0.10
Male never-smoker	50	25	0.30	0.44	0.34 (0.08 to 1.43)	0.13
Female ever-smoker	278	60	0.37	0.36	2.28 (0.78 to 6.65)	0.12
Female never-smoker	1041	260	0.35	0.36	1.27 (0.81 to 1.98)	0.29

Anti-citrullinated protein antibody (ACPA)+ and ACPA−, ACPA-positive and ACPA-negative rheumatoid arthritis (RA) patients, respectively.
*rs11203673 (T/C, T is the minor allele) and rs174803 (T/C, T is the minor allele) were genotyped for the test. Case subjects of Japanese sets (first and second) were combined for analysis.
MAF, minor allele frequency; *PADI4*, peptidyl arginine deiminase 4.

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