Extrapituitary prolactin promoter polymorphism in Czech patients with systemic lupus erythematosus and rheumatoid arthritis

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Prolactin (PRL) and its production by lymphocytes have been suggested to play a distinct role in the pathogenesis of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).^{1–2} PRL acts as a cytokine and influences the maturation and differentiation of immune cells.^{3–4} Extrapituitary PRL synthesis is regulated by an alternative promoter,⁵ which contains a single-nucleotide polymorphism (SNP) at the region –1149 G/T. Higher PRL mRNA expression is associated with the G allele in lymphocytes.⁶ High frequency of the G allele was described in patients with SLE,⁷ but was not confirmed in other work.⁸

We investigated –1149 G/T SNP in 156 patients with SLE and 173 patients with RA, and in 123 healthy individuals (control group). Patients with SLE consisted of 134 (85.9%) women and 22 (14.1%) men, with a mean age of 43.4 years. SLE diagnosis was determined using the American College of Rheumatology classification criteria.⁹ Patients with RA consisted of: 132 (76.3%) women and 41 (23.7%) men, with a mean age of

57.4 years; all fulfilled the RA diagnostic criteria.¹⁰ The control group consisted of 40 (32.5%) women and 83 (67.5%) men, with a mean age of 38.7 years. The study was approved by the ethics committee of The Third Medical Faculty, Charles University, Prague, Czech Republic.

The PCR–RFLP (restriction fragment length polymorphism) method was used for –1149 G/T SNP detection. During PCR, the 137 base pairs (bp) region of the PRL extrapituitary promoter was amplified by using the following primers: forward 5′-GCAGGTCAAGATAACCTGGA and reverse 5′-CATCTCAGAGTTGAATTATTTCCTT. For RFLP, *ApoI* restriction endonuclease was used. The genotypes identified were TT homozygote characterised by 120 and 17 bp, GG homozygote characterised by 85, 35 and 17 bp, and GT heterozygote characterised by 120, 85 and 35 bp + 17 bp DNA

Abbreviations: PRL, prolactin; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism

Table 1 Occurrence of genotype and allele frequencies of –1149 G/T single-nucleotide polymorphism of the extrapituitary prolactin promoter in Czech patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis and controls, and in patients with SLE according to specific organ involvement

Subject groups			–1149 G/T SNP of the extrapituitary prolactin promoter				
			Genotype			Allele frequency	
			GG	GT	TT	G	T
			Number of cases	%	%	%	
SLE	Pc ¹	156	34.60	44.90	20.50	0.57	0.43
			NS	NS	NS	NS	NS
Specific organ involvement							
Renal	With	63	31.10	52.40	17.50	0.56	0.44
	Without	93	37.60	39.80	22.60	0.57	0.43
	Pc ²		NS	NS	NS	NS	NS
Neuropsychiatric	With	33	30.30	51.50	18.20	0.56	0.44
	Without	123	35.80	43.10	21.10	0.57	0.43
	Pc ²		NS	NS	NS	NS	NS
Cardiac	With	35	28.60	45.70	25.70	0.51	0.49
	Without	121	36.40	44.60	19.00	0.59	0.41
	Pc ²		NS	NS	NS	NS	NS
Pulmonary	With	25	32.00	48.00	20.00	0.56	0.44
	Without	131	35.10	44.30	20.60	0.57	0.43
	Pc ²		NS	NS	NS	NS	NS
Articular	With	117	41.00	43.60	15.40	0.63	0.37
	Without	39	15.40	48.70	35.90	0.40	0.60
	Pc ²		NS	NS	NS	0.0086 [†]	0.0086 [‡]
Dermal	With	94	36.20	45.70	18.10	0.59	0.41
	Without	62	32.30	43.50	24.20	0.54	0.46
	Pc ²		NS	NS	NS	NS	NS
RA	Pc ³	173	32.40	56.10	11.50	0.60	0.40
			NS	0.039*	NS	NS	NS
Controls		123	39.80	41.50	18.70	0.61	0.39

Pc, P corrected values; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism.

Pc values were calculated by the χ^2 test from 2×2 contingency tables of the separate genotype occurrence or allele frequency of (1) each patient with SLE and control group (Pc¹), (2) presence or absence for the clinical feature (Pc²) and (3) each patient with RA and control group (Pc³). Bonferroni correction for multiple comparisons was used.

*OR 1.82, 95% CI 1.14 to 2.94.

†OR 2.56, 95% CI 1.51 to 4.33.

‡OR 0.39, 95% CI 0.23 to 0.6.

fragments. Results were evaluated by χ^2 test with Bonferroni correction.

There was no difference in genotype and allele frequencies in the SLE group compared with healthy Czech individuals (table 1). Our results support previous Italian findings,⁸ but differ from the UK report.⁷ However, with respect to the specific organ manifestation of SLE (table 1), we detected an association between the G allele and articular involvement ($P_c = 0.0086$, OR 2.56, 95% CI 1.51 to 4.33). Based on age when SLE was diagnosed, we observed a GG genotype frequency of 44.8% in the 21–40 years subgroup compared with 15.8% and 24.0% in the <20 years and >40 years subgroup, respectively ($P_c = 0.023$, OR 2.94, 95% CI 1.43 to 5.96). Additionally, we correlated the presence of alleles and genotypes of –1149 G/T SNP with antibodies against antinuclear antibody, double-stranded DNA, Sm, RNP, Ro and La, but no connection was found (data not shown).

Significantly higher heterozygote GT genotype was detected in the RA group compared with the controls ($P_c = 0.039$, OR 1.82, 95% CI 1.14 to 2.94). We observed no differences in homozygote genotypes or in allele frequencies between patients with RA and controls (table 1). Across the groups, no gender differences in genotype distribution or allele frequencies were identified (data not shown).

In conclusion, the presence of the G allele and GG genotype of the PRL extrapituitary promoter –1149 G/T SNP is associated with certain clinical features of SLE. The GT genotype is a predisposing genetic factor for RA. Further investigation on this is required.

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Doppler ultrasonographic characteristics of superficial and deep-flow signals in the knee joint pannus of patients with rheumatoid arthritis

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Increased blood flow signals on power Doppler ultrasonography in the proliferated synovium (pannus) of patients with rheumatoid arthritis (RA) have been used to evaluate articular inflammation.^{1–7} Fiocco *et al*⁸ reported that, in patients with RA after 3 months of etanercept treatment, superficial layer vascularity of the knee joint pannus⁸ was significantly reduced, and this occurred before deep-layer vascularity was affected. In all, 97 knee joints of 52 patients with RA were scanned longitudinally and transversely at suprapatellar recess; we measured the vascular resistance, resistance index (RI) and pulsatility index (PI) on spectral Doppler (with the lowest filter setting at 125 Hz) and 167 colour flow signals in the pannus on power Doppler (using a pulse repetition frequency of 1170–1220 Hz with maximum gain eliminating background noise). The flow signals were categorised into superficial flow signals (located in the superficial half of the pannus and fluid space) and deep flow signals (located in the deep

half of the pannus) of either capsular or supracortical pannus. The mean (SD) RI value (0.724 (0.125)) of the 102 superficial flow signals (87 capsular, 15 supracortical) was significantly lower ($p < 0.001$) than the mean (SD) RI value (0.872 (0.172)) of the 65 deep flow signals (43 capsular, 22 supracortical). The difference in mean PI values between superficial and deep signals was less significant ($p = 0.002$) than for mean RI values.

Subsequently, the flow signals in the joints were divided into a superficial pattern and a deep pattern on the basis of their dominant location in the pannus on both longitudinal and transverse scans. Following this, we compared 61 joints with superficial pattern signals and 36 joints with deep pattern signals with respect to the intensities of the colour flow signals (graded 0–3 by the modified Klausner's method),⁹ synovial effusion

Abbreviation: RA, rheumatoid arthritis

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