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Gene polymorphisms (G82S, 1704G/T, 2184A/G and 2245G/A) of the receptor of advanced glycation end products (RAGE) in plaque psoriasis

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Abstract Having in mind the relationships among oxidative stress, psoriasis and common disorders, the association between polymorphisms in the gene encoding the receptor for advanced glycation end products (RAGE) and plaque psoriasis, including patients with a personal history of diabetes mellitus, cardiovascular disorders, cancer and allergy, was investigated. The allele frequencies and genotype distribution combinations of the four polymorphisms in the RAGE gene (6p21.3, G82S, 1704G/T, 2184A/G and 2245A/G) were compared in a case-control study of 272 subjects (130 patients with plaque psoriasis and 142 healthy control subjects of comparable age and sex distribution). The polymerase chain reaction with subsequent restriction analysis was used for detection of genotype variants. There was a significantly higher frequency of the 2184G allele of the 2184A/G RAGE polymorphism in psoriatic patients than in the control subjects (odds ratio 2.18, 95% CI 1.32–3.59, *P*=0.001). The 2184G allele occurred more often in psoriatic patients with a negative history of cardiovascular diseases (odds ratio 2.38, 95% CI 1.35–4.18, *P*=0.001, *P*corr=0.004), in those with a negative history of diabetes mellitus (odds ratio 2.05, 95% CI 0.1.22–3.45, *P*=0.004, *P*corr=0.012) and in those with a negative history of cancer (odds ratio 1.97, 95% CI 1.17–3.31, P=0.007, Pcorr=0.014) compared with the corresponding control subjects. We conclude that the 2184G allele of the RAGE gene is a significant risk factor for plaque psoriasis. The risk is associated with the nonpresence of some common, especially cardiovascular, diseases in psoriatic patients.

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Introduction

Psoriasis is believed to be a multigenic disease the expression of which is partly dependent on external factors [1]. The pathogenesis of psoriasis is explained by abnormal regulation of keratinocyte growth and/or differentiation, and/or by skin inflammation. The functional importance of T cells as the likely major effector cells in the pathogenesis psoriasis is well known [2]. Psoriasis is further characterized by increased antioxidant activity as detected by an increase in the carbonylation of macromolecules in the dermis of psoriatic skin compared to the dermis of normal skin [3]. Oxidative stress in keratinocytes is considered a factor in an aetiopathogenic concept which considers psoriasis as a typical inflammatory process characterized by increased antioxidant activity and overexpression of apoptotic receptors [4].

Common diseases such as diabetes mellitus, cardiovascular disorders, cancer and allergy are considered multifactorial disorders with a high prevalence in the general population. Because of supposed relationships among oxidative stress, psoriasis and common disorders that are more frequent in psoriatic patients [5], associations of the polymorphisms in the gene coding for the receptor for advanced glycation end products (RAGE) in psoriatic and control subjects including those with a personal history of diabetes mellitus, cardiovascular disorders, cancer and allergy were investigated.

Methods

Subjects

The study group (*n*=130, 64 men, 66 women, aged 44±15 years) included patients with plaque psoriasis. The patients were diagnosed by an experienced dermatologist. The control group (*n*=142, 75 men and 67 women, aged 39±15 years) consisted of healthy subjects without an individual history of psoriasis. In the patient group, 79 (61%) had a positive family history of psoriasis and 104

Table 1 Primers used for four PCR assays in the RAGE gene (accession D28769 in GenBank)

Primer	RAGE gene	Sequences (from 5′ to 3′)	Position in RAGE
1RAGEG82S	Exon 3	GTAAGCGGGGCTCCTGTTGCA	7007–7027
2RAGEG82S		GGCCAAGGCTGGGGTTGAAGG	7383–7403
1RAGE1704	Intron 7	GGAGCCAGAAGGTGGAGCAGTAG	8223–8245
2RAGE1704		GTCTCACCGATGATGCTGATGATG	8627–8647
1RAGE2184	Intron 8	GGCCTCAGGACCAGGGAACCTACA	8551–8574
2RAGE2184		TTGGTCAGGCTGGTCTCGAACTCC	8929–8952
1RAGE2245	Intron 8	GCCCCATTCTGGCCTTATCCCTAA	8710–8733
2RAGE2245		CCACCATGCCTGGCTAATTTTGT	8982–9004
3RAGE2245		ACACTTTGGGAGGCTGCTGC	8888–8908

(80%) had type 1 psoriasis with early onset of the disease (<40 years of age). Those with a personal history of diabetes mellitus, cardiovascular disorders, cancer and allergy were included.

Informed consent was obtained from each patient prior to inclusion in the study. The Committee for Ethics of Medical Experiments on Human Subjects (Medical Faculty, Masaryk University, Brno) approved the study.

Genotyping

Genomic DNA was isolated from peripheral leucocytes by a standard technique using proteinase K. Based on the published RAGE sequence (GenBank D28769) [6], three of four detected RAGE polymorphisms were newly identified in our laboratory [7]. The G82S polymorphism was detected by polymerase chain reaction (PCR) with primers 1RAGEG82S and 2RAGEG82S (Table 1). Digestion with AluI (New England Biolabs, Beverley, Mass.) revealed fragments of length 123, 26 and 248 bp for the wild-type allele, and 123, 26, 67 and 181 bp for the mutated allele [8]. Polymorphism 1704G/T was detected by PCR with primers 1RAGE1704 and 2 RAGE1704 (Table 1) which were used to amplify a 425-bp product. A volume of 10 µl of product was digested with BfaI (New England Biolabs) for 5 h at 37ºC. The digestion produced fragments of length 240, 143 and 42 bp for the G allele, and 240 and 185 bp for the T allele [8].

Polymorphism 2184A/G was detected using primers 1RAGE2184 and 2RAGE2184 (Table 1). Digestion with BsmFI (New England Biolabs) for 4 h at 65ºC produced fragments of length 266 and 136 bp for the A allele, and 174, 136 and 92 bp for the G allele [8]. The RAGE polymorphism 2245G/A was detected by a two-step nested PCR using external primers 1RAGE 2245 and 2RAGE2245 to amplify a 294-bp product. The product (10 µl) was diluted to 500 µl, and this was used as a template (1 µl) for the second PCR reaction. The reaction was performed in a final volume of 15 µl using 1RAGE2245 and 3RAGE2245 primers (Table 1). The amplification-created restriction site was created for subsequent digestion with PstI (MBI Fermentas, Vilnius, Lithuania). Restriction analysis at 37ºC overnight provided fragments of length 116 bp for the G allele, and 95 and 21 bp for the A allele [9].

Statistical analysis

The significances of the differences from Hardy-Weinberg equilibria as well as in the genotype distributions and/or allelic frequencies among groups were tested using the χ^2 test. The odds ratios with 95% confidence intervals were calculated as usual. The probability of relative risk was calculated using Fisher's exact test. Holm's correction for multiple comparisons (*P*corr) was used where necessary.

Results

The genotypes of G82S, 1704G/T, 2184A/G and 2245 G/A RAGE polymorphisms were determined in 272 subjects (Table 2). Genotype distributions of all polymorphism

Table 2 Observed genotype distributions and allelic frequencies of G82S, 1704G/T, 2184A/G and 2245G/A polymorphisms in the RAGE gene in psoriatic and control subjects

Polymorphism	Genotypes	Genotype distribution			Alleles	Allele frequency			Homozygotes/heterozygotes		
		Psoriatics (*n*=130)	Controls (*n*=142)	Probability of difference		Psoriatics (*n*=130)	Controls (*n*=142)	Probability of difference	Psoriatics (*n*=130)	Controls (*n*=142)	Probability of difference
G82S	GG	120	138		G	0.962	0.986	0.07	120/10	138/4	0.06
	AG	10	4		A	0.038	0.014				
	AA	0	0								
1704G/T	GG	116	115	0.148	G	0.942	0.902	0.08	117/13	116/26	0.19
	GT	13	26		T	0.058	0.098				
	TT	1	1								
2184A/G	AA	69	101	0.006	A	0.735	0.827	0.009	77/53	109/33	0.001
	AG	53	33		G	0.265	0.173				
	GG	8	8								
2245G/A	GG	102	104		G	0.892	0.861	0.351	102/28	104/38	0.194
	AG	28	38		A	0.108	0.133				
	AA	0	0								

Table 3 Positive personal history of common diseases in psoriatic and control subjects

Disease	Psoriatics (*n*=130)	Controls (*n*=142)	*P* value
Diabetes mellitus	11 (8.6%)	7 (4.9%)	0.177
Cardiovascular	45 (34.6%)	7 (4.9%)	10^{-6}
Cancer	18 (13.8%)	1 (0.7%)	10^{-5}
Allergy	9 (6.9%)	2 (1.4%)	0.02
At least one	73 (56.2%)	14 (9.9%)	10^{-6}

were consistent with a Hardy-Weinberg equilibrium in both groups. The G82S, 1704G/T and 2245A/G RAGE polymorphisms were not associated with plaque psoriasis. A significantly greater 2184G allele frequency was observed in psoriatic patients than in control subjects (odds ratio 2.18, 95% CI 1.32–3.59, *P*=0.001, after correction for *n*=4 comparisons *P*corr=0.004). The number of A2184G heterozygotes in relation to the number of both homozygotes was higher among psoriatic patients (*P*= 0.001, *P*corr=0.004).

No correlations between alleles and/or genotypes of all the RAGE polymorphisms examined and a positive familial history or early onset of psoriasis (earlier than 40 years) were found. This negative result may have been because of a too-low frequency of some alleles with weak effects. The numbers of patients and controls with a personal history of common diseases are shown in Table 3. Cardiovascular disease and cancer were highly significantly more frequent among psoriatic patients than among control subjects.

The G82S, 1704G/T and 2245A/G RAGE polymorphisms were not associated with a personal history of the common diseases. On the other hand, the 2184G allele occurred more often in psoriatic patients with a negative history of cardiovascular disease (odds ratio 2.38, 95% CI 1.35–4.18, *P*=0.001, *P*corr=0.004), in those with a negative history of diabetes mellitus (odds ratio 2.05, 95% CI 0.1.22–3.45, *P*=0.004, *P*corr=0.012) and in those with a negative history of cancer (odds ratio 1.97, 95% CI 1.17–3.31, *P*=0.007, *P*corr=0.014) compared with the corresponding control subjects. When the subjects were divided according to the presence of at least one common disease compared to a completely negative personal history, the odds ratio for the 2184G allele of the RAGE gene in psoriatic patients compared with control subjects with a negative personal history of the common diseases was 2.13 (95% CI 1.15–3.96, *P*=0.012, *P*corr=0.012).

Taking the results together, we conclude that the significant relative risk of the 2184G allele of the RAGE polymorphism for plaque psoriasis is even more prominent in psoriatic patients with a negative personal history of cardiovascular disease compared with control subjects with a negative personal history of the diseases (Table 4).

Discussion

The genome region 6p21.3 is often related to psoriasis. Recent genome-wide linkage analyses have identified a locus encoding susceptibility to psoriasis and have placed this gene between markers D6S426 and D6S276 on chromosome 6p21.3 [10]. Susceptibility to psoriasis is linked to at least three different ancestral HLA haplotypes [11]. The strongest genetic association of early-onset psoriasis is found with the major histocompatibility complex (MHC) region, and specifically with HLA-Cw6 [12]. The allele octamer transcription factor-3B has been found to be more strongly associated with psoriasis vulgaris than Cw*0602. The increase in the octamer transcription factor-3B allele is independent of the linkage disequilibrium with Cw*0602 as this has also been found in Cw*0602-negative patients [13]. A significant association between alleles at the corneodesmosin gene (MHC S) and psoriasis has been detected in type 1a (early-onset) psoriasis [14, 15].

RAGE (6p21.3) [16, 17] is a multiligand member of the immunoglobulin superfamily of cell surface molecules. The receptor recognizes families of ligands with diverse structural features, not only advanced glycation end products (AGEs) but also amyloidogenic peptides/polypeptides, amphoterins and S100/calgranulins [18]. The RAGE-ligand interaction seems to be a propagation factor in a range of chronic disorders as indicated by the enhanced accumulation of ligands in diseased tissue. The RAGE is a central cell surface receptor for EN-RAGE (extracellular newly identified RAGE-binding protein) and related members of the S100/calgranulin superfamily [19]. Interaction of EN-RAGEs with cellular RAGE in the endothelium, on mononuclear phagocytes, and on lymphocytes triggers cellular activation, with generation of

Table 4 Distributions of genotypes of 2184A/G RAGE gene polymorphism in psoriatic and control subjects in relation to a personal history of cardiovascular disease

Subject group	History of cardiovascular disease	Genotype distribution				Allele frequency			Homozygotes/heterozygotes	
		AA	AG	GG	Probability of difference	A	G	Probability of difference		Probability of difference
Psoriasis	Negative (*n*=85)	42	35	8	0.01			0.003	50:35	0.009
	Positive (*n*=45)	27	18	10						
Control	Negative (*n*=135)	93	33	7					100:33	
	Positive (*n*=7)	6	0	1						

key proinflammatory mediators. Interestingly, several polymorphisms in some proinflammatory genes (TNFα, TNFβ and angiotensinogen) are associated with psoriasis [20, 21].

We have analysed the gene coding for RAGE in a previous study and described 14 new polymorphisms [8, 9]. So far, a statistically significant difference in allele distribution in the G28S polymorphism between diabetic subjects with skin microangiopathy and the control subjects has been found [7]. Recently, correlations between the polymorphisms in the RAGE gene examined and some antioxidant parameters (total carotenoids, γ-tocopherol, α-carotene, β-carotene, lutein, lycopene, α tocopherol) have been reported [9]. The RAGE polymorphisms 1704 G/T and 2184 A/G show a similar tendency in non-insulin-dependent diabetes mellitus patients and in comparable nondiabetic subjects. The homozygotes GG (1704G/T) and AA (2184A/G) are associated with higher levels of antioxidants in blood than the other genotypes. The allele 2184G associated with plaque psoriasis could therefore be associated with a worse antioxidant potential in its carriers which could contribute to the manifestation of psoriasis.

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