Letters to the Editors

Identification of the semaphorin receptor Plexin-A2 as a candidate gene for susceptibility to ankylosing spondylitis

Sirs,

Semaphorins are a large family of secreted and membrane bound proteins in which "sema" is the conserved cysteine-rich extracellular domain that mediates signal transduction (1). Two families of semaphorin receptors have been identified: neuropilins and plexins (1). Polymorphism rs6656560 in intron 3 of plexin A2 *(PLXNA2)* gene has been associated with rheumatoid arthritis (RA) (2). In the present study, we further investigated the association of rs6656560 *PLXNA2* polymorphism with RA and expanded, for first time, the genetic association study to ankylosing spondylitis (AS) and psoriatic arthritis (PsA).

One hundred and thirty-six unrelated patients with RA, 49 with AS, 29 with PsA, and 147 ethnic-matching healthy volunteers were enrolled in the study. RA patients satisfied the American College of Rheumatology (ACR) criteria (3), AS patients met the modified New York criteria for AS (4) and PsA patients satisfied classification criteria for PsA (Classification criteria for Psoriatic Arthritis, CASPAR) (5). All subjects gave informed written consent in accordance with Helsinki Declaration of 1975/83 and the local ethics committee granted approval.

Polymorphism rs6656560 was amplified using the following primer pair: rs6656560F: 5'-TGCAAGGTACTCTGTCCTATCTGGACT-3', rs6656560R: 5'-TCCTTCACAGGATCGTGGGGGCAA-3'. Restriction fragment length polymorphism assay was conducted using the restriction endonuclease *Bcl I*. The SPSS statistical package was used to test differences in polymorphism distribution between the studied groups. A difference at $p \leq 0.05$ was considered as statistically significant.

The studied polymorphism was in Hardy-Weinberg equilibrium in all the studied groups: controls (p=0.608), RA (p=0.071), AS (p=0.469), and PsA (p=0.535). Statistical significant difference was observed in the distribution of rs6656560 genotypes between AS patients and controls, but not between RA patients and controls or between PsA patients and controls (Table I). Furthermore, comparing the rs6656560 alleles' distribution between AS patients and controls the revealed difference was higher (Table I). Additionally, the variant rs6656560 was distributed differently between AS and RA patients (p=0.035), but not between AS and PsA patients (p=0.178) both of which belong to spondyloarthropathies (SpAs).

The genetic variability in *PLXNA2* gene may affect semaphorin role in bone development and homeostasis and as a result in AS manifestation (1). Chondrocytes, osteoblasts and osteoclast were reported to express *PLXNA2* and transmit Sema3A mediated signal modulating vascularisation of bone and the innervation of osteoblasts and osteoclasts during bone growth and re-modelling (6). Remodelling of bone was reported to cause square vertebral bodies in AS patients which seem to be the result of a combination of destructive osteitis and repair. Furthermore, osteoclasts, which have been reported to play critical role in inflammation-associated bone loss, show increased activity in AS and PsA compared to RA (7). In AS patients the number of osteoclastic foci was reported to be higher in areas with cartilage in comparison to areas without cartilage on the surface. Additionally, osteoclast activity was observed in parallel with T-cell response against a cartilage-derived antigen, which reveals a cartilage-directed autoimmune T-cell response in AS pathogenesis (7).

Moreover, it was reported a correlation of *PLXNA2* polymorphisms with vertebral fracture risk and bone mineral density in postmenopausal women (8). Low bone density, osteoporosis, and an increased rate of fractures, which may add to the hyperkyphosis, are also clinical features of AS.

Consequently, the reported here *PLXNA2* polymorphism association with AS may be attributed through alterations in *PLXNA2* interaction with Sema3A, Sema3B, and Sema6D that were associated with bone development and remodelling (9). Additional genetic association studies in other larger ethnic groups of patients are necessary to confirm the results of the present study and to add to the genetics of AS (10, 11).

Table I. The distribution of polymorphism PLXNA2 rs6656560 in RA, AS and PsA patients and controls.

	rs6656560			
Genotypes	GG	GA	AA	p-value*
RA n=136	39	77	20	0.183
AS n=49	24	19	6	0.041
PsA n=29	8	16	5	0.729
Controls n=147	44	70	33	-
Alleles	G	A	p-value*	OR (95% CI)
RA n=272	155	117	0.448	0.877 (0.629–1.222)
AS n=98	67	31	0.013	0.538 (0.331–0.872)
PsA n=58	32	26	0.886	0.944 (0.536–1.662)
Controls n=294	158	136		

*vs. controls.

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