PDCD1 polymorphisms are not associated with Takayasu's arteritis in Turkey

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ABSTRACT

Objectives. *Takayasu's arteritis (TA) is a chronic arterial inflammation of unknown etiology involving mainly the aorta and its major branches. Based on the associations of programmed death-1 (PD-1) protein encoding gene (PDCD1) with connective tissue diseases and vasculitides, PDCD1 polymorphisms are studied for susceptibility to TA in this study.*

Methods. *The study group is made up of TA patients (n=229) fulfilling the 1990 ACR classification criteria and compared to 193 healthy controls (HC). PD-1.3, PD-1.5 and PD-1.6 single nucleotide polymorphisms of PDCD1 gene are genotyped by polymerase chain reaction and restriction analysis (PCR-RFLP).*

Results. *The distribution of PD-1.5 polymorphism in TA patients and HC revealed a similar presence of TT genotype in patients and controls (13.3% vs. 11.4%). PD-1.3 and PD-1.6 were less polymorphic and did not differ between the groups. Rare AA genotype of PD-1.3 (1.4% vs. 1.0%) and AG genotype of PD-1.6 was again similarly (22.4% vs. 19.2%) present in TA and HC.*

Conclusion. *PD-1.3, 1.5 and 1.6 polymorphisms of PDCD1 gene, which were shown to be associated with various autoimmune disorders and vasculitides, are not associated with a susceptibility to TA in Turkish population.*

Introduction

Programmed cell-death 1 (PD-1) molecule is a member of B7/CD28 superfamily receptors that contain an immunoreceptor tyrosine-based inhibitory motif in its cytoplasmic tail and is expressed in activated T and B cells (1, 2). PD-1, after binding to its ligands PD-L1 or PD-L2, inhibits cytokine production and proliferation of pre-activated lymphocytes and is thought to be a negative regulator of autoimmunity (3).

A single-nucleotide polymorphism (SNP) in *PDCD1* gene, named PD-1.3 and located in intron 4 (rs11568821), was first described by Prokunina *et al.* to be associated with susceptibility to systemic lupus erythematosus (SLE) (4). An allele of SNP PD-1.3 is shown to alter a binding site for the runt-related transcription factor 1 (RUNX1) located in an intronic enhancer and is suggested to increase susceptibility to autoimmunity. The same group also reported the first association of PD-1.3A allele with rheumatoid arthritis (RA), even though only in patients negative for both rheumatoid factor (RF) and shared epitope (SE) alleles (5). Another SNP in PD-1.5C/T in exon 5 (rs2227981) was later shown to be associated with RA in Chinese patients from Taiwan (6). Further associations of *PDCD1* SNPs with RA and ankylosing spondylitis are also reported, but not confirmed in all populations (7-11). Similarly, previous associations of *PDCD1* with various vasculitides is also reported (12-14), but not always confirmed (15) .

Studies of *PDCD1* SNP frequencies are limited by a large variation among different ethnic groups (16). PD-1.3A is more common among Europeans, but non-polymorphic in Chinese, whereas PD-1.5C is significantly higher in Chinese and Korean populations (7, 17). Similarly, we previously demonstrated that *PTPN22*, while it is a second a major risk factor after "shared epitope" in Caucasian RA patients, is found to be unassociated with RA in Turkey, possibly due to its low presence in healthy population (18).

Takayasu's arteritis (TA), also known as pulseless disease, is a chronic granulomatous panarteritis characterised by the involvement of large vessels, especially the aorta and its major branches (19). Evidences of genetic susceptibility to TA were previously demonstrated for HLA-B alleles, B52, B39, and also for various cytokine polymorphisms (20, 21). Tissue specimens from the aorta of TA patients are infiltrated with T cells which have a restricted T cell repertory, typical of antigen-induced proliferation (22, 23). However, a low production of IL-2 by the peripheral blood $CD3^+$ T cells suggests that T cell activation may be defective in TA (24). PD-1, as a negative regulator of T cells, might be associated with this defect.
With this background, we aimed to investigate 3 common SNPs of *PDCD1* in TA, in patients from Turkey.

Materials and methods

Patients and controls

The study was designed as a case-control study enrolling 229 patients with TA (female/male: 208/21, mean age: 38.5 years). Patients, followed in the tertiary centres of Universities and State Hospital Rheumatology Clinics, were included according to the 1990 American College of Rheumatology criteria for the classification of TA (25), and were part of a clinical series published before (26). According to the angiographic classification defined at the International Conference on Takayasu's Arteritis in Tokyo in 1994, 38% (n=89) of the patients have type 1 vessel involvement, 8.3% type 2a (n=19), 2.2% type 2b (n=5), 3.9% type 3 (n=9), 3.9% type 4 (n=9) and 42.8% type 5 (n=98) (27). As controls, 193 healthy blood donors (HC, female/male; 98/95 mean age: 42.4 years) were recruited. All patients and controls were enrolled with Local Ethics Committee approval and provided their informed consent.
Among the previously described SNPs of *PDCD1* gene, PD-1.3 (rs11568821, G/A at intron 4), PD-1.5 (rs2227981, C/T, exon 5) and PD-1.6 (rs10204525, G/A, 3'-UTR) (GenBank accession no: AF363458) were selected to screen in both groups on the basis of the haplotypes of these SNPs (Table I). For genotyping, 60 ng of each DNA was amplified with the primer pairs listed in Table I with a programme of 2' at 96ºC, 35 cycles 20" at 96ºC, 20" at 56–58ºC and 30" at 72ºC, and an additional 2' at 72ºC. The products are digested with the respective restriction enzymes as described by the producers (Fermentas, Lithuania). Genotype and allele frequencies were compared between the patient and control groups by chi-square test.

Table I. Screened *PDCD1* gene polymorphisms, used primer sequences, lengths of PCR products (base pairs, used restriction enzymes and the digestion product lengths).

PD-1.3 (G/A)	5'-GCAGCAACCTCAAATCCCTAA-3' 5'-CATTGGAGACAGGAGAGCTTG-3'	330 bp	*Pst* I	G: 330 bp A: 50 bp + 280bp
PD-1.5 (C/T)	5'-GCTTTGGGCTTCTTGATGAG-3' 5'-GTGAGCTTCTTGAGGCAAA-3'	213 bp	*Pvu* II	C: 213 bp T: 54bp + 159bp
PD-1.6 (G/A)	5'- CCTCACACCACTCGGGAGA-3'5'- AGTGGGGGTGCAGTGTGT-3'	301 bp	*Nla* III	A: 301bp G: 137bp +164bp

Table II. PD-1.3 (G/A at intron 4), PD-1.5 (C/T, exon 5) and PD-1.6 (rs10204525, G/A, 3'-UTR) gene polymorphisms in TA and controls.

	TA		HC	
	n.	%	n.	%
PD-1.3 rs11568821	219		193	
AA	3	1.4	2	1.0
AG	43	19.6	38	19.7
GG	173	79.0	153	79.3
PD-1.5 rs2227981	226		193	
CC	97	42.9	71	36.8
CT	99	43.8	100	51.8
TT	30	13.3	22	11.4
PD-1.6 rs10204525	219		193	
AA	0	0	0	0
AG	49	22.4	37	19.2
GG	170	77.6	156	80.8

Results

The distribution of PD-1.5 polymorphism in TA patients and HC revealed a similar presence of TT (13.3% *vs*. 11.4%) and CT (43.8% *vs*. 51.8%) genotypes in both groups (Table II). PD-1.3 and PD-1.6 were less polymorphic and did not differ between the groups. Rare AA genotype of PD-1.3 (1.4% *vs*. 1.0%) and AG genotype of PD-1.6 were again similarly (22.4% *vs*. 19.2%) present in TA and HC. No association with angiographic type, prognosis or other clinical features and *PDCD1* polymorphisms was observed.

Discussion

As a granulomatous, chronic large-vessel vasculitides, adaptive immune response with a predominance of $CD4^+$ T cell infiltrations is possibly involved in the pathogenesis of TA. Polymorphism of PD-1, a crucial molecule of T cell activation, was, in this respect, a natural candidate for immunogenetic associations with TA. In a recent study investigating the role of PD-1.3A, patients homozygous for PD-1.3, but not the heterozygous ones, had reduced basal and induced PD-1 expression on activated $CD4^+$ T cells. In autologous mixed lymphocyte reactions, SLE patients had defective PD-1 induction on activated $CD4^+$ cells and abnormalities were more pronounced among homozygotes. PD-1 crosslinking suppressed proliferation and cytokine production in both normal and lupus T cells and addition of serum from patients with active SLE significantly ameliorated this effect on proliferation (28).
However, we did not observe any association with *PDCD1* polymorphisms with TA in the Turkish population in our study. Among the various *PDCD1* polymorphisms studied, PD-1.5 seems more polymorphic in Turkey with a very high C allele presence (63%), similar

to Korean, Spanish and Mexican populations (53–63%) (17). PD-1.3 and -1.6 polymorphisms were less polymorphic compared to other populations.

Three studies investigated *PDCD1* poymorphisms in vasculitides before. In one study, T allele of rs41386349, a SNP which we have not studied, is found to be associated with Kawasaki disease, whereas no association is observed with PD-1.5 (12). In Wegener's Granulomatosis (WG), co-occurrence of the PD-1.5T allele with an adhesion molecule polymorphism CTLA4 +49 AA homozygosity was less often present in patients compared to controls, which may lead to a hyperreactivity of T cells (13). However, in another study, no association was observed with WG and *PDCD1* polymorphisms (15). Interestingly, a lower PD-1.5 presence is reported to be associated with Vogt-Koyanagi-Harada disease, a granulomatous vasculitis with unknown etiology (14).

Our study has some limitations. All patients are followed in tertiary centres and may reflect a more severe disease spectrum. However, as TA is a rare disease, we think most patients suspected or diagnosed as TA are referred to specialised rheumatology centres in Turkey. Our gender ratio is not well-matched among TA and controls; however, no previous data suggest a gender bias in *PDCD1* studies. Finally, up to 130 SNPs are reported in *PDCD1* gene, we only studied 3 commom SNPs.

In conclusion, although some role of *PDCD1* polymorphisms is reported for various vasculitides especially in Caucasian populations, we observed no association of any *PDCD1* polymorphism with Takayasu's Arteritis in Turkey, similarly to *PTPN22* polymorphism, previously studied (29). These results possibly reflect the ethnical differences among various populations for auto-immunity related genes.

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References

1. ISHIDA Y, AGATA Y, SHIBAHARA K, HONJO T: Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *Embo J* 1992; 11: 3887-95.
2. AGATA Y, KAWASAKI A, NISHIMURA H *et al*.: Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol* 1996; 8: 765-72.
3. BENNETT F, LUXENBERG D, LING V *et al*.: Program death-1 engagement upon TCR activation has distinct effects on costimulation and cytokine-driven proliferation: attenuation of ICOS, IL-4, and IL-21, but not CD28, IL-7, and IL-15 responses. *J Immunol* 2003; 170: 711-8.
4. PROKUNINA L, CASTILLEJO-LOPEZ C, OBERG F *et al*.: A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nat Genet* 2002; 32: 666-9.
5. PROKUNINA L, PADYUKOV L, BENNET A *et al*.: Association of the PD-1.3A allele of the PDCD1 gene in patients with rheumatoid arthritis negative for rheumatoid factor and the shared epitope. *Arthritis Rheum* 2004; 50: 1770-3.
6. LIN SC, YEN JH, TSAI JJ *et al*.: Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. *Arthritis Rheum* 2004; 50: 770-5.
7. KONG EK, PROKUNINA-OLSSON L, WONG WH *et al*.: A new haplotype of PDCD1 is associated with rheumatoid arthritis in Hong Kong Chinese. *Arthritis Rheum* 2005; 52: 1058-62.
8. IWAMOTO T, IKARI K, INOUE E *et al*.: Failure to confirm association between PDCD1 polymorphisms and rheumatoid arthritis in a Japanese population. *J Hum Genet* 2007; 52: 557-60.
9. JAMES ES, HARNEY S, WORDSWORTH BP, COOKSON WO, DAVIS SJ, MOFFATT MF: PDCD1: a tissue-specific susceptibility locus for inherited inflammatory disorders. *Genes Immun* 2005; 6: 430-7.
10. WANG SC, LIN CH, LI RN *et al*.: Polymorphisms of genes for programmed cell death 1 ligands in patients with rheumatoid arthritis. *J Clin Immunol* 2007; 27: 563-7.
11. YANG Q, LIU Y, LIU D, ZHANG Y, MU K: Association of polymorphisms in the programmed cell death 1 (PD-1) and PD-1 ligand genes with ankylosing spondylitis in a Chinese population. *Clin Exp Rheumatol* 2011; 29: 13-8.
12. CHUN JK, KANG DW, YOO BW, SHIN JS, KIM DS: Programmed death-1 (PD-1) gene polymorphisms lodged in the genetic predispositions of Kawasaki Disease. *Eur J Pediatr* 2010; 169: 181-5.
13. SLOT MC, SOKOLOWSKA MG, SAVELKOULS KG, JANSSEN RG, DAMOISEAUX JG, TERVAERT JW: Immunoregulatory gene polymorphisms are associated with ANCA-related vasculitis. *Clin Immunol* 2008; 128: 39-45.
14. MENG Q, LIU X, YANG P *et al*.: PDCD1 genes may protect against extraocular manifestations in Chinese Han patients with Vogt-Koyanagi-Harada syndrome. *Mol Vis* 2009; 15: 386-92.
15. SAKTHIVEL P, GISCOMBE R, RAMANUJAM R, LEFVERT AK: Polymorphisms in PDCD1 gene are not associated with Wegener's granulomatosis. *Rheumatol Int* 2009; 29: 1247-50.
16. MORI M, YAMADA R, KOBAYASHI K, KAWAIDA R, YAMAMOTO K: Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. *J Hum Genet* 2005; 50: 264-6.
17. LEE SH, LEE YA, WOO DH *et al*.: Association of the programmed cell death 1 (PDCD1) gene polymorphism with ankylosing spondylitis in the Korean population. *Arthritis Res Ther* 2006; 8: R163.
18. SAHIN N, GUNDUZ F, INANC N, DIRESKENELI H, SARUHAN-DIRESKENELI G: No association of PTPN22 gene polymorphism with rheumatoid arthritis in Turkey. *Rheumatol Int* 2009; 30: 81-3.
19. MASON JC: Takayasu arteritis-advances in diagnosis and management. *Nat Rev Rheumatol* 2010; 6: 406-15.
20. KIMURA A, OTA M, KATSUYAMA Y *et al*.: Mapping of the HLA-linked genes controlling the susceptibility to Takayasu's arteritis. *Int J Cardiol* 2000; 75 (Suppl. 1): S105-10; discussion S111-2.
21. SARUHAN-DIRESKENELI G, BICAKCIGIL M, YILMAZ V *et al*.: Interleukin (IL)-12, IL-2, and IL-6 Gene Polymorphisms in Takayasu's Arteritis from Turkey. *Human Immunology* 2006; 67: 735-40.
22. INDER SJ, BOBRYSHEV YV, CHERIAN SM *et al*.: Immunophenotypic analysis of the aortic wall in Takayasu's arteritis: involvement

of lymphocytes, dendritic cells and granulocytes in immuno-inflammatory reactions. *Cardiovasc Surg* 2000; 8: 141-8.

23. SEKO Y, SATO O, TAKAGI A *et al*.: Restricted usage of T-cell receptor Valpha-Vbeta genes in infiltrating cells in aortic tissue of patients with Takayasu's arteritis. *Circulation* 1996; 93: 1788-90.
24. TRIPATHY NK, GUPTA PC, NITYANAND S: High TNF-alpha and low IL-2 producing T cells characterize active disease in Takayasu's arteritis. *Clin Immunol* 2006; 118: 154-8.
25. AREND WP, MICHEL BA, BLOCH DA *et al*.: The American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis. *Arthritis Rheum* 1990; 33: 1129-34.
26. BICAKCIGIL M, AKSU K, KAMALI S *et al*.: Takayasu's arteritis in Turkey - clinical and angiographic features of 248 patients. *Clin Exp Rheumatol* 2009; 27 (Suppl. 52): S59-64.
27. HATA A, NODA M, MORIWAKI R, NUMANO F: Angiographic findings of Takayasu arteritis: new classification. *Int J Cardiol* 1996; 54 Suppl: S155-63.
28. BERTSIAS GK, NAKOU M, CHOULAKI C *et al*.: Genetic, immunologic, and immunohistochemical analysis of the programmed death 1/programmed death ligand 1 pathway in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60: 207-18.
29. SAHIN N, AKSU K, KAMALI S *et al*.: PTPN22 gene polymorphism in Takayasu's arteritis. *Rheumatology* (Oxford) 2008; 47: 634-5.