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


For the Turkish Takayasu Study Group.

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.

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Key words: Takayasu’s arteritis, PDCD1, programmed cell death.
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 96°C, 35 cycles 20' at 96°C, 20’ at 56–58°C and 30” at 72°C, and an additional 2’ at 72°C. 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.

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 polymorphisms 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 4+9 AA homozygosy 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 common 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 ethnic differences among various populations for auto-immunity related genes.

The Turkish Takayasu Study Group’s affiliations:
1. Department of Rheumatology, Marmara University, Faculty of Medicine, Istanbul;
2. Department of Physiology, Istanbul University, Istanbul Faculty of Medicine, Istanbul;
3. Department of Rheumatology, Yeditepe University, Faculty of Medicine, Istanbul;
4. Department of Rheumatology, Ege University, Faculty of Medicine, Izmir;
5. Department of Rheumatology, Istanbul University, Istanbul Faculty of Medicine, Istanbul;
6. Department of Rheumatology, Ankara Numune Training and Research Hospital, Ankara;
7. Department of Rheumatology, Cukurova University, Faculty of Medicine, Adana;
8. Department of Rheumatology, Dokuz Eylül University, Faculty of Medicine, Dokuz Eylül University, Izmir;
9. Department of Rheumatology, Istanbul University, Cerrahpasa Faculty of Medicine, Istanbul;
10. Department of Rheumatology, Gaziantep University, Faculty of Medicine, Gaziantep;
11. Department of Rheumatology, Kocaeli University, Faculty of Medicine, Kocaeli;
12. Department of Rheumatology, Pamukkale University Faculty of Medicine, Denizli;
13. Department of Rheumatology, Süleyman Demirel University, Faculty of Medicine, Isparta;
14. Department of Rheumatology, Gazi University, Faculty of Medicine, Ankara;
15. Department of Rheumatology, Başkent University, Faculty of Medicine, Ankara, Turkey.

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