No association of granzyme B gene polymorphism with Behçet’s disease

Sirs,

Behçet’s disease (BD) is a recurrent systemic inflammatory disorder. Inflammation and immunological abnormalities triggered by microbial agents and environmental factors in genetically susceptible individuals are implicated in BD etiopathogenesis (1). Both innate and adaptive immune systems contribute to the pathogenesis. The genetic association between BD and HLA-B51 has been confirmed in many ethnic groups (2). However, further studies are needed to identify genes other than HLA-B conferring susceptibility to BD (3).

The association of HLA-B51 with BD implicates a role for class I molecules with the involvement of CD8+ cells in disease development as reported previously (4-5). Granzyme B (GzmB), a serine protease, is used by cytotoxic T cells and released from secretory granules leading to caspase activation and intracellular proteolysis in the target cells. Involvement of GzmB in the initiation of autoimmunity has also been implicated (6). When single nucleotide polymorphisms (SNP) of GzmB gene (GZMB) were screened, a common allele coding for a triple-mutated protein (R<sup>48</sup>H<sup>49</sup>Y<sup>50</sup>) has been identified. This variant protein of GZMB has been shown to lose its pro-apoptotic capacity when compared to the wild type Q<sup>48</sup>P<sup>49</sup>S<sup>50</sup> form. These three polymorphisms (A/G, C/G, T/C) are shown to be in linkage disequilibrium with each other (7). Although the functional impact of this polymorphism remains unclear (8), we hypothesized that a polymorphism of GZMB may be a candidate for susceptibility and investigated GZMB exon 2 Q48R polymorphism in BD patients.

BD patients (n=152; 59 women, 93 men, mean age: 38.6±10.3 years) diagnosed according to International Study Group for Behçet’s Disease’s criteria (9) are enrolled in the study. An approval of local ethical committee and informed consent of the patients are taken. Healthy controls (HC) (n=156; 76 women, 80 men, mean age 39.3±10.5 years) were selected with similar age to patients group. All patients had oral ulcers and the occurrence ratios of other symptoms were as follows; genital ulcerations: 82.5%, skin lesions: 95%, crythema nodosum: 65.5%, pathergy positivity: 89%, eye involvement: 38% and neurological involvement: 10%. HLA-B*51 was positive in 64% of the BD group which is in concordance with our previous data (10). GZMB exon 2 (rs8192917, A→G) non-synonymous coding SNP (Q48R) was screened by polymerase chain reaction-restriction fragment length polymorphism using the forward (5‘-gaattggaacccctctc-3′) and reverse (5‘-aagtgttccagggaggggttc-3′) primers and Exo I restriction enzyme. Allele and genotype frequencies were compared with a Chi-Square test.

The distribution of the genotypes of GZMB Q48R in HC group was in Hardy-Weinberg equilibrium. The screened SNP of GZMB was highly polymorphic with a minor allele frequency of 0.29 in both patients and controls. GZMB genotypes were evenly distributed in BD and controls (Table 1). In this attempt to identify a new BD susceptibility marker, we have investigated GZMB which was reported to be polymorphic. The association of BD with HLA-B51 implicating a cytotoxic T cell mediated mechanism could have been related to GzmB activity and a mutated variant of GzmB protein unable to induce apoptosis (7) could have contributed to this effect. However, the screened polymorphism was highly frequent in both groups and the distribution in controls was similar to other populations with frequencies of 25-30% of the polymorphic allele (7-8). As in both published studies the three polymorphisms encoding three distinct amino acid changes were in linkage disequilibrium, we have investigated one of the SNPs (Q48R) representing the variant RAH protein. This study is the first evaluation of a GZMB polymorphism as a disease susceptibility marker and provides a screening with relatively low power.

The present data however do not provide an evidence for the involvement of the GZMB polymorphism in BD.

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References


Table 1. The distribution of the genotypes and the allele frequencies of GZMB Q48R (rs8192917, A→G) in Behçet’s disease (BD) patients and healthy controls (HC).

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