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Association of HLA class I alleles with alopecia areata in Chinese Hans

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Summary

Background: Some studies suggested that human HLA status may potentiate development of the AA phenotype and exists ethic differences. No report has been published about HLA class I alleles associated with AA in Chinese Hans.
Objective: To study the distribution of HLA class I alleles and haplotypes in Chinese Hans AA patients and the relation of HLA class I profile with age of onset, severity, duration of current attack, past history and family history.
Methods: The polymerase chain reaction-sequence-specific primer (PCR-SSP) method was used to analyze the distribution of HLA class I alleles in 192 patients with AA and 252 healthy controls in Chinese Hans.
Results: The frequencies of HLA-A*02, -A*03, -B*18, -B*27, -B*52 and -Cw*0704 were significantly higher in patients than in controls. The A*2-B*18, A*2-B*27, A*2-B*52, A*2-Cw*0704, B*18-Cw*0704, B*27-Cw*0704, B*52-Cw*0704 were found as high-risk haplotypes in developing AA in this study. The HLA-A*02 and -A*03 were observed increased frequencies in patients less than 50% hair loss, and HLA-B*27 equally in patients of 50—99% hair loss, alopecia totalis and alopecia universalis. The frequencies of HLA-A*02 and -B*27 were significantly raised in recurrent patients, and ones of HLA-A*02, -A*03 and -B*27 similarly in patients without a positive family history.
Conclusion: This study demonstrated the positive association of HLA class I alleles and haplotypes with AA. There may be differences in genetic background in patients

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with different age of onset, grade of scalp hair loss, duration of current attack, a past history and a family history.

Aloplecia areata (AA) is characterized by discrete, well demarcated areas of non-scarring terminal hair alopecia and its clinical presentation is generally multifocal. It is estimated that AA affects 0.94% of patients in China [1] and 2—4% of patients in American [2] in average dermatology practices. AA is a disorder with a complex genetic aetiology with, most likely, polygenic susceptibility and severity loci interacting with environmental factors [1,3]. There is increasing consensus that AA is an organ-specific autoimmune disease directed against the hair follicle [4,5]. HLA alleles encode polymorphic protein chains that function to bind and present peptide antigens to T lymphocytes. HLA is now recognized as a major contributing factor for susceptibility to autoimmune diseases. Current research has focused on HLA type and immune function in alopecia patients [6,7]. It is thought that HLA status may potentiate development of the AA phenotype and exists ethic differences [8—11]. However, no report has been published about HLA alleles associated with AA in Chinese Hans.

To explore the possible involvement of HLA class I alleles in the pathogenesis of AA, we studied the distribution of HLA-A, -B, -C alleles and haplotype in patients with AA and healthy individuals, and the relation of HLA class I profile with the age of onset, severity of scalp hair loss, duration of current attack, a past history and a family history of AA using polymerase chain reaction with sequence-specific primers (PCR-SSP) assay in 192 patients with AA from Chinese Hans.

1. Materials and methods

1.1. Patients and controls

A total of 192 unrelated Han patients (97 males and 95 females) with AA were recruited consecutively from the outpatients at the Department of Dermatology, the First Affiliated-Hospital, Anhui Medical University. The patients ranged in age from 3 to 69 years with a mean age of 27.83 years. The healthy controls were comprised of 252 disease-free unrelated individuals, age-, sex- and ethnicity-matched with the patients from the same areas. All subjects gave their informed written consent before participation.

Patients were categorized according to: (1) age of onset (early onset: $<$16 year; late onset: $\geq$16 year), (2) duration of current attack (short duration: $<$1 year; long duration: $\geq$1 year), (3) severity of scalp hair loss (S1—S2: less than 50% hair loss; S3—S4: 50—99% hair loss; AU + AU: alopecia totalis and alopecia universalis, by Olsen et al. [12]), (4) past history of AA (primary: first onset of AA; recurrent: second or poly-onset of AA), (5) family history (positive: a patient's first and/or second-degree relatives had AA; negative: otherwise).

1.2. DNA preparation

Venous blood for HLA typing was collected in ethylenediamine tetraacetic acid (EDTA) anticoagulated tubes. Genomic DNA was extracted from these blood specimens by an improved salting-out method [13], and then dissolved in sterile double-distilled water for usage.

1.3. Amplification primers and PCR conditions

The amplifying primers were previously described by Bunce et al. [14,15]. Control primers giving rise to a 796 base-pair fragment from the third intron of HLA-DRB1 were included in all PCR reactions. The conditions and parameters of amplifying reactions were also referred to the previous description [14,15]. PCR reaction mixtures (10 μL) consisted of 100 ng genomic DNA, PCR buffer [50 mM KCl, 1.5 mM MgCl2, 10 mM Tris—HCl (pH 8.5), and 0.01% (v/v) Tween 20], 0.2 mM each of dNTP, 0.30 μM of the allele- and group-specific HLA class I primers, 0.06 μM of the control primers and 0.5 units of Taq polymerase (MBI Fermentas).

Preliminary denaturation was performed at 94 °C for 5 min, followed immediately by 30 cycles of 30 s of denaturation at 94 °C, 30 s of primer annealing at 55—62 °C and 40 s of primer extending at 72 °C in each cycle, followed by a single round of final extention at 72 °C for 10 min. PCR products were electrophoresed in 2.0% agarose gel containing 0.5 μg/ml ethidium bromide. Gels were run for 90 min at 5 V/cm in 0.5 $\times$ TBE buffer (89 mM Tris base, 89 mM boric acid and 2 mM EDTA, pH 8.0). Gels were visualized using Gel Documentation and Ana-