Original Article

An Investigation of the Relationship Between HLA-CW06 and Psoriasis in Iranian Patients Using Sequence-specific Primers

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A B S T R A C T

Background and Aims: Psoriasis is a chronic inflammation of the skin caused by the proliferation of inappropriately differentiated horn cells, resulting in plaque psoriasis formation. It is not often fatal, and it may lead to physical disabilities and severe mental stress in which professional and social activities could be highly affected. It is generally accepted that the human leukocyte antigen (HLA)-CW06 allele has a significant role in the onset of this disease.

Materials and Methods: This study determines the association between the HLA-CW06 allele and psoriasis. The present case-control study was conducted to evaluate the relation between HLA-CW06 and psoriasis in a population in Iran. This study was performed on 30 samples of patients with psoriasis.

Results: The results of polymerase chain reaction- sequence-specific primers for the detection of HLA-CW06 showed positive amplification in 7 out of 30 psoriasis patients as compared to 4 among 30 controls.

Conclusion: This study showed that due to different allele frequencies associated with psoriasis in different parts of the world, it seems that other genetic and epigenetic factors may be involved in this disease.

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Introduction

Psoriasis is a chronic inflammatory disease with a worldwide distribution, affecting 0.44% to 2.88% worldwide of the general population. The prevalence of psoriasis has increased, and it is the second-largest contributor to skin-related disability [1-4]. It is a heterogeneous disorder with distinguished but overlapping phenotypes. This difference may be due to racial factors. Psoriasis influences various organs past the skin and has been related to metabolic dysfunction and cardiovascular sicknesses [5-8]. The pathogenesis of psoriasis is not fully known, but genetic history's impact is a consensus issue [9, 10]. The human leukocyte antigen (HLA) located on chromosome 6p21 is notable for carrying the main hereditary elements in the vulnerability to psoriasis, whereas the critical segment seems to be a 300-kb distance at the centromeric end of HLA class I(6p21.33), named PSORS1[11-14]. The association of HLA and diseases is accepted. Many population studies offered to suggest proof of association of HLAs in additional than forty diseases [15, 16].

This study aimed to survey the HLA-CW6 distribution in Iranian psoriatic patients. HLA-Cw6 is known to be quite possibly the most emphatically related psoriasis susceptibility alleles. It is widely recognized that HLA-Cw6 is related to phenotypic highlights, for example, beginning stage psoriasis and guttate lesions[17]. Type I manifests early in life (onset < 40 years) and is possibly related to family history; type II onsets are later in life (onset > 40 years) and is more likely to be sporadic [18]. Stress, obesity, and streptococcal pharyngitis are generally seen in HLA-Cw6-positive patients [19, 20].

Materials and Methods

Research participants

The present case-control study was conducted to evaluate the relation between HLA-CW6 and psoriasis in a population in Iran. The target population is in northern Iran. This study was performed on 30 samples of patients with psoriasis. For the control group, 30 healthy individuals without any disease manifestation were collected, and age, sex, and geographical distribution were adjusted to the patient group. The sample size was calculated using the power calculator for case-control genetic association studies [21]. People in the control group should not have a family relationship with patients. At last, blood sampling was performed after obtaining their informed consent.

DNA extraction and genotyping

Peripheral blood samples of patient and control groups were collected in tubes with ethylenediaminetetraacetic acid as an anticoagulant. According to the manufacturer's instructions, DNAs were then extricated from whole-blood samples by a DNA extraction Kit (GENET BIO, Chungnam, Korea). DNA quality was checked by the NanoDrop micro-volume spectrophotometer (Thermo Scientific NanoDrop Products) and through the agarose gel electrophoresis. The classic sequence-specific primers (SSP) HLA-Cw06 standard kit (Bio-Rad, United States) was used in the study. The applied

primers for the polymerase chain reaction (PCR) amplification were as follows:

F: 5'-TACTACAACCAGAGCGAGGA-3'/

R: 5'-GGTCGCAGCCATACATCCA-3'–control primer F: 5'-TGCCAAGTGGAGCAC CCAA-3'/

R: 5'-GCATCTTGCTCTGTGCAGAT-3'.

PCR was performed on an Eppendorf thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: 95 °C for 5 min followed by 30 cycles of 96 °C for 25s, 65 °C for 50 s, 72 °C for 30 s, and a final extension of 72 °C for 1.5 min. In order to prevent the extraction buffer from any damage, the DNA extraction buffer should generally be stored at 4 °C. On top of that, for long-term DNA storage and to prevent any enzymatic and physical damage to the extraction supply, DNA would be kept at -20 °C.

Qualitative and quantitative analysis

The accuracy of DNA extraction is so important that the extracted DNA is qualitatively and then quantitatively analyzed for molecular investig-ations. Electrophoresis and spectrophotometry were the techniques followed for qualitative and quantitative analysis.

Statistical analysis

Allele frequencies in the study group and the controls were evaluated by direct counting. Data were tested for the efficiency between the observed and expected genotype frequencies, and no deviations from the Hardy–Weinberg equilibrium were found. Associations of particular alleles with psoriasis were expressed as odds ratios (OR) with 95 % confidence intervals (CI) calculated according to Woolf's formula [22].

Results

Existing case-control research studied a total of 60 volunteers. Among them, 30 patients with a history of psoriasis were classified as the case group. The results of PCR-SSP for the detection of HLA-CW06 showed positive amplification in 7 out of 30 psoriasis patients compared to 4 among 30 controls (Table 1). The electrophoresis image confirms the positive control PCR reaction (796 bp) and the desired allele (256 bp) (Fig. 1). According to the calculations, the OR was 1.97; due to the high number associated with psoriasis, we can say that the HLA allele affects this disease.

Discussion

Scientists estimate that about 10% of the world's population has at least one gene that causes psoriasis, but the prevalence of the disease in the world is only 2 to 3%. In this case, the combination of genes that cause psoriasis and environmental factors contribute to the disease occurrence [17]. About one-third of people with psoriasis have a family history of the disease. Studies on identical twins showed that if one of the twins has psoriasis, there is a 70% chance that the other twins will also get the disease.

Table 1. Comparison of the psoriasis types among HLA-Cw06-positive and HLA-Cw06-negative patients

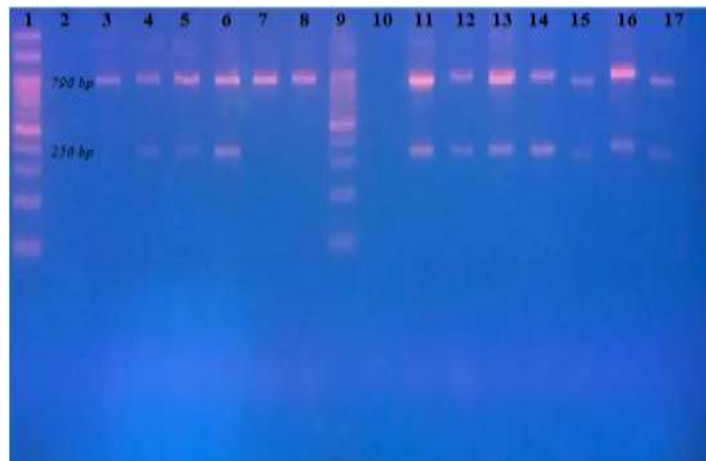|	Existence of risk factor	Absence of risk factor
Patients	A=7	**B=23**
Controls	C=4	**D=26**

Fig. 1. The positive control PCR reaction (796 bp) and the desired
allele (256 bp). The odds ratio was used to analyze the results.

On top of that, the probability for heterogeneous twins is 20%. These studies show the importance of genes' impacts on psoriasis and the influence of environmental factors on the incidence and occurrence of psoriasis [23-26]. Psoriasis has a vital genetic component, so many genes are associated with the disease, but these genes causing the disease are still unknown. Today, researchers have identified many chromosomal loci associated with psoriasis. Most of the identified effective genes are related to the immune system, especially T cells and the major histocompatibility complex [27, 28]. In general, nine chromosomal sites associated with psoriasis have been identified, known as psoriasis susceptibility sites 1 to 9 or, for short, PSORS1 to PSORS9. These chromosomal sites are genes for the inflammatory pathways in patients with psoriasis; these genes are mutated. Other genes have also been discovered linked to the development of psoriasis and have been significantly altered in people with psoriasis. Some of these genes cause the expression of inflammatory proteins affecting cells in the immune system that are involved in causing psoriasis. Changes in some of these genes are also seen in other autoimmune diseases[29-33]. The most important effect of genes on psoriasis is related to the PSORS1 locus, responsible for 35 to 50% of hereditary psoriasis. PSORS1 is responsible for regulating immune-stimulating genes or producing skin proteins that are overproduced in psoriasis. The HLA-CW06 gene is one of the essential genes in the PSORS1 locus [34, 35]. The HLA-C* 06 allele is the genetic variation related to the phenotypic highlights of psoriasis, which have been seen more than once. It is associated mainly with the beginning stage of the illness, as affirmed by Szczerkowska-Dobosz et al. [36]. This study examined the interplay between the HLA-C allele and psoriasis in patients with PCR-SSP in the Iranian population. Given the different allelic frequencies associated with psoriasis in different parts of the world, it seems that other genetic and epigenetic factors are also involved in this disease. Nonetheless, broad approvals are needed to test the consensus of our theory among various populations around the world.

Conclusion

Our study confirmed the strong positive association of psoriasis with HLA-C*06, observed mainly in Caucasoid populations and in several other ethnically different groups.

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Conflicts of Interest

The authors declare no conflicts of interest and financial support for the present original article.

Acknowledgments

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