Genotyping of HLA-B27 in Ankylosing Spondylitis Patients in Kazakhstan

Aizhibek Karina, BSc; Dariya Shafiee, MSc; Jeannette Kunz, PhD,
Nazarbayev University

Introduction

Spondyloarthritis, shortly SpA, is a group of inflammatory diseases that affect joints and the spine, and are associated with additional clinical manifestations, such as gut inflammation, psoriasis, and uveitis. The disease is highly associated with the HLA-B27 allele, nonetheless, the disease aetiology is not well understood and likely results from a combination of genetic and environmental factors. Some of the potential triggers that may play a role in the development of spondyloarthritis are:

Environmental factors: Exposure to certain environmental triggers and lifestyle choices, such as mechanical triggers and infections, may increase the risk of developing SpA diseases. Due to life events, stress may activate preexisting inflammatory arthritis as immune function alters in response. Moreover, according to a Swedish national control case study, infectious childhood hospitalizations, such as appendicitis, respiratory tract infections, and tonsillitis, later in life may show an association with the development of Ankylosing Spondylitis (AS), disease that cause inflammation in lower spine.

Genetics: HLA-B27 is the most well-known genetic marker associated with an increased risk of spondyloarthritis, but other genes may also play a role. As HLA-B27 part of the class I MHC group, it is responsible for immune response regulation by presenting short antigenic peptides for recognition of cytotoxic T lymphocytes. Yet, it is also highly linked to SpA, especially to ankylosing arthritis (AS) development with 95% of AS patients being HLA-B27 positive. Moreover, different alleles of the gene are also responsible for different ethnic groups. The highest associated subtypes in AS patients are HLA-B27:05 for Europeans and HLA-B27:04 for the Chinese population. And although this study does not provide the common subtypes of HLA-B27 in AS patients, it gives a clearer understanding of the correlation in the Kazakhstani population comparingly to the already existing data of some ethnicities.

Gender	Patient Number
Female	14
Male	53
Together	67

Mean average = 41.5 years
Age range = from 19 to 64 years

Table 1. *Patient Information*

Methods and Materials

Ankylosing spondylitis (AS) patients were recruited by rheumatologists at Astana Medical University. The overall number of patients was 67, with 14 female and 53 male patients. The mean average age of patients was 41.5 years, ranging from 19 to 64 years of age (Table 1). Participants underwent physical examination and provided medical history. Upon recruitment, patients met at least one of the diagnostic criteria: New York Criteria, ILAR criteria for ERA, and ASAS criteria for axial SpA.

For HLA-B27 Genotyping, the genomic DNA of 67 AS patients and 26 RA patient controls were used for in-house PCR. Separate primers for HLA-B27 and GAPDH (Figure 1) were synthesized at the National Center for Biotechnology (NCB) and used for PCR amplification of HLA-B27. The resulting PCR products were analyzed by gel electrophoresis on 1.5% agarose gel and exposed to BioRad ChemiDoc XRS+ System (cat# 1708265, BioRad). Two bands for HLA-B27 and GAPDH, which is a housekeeping gene and serves as the internal control, were expected at 250 bp and 450 bp, respectively.

	HLA-B27	GAPDH
FORWARD PRIMER	GGTCTCACACCCTCCAFAAT	ACCACAGTCCATGCCATCA
REVERSE PRIMER	GCGTCTCCTTCCCGTTCTCA	CCACCACCCTGTTGCTGT

Figure 1. *PCR Primers.*

Results

Genotyping of HLA-B27 in ankylosing spondylitis (AS) patients and rheumatoid arthritis (RA) patient controls was conducted by PCR. Up to this point, 67 AS patients and 26 RA controls underwent PCR genotyping to identify the status of HLA-B27. As predicted, Figure 2 demonstrates an HLA-B27 frequency of 82% for AS patients. Yet, the results of RA controls illustrate the positive status of the HLA-B27 allele with about 15%, while the rest 85% are negative.

The next figure shows a cohort of two ethnicities, Kazakhs (n=58) and Caucasians (n=9), which includes Russians and Ukrainians. Notably, the prevalence of the HLA-B27 allele between these groups presents a small difference in percentage. The data provides statistical significance as it shows that the prevalence of HLA-B27 and AS patients in different ethnicities is approximately the same.

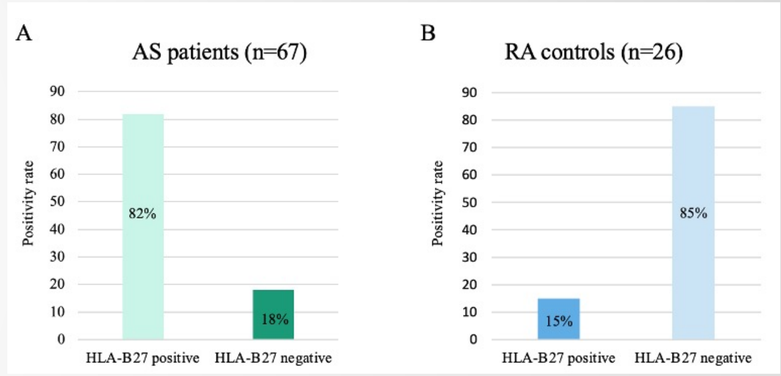

Figure 2. *HLA-B27 Genotyping results.*

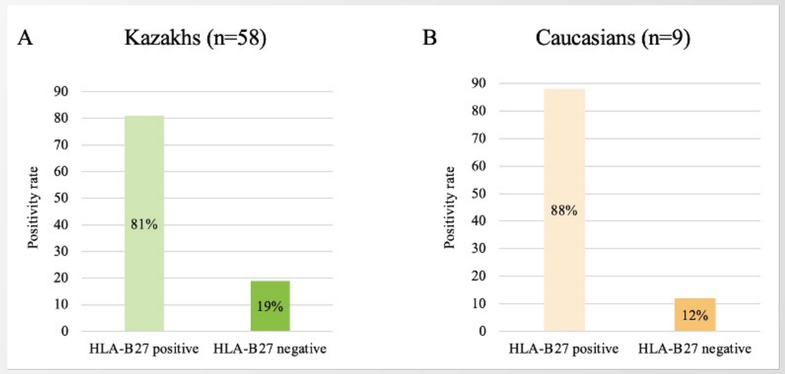

Figure 3. *Two subgroups within the AS Patients Group.*

Discussion

The PCR genotyping results of Kazakhstani AS patients and RA controls showed a good accord with published results with a 70-95% of positivity rate in AS patients, while RA controls showed the expected opposite data. Nevertheless, during the experiments, it is seen that in-house PCR has some limitations. Starting with lack of skillfulness that may lead to false-positive results. And secondly, in-house PCR processes a limited number of samples at a time, which can be time-consuming for a large amount of data. Nonetheless, it provides a cost-effective and reliable tool for genetic studies. Consequently, this study shows reliable data by providing PCR at least 3 times for each sample.

Moreover, the status of the HLA-B27 allele between the subgroups of ethnicities showed a 7% difference, which confirms that the association between HLA-B27 positivity and AS is significant also in Kazakhstan and independent of ethnicity. Hence, this research adds importance of HLA-B27 studies in AS patients around the world.

Contact

Name: Aizhibek Karina
Mail: aizhibek.karina@nu.edu.kz
ORCID: 0000-0003-4127-6268
Institution: Nazarbayev University

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