

Mutational Analysis of *DNase I* Gene in Pakistani SLE Patients

Manan A and Hussain N*

Department of Microbiology and Molecular Genetics, Quaid-e-Azam campus, University of the Punjab, Pakistan

Abstract

Systemic Lupus Erythematosus (SLE) is one of the classic examples of autoimmune diseases among human beings and is a rare disease in Pakistani population. Clinically, it is a quite diverse and complicated autoimmune disease in a sense that it involves multiple organs of the body and mimics other diseases as well. Mutational analysis of *DNase I* gene exon 8 was performed because most of the mutations were reported in these regions with reference to SLE. Genes were amplified by Step-down PCR technique in order to get the bands of our interest. Extracted DNA bands were sequenced to analyze mutations. Thirteen SLE samples showed a missense mutation in *DNase I* gene. The codon CAA changed to CGA (+2373 A>G) so the glutamine amino acid was replaced by arginine at position 244 of Deoxyribonuclease I protein.

Keywords: Systemic lupus erythematosus; *Deoxyribonuclease gene I*; Polymerase chain reaction

Introduction

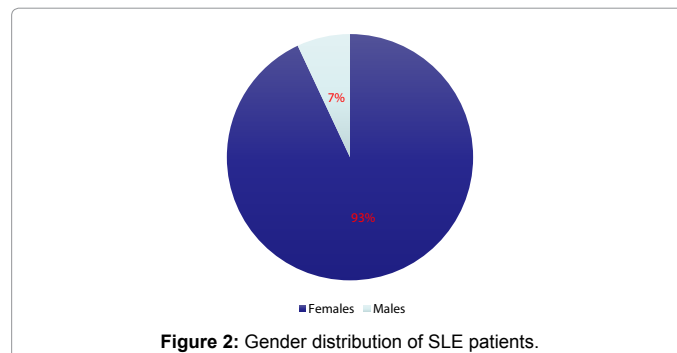
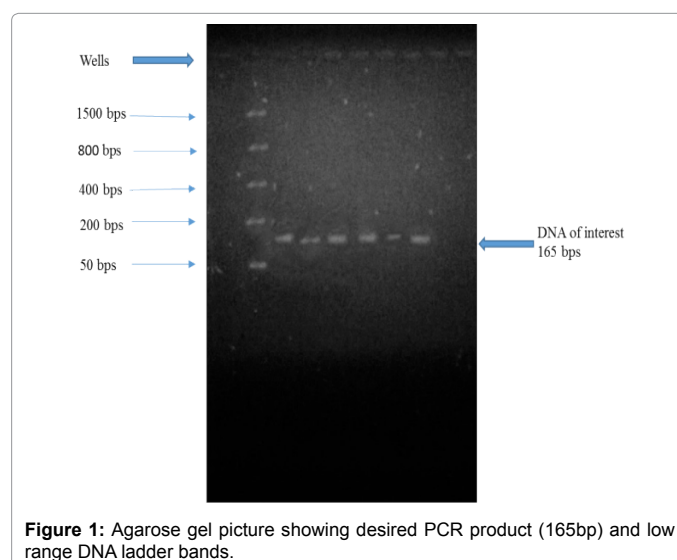
Systemic Lupus Erythematosus (SLE) is an autoimmune disorder of unknown etiology with clinical presentations of almost all organs and tissues. Even some investigators referred it as a syndrome due to its heterogeneity and broad spectrum clinical pictures [1]. *Deoxyribonuclease I* gene (*DNase I*), which is associated with the disposal of apoptotic nuclear debris, is located on the short arm of chromosome 16. This gene is 3.2 kb long with 9 exons separated by 8 introns. So it has been highlighted for its possible involvement in SLE pathogenesis. The defective enzyme production due to +2373 A to G mutation in exon 8 is considered to be a genetic risk factor for SLE patients in other populations [2]. The main objective of the present study was to analyze the mutations of *Deoxyribonuclease I* (*DNase I*) gene exon 8 in Pakistani SLE patients.

Materials and Methods

A total 42 SLE patients and 42 healthy controls were included in this study. This study focused mainly the population of Lahore present in the Punjab province of Pakistan. A total of 42 SLE patients were selected on the basis of American Society of Rheumatology (ACR) criteria from different hospitals of Lahore. Most of the SLE patients fulfilled 5 of the 11 ACR criteria. DNA was isolated by phenol chloroform DNA extraction method [3]. Then PCR was performed to amplify the exon 8 region of *DNase I* gene (Figure 1). PCR products were sequenced by dideoxy sequencing method. Bioinformatics (Chromas, EditSeq, BLAST) was applied to analyze mutations in the exon 8 region of *DNase I* gene. *DNase I* coding sequence (both mutated and non-mutated) was translated into protein using a DNA translator. Translated sequence was further analyzed by Phire ver 2 software [2].

Results

A total 42 SLE patients and 42 healthy controls were included in this study. 93% of the SLE patients were females while 7% were males, this may be because of the hormonal factors involved (Figure 2). Most of the SLE patients (49%) lie within the age group of 20-40 years (Figure 3). Most of the SLE patients fulfilled 5 of the 11 ACR criteria (Figure 4). In this study, 73% of the SLE patients were found to be positive for rheumatoid factor. SLE patients with positive rheumatoid factor have pain and swelling at lower and upper extremities which often limits the physical activity and interfere with rehabilitation activities such as transfer or movements. Photosensitivity was positive in 36% of the



*Corresponding author: Nageen Hussain, Assistant Professor, Department of Microbiology and Molecular Genetics, Quaid-e-Azam Campus, University of the Punjab, Pakistan, Tel: 0092 3224736630; E-mail: nageen1704@hotmail.com

Received: October 26, 2015; Accepted: May 12, 2016; Published: May 16, 2016

Citation: Manan A, Hussain N (2016) Mutational Analysis of *DNase I* Gene in Pakistani SLE Patients. J Clin Cell Immunol 7: 419. doi:10.4172/2155-9899-1000419

Copyright: © 2016 Manan A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

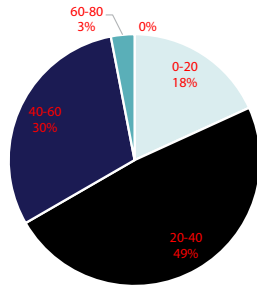


Figure 3: Frequency distribution of SLE patients according to age groups.

SLE patients. Discoid rash and malar rash were positive in 55% and 48% respectively. Hematological disorders were positive in 76% of SLE patients, but only 30% having neurological problems. 70% of the SLE patients with single missense mutation had arthralgia or arthritis and showed positive rheumatoid factor while 100% of such SLE patients had lupus nephritis (Figure 5).

Thirteen samples showed a missense mutation at position 3737 of *DNase I* gene (Figures 6 and 7). This allelic variant is not present in the normal Pakistani population (Figure 8). A was replaced by G so the codon CAA changed to CGA. The glutamine (a polar amino acid) was replaced by Arginine (a charged amino acid) at position 244 of

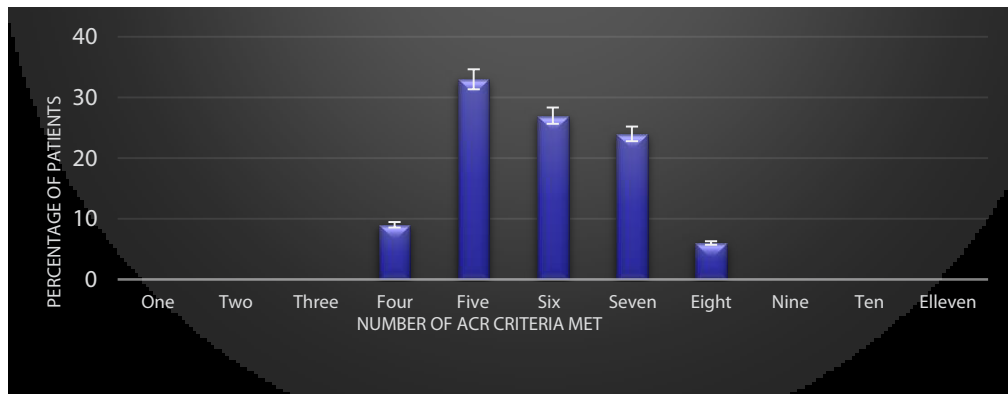


Figure 4: Percentage of ACR criteria met by diagnosed SLE patients.

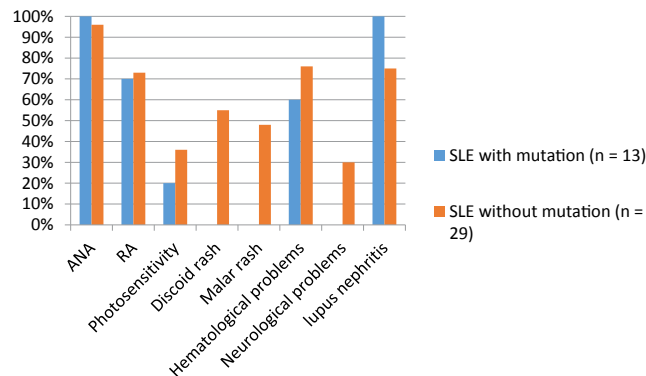


Figure 5: Clinical and laboratory features of SLE patients with and without the studied missense mutation.

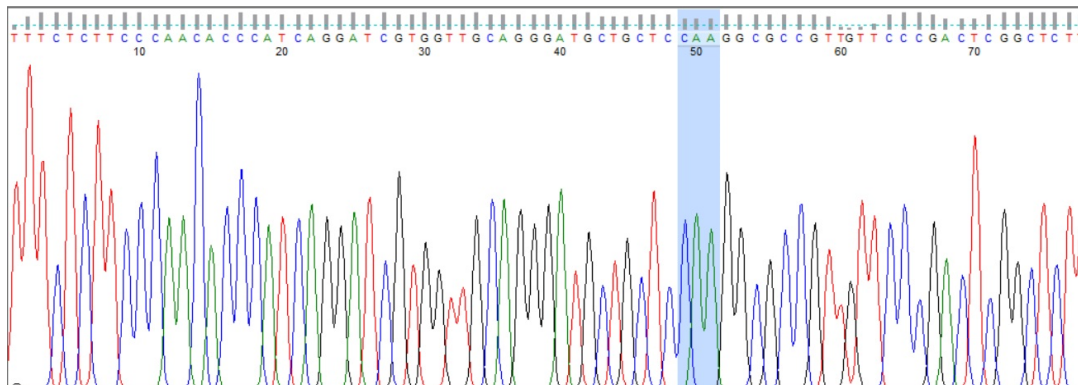


Figure 6a: Control sample showing the presence of "CAA" nucleotide sequence in the highlighted portion.

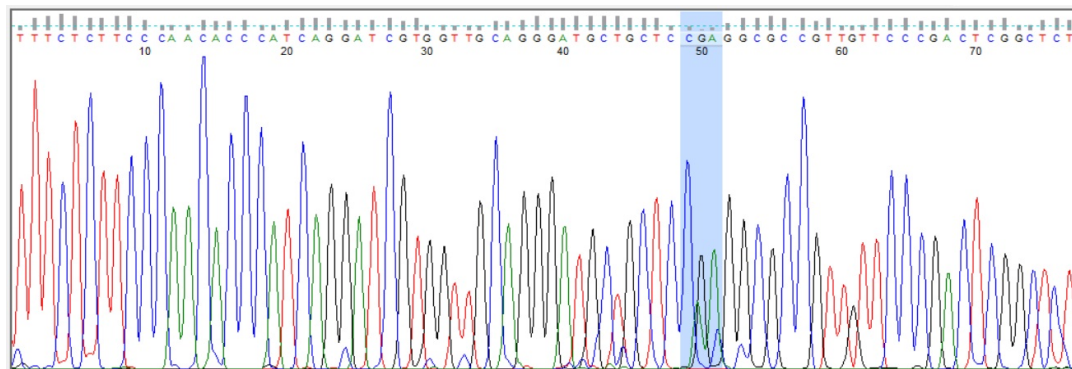


Figure 6b: Mutated sample showing the presence of “CGA” nucleotide sequence instead of “CAA” nucleotide in the highlighted portion.

Homo sapiens DNASE1 gene for deoxyribonuclease I, complete cds
Sequence ID: [dbj|D83195.1](#) Length: 4592 Number of Matches: 1

Range 1: 3688 to 3853 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
302 bits(163)	1e-78	165/166(99%)	0/166(0%)	Plus/Plus
Query 1	TTTCTCTTCCCAACACCCATCAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCCCGTT	60		
Sbjct 3688	TTTCTCTTCCCAACACCCATCAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCCCGTT	3747		
Query 61	GTTCCCGACTCGGCTCTTCCCTTTAACTTCCAGGCTGCCTATGGCCTGAGTGACCAACTG	120		
Sbjct 3748	GTTCCCGACTCGGCTCTTCCCTTTAACTTCCAGGCTGCCTATGGCCTGAGTGACCAACTG	3807		
Query 121	GTATGTGTCCTCCCTTGACAGCCACATGAGGATGGGACACAGGAG	166		
Sbjct 3808	GTATGTGTCCTCCCTTGACAGCCACATGAGGATGGGACACAGGAG	3853		

Figure 7: Missense mutation observed in SLE patients.

Homo sapiens DNASE1 gene for deoxyribonuclease I, complete cds
Sequence ID: [dbj|D83195.1](#) Length: 4592 Number of Matches: 1

Range 1: 3688 to 3853 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
307 bits(166)	3e-80	166/166(100%)	0/166(0%)	Plus/Plus
Query 1	TTTCTCTTCCCAACACCCATCAGGATCGTGGTTGCAGGGATGCTGCTCCAAGGCCCGTT	60		
Sbjct 3688	TTTCTCTTCCCAACACCCATCAGGATCGTGGTTGCAGGGATGCTGCTCCAAGGCCCGTT	3747		
Query 61	GTTCCCGACTCGGCTCTTCCCTTTAACTTCCAGGCTGCCTATGGCCTGAGTGACCAACTG	120		
Sbjct 3748	GTTCCCGACTCGGCTCTTCCCTTTAACTTCCAGGCTGCCTATGGCCTGAGTGACCAACTG	3807		
Query 121	GTATGTGTCCTCCCTTGACAGCCACATGAGGATGGGACACAGGAG	166		
Sbjct 3808	GTATGTGTCCTCCCTTGACAGCCACATGAGGATGGGACACAGGAG	3853		

Figure 8: Allelic variant is not present in the normal population.

KT037442	KT037443	KT037444	KT037445	KT037446
KT037447	KT037449	KT037450	KT037451	KT037452
KT037453	KT037454	KT037455		

Table 1: Accession numbers of the SLE samples showing novel mutation.

Deoxyribonuclease I protein. Accession numbers of mutated samples are shown in Table 1. There is a change in the tertiary structure of mutated DNase I protein as compared to non-mutated DNase I protein. Figures 9 and 10 represents the “*in silico*” simulation of protein changes. By using PyMOL viewer software, glutamine shows five hydrogen bridges with five different amino acids but in the mutated DNase I protein, Arginine at position 244 showed a reduced number of hydrogen bridges, with only two interactions with two different amino acids.

Discussion

In the present study, thirteen samples showed a missense mutation at position 3737 of DNase I gene. The codon CAA changed to CGA (+2373 A>G) so the glutamine was replaced by arginine at position 244 of Deoxyribonuclease I protein. This novel mutation in DNase I gene exon 8 may contribute to the pathogenicity of SLE as this type of allelic variant is not present in the Lahore healthy population. In this study, there is no evident consanguinity among the SLE patients carrying or not carrying

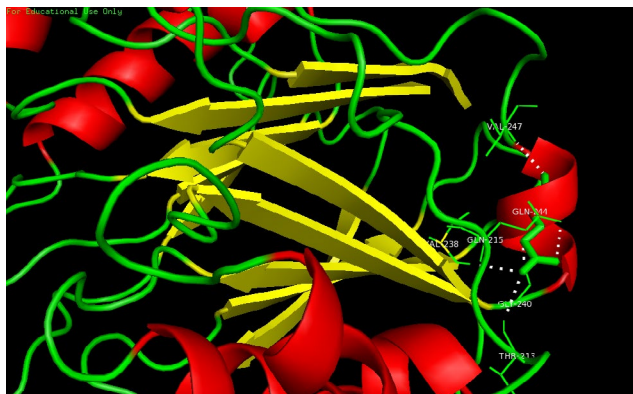


Figure 9: Normal DNase I protein with Gln at 244 position.

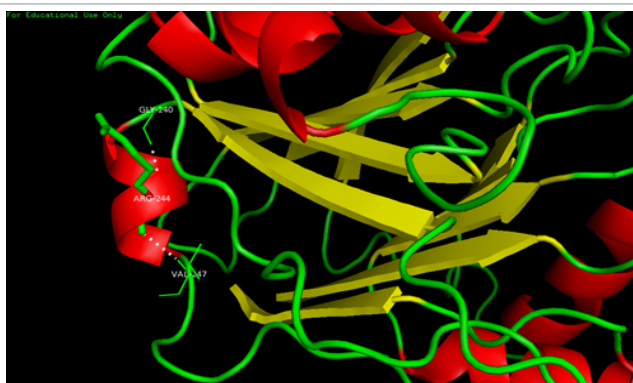


Figure 10: Mutated DNase I protein with Arg at 244 position.

the mutation. Still there is need to characterize SLE patients according to ethnicity because Lahore population is relatively homogenous, cousin marriages are common in Muslims so genome must be very unique. Thus our future plan is to continue this research on SLE families.

References

1. Shin HD, Park BL, Kim LH, Lee HS, Kim TY, et al. (2004) Common DNase I

polymorphism associated with autoantibody production among systemic lupus erythematosus patients. Hum Mol Gen 13: 2343-2350.

2. Tsokos GC (2011) Systemic Lupus Erythematosus. N Eng J Med 365: 2110-2121.

3. Miller S, Dykes D, Polesky H (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215.