Association of interleukin 7 receptor (IL7R) gene polymorphisms with multiple sclerosis in Kashmir: Preliminary report

Insha Zahoor[1#], Amrina Shafi[1a], Ravouf Asimi[2a]
[1]Department of Biotechnology, School of Biological Sciences, University of Kashmir, Hazratbal, J&K, India.
[2]Department of Neurology, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Soura, J&K, India.

E-mails:inshazahoor11@gmail.com, amrinashafi7@gmail.com, ravoufasimi@yahoo.co.in

[a]Both authors contributed equally
[#]Corresponding author: Dr. Insha Zahoor, Department of Biotechnology, School of Biological Sciences, Ground Floor, Science Block, University of Kashmir, Hazratbal, Srinagar, J&K, 190006, India. Tel.: +91-979-708-8530; E-mail: inshazahoor11@gmail.com

ABSTRACT *Multiple Sclerosis (MS) is a neurodegenerative condition affecting primarily central nervous system of the body. It is a multigenic condition and appears to be more prevalent in some ethnic groups than others. As far as its genetic background is concerned, interleukin 7 receptor (IL7R) gene seems to be a potential candidate owing to its role in proper functioning of the immune system especially in governing autoimmunity. The objective of this study was to investigate the role of IL7R exon 6 polymorphisms rs6897932 and rs201268331 in MS patients from Kashmir region of India. The preliminary findings indicated presence of C to T polymorphisms rs6897932 as well as rs201268331 in most of the MS samples as compared to controls, strongly suggesting possible role of IL7R in MS. However, further studies in large datasets from Kashmiri population are needed to validate the above findings.*

Keywords: MS; IL7R; Kashmir; India; Polymorphism

I. INTRODUCTION

Multiple sclerosis, also known as MS (OMIM 126200) is a chronic nervous system disease affecting nerves in the brain and spinal cord by causing axonal demyelination. It has a prevalence rate ranging from 2 to 150 across the globe, which strongly depends on the location or ethnicity, affecting mostly young adults, especially women [1, 2]. Even though the causes for MS remain obscure, studies have shown that there occur complex interactions between genetic and environmental factors [3-5]. Till 2007, it was believed that the inherited predisposition to this autoimmune disease was strongly linked to the Major Histocompatibility Complex (MHC) [6, 7]; however, there are other non-MHC genes as well

which have been found to be associated with its development [8]. The most widely studied non-MHC category gene, which has been identified to be associated with MS predisposition, is alpha (α or A) chain of interleukin 7 receptor (IL7R; OMIM 146661, Entrez Gene 3575) [9, 10]. It is located on short arm p of chromosome 5 at position 13 (5p13) and composes of γ-chain (IL7Rγ) and α-chain (IL7Rα), which are also known as CD132 and CD127, respectively [11, 12]. It plays a key role in maintaining immune cells by acting as a receptor for interleukin 7 (IL7) [11, 12]. This impelled us to explore the link between IL7R A and MS by screening its exon 6 for presence of C to T transitions rs6897932 and rs201268331 in ethnic cohort of Kashmiri patients as our population being pure and homogenous, makes it a right candidate for gene analysis study; thereby deciphering role of IL7R A in MS development.

II. MATERIALS AND METHODS

2.1 Participants

21 cases of MS were recruited from Kashmir region of India from the Department of Neurology, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Soura, Kashmir, India; following the proper ethical procedures of the hospital. Informed consent was acquired from all subjects prior to enrolment. All the cases of MS were relapsing-remitting (RR) type and diagnosis was made by the neurologist (RA) according to 2010 Revised McDonald Diagnostic criteria [13]. The local Institutional Ethics Committee of SKIMS reviewed and approved this study (No. SKIMS 1 31/IEC-SKIMS/2013-6380). All procedures performed with human participants were in accordance with the ethical standards of the institutional research committee and 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2 Sample collection and DNA extraction

5 ml of whole peripheral venous blood sample was collected in EDTA coated Vacutainer tubes (ADS Hitech Polymers, India) and stored at -80°C before processing for DNA extraction. Blood samples were also collected from healthy individuals from the same geographic area to serve as controls. Genomic DNA was extracted from the blood leukocytes by using DNeasy® Blood & Tissue Kit (Qiagen, Germany), according to the manufacturer's enclosed protocol and its integrity and specificity was checked on 0.8% agarose gel by comparing with 1 kb DNA ladder (Fermentas, USA). The concentration of extracted DNA

samples was determined by using NanoDrop™ Spectrophotometer (Thermo Fisher Scientific, USA) and stored at -20°C till further processing.

2.3 IL7R-A amplification

The amplification of IL7R A exon 6 was performed using the newly designed primer sequences (Forward Primer: 5' CCCACTGCATGGCTACTGAA 3' and Reverse Primer: 5' CTGACTGTCCGTGATCCCAC 3'). PCR was carried out in thermal cycler (Benchmark TC9639, USA), at respective annealing temperatures (Tm) of primer sets obtained after optimization, in a reaction volume of 50 µl containing 50-100 ng of genomic DNA, 0.2 mM dNTPs (Thermo Scientific, USA), 1x Phusion HF Buffer (New England Biolabs, Inc. USA), 1U of Phusion DNA Polymerase (New England Biolabs, Inc. USA) and 0.4 µM of each primer (Integrated DNA Technologies, India). Amplification was performed by initial denaturation at 98°C for 5 min, followed by 30 cycles of denaturation at 98°C for 30 s, annealing at 60°C for 45 s, extension at 72°C for 1 min and a final extension at 72°C for 5 min, after the last cycle. The specificity of amplified products was checked by analyzing on 1.5% agarose gel and comparing with 100 bp DNA ladder (Thermo Fisher Scientific, USA). Purification of PCR products was done by using the GenElute™ Gel Extraction Kit (Sigma-Aldrich, USA), according to the manufacturer's instructions and samples were then stored at -20°C till sequencing.

2.4 Sequencing

For exploration of C to T polymorphisms, the amplified products were sequenced commercially through the services of SciGenom Labs, Pvt., Ltd., Kerala, India (http://www.scigenom.com). Nucleotide sequences of the amplicons were viewed and analyzed for alterations by comparing with NCBI reference sequence using ClustalX Version 2 [14, 15], Chromas Pro Version 1.49 beta 2 (http://www.technelysium.com.au) and Finch TV (http://www.geospiza.com). Databases such as Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk), NCBI′s SNP (http://www.ncbi.nlm.nih.gov/snp) and PubMed databases (http://www.ncbi.nlm.nih.gov/pubmed) were consulted for the detection of variations.

III. RESULTS AND DISCUSSION

Our study comprised of 21 MS patients as well as unrelated controls from the Indian data set, covering the patients from its northern region Kashmir. Agarose gel electrophoresis revealed successful extraction of DNA from blood samples of both cases as well as controls (Fig. 1). The extracted DNA was used as a template for the amplification of IL7R A exon 6 and PCR products on analysis showed specific bands on the agarose gel as per expected size of 198 bp (Fig. 2).

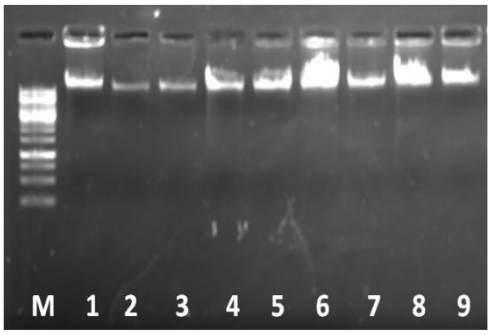

Figure 1. Representative 0.8% agarose gel showing DNA extracted from study subjects. 2 µl (100-300 ng/µl) of each sample was loaded in each well. [Lane M shows separation pattern of 1 kb ladder; and lanes 1 to 9 show genomic DNA].

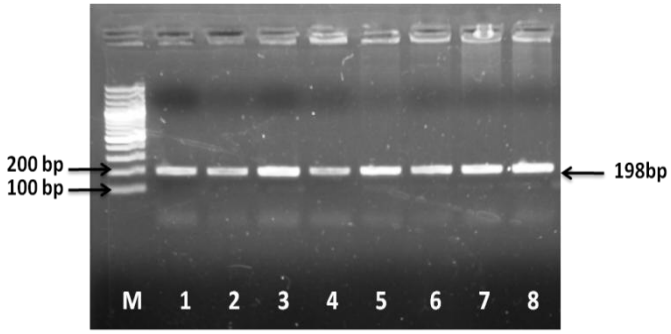

Figure 2. Representative 1.5% agarose gel showing specific PCR amplification product of IL7RA exon 6 (198 bp) in study subjects. 2 µl (100-200 ng/µl) of each sample was loaded in each well. [Lane M shows separation pattern of 100 bp ladder; and lanes 1 to 8 show desired product].

After sequence alignment, 12 (61.9%) MS samples out of 21 showed presence of C to T transition rs6897932 in the sequence and 7 (33.33%) out of 21 MS samples showed presence of rs201268331. Further, analysis of sequencing chromatograms also indicated overlapping peaks suggesting the possible variation (Fig. 3). Several control DNA samples were also

sequenced simultaneously to check for presence of any polymorphic variations. However, polymorphisms were found to be present predominantly in MS cases as compared to controls. The sequence analysis revealed that rs6897932 was present in the homozygous state while rs201268331 was present in the heterozygous state as revealed by the chromatograms (Fig. 3; Table 1). The characteristics of polymorphisms are given in Table 1.

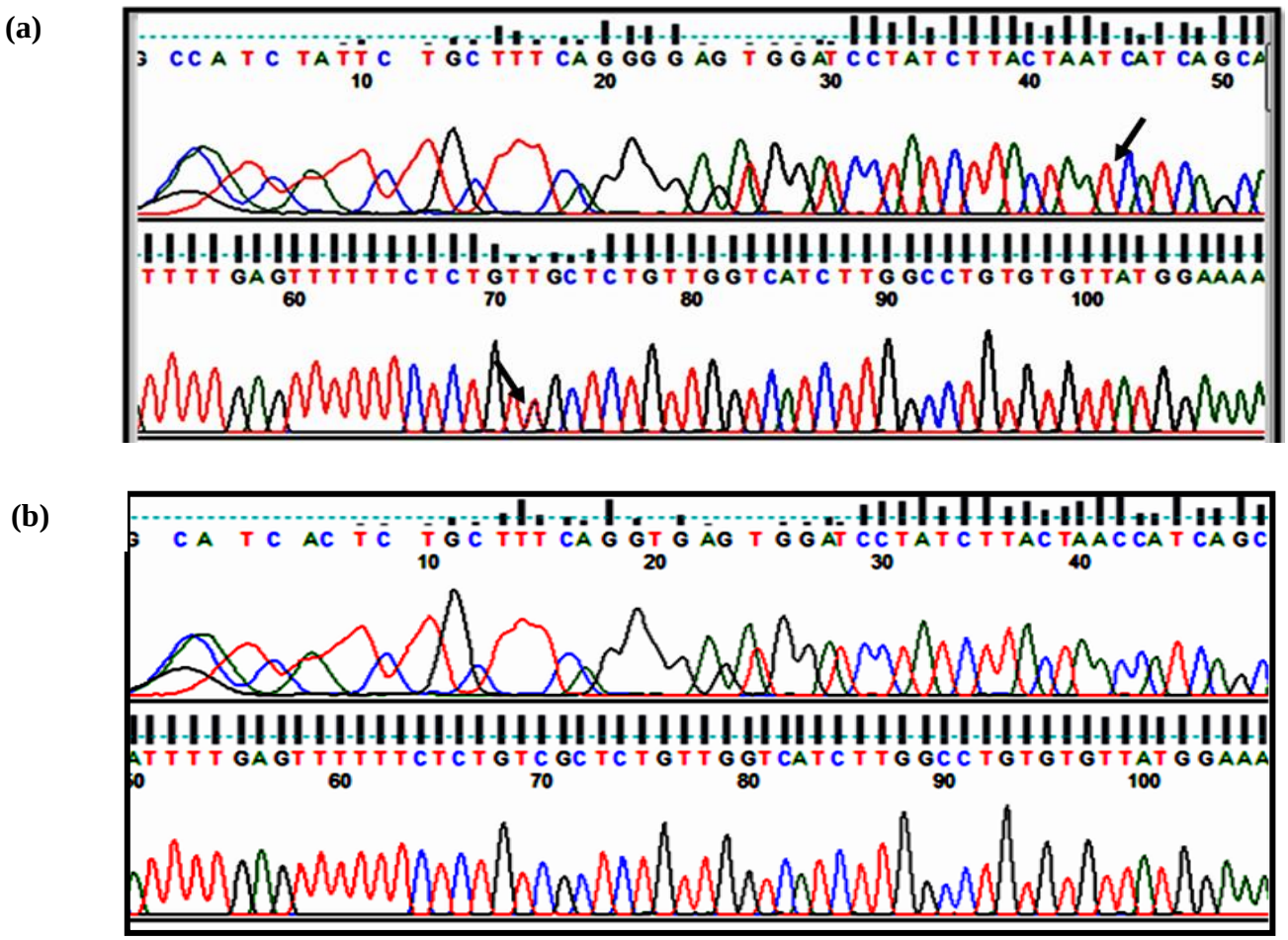

Figure 3. Representative sequence chromatograms of IL7R A exon 6 showing the presence of rs6897932 and rs201268331 (shown by black arrows) in MS patient (a) in contrast to corresponding normal sequence from control (b).

Table 1. Characteristics of IL7R A exon 6 polymorphisms

S. No.	SNP Type	SNP ID	Alleles	Codons
1.	Reported	rs6897932	C/T	aCc/aTc
2.	Reported	rs201268331	C/T	gtC/gtT

The epidemiology of MS has revealed geographical and ethnic variation in its prevalence rate, depicting the influence of local environmental conditions, genetics, and lifestyle on its susceptibility [2, 3]. Therefore, screening of MS candidate genes for variations can shed some light on predisposition to this chronic disease. In the present study, we investigated the association between non-MHC gene IL7R A exon 6 rs6897932 and rs201268331 with MS in Kashmir. Sequence analysis revealed significant presence of both polymorphisms in cases as compared to controls, which clearly shows its role in disease susceptibility in Kashmiri population. At the same time, the findings of our study are not solid due to insignificant sample size. These findings strongly suggest more or less similar pattern of genetic elements in MS across the globe; but, at the same time warrant an in-depth study to have a deeper understanding behind the divergence in geographic prevalence of this demyelinating disease globally.

IV. CONCLUSION

Our findings are not conclusive but just preliminary, indicating possible role ofIL7R A rs6897932 and rs201268331 in MS in Kashmir valley of India. However, nothing can be inferred presently which makes it mandatory to conduct further comprehensive studies with large sample number of both cases as well as controls from Kashmir.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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