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No evidence for a role of Ile587Val polymorphism of EIF2B5 gene in multiple sclerosis in Kashmir Valley of India

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory neurodegenerative disease of the nervous system with a profound genetic element. It is already known that alterations in Eukaryotic Translation Initiation Factor 2B (EIF2B) gene encoding the five subunits of eIF2B complex cause Vanishing White Matter (VWM) disease of the brain and emerging evidences have advocated certain resemblances between MS and VWM in terms of clinical and epidemiological characteristics, thus validating the association study between EIF2B and MS. Moreover, a recent study has implicated EIF2B5 Ile587Val (rs843358) polymorphism as a susceptibility factor for MS. In order to investigate the association of EIF2B5 Ile587Val polymorphism with MS susceptibility in Kashmir region in India, we screened EIF2B5 Exon 13 in 30 MS patients and 65 controls (a total of 95 participants). During the present course of study, we could not find statistically significant difference in the frequency of Ile587Val between MS patients and controls, thus indicating that such alteration does not appear to influence MS development in Kashmiri population. Our results provide evidence against a major role for Ile587Val polymorphism in MS susceptibility.

1. Introduction

Multiple sclerosis (MS) (OMIM 126200) is a chronic inflammatory disease of the nervous system in which the myelin sheath around the axons of the brain and spinal cord is damaged. Its prevalence is more common in young population between ages 20 and 40 and occurs more often in women than men [1,2]. It is characterized by wide continuum of symptoms and appears to be a multi-factorial disease reflecting variability in its prevalence among affected individuals from different populations [1–3]. Although the causes for MS still remain equivocal, mounting evidence suggests genetic risk factors to be largely responsible for its development [4–6] and it is supposed that it results from multiplex interactions between genes and the environment [7–10].

Genetic evidence indicates that variations in numerous genes influence MS development and variations in the Human Leukocyte Antigen (HLA) genes, particularly the HLA-DR beta 1 (DRB1) gene are the strongest genetic risk factors in MS development [11–13], although other genes have also been associated with an increased risk of developing MS like interleukin 7 receptor (IL-7R), interleukin 6 (IL6) and Protein Tyrosine Phosphatase Receptor Type C (CD45) [14–16]. Despite the fact that polymorphisms in dozens of non-HLA genes have been accounted to be associated with MS, still their confirmation has been complicated due to controversial data from different studies. There is some recent evidence that implies Eukaryotic Translation Initiation Factor 2B5 gene (EIF2B5) to be a susceptibility factor for MS development [17–19], albeit some reports have not revealed any association between EIF2B5 and MS in other populations [20,21]. EIF2B5 is a member of EIF2B 1–5 gene family which encodes the five non-identical subunits α, β, γ, δ and ε, respectively of eIF2B complex. Moreover, it is well-known that EIF2B genes are implicated in vanishing white matter disease (VWM) (OMIM 603896) of the brain [22,23]. eIF2B regulates translation initiation by converting eIF2-guanosine diphosphate (GDP) into eIF2-guanosine triphosphate (GTP), thus regenerating active eIF2 by exchange of GDP for GTP [24,25]. This exchange step is very crucial in the regulation of translation initiation under different conditions [24]. Among all members of EIF2B gene family, EIF2B5 is most imperative as it encodes the catalytic (epsilon) subunit of eIF2B complex and being largest and catalytic subunit, mutations in this gene may possibly influence the functioning of the whole complex [25].

In view of the fact that there exist certain similarities between VWM disease and MS, EIF2B5 gene has become an emerging player among the genetic risk factors involved in MS development [17,18,26]. This impelled us to conduct the study on Kashmiri patients with MS. There is lack of population-based studies available for MS on Kashmiri patients,

Abbreviations: CD45, protein tyrosine phosphatase receptor type C; DRB1, DR beta 1; eIF2, eukaryotic translation initiation factor 2; eIF2B, eukaryotic translation initiation factor 2B; EIF2B5, gene; eIF2B, protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate; HGMD, human gene mutation database; HLA, human leukocyte antigen; IL6, interleukin 6; IL-7R, interleukin 7 receptor; MS, multiple sclerosis; NCBI, national center for biotechnology information; SD, standard deviation; VWM, vanishing white matter disease.

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Table 1
Primers used for screening EIF2B5 gene.

Direction	Primer sequence (5′ to 3′)	Length	GC%	Tm (°C)	Product size (bp)
Forward (FP)	CCGGGAAGGTAGAGGCTTTC	20	60.00	59.82	341
Reverse (RP)	CATCTGTTGCAGGGGGAACT	20	55.00	59.96	

ours is the first one in this direction. To delineate the association of Ile587Val (rs843358) with MS, as reported by Ungaro et al., [17] and consequently corroborate the role of various reported variations of VWM in the etiology of MS in Kashmir Valley of India, we screened Exon 13 (coding sequence) for rs843358 and rs113994083 along with intronic sequence (Intron 12) adjacent to Exon 13 of EIF2B5 gene for rs843359 in 30 MS patients and 65 controls. Analysis of different variations in the present population may be helpful for diagnostic purposes and proper management of the disease.

2. Materials and methods

2.1. Study subjects

A total of 30 unrelated patients with MS registered for treatment from 2011 to 2014 at the Department of Neurology, Sher-I-Kashmir Institute of Medical Sciences (SKIMS) {the largest and the only tertiary care hospital in the Kashmir Valley of India} were recruited for the present study. Patients were diagnosed for MS according to revised McDonald criteria [27]. The present study stands approved by the Institutional Ethics Committee of SKIMS. Till date, there has been no prevalence study of MS in Kashmir Valley however, on the basis of hospital record (as provided by the hospital authorities) the number of MS patients received per year is on an average 5 to 8.

For selection of MS patients, inclusion criteria included clinico-radiological confirmed patients, while exclusion criteria included patients having autoimmune diseases other than MS and refusal from participating in the study. A questionnaire was prepared for collecting history of patients and it included age of the subject, sex, personal history, family history, dietary habits, marital status, socio-economic factors, history of disease onset and any associated complications. Clinical, biochemical and MRI details were recorded for all enrolled patients. To minimize the inter-individual variation, a limited number of trained researchers conducted the interviews and no proxies were used. A total of 65 age and sex matched healthy individuals were recruited consecutively from the same geographic area, to serve as controls. For selection of controls, inclusion criteria included subjects with no personal or familial history of MS, and no history of autoimmune disorders. Exclusion criteria included subjects with chronic diseases and refusal from participating in the study.

Blood sample was collected from the persons, who voluntarily participated in the present study. Informed consent was acquired from all participants prior to the study, in compliance with the ethical principles of Indian Council of Medical Research (ICMR), New Delhi, India. All subjects (patients and controls) were of Kashmiri origin.

Table 2
Demographic characteristics of MS patients and controls.

Characteristics	MS	Controls
N	30	65
Males (%)	10 (33.33)	20 (30.77)
Females (%)	20 (66.66)	45 (69.23)
Male/female (%)	0.5 (50)	0.44 (44.44)
Age in years (mean ± SD)	33.40 ± 6.245	32.06 ± 7.597
Age range in years	14–45	14–45

MS: multiple sclerosis; SD: standard deviation.

2.2. Genomic DNA extraction

5 ml of peripheral venous blood sample was collected by venipuncture from all participants in EDTA-containing Vacutainer tubes (ADS Hitech Polymers, Haridwar, India) and stored at −80 °C till further processing. The genomic DNA was extracted from blood by using DNeasy® Blood & Tissue Kit (Cat. No. 69504, Qiagen, Venlo, Netherlands), according to the manufacturer's instructions and its integrity was checked on 0.8% agarose gel stained with ethidium bromide by analyzing gel on ImageQuant LAS 4000 (GE Healthcare Life Sciences, Buckinghamshire, UK). The DNA concentrations were measured at 260 nm with NanoDrop™ 2000 UV–Visible Spectrophotometer (Thermo Scientific, Massachusetts, USA). DNA samples were stored at −20 °C until use.

2.3. Primer designing

For primer designing the referential genomic DNA sequence of EIF2B5 gene was derived from GenBank (Gene ID: 8893; GenBank ID: NG_015826.1), a gene sequence database at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) and Ensemble (Gene ID: ENSG00000145191; Transcript ID: ENST00000273783) (http://www.ensembl.org). With the help of NCBI/Primer-BLAST (http://www.ncbi.nlm.nih.gov/primer-blast), primers were designed to completely incorporate the exon, intron-exon boundaries and intronic sequence adjacent to exon. The details of the primers used in this study are shown in Table 1.

2.4. PCR amplification

PCR was carried out using Applied Biosystems thermal cycler. For optimization, gradient PCR was initially carried out at different temperatures covering annealing temperature (Tm) of both forward as well as reverse primer sets (Table 1). After obtaining the appropriate Tm for primer set, routine PCR was carried out in a reaction volume of 50 μl containing 100–200 ng of genomic DNA, 0.2 mM dNTPs (Thermo Scientific, Massachusetts, USA), 1× Standard Taq Reaction Buffer (New England Biolabs, Inc. Massachusetts, USA), 1 U of Taq DNA Polymerase (New England Biolabs, Inc. Massachusetts, USA) and 0.5 μM of each primer (Imperial Life Sciences, Gurgaon, India). Amplification was carried for 25 cycles, with initial denaturation at 95 °C for 5 min, followed by denaturation at 95 °C for 30 s, annealing at 58 °C for 45 s, extension

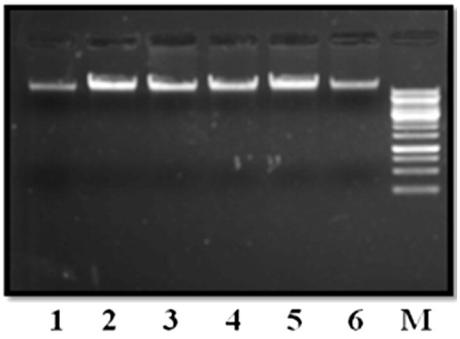

Fig. 1. Representative 0.8% agarose gel showing DNA extracted from blood samples of study subjects [lane M, separation pattern of 1 kb ladder; and lanes 1–6, genomic DNA].