Neuroscience Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Chondroitin sulfate β-1,4-*N*-acetylgalactosaminyltransferase-1 (ChGN-1) polymorphism: Association with progression of multiple sclerosis

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ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form
28 December 2015
Accepted 12 January 2016
Available online xxx

Keywords:
Proteoglycan
Multiple sclerosis
Demyelinating disease
Glycosyltransferase
Glycobiology
Sex differences

ABSTRACT

Chondroitin sulfate proteoglycans (CSPGs) are a constituent of the matrix of the central nervous system (CNS), likely participating as regulatory molecules in the process of demyelination, remyelination, axonal degeneration and regeneration in the CNS. *ChGn-1* is a key enzyme for production of CSPGs and knock-out mice of this gene showed better recovery from spinal cord injury. We hypothesized that the clinical course of multiple sclerosis (MS) is influenced by the level of expression of *ChGn-1* gene. We recruited 147 patients with MS and 181 healthy control subjects and analyzed single nucleotide polymorphisms (SNPs) of this gene. We found the coding SNP (cSNP: rs140161612) in approximately 10% of patients with MS as well as normal controls. The cSNP is changed from serine to leucine at position 126 (p.S126L). The expressed ChGn-1 mutant proteins exhibited no enzyme activities in COS-1 cells. In men, patients who had MS with S126L had a slower disease progression. This cSNP might be associated with the sex differences in clinical course of MS.

1. Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS) that is characterized by inflammation, demyelination, and axonal degeneration (Noseworthy et al., 2000). In Japan, the prevalence rate of MS was estimated to have increased by 4-fold, between 1972 and 2004, to 7.7 per 100,000 persons, with a male-to-female ratio of 1:2.9 (Osoegawa et al., 2009). The precise pathogenetic mechanisms of MS remain to be elucidated. In addition to immunological derangements, there are many known factors, such as metabolic disturbance, trauma, and others, involved in the pathogenesis of MS. Several researchers have reported that genetic factors underlie susceptibility, progression and relapse of MS (Comi et al., 2012; Mowry et al., 2013).

Chondroitin sulfate is a kind of proteoglycan. The chondroitin sulfate chain is present at the surface of almost all cells as well as in the extracellular matrix and covalently binds to core proteins to form chondroitin sulfate proteoglycans (CSPGs) (Koutsoudaki et al., 2010). The chondroitin sulfate chain consists of repeat units of glucuronic acid and *N*-acetylgalactosamine as a basic sugar chain structure and gains structural diversity *via* sulfate modification at its various sites by sulfotransferases with different substrate specificities (Haylock-Jacobs et al., 2011). Studies conducted till date have shown that CSPGs are abundant in the matrix of CNS and that they may play important roles in the formation of the neural network (Kucharova et al., 2011). The blocking effects of CNS regeneration by CSPGs have been reported. The effect of the change in carbohydrate structure of CSPG on the clinical course of experimental autoimmune encephalomyelitis (EAE), a disease model of MS, has recently been reported (Miyamoto et al., 2014). In addition, the association of CSPGs and the diseases in the peripheral

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http://dx.doi.org/10.1016/j.neures.2016.01.002
0168-0102/

nervous system (PNS) has also been reported (Saigoh et al., 2011; Izumikawa et al., 2013). CSPGs were reported to accumulate in remyelinating lesions and remyelination is a critical repair process in demyelinating diseases such as MS (Lau et al., 2012).

Chondroitin sulfate β-1,4-*N*-acetylgalactosaminyltransferase 1 (*ChGn1*) gene encodes a glycosyltransferase involved in the synthesis of chondroitin sulfate. It has recently been reported that *ChGn1* knockout mice recovered from spinal cord injury more completely than wild-type mice (Takeuchi et al., 2013). Therefore, the expression level of *ChGn1* may also influence the clinical course of neurological disorders including MS. We previously reported missense mutations of *ChGn-1* in two patients with neuropathy (Saigoh et al., 2011). Further investigation revealed the presence of another missense mutation of *ChGn-1*, which is more frequently detected but has no association with a certain phenotype. We then examined the clinical course of the patients with MS who had such missense mutation or cSNP.

2. Materials and methods

2.1. Subjects and patient populations

We recruited 147 patients (45 male and 102 female) with MS and 181 healthy control subjects (54 male and 127 female). All cases were of Japanese origin. We collected data on the study participants from our hospital's blood bank and collaborators' blood bank data. This study was approved by the internal review board of the Kinki University Faculty of Medicine, Kyushu University Graduate School of Medicine, and Ehime University Graduate School of Medicine. All patients provided written informed consent before participating in the study.

2.2. MS criteria and classification

The MS criteria used in the study were an age of 18–70 years, a diagnosis of MS according to the revised McDonald criteria (Polman et al., 2005), and a score of 0–5.5 on the Expanded Disability Status Scale (Kappos et al., 2010; Kurtzke, 1983) (EDSS, which ranges from 0 to 10, with higher scores indicating greater disability). The patients with positive anti-AQP4 antibody were excluded from the present study. The progression index was defined as the last EDSS divided by the disease duration.

2.3. Sequence analysis and p.S126L cSNP genotyping

Genomic DNA extraction and genotyping were performed as per standard protocols. Genomic DNA was extracted from whole blood using the QiaAmp Mini DNA Kit (Qiagen, Tokyo, Japan). PCR amplicons generated with oligonucleotide primers were digested from *ChGn-1* gene on the basis of GenBank sequence. We sequenced all exons and their boundaries, using the ABI Prism 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Genotyping of the selected p.S126L cSNP was performed using the 5′ nuclease assay technology for allelic discrimination using fluorogenic TaqMan® probes from Applied Biosystems (Foster City, CA, USA) through the Assay-on-Demand service. We also sequenced using the ABI Prism 3700 DNA analyzer (Applied Biosystems). The PCR amplicons generated using oligonucleotide primers were digested for the *ChGn-1* gene on the basis of the NCBI SNP database.

2.4. Statistical analysis

The relationships between MS and healthy controls were analyzed using the chi-square test and Fisher's exact test. These tests were also used to compare categorical variables appropriately, and odds ratios (ORs) and confidence intervals (CIs) were used for the assessment of risk factors. All *p* values were two tailed, and CIs were set at 95%. Significance was set at $p < 0.05$.

Table 1
The ChGn-1 SNP analysis of multiple sclerosis subtype. Q7

	SNP p.S126L	SNP p.S126S
SNP type		
Healthy controls	(*n* = 15)	(*n* = 166)
Male/female (ratio)	5/10 (1:2.0)	49/117 (1:2.38)
MS patients	(*n* = 13)	(*n* = 134)
Male/female (ratio)	5/8 (1:2.0)	40/94 (1:2.35)
MS subtype		
RRMS (*n* = 116)	4/5	29/76
PPMS (*n* = 16)	0/0	5/11
SPMS (*n* = 16)	1/2	6/8
Unknown (*n* = 41)	0/1	4/35

RRMS, relapsing–remitting MS; PPMS, primary progressive MS; SPMS, secondary progressive.

2.5. Expression of the soluble forms of ChGn-1 and the p.S126L ChGn-1 mutant and enzyme assays

The expression plasmids (6.0 μg each) were transfected into COS-1 cells on 100-mm plates using FuGENE 6 (Roche Molecular Biochemicals, Tokyo, Japan), according to the manufacturer's instructions. At 2 days after transfection, 1 ml of the culture medium was collected and incubated with 10 μl of HIS-Select Cobalt Affinity beads (Sigma, Tokyo, Japan) for 1 h at 4 °C. The beads recovered by centrifugation were washed and then resuspended in the assay buffer described below. GalNAcT transferase activity was assessed using polymer chondroitin (167 μg) as an acceptor. Reaction mixtures were incubated at 37 °C for 1 h, and radiolabeled products were then separated from UDP-[^{3}H]GalNAc by gel filtration using a syringe column, as described previously (Uyama et al., 2002).

2.6. Western blot analysis

After 2 days of culture, the culture medium was collected and incubated with 10 ml of HIS-SelectTMCobalt Affinity beads (Sigma) for 12 h at 4 °C. The beads recovered by centrifugation were washed with Tris-buffered saline (TBS) buffer containing Tween 20 and then resolved on 7.5% SDS-polyacrylamide gels, and proteins were transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was incubated for 1 h with an anti-His antibody (Takara, Otsu, Japan). The antibody was diluted 1:200 with 25 mM TBS. The bound antibody was detected with anti-mouse IgG conjugated with horseradish peroxidase (HRP).

3. Results

3.1. The cSNP analysis of patients with MS and healthy controls

In SNPs analysis of the *ChGn*-1 gene, we found coding p.S126L cSNP (c.377C/T: rs140161612) in patients with MS and healthy in controls. This cSNP frequency is 8.8% (13/147) in MS and 7.9% (15/181) control (Table 1). No significant difference was observed between controls and MS. No significant difference also was observed in the ChGn-1 SNP frequencies among MS subtypes (Table 1).

Please cite this article in press as: Saigoh, K., et al., Chondroitin sulfate β-1,4-*N*-acetylgalactosaminyltransferase-1 (ChGN-1) polymorphism: Association with progression of multiple sclerosis. Neurosci. Res. (2016), http://dx.doi.org/10.1016/j.neures.2016.01.002