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SHORT ORIGINAL COMMUNICATION

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Analysis of the expression level of α-synuclein mRNA using postmortem brain samples from pathologically confirmed cases of multiple system atrophy

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Abstract To determine whether multiple system atrophy (MSA) is associated with altered expression levels of the α-synuclein messenger RNA (mRNA), we performed quantitative reverse transcription polymerase chain reaction for α-synuclein mRNA using postmortem brain samples from 11 cases of MSA and 14 age-matched control subjects. The brain specimens used in this study contained both the gray matter and white matter, which were dissected from the frontal, temporal or occipital lobe. The expression levels of α-synuclein mRNA in the brain specimens of MSA cases were not different from those of the control subjects. These results suggest that the transcriptional regulation of the α-synuclein gene is unlikely to be affected in MSA brains.

Keywords α-Synuclein · Gene expression · Multiple system atrophy · Reverse transcription · Quantitative polymerase chain reaction

Introduction

Multiple system atrophy (MSA) is a sporadic, adult-onset neurodegenerative disease characterized clinically by various combinations of parkinsonism, cerebellar ataxia and autonomic failure [3]. A definite diagnosis of MSA requires pathological confirmation of the presence of characteristic glial cytoplasmic inclusions (GCIs) in the affected structures [8]. GCIs in MSA have been demonstrated to be strongly immunoreactive for antibodies against α-synuclein [11]. Furthermore, it has been shown that accumulation of α-synuclein in the oligodendroglia, as well as in neurons, is a specific cytopathological feature of MSA [10], forming the basis for the current hypothesis that α-synucleinopathy underlies the pathogenesis of MSA.

We recently found that there was no mutation in the entire coding region of the α-synuclein gene in 11 pathologically confirmed cases of MSA [7], indicating that other mechanisms involved in α-synucleinopathy promote the development of MSA. Recently, an increase in total α-synuclein immunoreactivity widely distributed in the cerebral cortex and white matter of MSA cases has been identified [2], suggesting that there is increased expression of α-synuclein messenger RNA (mRNA) in cases of MSA. With this background, we performed a quantitative reverse transcription polymerase chain reaction (PCR) [4] to determine the expression levels of α-synuclein mRNA in the cerebral cortex and cortical white matter of pathologically confirmed cases of MSA.

Materials and methods

Eleven autopsied cases of MSA (aged 56–72 years; mean age 63.2 years), which have been reported previously [7], were examined in this study. Fourteen age-matched autopsied cases (aged 49–77 years; mean age 63.7 years) without any neurological diseases were examined as controls. Brain specimens of MSA cases were taken from the frontal (three cases), temporal (one case) or occipital lobe (seven cases), and those of the control subjects were taken from the frontal lobe. The brain specimens were stored at –80°C prior to RNA extraction. Total RNAs were extracted from 3 g of frozen tissue containing both the gray matter and white matter as previously reported [5]. We did not use brain structures related to neurodegeneration of MSA because these were necessary for neuropathological diagnosis of MSA. We are also concerned that neuronal loss could subsequently reduce the amount of α-synuclein mRNA in those structures, thereby being less informative on the pathogenesis of MSA.

Primers and probes were designed (Table 1) for the TaqMan-PCR technique [4], which has recently been established as a rapid and sensitive technique for quantitation of gene expression. The probes were labeled with FAM and TAMURA at their 5′ and

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Table 1 Sequences and exon localizations of primers and probes (*CNP* 2′,3′-cyclic nucleotide 3′-phosphodiesterase, *UCH-L1* neuron-specific ubiquitin C-terminal hydrolase, ▼ site of the junction of exons)

Primers and probes	Oligonucleotide sequence	Exon	Position on cDNA
α-synuclein-forward	CCAGTTGGGCAAG▼AATGAAGAA	e4–e5	346–367
α-synuclein-reverse	CTTGATACCCTTC▼CTCAGAAGGC	e5–e6	455–433
α-synuclein-probe	AATTCTGGAAGATATGCCTGTGGATCCTGA	e5	385–414
CNP-forward	AGGAGCTGCGACAAT▼TCGT	e1–e2	615–633
CNP-reverse	TTTAACACtATCTTGTTGAGCGT	e2–e3	777–756
CNP-probe	AAGAGACCCCCAGGCGTGCTGCATTGCACA	e2	683–712
UCH-L1-forward	TGAACTTG▼ATGGACGAATGCC	e7–e8	518–539
UCH-L1-reverse	CAGCGTC▼CTTCAGCAGGGT	e8–e9	581–561
UCH-L1-probe	TTTCCGGTGAACCATGGCGCC	e8	541–559

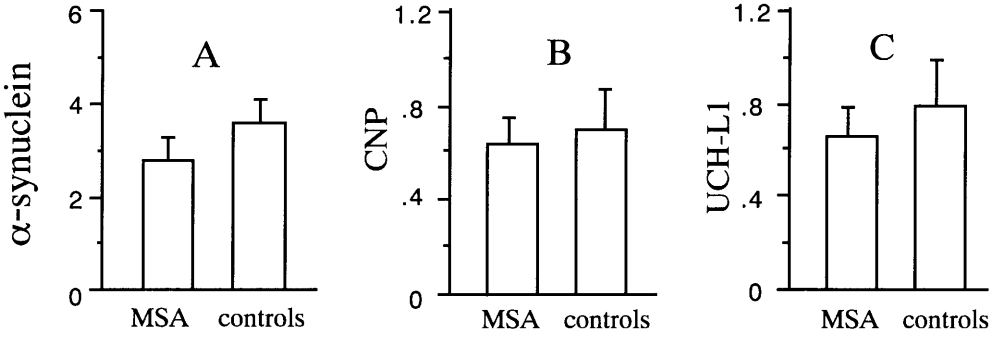

Fig. 1A–C The expression levels of α-synuclein, 2′,3′-cyclic nucleotide 3′-phosphodiesterase (*CNP*) and neuron-specific ubiquitin C-terminal hydrolase (*UCH-L1*, also known as PGP9.5) mRNA in the autopsied brain specimens of multiple system atrophy (*MSA*) (n=11) and of control subjects (n=14). The expression levels of α-synuclein (**A**), CNP (**B**) and UCH-L1 mRNA (**C**) in MSA cases are not different from those in the control subjects. All values are shown as the mean ±SEM

3′ ends, respectively. The primer pairs for α-synuclein were designed based on the published nucleotide sequence of α-synuclein mRNA [9]. To compare the expression levels of the markers for the oligodendroglia and neurons between the MSA cases and the control subjects, probes and primers were also designed based on the published nucleotide sequences of 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) [6] and neuron-specific ubiquitin C-terminal hydrolase (UCH-L1, also known as PGP9.5) [1], respectively. To avoid the inclusion of genomic DNAs in the amplification, the primers were designed to include a junction with exons in the target mRNA.

Reverse transcription (RT) was performed in a reaction volume of 20 μl containing 1 μg each of total RNAs from the specimens, random hexamers and the Superscript preamplification system (Gibco BRL), according to the manufacturer's instructions. Aliquots of 5 μl of the RT products were then subjected to quantitative PCR using a TaqMan PCR core reagent kit (Perkin-Elmer). The reaction mixture contained 1× TaqMan buffer A (containing ROX-labeled passive reference dye), 5.5 mM $MgCl_2$, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 400 μM dUTP, 200 nM of each primer, 100 nM probe, 0.025 U/μl AmpliTaq Gold DNA polymerase, 0.01 U/μl uracil-*N*-glycosylase and 5 μl of RT products in a total volume of 45 μl. To determine the expression levels of the endogenous control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) detection reagents (Perkin-Elmer) were used for the quantitation of GAPDH mRNA. The thermal cycling protocol consisted of one cycle each at 50°C for 2 min and at 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. The thermal cycling and fluorescence detection were performed using an ABI Prism 7700 Sequence detection system (PE Applied Biosystems). The cycle number at which each PCR reached a significant threshold during the log phase of the amplification was used as a quantitative measure of the target product [4]. The value calculated from the threshold for each target mRNA was normalized using those of GAPDH mRNA.

Mann-Whitney's U-test was used to determine the statistical differences in the expression level of mRNA between the two groups. P values less than 0.05 were considered to indicate a significant difference.

Results and discussion

Fig. 1 shows the results of quantitative RT-PCR analysis using the ABI Prism 7700 Sequence detection system. The expression levels of α-synuclein mRNA in the autopsied brain specimens from 11 MSA cases were not different from those in the 14 controls (P=0.14). Moreover, no differences were found in the expression levels of CNP or UCH-L1 mRNA between the two groups (P>0.5).

Given the fact that there is accumulation of α-synuclein in both oligodendroglia and neurons in cases of MSA [10], the expression levels of α-synuclein mRNA in a certain quantity of oligodendroglia and neurons should be evaluated with regard to the pathogenesis of MSA. In this study, the number of oligodendroglia and neurons in the brain specimens were comparable between the MSA cases and the control subjects, because the expression levels of their markers were not different between the two groups. These results suggest that the expression levels of α-synuclein mRNA in the oligodendroglia and neurons are not altered in the brain specimens from MSA cases. However, further quantitation of α-synuclein mRNA in cells isolated from vulnerable regions is needed to elucidate the mechanism involved in the cytopathological feature of MSA [10]. Moreover, the post-translational modi-

fications of α-synuclein should also be studied with regard to the disease process of MSA.

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