

Human Endogenous Retrovirus W and Motor Neurone Disease

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Abstract

Background

Enteroviral infections and mutations of superoxide dismutase 1 gene (SOD 1) have been implicated in the pathogenesis of motor neurone disease. This study was done to determine if sequences of enteroviruses could be detected in muscles, CSF, and plasma of subjects with motor neurone disease, and if there is any change in the expression of SOD 1 gene and human endogenous retrovirus W (HERV W) gene, which modulates SOD 1 gene, in motor neurone disease.

Method

Two muscles, one from the most symptomatic limb and one from the least symptomatic limb, were biopsied from each case, while one muscle was biopsied from each control. Plasma and CSF were collected from cases and controls. Sequences of enteroviruses, SOD 1 and human endogenous retroviral genes were amplified using real-time PCR.

Results

Ten subjects with motor neurone disease and six controls were studied. Mean ages were, 57 years (SD 8, range 46 - 68, median 57) for cases, and 43 years (SD 13, range 24 - 62, median 44) for controls. Sequences of enteroviruses were not detected in muscles of cases and controls using qualitative PCR. Sequences of echovirus types 6

were not detected in muscles, plasma, and CSF of cases and controls, using real time PCR.

For SOD 1 gene, mean levels of transcripts were; 7.0 (SD 0.5, range 6.5 - 7.7, median, 6.9) for the affected limb of cases, 7.3 (SD 1.3, range 4.3 - 8.9, median, 7.5) for the unaffected limbs of cases, and 7.4 (SD 0.5, range 6.8 - 8.0, median, 7.4) for controls. There was no difference in the expression of superoxide dismutase 1 gene between muscles of the affected and unaffected limbs, $P = 0.11$, and between the affected limbs and controls, $P = 0.15$.

For HERV-W env gene, mean level of transcripts were; 15.4 (SD 1.4, range 12.5 - 16.6, median 15.0) for most affected muscles of cases, 16.01 (SD 1.5, range 12.4 - 17.7, median 16.4) for most unaffected muscles of cases, but 16.7 (SD 0.9, range 15.7 - 18.3, median 16.5) for controls. For HERV - W gag gene, mean level of transcripts were; 10.5 (SD 1.3, range 7.9 - 12.2, median 10.4) for the most affected muscles of cases, 11.7 (SD 1.6, range 8 - 13.8, median 11.8) for the most unaffected muscles, but 12.3 (SD 0.7, range 11.5 - 13.1, median 6.9) for controls.

The difference in the level of expression of HERV - W env gene between the affected limbs and unaffected limbs was not significant, $P = 0.06$, but the difference between the affected limbs and control was significant, $P = 0.02$. The difference in the level of expression of HERV - W gag genes between the affected and unaffected limbs was not significant, $P = 0.05$, but between the affected limbs and controls was significant, $P = 0.005$.

Conclusion

The absence of sequences of enteroviruses in muscles, plasma, and CSF of subjects suggests that enteroviruses are not implicated in the pathogenesis of motor neurone disease in this study. Elevated expression of HERV W env and gag genes, which modulate SOD 1 gene, suggests that this human endogenous retrovirus may be involved in pathogenesis, or may be a marker of disease.

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Introduction

Motor neurone disease is a clinical syndrome, which has several variants like amyotrophic lateral sclerosis (ALS), primary lateral sclerosis, progressive bulbar palsy, and spinal muscular atrophy(1, 2). Although several putative agents(3), which include neurotoxins, glutamate toxicity(4), oxidative stress, and trauma(5, 6), have been implicated in the causation of motor neurone disease, mutations of superoxide dismutase 1 gene (SOD 1), which is present in 20 % of familial ALS(3), is the only known definite cause of motor neurone disease. It has been shown that expression of human endogenous retrovirus W (HERV-W) *env* gene product, syncytin, which modulates SOD 1 gene in the placenta, is increased in neurological syndromes(7).

Persistent infection with neurotropic viruses(8), particularly enteroviruses(9, 10), has been suggested as a possible cause of motor neurone disease. Although sequences of enteroviral RNA were demonstrated in neuronal cell bodies of anterior horns of cases of ALS in one study(11), studies that have not demonstrated evidence of enteroviral infections(12, 13) have questioned the role of enteroviruses in motor neurone disease. This study was done to determine if sequences of enteroviral RNA are present in muscles, plasma, and CSF of cases of motor neurone disease, and if there is alteration of expression of SOD 1 and HERV W genes in

Patients and Methods

Cases of motor neurone disease and controls were seen and diagnosed in the Department of Neurology, Karolinska Hospital, Stockholm, Sweden, using the Escorial criteria. Two muscles, one from the limb where symptoms and signs were most severe and one from the limb where symptoms and signs were least severe, were biopsied for each case, while only one muscle was biopsied for each control. CSF and plasma were collected from cases and controls. All samples, which were coded to blind the status of the subjects during analysis, were stored at 70°C.

RNeasy Fibrous Mini kit (Qiagen, Hilde, Germany) was used to extract total RNA from muscles. Briefly 30 mg of muscle was disrupted and homogenized with a high speed rotor homogenizer in 300 μ l of lysis buffer. Inter-sample contamination was avoided by washing the rotor sequentially in water, 1 M HCl, 1 M NaOH, 1 M Tris-HCl, and de-ionised water, between samples. Qiagen Viral RNA Kit was used to extract viral RNA from CSF and plasma. All RNA samples were eluted in 50 μ l of nuclease-free water. Contaminating genomic DNA was removed by treating 250 ng (muscle tissue) or 16 μ l of RNA (CSF and plasma) with DNase I (Invitrogen, Groningen, The Netherlands). Random primers were used to generate cDNA in 40 μ l reaction volume using Superscript II (Invitrogen) reagents.

Table PCR Primer Sequences

Gene	Polarity	Sequence 5' to 3'	Type of PCR
Human GAPDH	Forward	GAA GGT GAA GGT CGG AGT CA	Qualitative
	Reverse	AAT GAA GGG GTC ATT GAT GG	
Enterovirus pan primers	Forward	GTC ACC ATA AGC AGC CAT TG	Qualitative
	Reverse	GCT GTT TCC ACG GCT AAA AG	
Echovirus	Forward	CAA GCA CTT CTG TTT CCC CGG	Real time
	Reverse	ATT GTC ACC ATA AGC GCC A	
HERV-W env	Forward	CCA ATG CAT CAG GGT GGG TAA C	Real time
	Reverse	GAG GTA CCA CAG ACA AAA AAT ATT CCT	
HERV-W gag	Forward	TCA GGT CAA CAA TAG GAT GAC AAC A	Real time
	Reverse	CAA TGA GGG TCT ACA CTG GGA ACT	
Superoxide dismutase 1	Forward	GCGTGGCCTAGCGAGTTATG	Real time
	Reverse	TCCTTCTGCTCGAAATTGATGA	

1 μ M of pan-enterovirus primers (Table), which were directed at the conserved 5' untranslated region of the enterovirus genome, was used in 25 μ l PCR reactions (Titanium Taq cDNA polymerase mix, Clontech, Palo Alt, CA, USA), which contained 1 μ l of cDNA from CSF, plasma, and muscle-tissue. After 40 cycles of 94°C for 30 s and 68°C for 1 min, amplicons were visualized by SYBR Gold staining after electrophoretic separation in 2% agarose gels.

For real-time PCR, primers for transcripts of GAPDH, SOD 1, HERV-W, and Echovirus type 6 (Table) were designed using Primer Express (Applied Biosystems, Foster City, CA, USA). 25 μ l reactions, in triplicates, were run for each transcript using the Platinum SYBR Green qPCR mix, which contain UDG, according to the manufacturer's instructions (Invitrogen) in the following cycling conditions; 50°C for 2 min, 95°C for 2 min followed by 45 cycles of 95°C for 15 s, and 60°C for 30 s in ABI Prism 7000 instrument (Applied Biosystems). Dissociation curves were generated for all assays.

The level of expression of GAPDH was used as endogenous control to compare levels of expression of SOD 1 and HERV W genes. Absolute values of gene expression were determined using $2^{-\Delta\Delta Ct}$ method. Levels of expression of SOD 1 and HERV W *env* and *gag* genes in the affected limbs of subjects were compared with that of the unaffected limbs and of controls using the Mann-Whitney U test.

Results

Ten subjects with motor neurone disease and six controls were studied. Mean ages were, 57 years (SD 8, range 46 - 68, median 57) for cases, and 43 years (SD 13, range 24 - 62, median 44) for controls. Sequences of enteroviruses were not detected in muscles of cases and controls using qualitative PCR. Sequences of echovirus types 6 were not detected in muscles, plasma, and CSF of cases and controls, using real time PCR.

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Discussion

This study did not show evidence of enteroviral infection in muscle, CSF, and plasma of subjects with motor neurone disease. Enteroviral infection was implicated in the pathogenesis of motor neurone disease following the finding of a study(11), which showed sequences of enteroviruses in archival specimens of spinal cord of motor neurone disease subjects. Reservations about a causal role for persistent enteroviral infection and motor neurone disease have been expressed largely because there was no evidence of inflammation in the spinal cords that were studied(14). Although spinal cords were not examined directly in this study absence of sequences of enteroviruses from muscles, CSF, and plasma makes the possibility of enteroviruses in the subjects very remote. Furthermore, the presence of enteroviruses in archival specimens of spinal cord of motor neurone disease subjects has not been replicated(15).

Oxidative stress, which follows toxic gain of function of mutated SOD 1 gene(16), has been

suggested as contributory to or the major mechanism of development of a subset of motor neurone disease(17, 18). In this study levels of expression of SOD 1 gene were comparable in cases and controls. This finding is not surprising since only 2 % of all cases of motor neurone disease have been shown to be related to pathology of SOD 1 gene(3). The function of SOD 1 gene in human placenta has been shown to be modulated by syncytin, the gene product of HERV W *env* gene(19). The level of expression of SOD 1 gene correlates inversely with the level of expression of HERV W *env* gene(19). In this study, however, SOD 1 in muscles does not appear to be modulated by the levels of HERV-W *env* gene.

Elevated levels of expression of HERV-W genes have been reported in multiple sclerosis(20) and schizophrenia(7), but not in motor neurone disease. The finding of elevated transcripts of *env* and *gag* genes in this study, adds motor neurone disease to neurological diseases that have been associated with HERV W. Although it has been suggested that the *env* and *gag* genes of HERV could be pathogenic(21), the role of gene products of HERV W in neurological disease is yet to be clarified. It is possible that it is a non-specific marker of disease. However, since epidemiological studies have associated trauma with motor neurone disease, a pathological process which is linked to muscles may trigger degeneration of anterior horn cells.

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