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# Cerebrospinal fluid detection of enterovirus genome in ALS: A study of 242 patients and 354 controls

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## Abstract

Detection of enterovirus (EV) in the spinal cord of patients with amyotrophic lateral sclerosis (ALS) has been reported on post mortem central nervous system tissues. In cases of persistent infection, it is very likely that the EV genome might be detected in the cerebrospinal fluid (CSF). A study was conducted in seven French amyotrophic lateral sclerosis (ALS) centres between 1997 and 2002. A total of 242 ALS patients and 354 age- and sex-matched controls (non-ALS patients) were enrolled. A sensitive RT-PCR method was performed on the CSF to assess the presence of EV RNA; 14.5% of ALS patients were positive compared to 7.6% of controls ( $\chi^2$  value, 5.31;  $p = 0.02$ ). Although EV infection has a seasonal pattern, we observed no seasonality in positive detection of the EV genome among ALS patients. There was no significant relationship among ALS patients between the initial clinical form or survival and the result of the RT-PCR. These findings suggest a relationship between the presence of EV sequences in CSF and ALS. Our study is consistent with the hypothesis that persistent EV infection can be one of the multiple factors involved in the development of ALS.

**Key words:** *Case control studies, viral infections, amyotrophic lateral sclerosis, cerebrospinal fluid*

## Introduction

Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of upper and lower motor neurons. The aetiology of ALS is still unknown, but different hypotheses have been suggested such as defect of superoxide dismutase gene (1), autoimmunity (2) or neuronal destruction related to glutamate excitotoxicity (3–5). Thus, ALS is a multifactorial disease in which environmental factors (6) may have a role, including viral infections.

Motor neuron disease has been related to picornavirus infections. Among these viruses, poliovirus is responsible for poliomyelitis and the subsequent post-polio syndrome (7). Other picornaviruses of the enterovirus (EV) genus have been involved in acute infections leading to chronic diseases, in conjunction with their ability to develop pseudo-autoimmune response, or persistence in infected cells in a limited number of patients (8,9). These EVs are responsible for mild infections that are mainly aseptic meningi-

tis, with annual epidemics in temperate countries. However, EV is also responsible for severe epidemics with life-threatening diseases (e.g. EV71) (10).

Recent studies have provided *in vitro* data suggesting the development of chronic EV or poliovirus infection in human CNS cells, suggesting that EV can be responsible for persistent infections in the CNS (11,12). In the case of ALS, a persistent virus in the CNS could act as a potential additional causal factor in the development of this disease. This hypothesis is supported by *in vitro* studies showing that EV persistent infection has a major impact on the infected cell, including altered astrocytic glutamate transporter (EAAT-2), mitochondrial MnSOD activity and resistance to oxidative stress (4,5). These factors have been reported in ALS patients (13).

Recent post mortem studies assessing a relationship between ALS and EV infection showed contradictory results using various kinds of tissues (brain, spinal cord, fixed or frozen material) (14–17). Collection of post mortem spinal cord tissue is of limited access. In addition, viral detection in such material can be impaired due to tissue necrosis. CSF is the best surrogate for spinal cord tissue for the detection of an infectious agent putatively involved in a neurological disorder. Moreover, CSF sampling can be easily performed *in vivo* at the time of the diagnosis. To better determine the role of EV infection in ALS, we therefore performed a French multicentre study aimed at detecting by RT-PCR the presence of EV RNA in the CSF from a large cohort of ALS patients and controls.

## Patients and methods

### *ALS patients and controls*

CSF sampling in ALS cases was performed as part of the diagnosis procedure. Patients and controls were informed that further research analysis could be performed on the CSF sample. The consent form specified that research used molecular genetics and could take several years before results might be available and that all information regarding the patient was handled anonymously.

The following clinical parameters were recorded for each patient: age, sex, initial clinical presentation, duration between onset and, respectively – diagnosis, CSF sampling and death, sporadic or familial form. The familial forms presented with two or three ALS cases in the same family (within relatives of first and second degree). All these patients were negative for SOD1 mutation.

CSF specimens were collected from ALS patients between 1997 and 2002 according to the revised El Escorial criteria (18). All patients were admitted for hospitalization in one of the seven participating ALS centres representing the main geographical areas of France. Patients' inclusion was considered on the basis of possible or suspected ALS when addressed

to the centre. The final diagnosis was further confirmed by long-term follow-up. Thereafter, only patients with probable or certain ALS diagnosis were retained and, thus, patients whose diagnosis was not confirmed were excluded from the initial group of ALS patients. These non-ALS patients corresponded mostly to motor neuropathy with conduction block, pure lower motor neuron syndrome, spinal muscular atrophy or other peripheral neuropathies. These patients were subsequently included in the control group. From the initial cohort of 318 patients, 242 were confirmed as ALS patients with probable or certain diagnosis, at long-term follow-up.

The control group (non-ALS patients) included: acute headache with normal lumbar puncture, hydrocephalus, brain tumour, central nervous system traumatism with loss of consciousness, cerebral haemorrhage (complication of chronic hypertension or vascular malformation with hospitalization in critical care or neurosurgical unit, without diagnosis of haemorrhagic encephalitis) and non-ALS peripheral neuropathies. Exclusion criteria were infectious, neurodegenerative and central inflammatory diseases (such as multiple sclerosis). The control group had well established diagnosis, without any borderline forms with ALS. We excluded neurodegenerative diseases to avoid confounding factors such as borderline forms and associated forms between ALS and extrapyramidal syndrome or dementia. Control CSF samples were all collected in Lyon at the Pierre Wertheimer Neurological Hospital since no geographical differences in EV infection frequency have been reported in France (19). CSF sampling was peri-operative for the brain tumour control group. Furthermore, patients' and controls' CSF samples were collected with the same seasonal distribution regarding the epidemiological features of EV infections (from April to October) (19).

After lumbar puncture, the CSF was collected in a sterile tube prepared specifically for that purpose. CSF samples for ALS patients and controls were frozen at  $-20^{\circ}\text{C}$  and then sent in dry ice to the Laboratory of Virology of Lyon University for further analysis as soon as they were obtained. Patients' and controls' CSF specimens were then handled anonymously. At the Laboratory of Virology, they were kept at  $-80^{\circ}\text{C}$  until they were analysed. All of the CSF samples (ALS and controls) were defrosted only once for analysis. Three hundred and fifty-four controls collected from August 2003 to July 2004 were tested for EV within the year. ALS CSF samples were collected from 1997 to 2002 and concurrently analysed with controls (ALS and controls CSF were tested within the same RT-PCR runs after a blinding procedure) from July to November 2004. The RT-PCR method used for this study corresponded to the diagnosis procedure current at this time in the Laboratory of Virology of Lyon University. All of the RT-PCR tests were specifically performed by two technicians of the laboratory.

## RNA extraction and RT-PCR

RNA was extracted using the Tri-reagent method (Sigma Aldrich, France) according to the manufacturer's instructions. Each air-dried RNA pellet was reconstituted in a reaction mixture containing 1  $\mu$ M of EV specific primer RT-EV (5'-CACT/CGGATG-GCC-3', position 760 to 771) for reverse transcription. The cDNA was then subjected to PCR with primers that recognize the 5' non-translated region of all known EV: EV-NC1 (5'-CTCCGGCCCCCT-GAATGCG-3', position 572-590) and EV3 (5'-ATTGTCACCATAA GCAGCCA-3', position 707-726) as previously described (19). Ten  $\mu$ l of the PCR products was subjected to electrophoresis in an agarose gel to observe a band of 154 bp consistent with EV. The RT-PCR assay we used was efficient in detecting viral RNA in the CSF because of a high sensitivity estimated at 3-10 RNA copies by sample (20). The sensitivity and specificity of this technique has been favourably evaluated in the various QCMD programs.

Control of positive detection was systematically carried out. A second extraction of the CSF was performed, and a new RT-PCR run was carried out using the same procedure. Discrepant results were controlled a third time. Hence, a positive result consisted of two positive RT-PCR reactions obtained from two totally independent RT-PCR tests carried out on two independent RNA extractions from the same CSF.

To avoid putative contamination by carry-over of amplicons, the extraction, sample mix, PCR and gels were performed in different technical rooms. Moreover, one negative control was tested every five specimens, and a run was considered as non-interpretable if one negative control was positive.

## Statistical analysis

Student's *t*-tests for continuous covariates and Pearson's  $\chi^2$  tests were used to compare the characteristics between controls and ALS patients, and a Pearson's  $\chi^2$  test was used to compare the proportion of patients being positive for CSF EV RT-PCR. A second test,

Fisher's exact test for  $m \times 2$  contingency table was used to test the difference in EV RNA detection between the  $m$  control groups for more accuracy in small groups. Log-rank tests were used to study the survival of ALS patients according to initial clinical form. Differences were considered as significant when  $p < 0.05$ . Data analyses were made using R version 2.6.1 (The R Foundation for Statistical Computing).

## Results

Among the 242 patients with confirmed ALS, 156 were males and 86 females. Of these patients, 231 were deceased at the time of final study analysis. The initial form was spinal in 174 patients (99, lower limbs; 75, upper limbs), bulbar in 63 patients and undetermined in five patients. Fifteen patients presented with a familial form, 227 were sporadic. Mean age at diagnosis was  $58.9 \pm 12.4$  (SD) years and mean duration from onset to diagnosis was  $12.9 \pm 19.1$  months and from onset to CSF sampling was  $10.5 \pm 18.3$  months. Mean age at CSF sampling was  $58.4$  years  $\pm 12.2$  years. Mean duration from onset to death was  $38.8 \pm 29.4$  months in the 231 deceased patients. Mean survival time was  $50.8 \pm 7.6$  months for spinal lower limbs forms,  $54.2 \pm 5.7$  months for spinal upper limbs forms and  $33.5 \pm 2.4$  months for bulbar forms. There was a significant difference in survival between spinal and bulbar initial forms ( $p = 0.01$ , log-rank test).

CSF from 354 controls was collected (212 males/142 females; mean age  $56.6 \pm 15.8$  years). There was no statistical significance between group differences for age and sex (*t*-test). CSF parameters (protein level and cellular count) were within normal range for ALS patients and the control population.

35/242 (14.5%) ALS patients were positive for CSF EV RT-PCR, compared to 27/354 (7.6%) controls ( $\chi^2$  value, 5.31;  $p = 0.02$ ). There was no significant relationship among ALS patients between the initial clinical form, age or sex and the result of the RT-PCR (Table I). None of the familial ALS forms was positive for EV RT-PCR. Positive or negative RT-PCR results had no significant effect on ALS patients' survival (Table I).

Table I. Clinical profile of ALS patients according to RT-PCR results.

ALS	RT-PCR+ 35/242 cases	RT-PCR- 207/242 cases	<i>p</i> -value
Mean age $\pm$ SD (years) at CSF sampling	$58.7 \pm 15.42$	$58.40 \pm 15.22$	0.64
Sex ratio (M/F)	23/12	133/74	1.00
Initial ALS form*:			
No. of spinal lower limbs onset	14	85	0.38
No. of spinal upper limbs onset	8	67	0.38
No. of bulbar onset	12	51	0.47
Mean disease duration $\pm$ SD (months):			
from onset to diagnosis	$13.88 \pm 31.67$	$9.97 \pm 14.83$	0.48
from onset to death	$36.53 \pm 4.11$	$29.40 \pm 1.75$	0.58

SD, standard deviation; M, male; F, female; No., number.

\*The initial form was undetermined in five patients (one patient in the RT-PCR+ group, four patients in the RT-PCR- group).

Table II. Enterovirus genome detection among the different control subgroups.

CSF controls	RT-PCR+
Acute headache	4/38 (10.5%)
Hydrocephalus	4/44 (9.1%)
Brain tumour	6/90 (6.7%)
Central nervous system traumatism with loss of consciousness	5/57 (8.8%)
Cerebral haemorrhage	3/58 (5.2%)
Non-ALS peripheral neuropathies	5/68 (7.3%)
Total	27/354 (7.6%)

Table II presents the number and percentage of positive EV genome detection among the different control subgroups. There was no statistical difference in EV RNA detection between the different control groups ( $p = 0.91$ , Fisher's exact test).

Figure 1 shows the seasonal distribution of the percentage of positive EV detection in ALS patients and in controls. This demonstrates that the percentage of positive CSF samples is higher in ALS whatever the season and is independent from a seasonal effect. Each positive EV RT-PCR in a CSF sample has been checked twice. Thus, all of the 63 initial positive EV detections (ALS patients and controls) were reanalysed: 6.3% of them ( $n = 4$  CSF samples corresponding to one ALS patient and three controls) showed discrepant results (negative result after reanalysis). They were thus tested a third time. Finally, only one initially positive result for a control was considered as negative because of two successive negative reanalyses (1.6%), giving a total of 62 positives.

## Discussion

The major finding of this study is a statistically significant higher detection of EV sequences in CSF of ALS patients compared to controls.

One of the most important issues in this study is the accuracy of the clinical diagnosis of ALS. To this end, we decided to perform a long-term follow-up (five years or more), which allowed us to more precisely assess the diagnosis of probable or certain ALS according to the El Escorial criteria (19). The epidemiological and clinical profiles of our ALS patients were comparable with those of the general ALS population data available in the literature.

EVs are endemic worldwide. They are present in temperate climates at low levels in winter and spring but can be responsible for epidemics in summer and autumn. Consistent with these data, our surveillance in France showed a seasonal pattern of distribution, with the circulation of EV peaking in summer and slowly decreasing in the early autumn. This refers to the years 2000–2004 (20). This reported pattern is identical to the survey records from the years 1997–2000 and 2004–2007 (Lina, unpublished data). With reference to this pattern of circulation of EV, our study did not show a seasonal effect in the positive detection of RNA sequences both in ALS and in control populations.

The EV generic RT-PCR we used for this study detects the highly conserved 5' NTR region of the viral genome. This conserved region cannot be used to identify the EV serotype detected. With this technique we detected EV RNA sequences in 14.5% of proven ALS cases. Conversely, 7.6% of positive RNA detection was also observed in the CSF of controls.

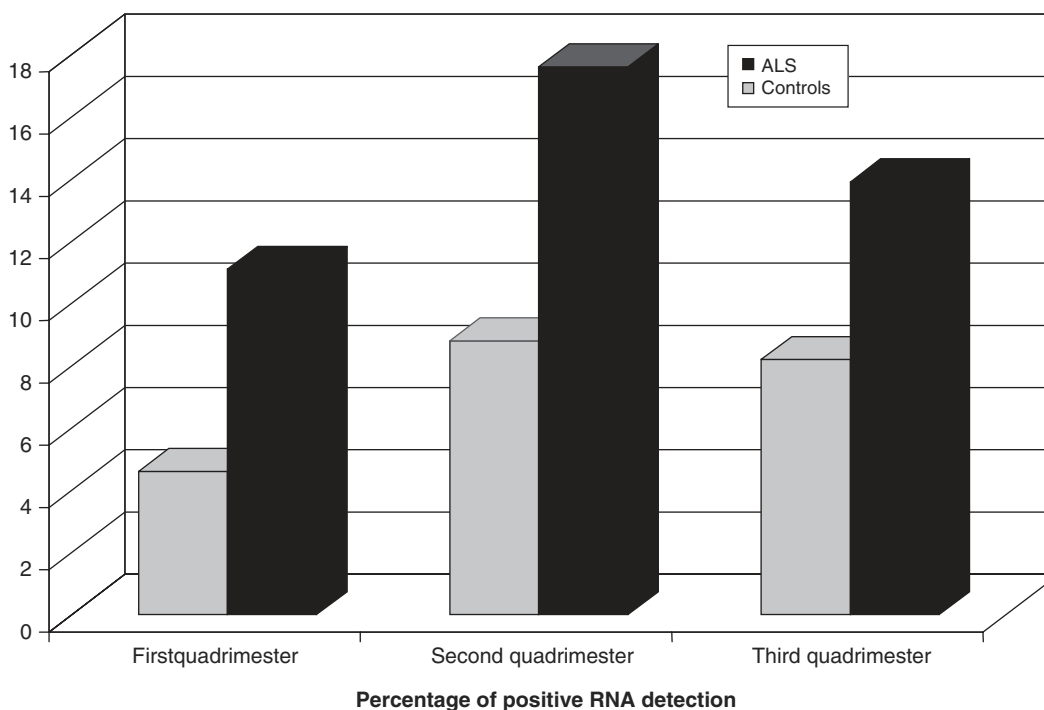


Figure 1. Positive EV RNA genome detection per quadrimester.

This was much higher than expected. This positive detection in both groups was not linked to any obvious acute ongoing infection of the CNS. This high level of detection in the control group may have at least two explanations.

First, although EVs are among the most common viruses found in humans with a high tropism for the CNS, they can be responsible for asymptomatic infections. Their detection could reflect asymptomatic infections in the CSF of patients.

Secondly, carry-over of PCR products can result in contamination of the specimens. This contamination cannot be ruled out in the present study. In the control patients with a positive detection, only a small amount of PCR product was detected; this amount of product precluded sequencing that would have been very informative to assess for/against contamination. Since no real-time RT-PCR has been conducted, we could not compare the viral load between ALS and control CSFs – this would also have been very informative. Overall, further research with the use of real-time PCR with typing capacities is needed to clarify this point.

As stressed above, EVs are known for their high tropism for motor neurons (i.e. poliovirus, enterovirus 71). Recent data confirm that these viruses can be responsible for chronic disorders whose mechanisms remain to be deciphered (21). Regarding motor neuron diseases, several studies found poliovirus in CSF of patients with post-polio myelitis syndrome suggesting the virus's ability to initiate persistent infection of the CNS (22). This result was supported by recent studies using permissive transgenic mice, mixed brain cultures and neuron cultures that showed that chronic poliovirus infection can induce cell-specific effects with subsequent neuronal apoptosis (22).

In ALS, several studies with EV-specific RT-PCR were performed on post mortem CNS tissues (14–17). Some demonstrated a high rate of detection of EV in ALS cases compared to controls, but not others. The retrospective testing of archived tissues, the different techniques used for collection, fixation, storage, tissue sampling, RT-PCR and the lack of internal controls could explain differences in the results reported. In one of our previous studies, echovirus 6 related sequences (a subtype of EV) were detected in spinal cord of ALS patients (5). To support a putative EV chronic infection, we subsequently developed an in vitro model of echovirus 6 chronic infection in a human glial precursor cell line (12). In this cell line, echovirus 6 chronic infection was responsible for a reduction of glutamate uptake by the cell due to a lack of EAAT2 protein expression, as described in ALS patients (5). Hence, there is some evidence for a putative link between EV chronic infection and the glutamate-mediated excitotoxicity for motor neurons observed during ALS. Furthermore, in our in vitro study, the cells chronically infected by an EV had a dramatic down-regulation

of the expression of mitochondrial manganese superoxide dismutase (mnSOD), leading to vulnerability to oxidative stress. This vulnerability resulted in progressive cell death, analogous to that observed in neurodegenerative diseases.

The present findings do not allow to test in daily practice patients' susceptibility to develop ALS by assessing CSF samples for EV, because of the relatively high percentage of positive findings in controls. Ideally, combined detection of EV in spinal cord and CSF samples in the same patient is required. This was not applicable in this study. Nevertheless, our study suggests that the presence of EV RNA in the CSF of ALS patients may be one factor, among others, of this illness. Similarly, when considering genetic factors in ALS, specific mutations are found only in a small number of familial and sporadic cases (24–26). The small number of familial ALS patients does not allow us to draw conclusions on the EV RT-PCR results in this specific group.

EV infection gives sensibility to the pathology in a subgroup of patients in our study, with characteristics that still need to be determined. In our study, we have not found clinical particularities in the positive EV genome detection group as initial clinical presentation, duration of disease between onset and diagnosis or between first sign and death. We did not look for other factors such as lower versus upper motor neuron involvement. At the present time, even though our findings are consistent with an environmental factor that is distinct from the genetic causes already mentioned, the aetiology of ALS still remains undetermined in the majority of cases. Such observation can be drawn in other neurodegenerative diseases such as Parkinson's disease.

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**Declaration of interest:** The authors report no conflicts of interest.

## References

1. Cluskey S, Ramsden DB. Mechanisms of neurodegeneration in amyotrophic lateral sclerosis. *Mol Pathol.* 2001;54: 386–92.

2. Niebroj-Dobosz I, Jamrozik Z, Janik P, Hausmanowa-Petrusewicz I, Kwieciński H. Anti-neural antibodies in serum and cerebrospinal fluid of amyotrophic lateral sclerosis patients. *Acta Neurol Scand.* 1999;100:238–43.
3. van Damme P, Dewil M, Robberecht W, van den Bosch L. Excitotoxicity and amyotrophic lateral sclerosis. *Neurodegener Dis.* 2005;2:147–59.
4. Berger MM, Jia XY, Legay V, Aymard M, Tilles JG, Lina B. Nutrition- and virus-induced stress represses the expression of manganese superoxide dismutase in vitro. *Exp Biol Med.* 2004;229:843–9.
5. Legay V, Deleage C, Beaulieux F, Giraudon P, Aymard M, Lina B. Impaired glutamate uptake and EEAT2 down-regulation in an enterovirus chronically infected human glial cell line. *Eur J Neurosci.* 2003;17:1820–8.
6. Sutedja NA, Veldink JH, Fischer K, Kromhout H, Heederik D, Huisman MH, et al. Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. *Amyotroph Lateral Scler.* 2008;8:1–20.
7. Mulder DW, Rosenbaum RA, Layton DD Jr. Late progression of poliomyelitis or forme fruste amyotrophic lateral sclerosis? *Mayo Clin Proc.* 1972;47:756–61.
8. Roivainen M. Enteroviruses: new findings on the role of enteroviruses in type 1 diabetes. *Int J Biochem Cell Biol.* 2006;38:721–5.
9. Andréoletti L, Ventéo L, Douche-Aourik F, Canas F, Lorin de la Grandmaison G, Jacques J, et al. Active Coxsackieviral B infection is associated with disruption of dystrophin in endomyocardial tissue of patients who died suddenly of acute myocardial infarction. *J Am Coll Cardiol.* 2007;50:2207–14.
10. Chang LY, Huang LM, Gau SS, Wu YY, Hsia SH, Fan TY, et al. Neurodevelopment and cognition in children after enterovirus 71 infection. *N Engl J Med.* 2007;356:1226–34.
11. Destombes J, Couderc T, Thiesson D, Girard S, Wilt SG, Blondel B. Persistent poliovirus infection in mouse motor neurons. *J Virol.* 1997;71:1621–8.
12. Beaulieux F, Zreik Y, Deleage C, Sauvinet V, Legay V, Giraudon P, et al. Cumulative mutations in the genome of echovirus 6 during establishment of a chronic infection in precursors of glial cells. *Virus Genes.* 2005;30:103–12.
13. Liu R, Li B, Flanagan SW, Oberley LW, Gozal D, Qiu M. Increased mitochondrial antioxidative activity or decreased oxygen free radical propagation prevents mutant SOD1-mediated motor neuron cell death and increases amyotrophic lateral sclerosis-like transgenic mouse survival. *J Neurochem.* 2002;80:488–500.
14. Muir P, Nicholson F, Spencer GT, Ajetonmobi JF, Starkey WG, Khan M, et al. Enterovirus infection of the central nervous system of humans: lack of association with chronic neurological disease. *J Gen Virol.* 1996;77:1469–76.
15. Berger MM, Kopp N, Vital C, Redl B, Aymard M, Lina B. Detection and cellular localization of enterovirus RNA sequences in spinal cord of patients with ALS. *Neurology.* 2000;54:20–5.
16. Walker M, Schlaberg R, Hays AP, Bowser R, Lipkin WI. Absence of echovirus sequences in brain and spinal cord of amyotrophic lateral sclerosis patients. *Ann Neurol.* 2001;49:249–53.
17. Nix WA, Berger MM, Oberste MS, Brooks BR, Mckennayasek DM, Brown RH, et al. Failure to detect enterovirus in the spinal cord of ALS patients using a sensitive RT-PCR method. *Neurology.* 2004;62:1372–7.
18. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial Revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord.* 2000;1:293–9.
19. Lina B, Pozzetto B, Andreoletti L, Beguier E, Bourlet T, Dussaix E, et al. Multicentre evaluation of a commercially available PCR assay in a panel of cerebrospinal fluid specimens. *J Clin Microbiol.* 1996;34:3002–6.
20. Antona D, Lévêque N, Chomel JJ, Dubrou S, Lévy-Bruhl D, Lina B. Surveillance of enteroviruses in France 2000–2004. *Eur J Clin Microbiol Infect Dis.* 2007;26:403–12.
21. Chapman NM, Kim KS. Persistent Coxsackievirus infection: enterovirus persistence in chronic myocarditis and dilated cardiomyopathy. *Curr Top Microbiol Immunol.* 2008;323:275–92.
22. Pallansch MA, Roos RP. Enteroviruses: polioviruses, Coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, editors. *Fields virology*. Philadelphia: Lippincott Williams and Wilkins; 2001. pp. 723–75.
23. Daley JK, Gechman LA, Skipworth J, Rall GF. Poliovirus replication and spread in primary neuron cultures. *Virology.* 2005;340:10–20.
24. Schymick JC, Talbot K, Traynor BJ. Genetics of sporadic amyotrophic lateral sclerosis. *Hum Mol Genet.* 2007;16:233–42.
25. Liscic RM, Grinberg LT, Zidar J, Gitcho MA, Cairns NJ. ALS and FTLD: two faces of TDP-43 proteinopathy. *Eur J Neurol.* 2008;15:772–80.
26. Valdmanis PN, Rouleau GA. Genetics of familial amyotrophic lateral sclerosis. *Neurology.* 2008;70:144–52.