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Short communication

Detection of Borna disease virus genome in normal human brain tissue

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Abstract

Borna disease virus (BDV), a neurotropic virus naturally infecting horses and sheep, has been suggested to be associated with human psychiatric disorders. Thus far no extensive studies have been done, providing the evidence of BDV genome in normal human brain tissue. We therefore examined four brain regions of 30 normal autopsy brains for BDV p24 genome. By highly sensitive nested reverse transcriptase (RT)-mediated PCR analysis, we found positive PCR products in two brains: one in frontal and temporal cortices and hippocampus and another in frontal cortex and olfactory bulb. Our results suggest that BDV can infect human brain tissue latently, without causing an apparent neuropsychiatric disorder. © 1997 Elsevier Science B.V.

Keywords: Borna disease virus; p24; Nested RT-PCR; Brain; Olfactory bulb; Schizophrenia; Mood disorder

Borna disease virus (BDV) is a neurotropic virus naturally infecting horses and sheep (for a review, see [15]). A large body of serological evidence has suggested that infection with BDV or a related agent may be associated with psychiatric disorders, including major depression and schizophrenia [1–4,9,13,14,17,18]. Recently RNA specific to BDV p24 and p40 genome has been detected in peripheral blood mononuclear cells from both normal and psychiatric individuals, by use of a highly sensitive nested reverse transcriptase-polymerase chain reaction (RT-PCR) method [5,11]. Here again, the prevalence of BDV p24 and p40 RNA was higher in psychiatric patients than in controls. Infectious human BDV has been isolated from blood cells of three patients with mood disorders [6]. These data implicate that BDV or a related agent infects human blood cells and the agent might be associated with some portions of patients with psychiatric disorders. Very recently, de la Torre and colleagues reported for the first time the presence of BDV genome in human brain tissue: hippocampi of three patients with hippocampal sclerosis harbored BDV p40 RNA [8]. Based on their finding of the lack of PCR products in hippocampal tissue of patients with Alzheimer's disease and normal controls, they suggested the causal association of BDV with this disorder. Another study has also failed to detect BDV genome in a limited number of control cases [16]. No study has so far been done looking for BDV genome in defined brain regions of a relatively large number of normal autopsy controls. BDV is highly neurotropic and it causes a latent infection in animal brains [12]. We therefore attempted to search for the footprint of BDV p24 genome in four regions of 30 postmortem brains by the sensitive nested RT-PCR method.

Tissue samples from frontal and temporal cortices, hippocampus and olfactory bulb were taken from 30 cadavers at autopsy which was performed at the Tokyo Metropolitan Medical Examiner's Office. The present study was approved by the research committee of the Medical Examiner's Office. The postmortem time was between 6 and 31 h, and the mean age at death of the individuals was 54 years, ranging from one year to 88 years (see Table 1). Dissected tissues were kept frozen at −80°C until used. RNA extraction was carried out according to the acid guanidinium thiocyanate–phenol–chloroform (AGPC) method [7]. The quality of all the RNA samples was monitored by amplification of human β-actin mRNA by

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Table 1
Detection of BDV p24 genome in human brain

No.	Case	Sex	Age	F	T	H	O
1	TIP001	M	56	−	−	−	−
2	TIP002	M	48	−	−	−	−
3	TIP003	F	25	−	−	−	−
4	TIP004	M	64	−	−	−	−
5	TIP005	F	24	−	−	−	−
6	TIP006	M	26	−	−	−	−
7	TIP007	M	61	−	−	−	−
8	TIP008	M	60	−	−	−	−
9	TIP009	M	33	−	−	−	−
10	TIP010	M	52	−	−	−	−
11	TIP011	F	79	−	−	−	−
12	TIP012	F	88	−	−	−	−
13	TIP013	M	44	−	−	−	−
14	TIP014	M	44	−	−	−	−
15	TIP015	M	1	−	−	−	−
16	TIP016	M	64	−	−	−	−
17	TIP018	M	45	−	−	−	−
18	TIP019	M	60	−	−	−	−
19	TIP020	M	51	−	−	−	−
20	TIP031	F	70	−	−	−	−
21	TIP032	F	47	−	−	−	−
22	TIP033	M	56	+	+ +	+	−
23	TIP034	M	60	−	−	−	−
24	TIP035	M	50	+ +	−	−	+
25	TIP036	F	74	−	−	−	−
26	TIP037	M	69	−	−	−	−
27	TIP038	M	67	−	−	−	−
28	TIP043	M	31	−	−	−	−
29	TIP044	M	67	−	−	−	−
30	TIP045	M	68	−	−	−	−

Intensity of PCR product staining: (+ +) strong; (+) moderate; (−) none.

using a primer set of 5′-TCTACAATGAGCTGCGTGTG-3′ and 5′-TACATGGCTGGGGTGTTGAA-3′. The nested RT-PCR analysis for BDV p24 RNA was done according to the procedure described elsewhere [11], with a modification. The Gene Amp RNA PCR kit (The Perkin-Elmer Corporation, Foster City, CA) was used instead of the EZ rTth RNA PCR kit (The Perkin-Elmer Corporation). The first primer set was 5′-TGACCCAACCAGTAGACCA-3′ and 5′-GTCCCATTCATCCGTTGTC-3′, and the second nested set was 5′-TCAGACCCAGACCAGCGAA-3′ and 5′-AGCTGGGGATAAATGCGCG-3′ as described [11]. The RNA extracted from the MDCK cells persistently infected with BDV (strain He/80-2) [10], provided by Prof. R. Rott of the University of Giessen, Germany, served as the positive control.

We first checked the quality of RNA samples prepared from brains of cadavers with varying postmortem intervals. Although ribosomal RNAs were disintegrated in some degree as revealed by agarose–formaldehyde gel electrophoresis and ethidium bromide staining (data not shown), all the samples gave the positive PCR products by amplification of β-actin mRNA (Fig. 1). The positive PCR products for BDV p24 with the expected size of 374 bp

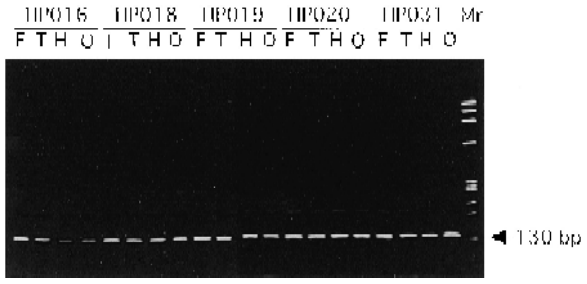

Fig. 1. Amplification of β-actin mRNA by RT-PCR with the RNA extracted from postmortem human brains. The result of 20 samples are shown here, representatively. F, frontal cortex; T, temporal cortex; H, hippocampus; O, olfactory bulb; Mr, DNA marker. Arrow indicates PCR products with an expected size of 130 bp.

were obtained in two out of 30 cases examined (Table 1). They were found with RNA extracted from the frontal and temporal cortices and hippocampus in one case (TIP033), and with RNA from the frontal cortex and olfactory bulb in another case (TIP035) (Fig. 2). The specificity of PCR products was verified by direct DNA sequencing. The PCR products from frontal cortex (TIP033) and olfactory bulb (TIP035) showed the homologous sequence to that of the corresponding portion of p24 RNA of BDV (strain He/80-2). A retrospective survey revealed that both the subjects died of myocardial infarction, and no neuropsychiatric problems were recorded.

Our results are of value in three respects. Firstly, they indicate for the first time that BDV genome can be found in normal human autopsy brain tissues. The question of whether complete BDV RNA is present in the brain awaits further studies using the similar method for BDV genomes other than p24. The examination for p40, a nucleoprotein of BDV, is currently underway in the same samples. de la Torre et al. have found BDV p40 RNA in hippocampus of patients with hippocampal sclerosis and no PCR product was detected in this tissue from patients with Alzheimer's disease and normal controls. Since the number of controls was not mentioned in that report and since they examined

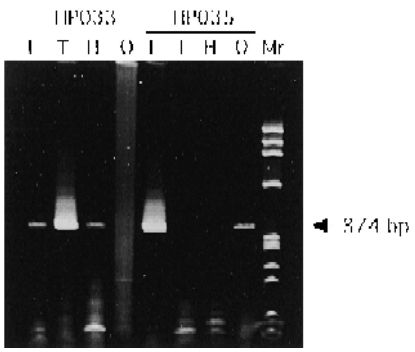

Fig. 2. Nested RT-PCR analysis for BDV p24 RNA. The results of two positive cases (TIP033 and 035) are shown. F, frontal cortex; T, temporal cortex; H, hippocampus; O, olfactory bulb; Mr, DNA marker. Arrow indicates PCR products with an expected size of 374 bp.

BDV p40 RNA instead of p24 RNA, it is impossible to compare their results with ours. If we confine our results to hippocampus alone, then the prevalence of positivity for BDV p24 genome in normal brain is 3.3% (1/30).

Secondly, our results show that not all four brain regions harbor the fragments of BDV p24 RNA in a single 'positive' individual. For example (TIP035), we could not detect the PCR products in hippocampus and temporal cortex, while the positive products were obtained with RNA extracted from frontal cortex and olfactory bulb. Given the huge volume of human brain, the caution should be kept in mind that a sampling error could occur when one studies BDV infection in human brain by the method used here.

Finally, the finding that BDV p24 RNA was detected in the human olfactory bulb was of particular interest. In infected horses, nasal secretions contain infectious agent [19], and it is generally suspected that natural BDV infection, either apparent or inapparent, may be mediated through the nasal root. The presence of viral genome in human olfactory bulb might suggest that the nasal root of transmission of BDV or a related agent may occur in human beings. Further extended examination with this highly sensitive nested RT-PCR of human autopsy brain tissue from both controls and psychiatric patients may help clarify the problem of a possible contribution of BDV to the pathogenesis of certain mental disorders.

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