

The neurological and behavioural consequences of virus infection in humans are well recognised and neurotropic viruses have been suggested as the cause of certain psychiatric conditions. Here, Richard Collins reviews the association, first postulated in 1985, between a virus disease named after a town in Germany and mental illness.

# Borna disease virus and mental illness

## In search of the missing link

**Some 20 years ago infection with a rare neurotropic virus was linked with psychiatric illness.<sup>1</sup> Since then, evidence has accumulated both for and against this controversial theory. So, what is the current status of research in this area and can mental illness really be infectious?**

The idea that mental illness has an infectious component has a long history in scientific circles. From about the middle of the 19th century, the infectious nature of 'insanity' was considered highly likely by many authors.<sup>2</sup> However, little progress in understanding the biological causes of mental disease was made as the psychoanalytical school of theorists, dominated by Sigmund Freud, considered that serious mental illness was a manifestation of childhood psychological trauma. The other major psychological theorist of the early 20th century, Carl Gustav Jung, was interested in the possibility that an unknown 'brain toxin' might be the cause of schizophrenia.

The continued study of slow (eg measles, rubella, HIV-1) and latent (eg herpes simplex, varicella, Epstein-Barr) virus infections has permitted their long-term effect on the central nervous system (CNS) to be determined. Many studies have examined the role of these viruses in the onset and progression of various psychiatric illnesses and the findings have been mixed.<sup>1</sup>

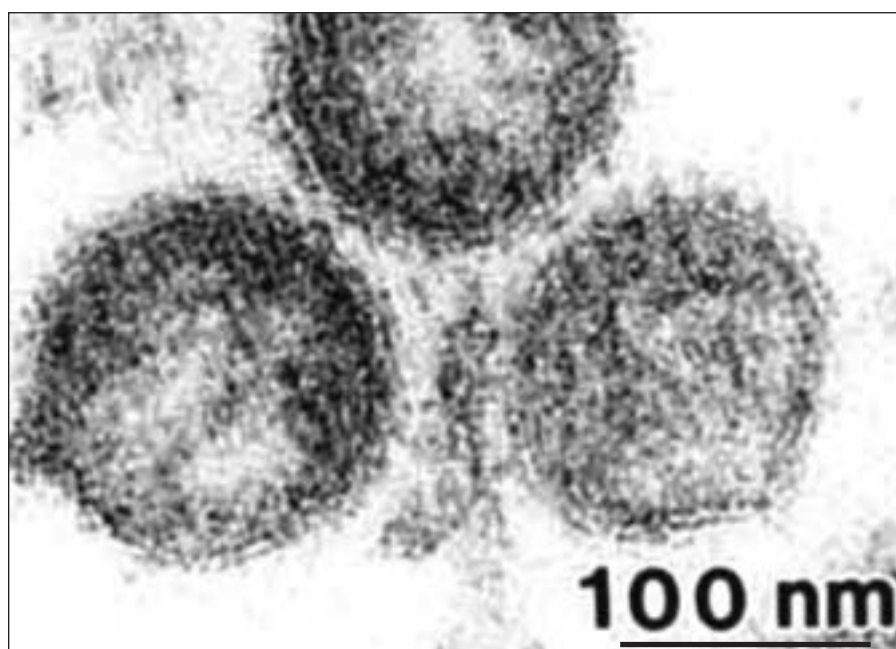
The neurological and behavioural consequences of virus infection in humans are well recognised. They can be seen in their gross form in rabies and in the dementia associated with acquired immune deficiency syndrome (AIDS), where abnormal behaviour and confusion are common symptoms. Therefore, it should come as no surprise that infection with neurotropic

viruses has been postulated as the cause of certain psychiatric illnesses.

### Borna disease virus

Borna disease virus (BDV) is named after the town of Borna, about 50 km south of Leipzig in central eastern Germany. A disease characteristic of Borna disease has been described in Germany since the early 1800s; however, it gained wider prominence after an epidemic in horses that was associated with significant neurological manifestations occurred near the town in 1885.

Borna disease virus is a spherical, enveloped virus about 90–130 nm in diameter (Fig 1). The viral genome comprises a linear, non-segmented, negative-sense, single-stranded RNA approximately 8900 nucleotides in length. Comparison of the complete genome of BDV isolates obtained at different times and from different species indicates that they are very highly conserved (Fig 2). The virus produces at least six major proteins (Table 1), is highly neurotropic and reproduces in the CNS, causing severe neurological and behavioural disturbances.



**Fig 1.** High magnification transmission electron micrograph of extracellular virus-like particles from persistently BDV-infected cells. Modified from ref 3.

# ‘The idea that mental illness has an infectious component has a long history in scientific circles’

A unique characteristic of BVD among the negative-sense, single-stranded RNA viruses is the fact that it reproduces in the cell nucleus.

The principal natural hosts are horses and sheep, but the virus has been detected in a wide range of animals including cattle, birds, rabbits and cats. Essentially, any warm-blooded vertebrate can be considered a potential host. Neither the reservoir nor the mode of transmission of natural infection is known.<sup>4</sup> In the laboratory, the Lewis rat has been the most widely used experimental host.

Natural transmission may occur through direct contact with infectious saliva, nasal or conjunctival fluids, as BDV-specific RNA has been detected in these secretions.<sup>5</sup> Alternatively, contaminated food and water may be a source of infection. Evidence suggests that BDV enters the CNS through nerve endings in the nose and throat.

## Borna disease virus and mental illness

The first association between BDV and mental illness involved the detection of BDV-specific antibodies in the serum of psychiatric patients.<sup>1</sup> Using an indirect immunofluorescence assay, it was shown that 16 (1.6%) out of 979 psychiatric patients were seropositive. None of the healthy volunteers in the control group ( $n=200$ ) were seropositive. The seropositive patients were characterised mainly by histories of affective disorders. Similar studies identified BDV-specific antibodies in 12 (4.5%) out of 265 patients with unipolar or bipolar depression. Once again, none of the healthy volunteers in the control group ( $n=105$ ) were seropositive.<sup>6</sup>

In contrast, viral nucleic acids specific to BDV were not detected by a polymerase chain reaction (PCR) technique in various samples (hippocampus, cerebrospinal fluid and peripheral blood mononuclear cells (PBMC)) taken from schizophrenic and non-schizophrenic controls.<sup>7</sup> However, 22 (37%) out of 60 patients in the psychiatric wards of a Japanese hospital were positive for BDV RNA using a more sensitive nested reverse-transcriptase PCR (RT-PCR) technique on PBMC samples.<sup>8</sup> These data are supported by other studies that used similar techniques. For example, 10 (37%) out of 27 selected patients with psychiatric disorders had detectable levels of BDV RNA in PBMC samples. In contrast, detectable levels were

**Table 1.** Major proteins in Borna disease virus.

PROTEIN	SIZE (KDA)	PUTATIVE FUNCTION
NP (p40)	41	Coat protein, nucleocapsid, major antigen
p10	9	Nuclear replication/transcription (?)
p24	22	Phosphoprotein, major antigen
M	16	Matrix protein, stabilises virus shape
G	56	Glycoprotein, envelope protein necessary for infectivity
L	180	RNA-dependent RNA polymerase

**Table 2.** Prevalence of BDV antibodies in various mental illnesses.

CONDITION	PREVALENCE (%)	
	DISEASE	CONTROL
Psychiatric (various)	0.6–30	0–11.1
Affective disorder	4.2–37	0–16
Schizophrenia	14–32	1.5–20
Extracted from ref 4.		

**Table 3.** Prevalence of BDV RNA in various mental illnesses.

CONDITION	PREVALENCE (%)	
	DISEASE	CONTROL
Psychiatric (various)	9.4–66.7	0–15.4
Affective disorder	0–33.3	0
Schizophrenia	0–63.6	0
Extracted from refs 4 and 9.		

found in only two (15.4%) out of 13 healthy controls.<sup>9</sup>

## Borna disease virus and other illnesses

Borna disease virus has been associated with several non-psychiatric illnesses. These include chronic fatigue syndrome (6/25 cases), multiple sclerosis (15/114 cases vs 10/483 controls), HIV (36/460 cases vs 11/540 controls) and schistosomiasis/malaria (19/193 cases vs 10/483 controls).<sup>4</sup> These studies used a variety of diagnostic techniques including Western blot, immunofluorescence and immunoprecipitation.

## Public health threat

Viral infections in the CNS often are difficult to diagnose. Existing techniques are either insensitive and slow (eg virus culture) or highly invasive (eg brain biopsy) and cannot easily be used to compare the accuracy of newer techniques such as RT-PCR.

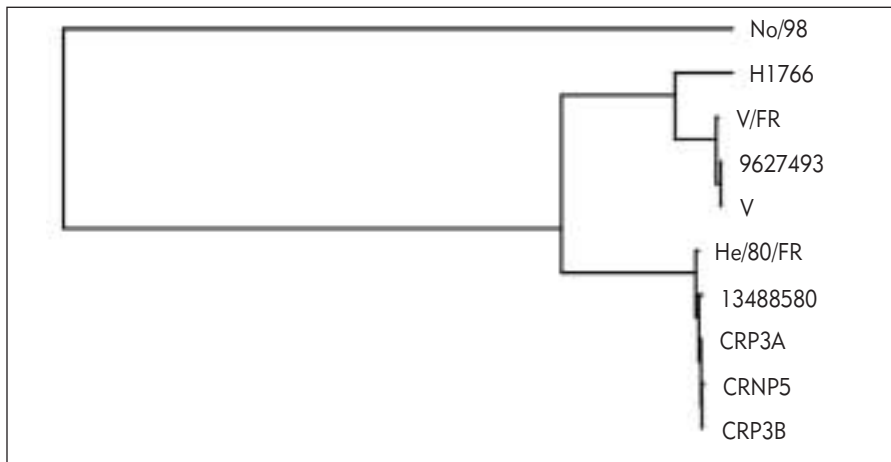
Using a nested RT-PCR technique on PBMC from Japanese blood donors, it was possible to detect BDV RNA in eight (4.7%) out of 172 samples tested. In addition, anti-BDV antibodies were found in 1% of the samples.<sup>10</sup> Taken together, these findings suggest a potentially huge public health problem that should alert the authorities to conduct more widespread testing.

However, a much larger study of Scottish blood donors indicated that the public health threat from BDV in donated blood is minimal, as no BDV RNA was detected in PBMC pools representing 25,000 plasma donors.<sup>11</sup>

The possibility of human-to-human transmission of BDV has been raised by the finding that BDV antibody seroprevalence is significantly higher in mental health workers than in a normal control group (15% vs 1.4%).<sup>12</sup> The same study confirmed the significantly higher seroprevalence of BDV antibodies in schizophrenic patients compared with normal controls (14% vs 1.4%). One possible scenario for this transmission is the ingestion/inhalation by nursing staff of aerosolised infectious particles exhaled/secreted by infected patients.

## Assays for Borna disease virus Serological

The primary serological assays for BDV involve detection of the NP (p40) nucleocapsid coat protein and the p24 phosphoprotein, which comprise the major viral antigens. Western blot, immunofluorescence and enzyme-linked immunosorbent assay (ELISA) protocols have all been used to detect BDV antigens and antibodies with varying degrees of sensitivity (Table 2).



**Fig 2.** An unrooted phylogenetic tree of 10 different complete BDV genomes constructed with Treefinder ([www.treefinder.de](http://www.treefinder.de)). Considerable sequence similarity is apparent despite differences in the species from which the viruses were isolated and the time they were isolated. The BDV isolates are indicated by strain or GenBank accession number.

‘The principal natural hosts are horses and sheep, but BDV has been detected in a wide range of animals including cattle, birds, rabbits and cats’

### Virological

Vero (an African green monkey kidney-derived cell line) and Madin-Darby canine kidney (MDCK) are cell lines that are commonly susceptible to infection with BDV. They can be used to confirm the presence of viable virus in a sample or to produce large quantities of the virus for further study.

### Nucleic acid tests

Nested RT-PCR soon became the standard nucleic acid test to detect BDV RNA (Table 3). However, a single-tube RT-PCR (st RT-PCR) has been developed to detect BDV genomic stranded RNA that is equivalent in sensitivity to the nested RT-PCR technique but with a reduced risk of contamination.<sup>13</sup> In this study, 10 copies of the template were amplified by the st RT-PCR method, and it was used to quantify the amount of genomic RNA present in BDV-persistently infected MDCK cells. Results showed that approximately 100 copies of BDV genomic RNA were present in each infected cell.

Elsewhere, RT-PCR was used to demonstrate the persistence of BDV RNA in six blood samples taken from a psychiatric patient over a period of eight months.<sup>14</sup> In conclusion, the authors point out that “The presence of BDV RNA in blood does not ... prove that BDV causes psychiatric symptoms, especially since presence of the virus cannot be demonstrated in the brain at the same time for obvious reasons.”

A selective PCR method for messenger RNA (mRNA) was used to distinguish target mRNA from the genomic DNA of host cells in a technique that relied on the use of dNTP

analogues.<sup>15</sup> The dNTP analogues were incorporated in complementary DNA (cDNA) formed using mRNA as a template during a standard reverse transcription step. Unlike genomic DNA, the cDNA/mRNA hybrid denatures at about 85 °C. The dNTP-containing strand was then selectively amplified at the next PCR step.

The RT-PCR technique, particularly nested RT-PCR, is prone to the production of false-positive results and artifacts due to inadvertent introduction of templates from laboratory isolates or cross-contamination of samples. The similarity of human BDV isolates detected by RT-PCR to known animal and tissue culture isolates suggests that many, if not all, are low-level contaminants.<sup>4</sup> False-negative RT-PCR results may occur due to the presence of inhibitors in the sample.

However, better control of RT-PCR is possible using internal molecular standards co-amplified during the assay. One such example uses the same primer recognition sites as do the viral nucleic acids, flanking a heterologous DNA fragment of distinct molecular weight, allowing easy discrimination of the target from the internal standard.<sup>16</sup> This technique was used to show that neuronal tissue contained significant larger amounts of PCR inhibitory substances than was present in blood.

Sensitive quantitative nucleic acid assays for BDV (eg TaqMan and LightCycler) have not been described in the literature.

### Inconsistent study results

Inconsistency is a major discrepancy seen between many of the reported studies of BDV

and mental illness. This may be due to the varying sensitivities of the multiple technologies used and their susceptibility to false-negative and false-positive results. False-positive serological results can be attributed, in part, to the purity of the BDV proteins used as antigens. Contamination of antigen preparations with *Escherichia coli*, MDCK or Vero cell-derived antigens of the same molecular weight as BDV NP (p40) or p24 during preparation would result in a false-positive reaction.

Using high-purity (>98%) BDV antigens, Fukuda *et al.* were unable to verify an association between BDV and either mood disorders or schizophrenia using Western blot, electrochemiluminescence immunoassay, immunofluorescence and T-cell proliferation assays.<sup>17</sup> In another study, the association between BDV-positive serology and mental illness was further challenged by the finding that many human BDV-reactive antibodies showed only low avidity and might therefore be cross-reacting antibodies.<sup>18</sup>

### Antiviral therapy

If BDV does cause mental illness then could antiviral therapy be an appropriate treatment for certain conditions? There are several issues to consider here. First, very few of the available antiviral drugs target the BDV-like viruses (negative-sense, single-stranded genomes). Second, BDV is highly neurotropic and therefore any antiviral compound would have to cross the blood-brain barrier to exert an effect.

The use of antiviral compounds in schizophrenics has been reported with mixed results. Studies have shown that acyclovir fails to alleviate symptoms but that valacyclovir reduces symptoms in cytomegalovirus-seropositive individuals.<sup>19,20</sup>

In BDV, the most promising antiviral agents appear to be amantadine and ribavirin, which are effective against, among others, the single-stranded RNA influenza A virus and the severe acute respiratory syndrome (SARS)-coronavirus, respectively. Studies have shown that amantadine does not have direct antiviral activity;<sup>21</sup> however, it has been used to treat depressed individuals with concomitant BDV infection, and has produced significant results.<sup>22</sup> Ribavirin has anti-BDV activity in persistently-infected cells *in vitro* and when used intracerebrally.<sup>23,24</sup> No studies on the anti-BDV activity of this compound in humans have been conducted.

Interferon- $\alpha$  is effective against BDV in persistently-infected cells in culture.<sup>25</sup> In infected mouse cerebellar slices, interferon- $\gamma$  inhibits BDV replication by protecting non-infected cells from infection rather than by clearing the virus from infected cells.<sup>26</sup>

Finally, nucleoside analogues, immunosuppressants and cell signalling inhibitors have been shown to inhibit BDV *in vitro* or *in vivo*.<sup>27-29</sup> and neutralising monoclonal antibodies are another approach to treating BDV infection.<sup>30</sup>

# 'Onset and degree of mental illness is likely to be a subtle interplay between many different genes and the environment, including a past history of infection'

Clearly, antiviral therapy for BDV infection is either non-specific or still in the experimental stage.

## The search continues

There is clear evidence to suggest that certain neurotropic viruses affect behaviour. In addition, there is persistent evidence that viral infection influences the onset and severity of various mental illnesses. Borna disease virus infection in mentally ill patients has been demonstrated by serological and nucleic acid assay and by virion isolation. Despite this, however, unequivocal evidence that BDV is the causative agent of any mental illness remains elusive. Early associations were plagued by insensitive assays and inappropriate study design.

The onset and degree of mental illness is likely to be a subtle interplay between many different genes and the environment, including a past history of infection. Viral infections induce a wide range of physiological responses, including cytokine expression, which are known to modulate gene expression. These, in turn, may affect behaviour directly or alter an individual's susceptibility to mental illness. Regrettably, Jung's 'brain toxin', or any other direct cause for schizophrenia or other mental illness, remains as elusive today as it was at the beginning of the 20th century. The search for the missing link continues.

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