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Borna disease virus in brains of North American and European people with schizophrenia and bipolar disorder

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The pathogenesis of schizophrenia is unknown. Although a genetic component is likely, discordance for disease in 50% of monozygotic twins and epidemiological studies suggest that there are environmental co-factors.[1] Borna disease virus (BDV) is an RNA virus characterised by low productivity, non-cytolytic replication, broad host range, neurotropism, and the potential to alter movement and behaviour through selective effects on brain monoamine circuits.[2] BDV RNA has been detected in peripheral blood mononuclear cells of patients with neuropsychiatric disorders in Germany and Japan with prevalence rates for schizophrenia ranging from 12% to 64%.[1]

We examined the prevalence of BDV P gene mRNA in postmortem brain samples from 75 North American and European individuals with various brain disorders and healthy controls by means of nested reverse transcription PCR experiments (table). On the basis of knowledge of BDV tropism in animals, samples were taken from limbic structures (prefrontal cortex, hippocampus, amygdala, basal ganglia, cingulate gyrus) to increase the probability of detecting BDV RNA. At the University of California Irvine (UCI), P gene transcripts were detected in ten people: eight with schizophrenia (two from Germany, two from Southern California, USA, and four from New England, USA; and two with bipolar disorder (from New England). None were detected in brains of people with multiple sclerosis, Alzheimer's Disease, Parkinson's Disease, or any of the controls.

Samples from 13 of the 75 brains examined were sent from New England to the University of Nevada Reno (UNR) for independent analysis. Amplification products were obtained from two of five brains positive at UCI. One sample from a patient with schizophrenia negative at UCI was positive at UNR. Detection was RT-dependent indicating that products did not represent amplification of DNA from plasmids or products of other RT-PCR reactions. All products were of the expected length and hybridised with internal P-gene probes. Considering assays at both UCI and UNR, BDV nucleic acids were present in brains of nine of 17 patients with schizophrenia (53%, 95% CI: 0·27–0·78) and two of five patients with bipolar disorder (40%, 95% CI: 0·05–0·86). The amplification products had sequence divergence in the range of one of four nucleotides with respect to previously reported human isolates[1] or animal isolate He/80-1.[3]

Diagnosis (DSM IV)	BDV+/total	m/f	Mean age at death in years (SD)
Schizophrenia (295·xx)	9/17	15/2	60·6 (18·0)
Bipolar disorder (296·xxx)	2/5	3/2	53·8 (22·9)
Major depressive disorder (296·30)	0/6	1/5	72·3 (16·7)
Psychotic disorder not otherwise specified (298·9)	0/2	2/0	67·0 (17·0)
Alzheimer's disease	0/19	8/11	81·8 (6·1)
Parkinson's disease	0/11	7/4	71·5 (6·9)
Multiple sclerosis	0/5	1/4	60·0 (11·7)
No neurological disease	0/10	6/4	67·1 (11·4)
Total	11/75	43/32	

Borna disease virus in human brains

The nested RT-PCR method we used is sensitive to 100 copies of RNA template yet functions near the threshold for detection of BDV nucleic acids. This limitation together with differences in viral nucleic-acid distribution in brain may account for discordance between laboratories with some samples. The observation that sequences of P gene messages from brain were highly conserved with respect to previously described isolates might argue that positive samples represent amplification of low-level contaminants. However, samples sent directly from brain banks to two independent laboratories yielded similar results. Furthermore, amplification was RT-dependent. The finding of sequence conservation is consistent with previous analyses of well-characterised isolates from different host species and geographic locations.[1]

In non-human species, BDV causes either an immune-mediated disease with profound disturbances in monoamine metabolism, movement, and behaviour, or persistent infection with disturbances in brain development and subtle abnormalities in learning and behaviour.[2] The capacity for BDV to alter neural circuits in non-human species that are also reported to be impaired in schizophrenia suggests the possibility of common pathways for brain dysfunction. However, BDV and BDV mRNA can be detected in brains of non-human species without recourse to cocultivation or amplification. Thus, if BDV can be implicated as a primary or co-factor in the pathogenesis of schizophrenia, the mechanisms in human beings are likely to be different.

We cannot exclude the possibility that BDV in the brains of people with schizophrenia reflects the influence of psychotropic drugs on BDV-gene expression, or increased probability of exposure to infectious agents including BDV, particularly in that subgroup of patients with lower socioeconomic status. These questions and others including identification of reservoirs for BDV and modes for natural transmission need further study.

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A detailed description of the study may be found at http://www.ucihs.uci.edu/neurovirol

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Protein kinase C expression in salivary gland acinar epithelial cells in Sjögren's syndrome

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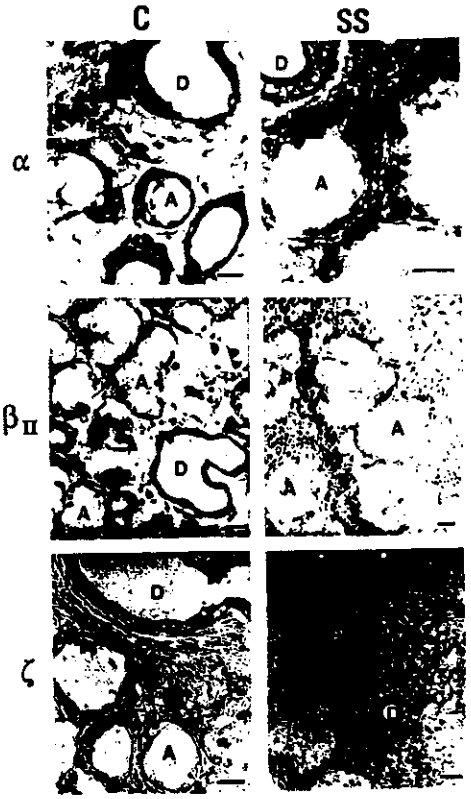
Sjögren's syndrome (SS) is associated with autoimmune features, such as a focal adenitis and SS-A/Ro and SS-B/La autoantibodies,[1] but the mechanisms underlying the diminished function of exocrine glands is unknown. A characteristic feature of salivary-gland tissue in SS is focal adenitis, which is not found in all labial salivary glands or in all lobules of individual glands. This focal and local involvement contrasts with the functional impairment of the salivary gland. It has been shown that in an animal model for SS (NOD mice) sialopenia develops independently of lymphocyte infiltration,[2] suggesting that defective intracellular signalling, rather than lymphocyte-mediated destruction of the acinar epithelial cells, is responsible for the sialopenia.

We postulated that acinar epithelial cells in SS have a diminished capacity to respond to stimuli, such as activation of α_1-adrenergic, M1 and M3 type muscarinic, and NK-1-type substance P receptors. All these receptors converge on one common intracellular signalling pathway—protein-kinase C (PKC). Because formation of the saliva depends on the normal function of the acinar epithelial cells and is partly dependent on PKC,[3] we looked for the expression of PKC in patients with SS compared with controls.

Seven patients with primary SS and six controls gave informed consent to a labial salivary gland biopsy. The diagnosis of SS was made with EU criteria.[4] The specimens were labelled with both mouse monoclonal and rabbit polyclonal antibodies which recognise PKC α, β_I, β_{II}, γ, δ, ϵ, and ζ isoforms. Binding of the primary antibodies was visualised by avidin-biotin-peroxidase complex staining and western blotting.

In control sections, PKC α and β_{II} isoforms were found in acinar and ductal epithelial cells. PKC ζ and β_{II} isoforms were found in myoepithelial cells, which surround the acini but are located inside the basal lamina. PKC ζ isoform was also found in ductal epithelial cells. By contrast, in the SS sections, PKC β_{II} isoform showed no detectable staining and α isoform staining was weak in the acinar epithelial cells.



Protein kinase C isoforms in control (C) and SS labial salivary glands.
Notice the defect and/or weak expression of PKC β_{II} and α in acinar epithelial cells (a) and PKC β_{II} and ζ in myoepithelial cells (arrows) in patients compared with controls. PKC staining of the ductal epithelial cells (d) is similar in patients and controls. In addition to the structural salivary gland cells, some immigrant cells, such as periductal lymphocytes and mast cells, show positive staining. Bar=50 μm.

Furthermore, ζ and β_{II} isoforms did not show any staining in myoepithelial cells in patients (figure). However, α, β_{II} and ζ isoform staining of the ductal epithelial cells was similar in patients and in controls. Acinar and ductal epithelial cells of patients and controls did not contain any detectable PKC γ, β_I, δ, or ϵ isoform immunoreactivity. Western blotting confirmed the presence of PKC α, β_{II}, and ζ isoforms in salivary tissue.

Our findings show a defect in the signal-transduction system of the acinar epithelial cell due to PKC isoform deficiencies. PKC is a family of enzymes that modulate protein activity via serine/threonine phosphorylation.[5] Conventional PKC isoforms (α, β_I, β_{II}, and γ) depend on calcium diacylglycerol for full activation. Unusual isoforms (δ, μ, ϵ, η, and θ) do not need calcium for activation. A third class, so called atypical PKC isoforms (ζ, λ, and ι) require phosphatidylserine but not calcium or diacylglycerol.[5] In the present study two conventional (α, β_{II}) and one phosphatidylserine-dependent (ζ) PKC isoforms were found to be defectively expressed in the acinar epithelial and myoepithelial cells in patients with SS.

Activation of the PKC enzyme by stimuli from hormones, neurotransmitters, and growth factors is essential for cell proliferation, differentiation, membrane transport, and gene expression.[5] In salivary-gland acinar cells, activation of M1 and M3, α_1, or NK-1 receptors by acethylcholine, noradrenaline, or substance P, respectively, is linked to phospholipase C via GTP-dependent proteins. This leads to the hydrolysis of phosphatidylinositol 4,5-biphosphate to inositol 1,4,5-trisphosphate and *sn*-1,2-diacylglycerol, which raise intracellular free (Ca^{2+}) concentration and activate PKC, respectively.[5] The elevation of intracellular (Ca^{2+})