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Detection of Antibodies against Borna Disease Virus Proteins in an Autistic Child and Her Mother

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Borna viruses are phylogenetically diverse, non-segmented, negative-strand RNA viruses that infect various animals. Although Borna disease virus (BDV) is a prototypical mammalian bornavirus that causes neurobehavioral disorders in rodents (1), the association between BDV and human psychiatric disorders remains controversial (2). Recently, bornaviruses related to BDV were discovered in birds (i.e., avian bornaviruses) (3). Furthermore, fatal encephalitis was reported in human cases of infection with a novel bornavirus (variegated squirrel bornavirus [VSBV]) (4). These observations indicate that bornavirus is a potent zoonotic agent, highlighting the importance of reevaluation of bornavirus surveys.

Serological analysis is the only reliable antemortem method of investigating bornavirus prevalence. However, inter-laboratory variations in assessing epidemiological assay results have led to inconsistent findings regarding the bornavirus prevalence (5). Overcoming this situation is the first step of reevaluation of bornavirus surveys; therefore, we designed a diagnostic procedure for evaluating bornavirus epidemiology as follows: first, the presence of BDV-reactive antibodies is evaluated by using an indirect immunofluorescence assay (IFA), the most reliable assay for detecting these antibodies (2,5). Second, BDV-reactive samples are evaluated to confirm the presence of antibodies against linear and conformational BDV epitopes by using Western blotting (WB) and a radioligand assay (RLA), respectively (6). The presence of BDV-reactive antibodies should be confirmed for at least 2 BDV antigens to reduce the risk of false-positive results. By using this procedure, we determined that an autistic child and her mother had BDV-reactive antibodies in their serum.

A Japanese girl was the first-born child of a mother with depression (35 years old, receiving paroxetine) and a mentally healthy father. The child was delivered vaginally with labor induction at 36 weeks of gestation, had a birth weight of 2,734 g, and did not experience asphyxia. The mother received immunosuppressants after undergoing kidney transplantation at the age of 27 years. The child's early development was normal. At the age of one year, she experienced a febrile seizure. Her developmental quotient at the age of 2 years was 113 according to the Kyoto Scale of Psychological Development, which calculates a child's developmental level by dividing the developmental age by the chronological age and multiplying the result by 100. However, the child occasionally failed to notice other children's responses and could not cooperate with other children. She also exhibited echolalia, strong separation anxiety, stereotypical behaviors, and hypersensitivity to trivial changes and sounds. Based on these findings, she was diagnosed as having Asperger's syndrome at the age of 3 years. At the age of 5 years, her scores for special working memory, abilities of retention and manipulation of visuospatial information, and the Stockings of Cambridge task (estimates planning ability) were normal. However, her score for intra-extra dimensional set shift, which estimates rule comprehension and flexible responses to its change, was very low for her age (assessed by using the Cambridge Neuropsychological Test Automated Battery of tests, Cambridge Cognition Ltd, Cambridge, UK).

At the age of 2 years, the child was examined for BDV-reactive antibodies. This study was approved by the institutional review boards of Kyoto University and Osaka University, and informed consent was obtained. In BDV-infected oligodendroglioma (OL/BDV) cells, BDV forms a BDV-specific dot structure (viral speckles of transcripts [vSPOTs]) in the nucleus. Paraformaldehyde-fixed cells were incubated with the sera for 2 h, followed by incubation with secondary antibodies for 1 h. Staining of the child's serum revealed faint signals at vSPOTs, which were stained using an antibody against N protein, a vSPOT component, only in OL/BDV cells, suggesting that her serum specifically recognized BDV antigens (Fig. 1A). The secondary antibodies alone did not stain for vSPOTs; thus, the possibility of cross-reactivity was excluded. We then

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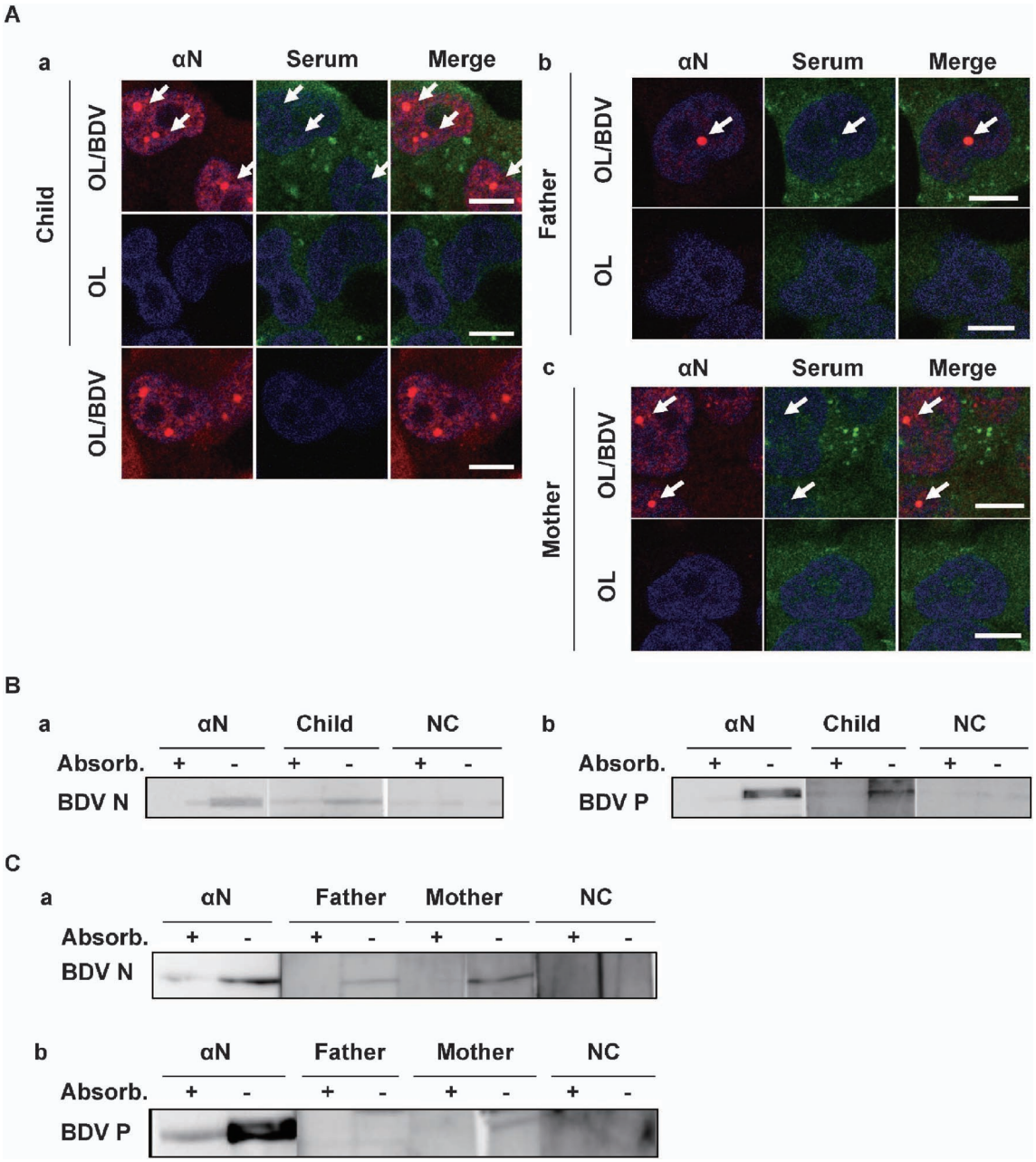

Fig. 1. BDV-reactive antibodies from an autistic child and her parents. (A) Evaluating serum reactions to vSPOTs via IFA. OL/BDV and OL cells were stained using an anti-N protein antibody with serum from the child (a), her father (b) or mother (c), or without serum (a, bottom). The sera stained vSPOTs in OL/BDV cells (arrows). Bars, 5 μm. (B, C) Evaluating reactions of serum from the child (B) and her parents (C) to N (a) and P (b) proteins using WB. For each sample, we compared results with (+) and without (−) preabsorption to recombinant N and P proteins, respectively. NC indicates the blot with an unrelated individual's serum.

evaluated her serum for antibodies against recombinant N and P proteins by using WB and RLA, as described previously (6). Purified recombinant proteins were similarly resolved by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subjected to WB. The child's non-preabsorbed serum recognized both N and P proteins (Fig. 1B). The specificities of the assays were confirmed by using serum from an unrelated individual (NC) and serum pre-absorbed with the recombinant proteins. For RLA, ^{35}S-methionine-labeled proteins were produced and precipitated with sera. The amount of the precipitated protein was quantified. The results were expressed as the ''anti-BDV index'' ([count per minute of the sample serum]/[that of the normal pooled serum]). The child's serum had an anti-BDV-P index that was +3 standard deviations higher than the mean for normal pooled sera (cutoff: mean +2 standard deviations), confirming the presence of antibodies against P protein.

Next, we evaluated the parents' sera by using IFA, which revealed faint signals at vSPOTs for both parents (Fig. 1A). WB and RLA confirmed the presence of antibodies to both N and P proteins in the mother's serum, whereas only WB detected antibodies against N protein in the father's serum (Fig. 1C and data not shown). These results indicated that at least the child

and her mother had BDV-reactive antibodies.

To the best of our knowledge, this is the first case of detection of BDV-reactive antibodies in an autistic child. BDV-reactive antibody titers are usually very low, indicating the possibility that these antibodies may recognize antigens from non-BDV bornaviruses (5). For example, the N and P gene sequences of VSBV (accession No. LN713681) are 87% and 75% identical to those of BDV, respectively, whereas those of avian bornavirus (accession No. JX065209) share 73% and 62% amino acid identity, respectively. BDV-reactive antibodies are identified in patients with VSBV infection, and antibodies against BDV proteins can cross-react with avian bornaviruses (4,7). Thus, the present case may have involved non-BDV bornavirus infection. We detected BDV-reactive antibodies as early as at the age of 2 years, suggesting that the child might have been infected early in her life. As the mother had received immunosuppressive therapy, which may have conferred a predisposition to bornavirus infection, the child's infection might have occurred via mother-to-child transmission. Consistently, previous studies reported vertical BDV transmission in horses and mice (8,9). Therefore, the present case might be a unique human case of vertical bornavirus transmission.

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Conflict of interest None to declare.

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