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Serologic Markers of Lyme Disease in Children with Autism

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To the Editor

A proposed link between Lyme disease and autism has garnered considerable attention.[1,2] Among individuals with autism spectrum disorders, rates of seropositivity for Lyme disease of greater than 20% have been reported.[1] However, controlled studies to assess serological evidence of infection with *Borrelia burgdorferi* (the causative agent of Lyme disease) in patients with autism are lacking.

Serological evidence of infection with *B burgdorferi* is essential for diagnosing Lyme disease, except in cases of typical erythema migrans skin lesions. To evaluate the suggestion that autism is commonly linked to Lyme disease, we performed Lyme disease serological testing on serum samples from children with autism and those without autism.

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Study concept and design: Alaedini.
Acquisition of data: Ajamian.
Analysis and interpretation of data: Ajamian, Kosofsky, Wormser, Rajadhyaksha, Alaedini.
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METHODS

Serum samples from 120 children aged 2 through 18 years with autism and those without autism were acquired from the Autism Genetic Resource Exchange (AGRE) (37 with autism and 27 unaffected siblings) and the Weill Cornell Autism Research Program (WCARP) (33 with autism, 8 unaffected siblings, and 15 unrelated healthy controls). All WCARP and some unselected AGRE sites collected serum samples; all available serum samples were included.

Patients from the AGRE program met diagnostic criteria for autism based on both the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview, Revised, whereas WCARP patients met criteria for autism based only on the ADOS. Participants in the AGRE program have been recruited primarily from the northeastern and western United States; serum samples for this study were collected from August 31, 1999, through April 25, 2001.

The WCARP serum samples were from participants who resided primarily in Connecticut, New Jersey, and New York, and were collected from May 19, 2010, through March 7, 2012. Screening questionnaires were used to evaluate the general health of unrelated controls.

Written informed consent was obtained for all study participants from a parent or guardian. Serum samples from 2 patients with culture-confirmed early Lyme disease were used as positive controls. Specimens were kept at −80°C to maintain stability. This study was approved by the institutional review board of Columbia University Medical Center.

Testing for antibodies to *B burgdorferi* was performed according to the 2-tier algorithm recommended by the US Centers for Disease Control and Prevention.[3] Initial screening for anti– *B burgdorferi* immunoglobulin G and M antibodies was performed with separate enzyme-linked immunosorbent assays (ELISAs), according to the manufacturer's protocols (Euroimmun). Specimens classified as borderline or positive were further tested by Western blotting for IgG or IgM antibodies to electrophoresis-separated *B burgdorferi* strain B31 proteins (Euroimmun).[4]

Assuming 1% or lower seroprevalence in controls, and at least 20% seroprevalence in cases as suggested, the sample size in this study would provide greater than 90% power with an α level of .05. Differences between groups were analyzed using the 2-tailed Fisher exact test; *P* values of less than .05 were considered to be statistically significant. Binomial distribution confidence intervals were determined by the Clopper-Pearson exact method.

RESULTS

Seventy children with autism (58 male; mean [SD] age, 7.2 [3.6] years) and 50 unaffected controls (32 male; mean age, 9.0 [4.0] years) were included. Of the patients with autism, 1 was positive by ELISA for anti– *B burgdorferi* IgG, whereas 4 were borderline by ELISA for IgM. Of the 50 children in the unaffected control group, 4 were positive and 1 was borderline for IgG by ELISA, whereas 1 was positive by ELISA for IgM.

All serum samples that were positive or borderline by ELISA were further analyzed using Western blot and were found to be negative for anti– *B burgdorferi* antibody reactivity (Table 1 and Table 2). The 95% confidence interval for seroprevalence in children with autism and in unaffected controls was 0% to 5.1%.

DISCUSSION

None of the children with autism or unaffected controls had serological evidence of Lyme disease by 2-tier testing. A potential limitation of this study is the lack of information about lifestyle for patients and controls, including time spent outdoors.

The data do not address whether Lyme disease may cause autism-like behavioral deficits in some cases. However, the study's sample size is large enough to effectively rule out the suggested high rates of Lyme disease or associated seroprevalence among affected children.

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Table 1

Serum Immunoglobulin G Antibody Reactivity to *Borrelia burgdorferi* Protein Bands as Determined by Western Blotting in Patients and Controls Who Were Positive or Borderline for IgG by Enzyme-Linked Immunosorbent Assay[a]

		Western Blot Band[b]											
Serum Sample No.	**Group**	**p18**	**p25**	**p28**	**p30**	**p31**	**p34**	**p39**	**p41**	**p45**	**p58**	**p66**	**p93**
1	Autism[c]								+			+	
2	Unaffected control[c]				+				+				
3	Unaffected control[c]								+				
4	Unaffected control[c]								+		+		
5	Unaffected control[c]	+							+			+	+
6	Unaffected control[c]								+				
12	Lyme disease control[d]	+	+					+	+	+		+	
13	Lyme disease control[c]		+						+	+			

[a] None of the children with autism or unaffected controls had serological evidence of Lyme disease by 2-tier testing. The 95% confidence interval for IgG seroprevalence in children with autism and in unaffected controls was 0% to 5.1%.

[b] According to Centers for Disease Control and Prevention testing criteria, an IgG immunoblot was considered positive if 5 or more of the 10 following protein bands reacted positively: p18, p25 (OspC), p28, p30, p39 (BmpA), p41(FlaB), p45, p58, p66, and p93.[3]

[c] Individual did not meet IgG seropositivity criteria for Lyme disease.

[d] Individual met IgG seropositivity criteria for Lyme disease.

Table 2

Serum Immunoglobulin M Antibody Reactivity to *Borrelia burgdorferi* Protein Bands as Determined by Western Blotting in Patients and Controls Who Were Positive or Borderline for IgM by Enzyme-Linked Immunosorbent Assay[a]

		Western Blot Band[b]		
Serum Sample No.	**Group**	**p25**	**p39**	**p41**
7	Autism[c]			+
8	Autism[c]			+
9	Autism[c]			+
10	Autism[c]			
11	Unaffected control[c]			
12	Lyme disease control[d]	+		+
13	Lyme disease control[d]	+	+	+

[a] None of the children with autism or unaffected controls had serological evidence of Lyme disease by 2-tier testing. The 95% confidence interval for IgM seroprevalence in children with autism and in unaffected controls was 0% to 5.1%.

[b] According to Centers for Disease Control and Prevention testing criteria, an IgM immunoblot was considered positive if 2 of the 3 following protein bands reacted positively: p25 (OspC), p39 (Bmp A), and p41 (FlaB).[3]

[c] Individual did not meet IgM seropositivity criteria for Lyme disease.

[d] Individual met IgM seropositivity criteria for Lyme disease.