hypothesis that unobserved patient characteristics are responsible for lower cardiac risk among patients taking atenolol.

It instead tests whether there is an association between atenolol use and risk factors for retained instruments such as obesity, unplanned or altered operations,[2] and staff adherence to operative checklists. However, these biases are not likely to coexist with other unobserved patient characteristics that confound the association between atenolol use and perioperative cardiac risk (eg, socioeconomic or clinical characteristics not accounted for in the analysis).

Therefore, a test of whether atenolol use is associated with higher rates of retained foreign objects after surgery would not be a useful falsification test.

We have centered our discussion on an observational study of perioperative β blockade, but note that in this particular case, we do not need to rely on observational evidence. A well-done randomized trial has tested whether long-acting perioperative β blockade is better than placebo and yielded negative results.[3]

In short, in much the same way observational studies are useful only when used appropriately (eg, when prospective designs are not feasible), falsification testing is valuable only if and when it is applied thoughtfully.

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Conflict of Interest Disclosures: The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

1. Redelmeier D, Scales D, Kopp A. Beta blockers for elective surgery in elderly patients: population based, retrospective cohort study. *BMJ*. 2005;331(7522):932.
2. Gawande AA, Studdert DM, Orav EJ, Brennan TA, Zinner MJ. Risk factors for retained instruments and sponges after surgery. *N Engl J Med*. 2003;348(3):229-235.
3. Devereaux PJ, Yang H, Yusuf S, et al; POISE Study Group. Effects of extended-release metoprolol succinate in patients undergoing non-cardiac surgery (POISE trial): a randomised controlled trial. *Lancet*. 2008;371(9627):1839-1847.

RESEARCH LETTER

Serologic Markers of Lyme Disease in Children With Autism

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.

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]

Serum Sample No.	Group	Western Blot Band[b]											
		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]

Serum Sample No.	Group	Western Blot Band[b]		
		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.

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Author Contributions: Dr Alaedini had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Alaedini.
Acquisition of data: Ajamian.
Analysis and interpretation of data: Ajamian, Kosofsky, Wormser, Rajadhyaksha, Alaedini.
Drafting of the manuscript: Ajamian, Alaedini.
Critical revision of the manuscript for important intellectual content: Ajamian, Kosofsky, Wormser, Rajadhyaksha, Alaedini.
Statistical analysis: Ajamian, Alaedini.
Obtained funding: Alaedini.
Administrative, technical, or material support: Ajamian, Kosofsky, Wormser, Rajadhyaksha, Alaedini.
Study supervision: Alaedini.
Conflict of Interest Disclosures: The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Wormser reported receiving grants from the Centers for Disease Control and Prevention, the National Institutes of Health, Immunetics Inc, Bio-Rad, DiaSorin Inc, and BioMerieux for research related to Lyme disease; holding stock in Abbott; providing expert witness testimony in malpractice cases involving Lyme disease; serving as unpaid board member at the American Lyme Disease Foundation; serving as an expert witness regarding Lyme disease in a disciplinary action for the Missouri Board of Registration for the Healing Arts; serving as a consultant to Baxter for Lyme vaccine development; and receiving reimbursement for travel expenses from the American Society for Microbiology. Dr Rajadhyaksha reported receiving a grant from The Hartwell Foundation for research related to autism. Dr Alaedini reported receiving grants from the National Institutes of Health, the Department of Defense, and the Lyme Research Alliance for research related to Lyme disease or autism. No other disclosures were reported.
Funding/Support: This study was supported in part by grant W81XWH 10-1-0887 from the Department of Defense and grant 1R56 AI093763-01 from the National Institutes of Health (awarded to Dr Alaedini). The Autism Genetic Resource Exchange (AGRE) is a program of Autism Speaks and is supported, in part, by grant 1U24MH081810 from the National Institute of Mental Health (awarded to Clara M. Lajonchere). The Weill Cornell Autism Research Program (WCARP) is supported in part through funding from the Clinical and Translational Science Center of the Weill Cornell Medical College and by National Institutes of Health grant UL1 TR000457-06.
Role of the Sponsors: The funding agencies had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.
Additional Contributions: We thank Nga M. Lau, MD (Department of Medicine, Columbia University Medical Center), Joseph J. Higgins, MD (Department of Pediatrics, Weill Cornell Medical College), Mary J. Ward, PhD (Department of Pediatrics, Weill Cornell Medical College), Peter H. Green, MD (Department of Medicine, Columbia University Medical Center), and Kaleb Yohay, MD (Department of Pediatrics, Weill Cornell Medical College) for their involvement in recruitment or clinical assessment of study participants. We thank the Weill Cornell Autism Research Program (WCARP) and the participating WCARP families. We thank the Autism Genetic Resource Exchange (AGRE) Consortium and the participating AGRE families. No compensation was received by any of these individuals.

1. Bransfield RC, Wulfman JS, Harvey WT, Usman AI. The association between tick-borne infections, Lyme borreliosis and autism spectrum disorders. *Med Hypotheses*. 2008;70(5):967-974.
2. Kuhn M, Grave S, Bransfield R, Harris S. Long term antibiotic therapy may be an effective treatment for children co-morbid with Lyme disease and autism spectrum disorder. *Med Hypotheses*. 2012;78(5):606-615.
3. Centers for Disease Control and Prevention (CDC). Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep*. 1995;44 (31):590-591.
4. Chandra A, Latov N, Wormser GP, Marques AR, Alaedini A. Epitope mapping of antibodies to VlsE protein of *Borrelia burgdorferi* in post-Lyme disease syndrome. *Clin Immunol*. 2011;141(1):103-110.

CORRECTIONS

Incomplete Conflict of Interest Disclosures: In the Preliminary Communication entitled "Effects of Fructose vs Glucose on Regional Cerebral Blood Flow in Brain Regions Involved With Appetite and Reward Pathways" published in the January 2, 2013, issue of *JAMA* (2013;309[1]:63-70), information reported by the authors for the Conflict of Interest Disclosures section was inadvertently omitted. The text in that section should have read as follows: "All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Sinha reported receiving a grant from and being a consultant to the National Institutes of Health; and being a scientific advisory board member for Embera Neurotherapeutics. Dr Sherwin reported receiving grants from the National Institute of Diabetes and Digestive and Kidney Diseases and the Juvenile Diabetes Research Foundation; serving on scientific advisory boards for Amylin Corp, Merck, Pfizer, Janssen, and Insulet; serving on data and safety monitoring boards for Novartis and MannKind; being a consultant to Bristol-Myers Squibb, Eli Lilly, and McKinsey & Company; owning stock in Insulet; and receiving payment for lectures from Merck. Dr Page reported no disclosures." This article has been corrected online.

Clarification of Statement: In the Editorial entitled "Promoting Quality Surgical Care: The Next Steps" published in the February 27, 2013, issue of *JAMA* (2013; 309[8]:827-828), a statement requires clarification. In the fourth full paragraph, the last sentence of this paragraph should read "The CMS already requires that Medicare beneficiaries receive care at institutions accredited by approved accreditation organizations such as the Joint Commission." This article has been corrected online.