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LACK OF ASSOCIATION BETWEEN AUTISM AND ANTI-GM1 GANGLIOSIDE ANTIBODY

Forty of 54 children with autism were reported to have an elevated antibody response to GM1 ganglioside that correlated with disease severity.¹ Anti-ganglioside autoantibodies, especially those directed at GM1, are known to be associated with and play a pathogenic role in some immune-mediated peripheral neuropathies.^{2,3} The presumed link between autism and anti-GM1 antibodies, therefore, implies that testing may identify a sizable subset of patients who would benefit from immunomodulatory therapy. To evaluate the proposed association between autism and anti-GM1 antibodies, we performed serologic testing on children diagnosed with autism by strict clinical criteria and those without autism, using a standard and validated immunoassay protocol.

Methods. Study population. Serum samples were from 181 children, including 70 with autism, 35 unaffected siblings of similar ages within the families of autistic children, and 76 age-matched unrelated healthy controls (table). Samples were obtained from the Autism Genetic Resource Exchange (AGRE) (37 autism and 27 unaffected siblings), the Weill Cornell Autism Research Program (WCARP) (33 autism, 8 unaffected siblings, and 14 unrelated healthy controls), and Uppsala University (62 unrelated healthy controls). AGRE patients met diagnostic criteria for autism based on both the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview, Revised (ADI-R), whereas WCARP patients met criteria for autism based on the ADOS. All available serum samples were included. Serum from a patient diagnosed with multifocal motor neuropathy (MMN) with elevated immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to GM1 was used as positive control. Specimens were kept at -80°C to maintain stability.

Standard protocol approvals, registrations, and patient consents. Written informed consent was obtained for all study participants. The consent procedures were approved by the institutional review boards of the involved organizations. This specific study was approved by the institutional review board of Columbia University Medical Center.

Measurement of anti-GM1 ganglioside antibodies. IgG and IgM antibodies to GM1 were measured by ELISA as previously described,⁴ with minor modifications. Ninety-six-well round-bottom polystyrene plates (Nunc, Penfield, NY) were coated with 0.5 µg/well of GM1 ganglioside (Sigma-Aldrich, St. Louis, MO) in methanol. Wells that were not coated with antigen served as controls. After methanol evaporation, wells were blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS) for 2 hours at 4°C. Sera were diluted serially in blocking buffer, starting at 1:100 for IgG and at 1:400 for IgM measurements. Wells were filled with 50 µL of diluted sera in duplicate (in both antigen-coated and noncoated wells), incubated overnight at 4°C, and then washed with PBS. Each plate contained the MMN positive control sample. Wells were incubated with 50 µL of horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Amersham Biosciences, Amersham, UK), at 1:2,000, or HRP-conjugated goat anti-human IgM (ICN Biomedicals, Irvine, CA), at 1:1,000 (2 hours at 4°C). Wells were washed, followed by the addition of 50 µL of developing solution.⁴ Plates were incubated at room temperature for 30 minutes before measuring optical density (OD) at 450 nm. The OD values of antigen-coated wells for each sample were corrected for nonspecific binding by subtracting the OD of the associated noncoated wells. Data were normalized according to the mean value of the positive control duplicate on each plate. The titer for each sample was assigned as the highest dilution at which the OD reading was 0.1 unit greater than in the corresponding control wells.

Table Demographic characteristics and experimental data for the study cohorts						
Group	No. of individuals	Mean ± SD age, y	Male sex, n (%)	White race, n (%)	Anti-GM1 IgG ^a	Anti-GM1 IgM ^a
Autism	70	7.2 ± 3.6	58 (83)	44 (63)	A: 0.095 ± 0.035	A: 0.053 ± 0.008
					C: 0.110 ± 0.027	C: 0.052 ± 0.014
Unaffected sibling	35	8.9 ± 3.9	23 (66)	29 (83)	A: 0.097 ± 0.036	A: 0.051 ± 0.008
					C: 0.118 ± 0.040	C: 0.054 ± 0.016
Unrelated healthy	76	8.8 ± 3.7	59 (77)	70 (92)	A: 0.085 ± 0.027	A: 0.052 ± 0.008
					C: 0.100 ± 0.032	C: 0.051 ± 0.009

Abbreviations: A = antigen-coated wells; C = control noncoated wells; IgG = immunoglobulin G; IgM = immunoglobulin M; OD = optical density.
^a As determined by ELISA. Values indicate mean normalized OD ± SD. Serum dilutions were at 1:100 for IgG and at 1:400 for IgM measurements.

Data analysis. The differences between groups were analyzed by the 2-tailed Fisher exact test; *p* values less than 0.05 were considered to be statistically significant. Binomial distribution confidence intervals (CIs) were determined by the Clopper-Pearson exact method.

Results. One autistic child and one pediatric healthy control were found to have a titer of 100 for IgG anti-GM1 antibody. All other sera were negative at the lowest titers examined for IgG and IgM antibodies to GM1. No differences in the frequency of seropositivity for anti-GM1 antibodies were found between patients and controls. The 95% CIs for the rate of positivity in children with autism and in unaffected controls were 0% to 7.7% for IgG and 0% to 5.1% for IgM anti-GM1 antibodies.

Discussion. The assay methodology in the present study was based on the widely accepted standard ELISA procedure for the detection of IgG and IgM anti-ganglioside antibodies, which has been utilized and validated in numerous studies by us and other groups.⁴⁻⁷ In addition, all patients in this report were identified as having autism based on detailed neuropsychological testing using either the ADOS or both the ADOS and the ADI-R. The results of this case-control study provide strong evidence against association of autism with increased antibody reactivity to GM1 ganglioside.

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Author contributions: S.M. carried out experiments, acquired data, performed statistical analyses, drafted the initial manuscript, and approved the final manuscript as submitted. N.M.L. assisted in acquisition of study samples and clinical data, reviewed and revised the manuscript, and approved the final manuscript as submitted. P.H.G. assisted in acquisition of study samples and clinical data, reviewed and revised the manuscript, and approved the final manuscript as submitted. D.H. assisted in acquisition of study samples and clinical data, reviewed and revised the manuscript, and approved the final manuscript as submitted. J.J.H. assisted in acquisition of study samples and clinical data, directed the Weill Cornell Autism Research Program, reviewed and revised the manuscript, and approved the final manuscript as submitted. A.M.R. assisted in acquisition of study samples and clinical data, reviewed and revised the manuscript, and approved the final manuscript as submitted. A.A. obtained funding for the study, conceptualized and designed

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