

Esophageal Motility Dysfunction in Children With Rett Syndrome, Gastroesophageal Reflux, and Dysphagia

KEY WORDS: Rett syndrome, manometry, gastroesophageal reflux disease (GERD), dysphagia

ABSTRACT

Background: Rett syndrome is a neurodevelopmental disorder associated with gastroesophageal reflux disease (GERD) and dysphagia.

Objective: Correlate esophageal motility disturbances with symptoms of GERD and dysphagia and with *MECP2* gene mutations in children with Rett syndrome.

Study Design: Thirty-two consecutive Rett patients with a mean (range) age of 6.2 (2.3-14) years with prior history of feeding problems underwent esophageal manometry. Lower esophageal sphincter

(LES) pressure and percent relaxation, mean peak esophageal body contractions, and percent of swallows with abnormal peristalsis were quantified.

Results: Patients with GERD ($n = 13$) or dysphagia ($n = 11$) had 37% and 34% of swallows followed by abnormal esophageal peristalsis, respectively, compared to 8% in patients without symptoms ($P < 0.01$). Patients with fundoplication ($n = 4$) had 45% of swallows followed by abnormal esophageal peristalsis versus 17% in those without fundoplication ($P < 0.05$). Fundoplication patients showed higher LES resting pressures (35 mmHg vs 23.5 mmHg, $P < 0.01$) compared with patients without previous anti-reflux surgery. There was no association between esophageal motor disturbances and *MECP2* mutations.

Table 1. Percentage abnormal esophageal peristalsis during esophageal manometry after swallows in patients with Rett syndrome. Asymptomatic patients served as controls. Subjects with symptoms of GERD or dysphagia were compared to controls. Patients with GERD only (no dysphagia), dysphagia only (no GERD), and patients with both GERD and dysphagia were also compared to controls.

Symptoms	Abnormal Peristalsis (%)	P Value
Controls (n = 15)	8	
GERD (n = 13)	37	0.004
Dysphagia (n = 11)	34	0.007
GERD only (n = 5)	31	0.02
Dysphagia only (n = 3)	18	0.16
Both GERD and dysphagia (n = 8)	41	0.003

Conclusions: Decreased esophageal peristalsis is a common finding in Rett patients with symptoms of GERD and dysphagia with or without fundoplication. Prospective studies are needed to determine if esophageal manometry should be used to screen Rett patients for esophageal motility dysfunction before anti-reflux procedures.

INTRODUCTION

Rett syndrome is a neurodevelopmental disorder affecting postnatal brain growth that characteristically occurs in females with a prevalence of 1:10,000 to 1:22,000.¹ In approximately 70%-80% of patients, clinical features of Rett syndrome are associated with mutations in the methyl CpG binding protein 2 (*MECP2*) gene located in the chromosome Xq28 region.²⁻⁴ Failure of brain growth results in developmental regression and is accompanied by seizures, respiratory irregularities, and severe mental retardation. Patients also have significant autonomic involvement resulting in gastrointestinal (GI) abnormalities and peripheral vasomotor instability.^{5,6} The common GI manifestations seen in Rett syndrome include gastroesophageal reflux disease (GERD), dysphagia, feeding impairment that often results in failure to thrive, and constipation. We have previously shown that patients with mutations closer to the *MECP2* amino-

terminus (proximal) had a greater number of gastrointestinal problems and often required early gastrostomy for severe malnutrition.⁷

The primary GI neuromuscular or motility mechanism underlying GERD and dysphagia in Rett patients has not yet been defined. It is unclear if the pathophysiology of GERD in patients with Rett syndrome can simply be explained through transient relaxations of the lower esophageal sphincter (TLESRs).⁸⁻¹¹ Gastroesophageal reflux disease and dysphagia may also be influenced by disordered esophageal body peristalsis resulting in impaired clearance of acid from the esophagus or disruption of bolus transit, respectively. Although dysmotility of the esophagus may occur as a secondary phenomenon related to esophageal injury from chronic exposure to refluxed gastric contents, an underlying inherent motility disorder of the esophageal body may contribute to symptoms of GERD and dysphagia in children with neurologic impairment.¹²⁻¹⁴

Antireflux surgery has been a mainstay of treatment for severe GERD in children since the 1960s.^{15,16} A review of the Pediatric Health Information Survey database has shown an upward trend in the rate of fundoplication.¹⁷ Among these patients, 14% underwent fundoplication without a prior gastroenterology consultation.^{17,18} Common side effects

Table 2. Classification of patients by mutation type and GI symptoms. Number of patients with proximal, distal, and no mutation of the *MECP2* gene and the clinical GI symptoms for each mutation are listed. Specific mutation types are noted for each category.

	Number of Patients	Reflux	Failure to Thrive	Dysphagia	Constipation
Proximal					
T158M	8	4	7	3	6
R133C	4	0	1	1	0
R168X	1	1	1	1	1
Deletion exon 3 & 4	1	0	1	0	1
1164 del/A140V	1	1	0	0	1
Distal					
R270X	4	1	2	3	3
R306H	2	1	2	0	1
R306C	2	2	1	1	1
R294X	2	1	1	0	1
R255X	1	0	0	0	1
P322L	1	0	0	0	0
Del 1152-1192	1	1	0	0	0
996_997 ins A	1	1	1	1	0
807 del	1	1	1	1	1
1163 del 26	1	1	1	0	1
1161 del 6	1	0	0	0	0
No mutation	2	0	2	0	0

related to the surgery include: dysphagia, gagging and retching, nausea, and abdominal distension. Postoperative complications or procedure failure can be as high as 25% in neurologically impaired children and may reflect an inherent esophageal or gastric motility disorder.¹⁹⁻²⁶

Studies defining the manometric characteristics of the esophagus in neurologically impaired children before and after fundoplication are limited.²⁷ Since children with Rett syndrome demonstrate a high incidence of GERD and dysphagia in the setting of severe neurological impairment, it was the objective of this study to better define esophageal neuromuscular function in these children. We also assessed esophageal motility in Rett patients with previous fundoplication. Finally, we examined the association between patients' genotype

in terms of mutations at the *MECP2* gene and esophageal symptoms and manometric findings.

PATIENTS AND METHODS

Patients

Thirty-two consecutive patients with Rett syndrome who were seen between September 2004 and March 2006 were included in this study. These patients had a history of feeding problems and were referred for neurologic assessment either to the Johns Hopkins University Children's Center or the Kennedy-Kreiger Institute. Feeding problems were defined as inability to consume adequate calories orally, prolonged feeding time, or food refusal/selectivity. Gastroesophageal reflux disease was defined based on prior clinical assessment and diagnostic evaluation of patient's referring physician. Children

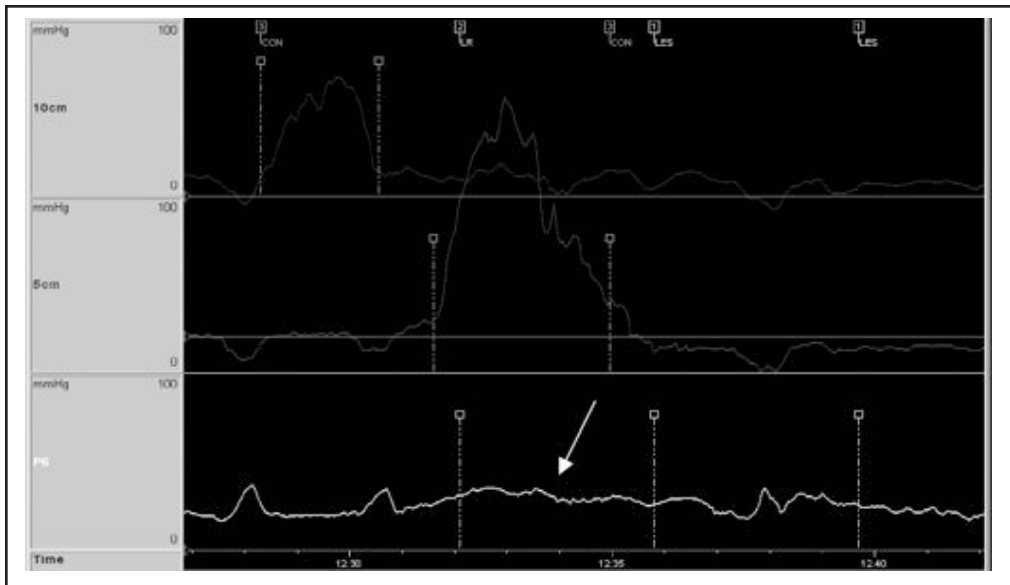


Figure 1. The lower panel shows failure of LES relaxation in a child with Rett syndrome and symptoms of GERD and dysphagia. The mean LES resting pressure for this patient was approximately 26 mmHg (arrow). The top and middle panels show esophageal peristalsis immediately after a wet swallow. Time is represented in minutes.

were considered to have GERD for symptoms of effortless regurgitation or vomiting and improvement of symptoms after acid suppression, a history of an abnormal pH probe, or endoscopic evidence of esophagitis. A diagnosis of dysphagia was based on evaluation by a pediatric gastroenterologist in consultation with a speech pathologist. Children were considered to have dysphagia if they exhibited symptoms of gagging or choking with oral intake, complaints of pain with eating, or an abnormal video fluoroscopic swallow study performed with a speech pathologist. A diagnosis of failure to thrive was made by a pediatric gastroenterologist in consultation with a pediatric dietitian using Waterlow's criteria.²⁸ A history of prior gastrostomy or Nissen fundoplication and gastrostomy was also noted for all patients. All subjects underwent *MECP2* mutation analysis and esophageal manometry testing. The study was approved by The Johns Hopkins University Institutional Review Board.

Esophageal Manometry

Esophageal manometry was performed without sedation after a 4-hour fasting period. A flexible 4-channel Koningberg solid-state catheter (Medical Measurement Systems USA, Inc. [MMS], Dover, New Hampshire, USA) was utilized. Pressure results from the esophageal body and LES were analyzed using MMS computer software. Patients were studied for approximately 20-30 minutes. Pressure readings were obtained at the LES and the esophageal body at distances 3-5 cm and 5-10 cm proximal to the LES based on the size of the patient. Pressure results after approximately 5-10 wet swallows (minimum 3 mL of water or juice) were recorded in mmHg.

The following manometry results were quantified: LES resting pressure, mean percent LES relaxation after all swallows, mean LES residual pressure after all swallows, mean peak amplitude for esophageal contractions after all swallows, and percent of swallows fol-

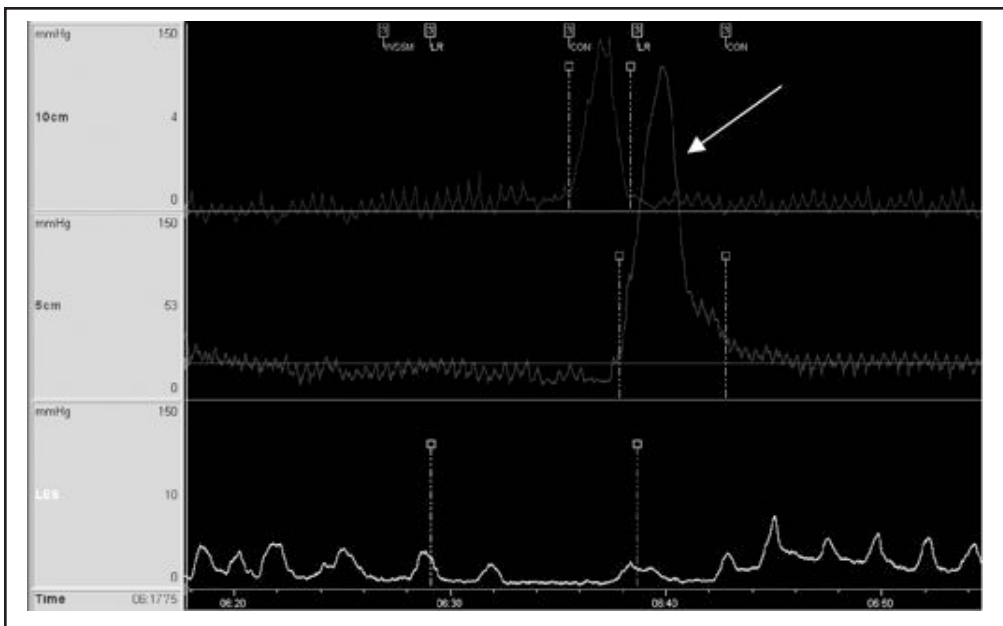


Figure 2. The top and middle panels show esophageal peristalsis immediately after a wet swallow with abnormally high amplitude esophageal body contractions in a child with Rett syndrome, prior fundoplication and symptoms of dysphagia. The arrow in the middle panel depicts an esophageal contraction in excess of 250 mmHg (arrow). Lower esophageal sphincter relaxation is shown in the lower panel. Time is represented in minutes.

lowed by abnormal esophageal peristalsis. Lower esophageal sphincter resting pressures from 6-25 mmHg were considered normal; LES relaxation less than 90% and LES residual pressures greater than or equal to 8 mmHg were considered abnormal. Esophageal peristalsis was considered abnormal if less than 80% of waves propagated in an antegrade direction, or if the peak amplitude of contraction was greater than 100 mmHg or less than 30 mmHg.

Genomic DNA Isolation, PCR, Genotyping, and Mutation Analysis of *MECP2*

Genomic DNA was isolated from peripheral blood samples or lymphoblast cell lines using methods described by Miller et al.²⁹ Mutation analysis was performed using a combination of denaturing high pressure liquid chromatography (DHPLC)³⁰ and direct sequencing.⁴ Patients, who initially

tested negative for *MECP2* mutations, were detected to have large deletions in the gene by Multiplex Ligation-Dependent Probe Amplification (MLPA) technology.³¹ Mutations defined as proximal mutations occurred closer to the amino-terminus, prior to amino acid 255, compared with mutations toward the carboxyl-terminus (distal).

Statistical Analysis

All analyses were performed using the statistical software package STATA version 8.0. We used Student *t*-tests for continuous outcomes and chi-squared analyses for categorical and dichotomous outcome data. A *P* value <0.05 was considered statistically significant.

RESULTS

Demographics

All patients in this study (n = 32) were female. The mean (range) age of the 32 patients was 6.2 (2.3-14) years. The mean

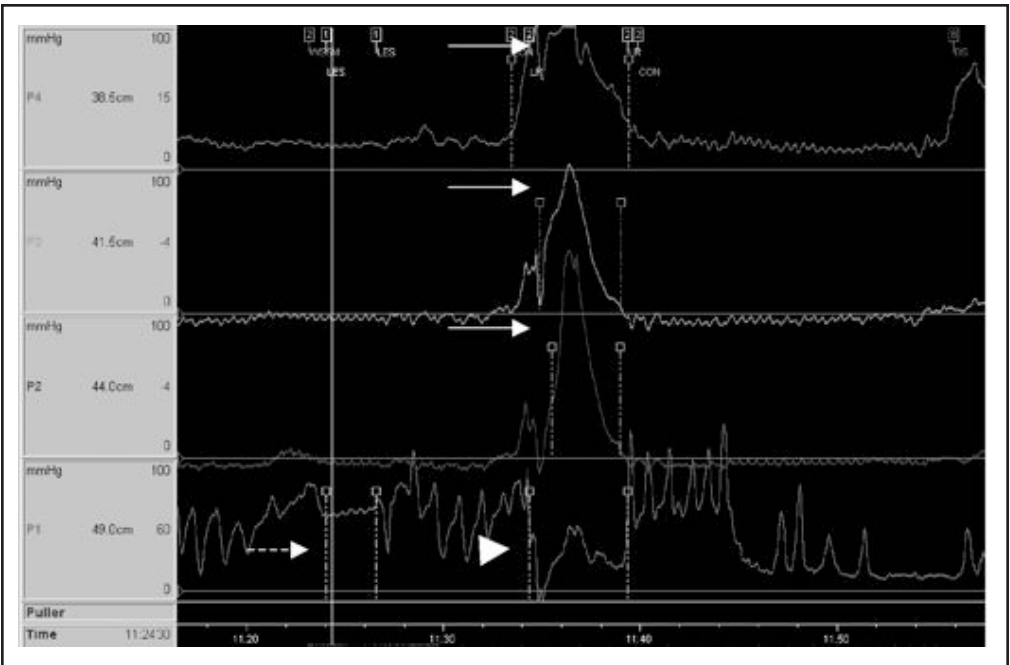


Figure 3. The upper 3 panels show simultaneous esophageal body contractions (arrows) in a patient with Rett syndrome immediately after a wet swallow. The lower panel shows an elevated LES resting pressure of approximately 62 mmHg (dashed arrow) with LES relaxation (arrowhead). Time is represented in minutes.

(range) age of patients at time of diagnosis of Rett syndrome was 2.8 (0.8-8.8) years. A clinical history of GERD was documented in 13 (40.6%), dysphagia in 11 (34.4%), failure to thrive in 21 (65.6%), and constipation in 17 (53.1%) patients. There were 15 patients without symptoms of GERD and dysphagia, and 8 with both GERD and dysphagia. A gastrostomy tube had been placed in 3 (8.8%) and a combined Nissen fundoplication and gastrostomy tube placement had been performed in 4 patients (11.8%).

Esophageal Manometry

High quality esophageal motility tracings were achieved in 30 of the 32 patients with Rett syndrome. The percentage of peristaltic contractions was not calculated in 2 patients due to artifact. The patients averaged 6.7 swallows per study (range 2-17 swallows).

Only 8 (25%) patients demonstrated

completely normal LES and esophageal peristalsis. Abnormal LES relaxation was demonstrated in 12 (37.5%) of the patients. The degree of incomplete relaxation ranged from 38% to 88%. Figure 1 shows an example of incomplete relaxation of the LES in a patient.

Abnormally high residual pressures were found in 6 (18.8%) of the patients, and these pressures ranged from 8 to 16 mmHg. In 9 patients (28.1%), LES resting pressures were abnormally high, ranging from 26-49 mmHg. No patients exhibited LES pressures below normal. Studies were limited to 30 minutes, and during this period of time TLESRs were not observed.

After wet swallows, 16 (53.3%) of the patients demonstrated abnormally high pressures in the esophageal body at either 3-5 or 5-10 cm proximal to the LES. The pressures ranged from 101-201 mmHg (Figure 2). One patient demonstrated mean pressure amplitude in the

esophageal body of 326 mmHg. This observation was reproduced on a subsequent manometric study performed for persistent symptoms of dysphagia. Nine (28%) of the patients showed abnormal propagation of esophageal waves (Figure 3). Two patients underwent repeat manometry testing 6 months later for persistent dysphagia, and abnormalities in esophageal pressures and LES function were persistent and similar compared to the original study.

Esophageal Manometry Results in Patients With and Without GERD and Dysphagia Symptoms

Patients without symptoms of GERD and dysphagia ($n = 15$) had 8% of wet swallows followed by abnormal peristalsis. Patients with GERD or dysphagia had 37% and 34% of wet swallows followed by abnormal peristalsis, respectively ($P < 0.01$). In patients with GERD alone ($n = 5$), 31% of wet swallows were followed by abnormal peristalsis ($P < 0.03$). In those with dysphagia alone ($n = 3$), 18% of wet swallows were followed by abnormal peristalsis ($P < 0.2$). In patients with both GERD and dysphagia ($n = 8$), 41% of patients demonstrated abnormal peristalsis ($P < 0.004$) (Table 1). Mean LES resting pressure was almost identical for patients with GERD (25.5 mmHg) and dysphagia (25.7 mmHg), respectively (normal 6-25 mmHg). There was no association between failure to thrive or constipation and abnormal manometry.

Esophageal Motility After Nissen Fundoplication

Patients with a prior fundoplication had 45% of wet swallows followed by abnormal peristalsis compared to 17% in those without a fundoplication ($P < 0.05$). Patients with a fundoplication also demonstrated increased LES resting pressure (35 mmHg) compared to 23.5 mmHg in patients without fundoplica-

tion ($P < 0.01$). There was a trend towards abnormal LES relaxation in patients with fundoplication (70.5% relaxation versus 87.6%, $P < 0.09$). Among the 4 patients with a fundoplication, 3 had symptoms of dysphagia and 3 were being treated with ranitidine for persistent symptoms of GERD at the time of esophageal manometric assessment.

Genetics

Seventeen patients (50%) had a distal mutation, 15 (44.1%) had a proximal *MECP2* mutation, and 2 (5.9%) had no mutation. A total of 8 patients (23.5%) had the T158M mutation, the most common genotype in the population studied. There was no statistical association found between symptoms of GERD, failure to thrive, dysphagia, or constipation and mutation class (distal or proximal). In addition, there was no correlation between any individual mutation types and clinical variable (Table 2).

DISCUSSION

Children with various neurologic impairments have an increased risk of GERD compared with children who are neurologically normal.³² The explanation for increased GERD in neurologically impaired children is not well defined. Children with Rett syndrome are often profoundly neurologically disabled and have autonomic dysfunction, which has been associated with several gastrointestinal manifestations such as GERD, dysphagia, failure to thrive, and constipation. The results of this study suggest that abnormalities in esophageal peristalsis may represent another component in the spectrum of motility changes exacerbated by acid and non-acid reflux. Poor esophageal peristalsis may play a role in impaired esophageal clearance resulting in a higher incidence of dysphagia and persistent GERD among

patients with Rett syndrome. We found abnormalities in peristalsis after 37% of wet swallows in patients with GERD and 34% of wet swallows in patients with dysphagia compared to 8% in asymptomatic subjects. The abnormal esophageal contractions were simultaneous, retrograde, or non-transmitted, and were often high-amplitude.

In a study in 1999, Rett patients with GERD and dysphagia were evaluated by an oral feeding assessment by a speech pathologist, a videofluoroscopic swallow study, and an upper gastrointestinal series.⁶ Esophageal dysmotility was found in 46% of patients. The esophageal motility abnormalities observed by x-rays were absence of primary or secondary peristaltic waves, esophageal atony, and esophageal spasm. Using more sensitive esophageal manometric recordings we found about 75% of our patients demonstrated esophageal motility abnormalities in either abnormal LES or esophageal peristalsis.

These abnormalities of the esophageal body raise the possibility of an additional neuromuscular mechanism for GERD and dysphagia in Rett patients. Sondheimer et al³² attributed symptoms of GERD to hypotensive LES in neurologically impaired children. In their study, patients with clinical GERD had a mean basal LES pressure almost 17 mmHg less than those without GERD (12.5 mmHg versus 29.0 mmHg, respectively), and 27% of patients demonstrated failure of esophageal peristalsis. In our study, 28% of Rett patients had abnormal esophageal peristalsis. However, the LES pressure was normal or increased in our Rett patients with GERD and dysphagia. Thus, in Rett syndrome, abnormal peristalsis in the esophageal body may contribute to GERD and dysphagia. Poor esophageal peristalsis may further impair clearance of acid or non-acid refluxate, thereby

explaining the persistence of symptoms despite maximal medical management.

Another unusual esophageal motility observation in Rett patients was high amplitude esophageal contractions after swallowing. Some patients exhibited mean pressures over 150 mmHg and as high as 326 mmHg. There was no statistical association between these high pressures and symptoms of GERD and dysphagia, Nissen fundoplication, or abnormal esophageal peristalsis. The significance of these high pressure contractions is unclear, but may be attributed to the underlying autonomic nervous system dysfunction in Rett syndrome. High amplitude esophageal contractions are not characteristic of routine GERD and support the notion that other mechanisms need to be considered in Rett syndrome and other neurologically affected children.

Among the 4 patients who had a previous fundoplication, all had abnormal esophageal peristalsis, and 3 of the 4 had symptoms of dysphagia. Preoperative manometric data were not available on these patients to determine an inherent esophageal dysmotility prior to surgery. The symptoms described in our postfundoplication Rett patients are consistent with symptoms commonly observed in children with neurologic impairment, respiratory disease, and previous anatomic anomalies such as esophageal atresia.³³⁻³⁸ The incidence of late postfundoplication complications is approximately 26% for neurologically impaired children compared to 12% for neurologically normal children.³⁴ Postoperative mortality is high in neurologically impaired children and ranges from 6% to 12%.^{34,35,39,40} Since these postfundoplication complications do not appear as frequently in neurologically normal children⁴¹⁻⁴³ or adults,⁴⁴⁻⁴⁹ manometry has not been considered mandatory in these patients. Our results in post-fundoplication raise the concern that some patients

with Rett syndrome may demonstrate primary abnormalities in esophageal peristalsis and may benefit from pre-fundoplication manometry testing to determine if alternative methods to treat reflux should be considered in these children. Future prospective esophageal motility studies before and after fundoplication are needed to address this possibility.

Diffuse neuromuscular dysfunction of the GI tract may explain the discrepancy in postfundoplication outcomes in neurologically abnormal compared to neurologically intact children. Abnormal esophageal motility related to the LES and antroduodenal motility has been observed in animal models¹² and in children with brain injury.^{12-14,50} Delayed gastric emptying in neurologically impaired children has also been well-described.^{36,51,52} Indeed, when delayed gastric emptying is identified preoperatively, an improved clinical outcome is often observed post-Nissen fundoplication when combined with pyloroplasty.³⁶

An association between abnormal esophageal motility and a patient's *MECP2* genotype was not found in this study. Naidu et al⁷ demonstrated that patients with more proximal mutations in *MECP2* had a greater number of GI symptoms (GERD, dysphagia, failure to thrive, and constipation) than those with distal *MECP2* mutations. Our failure to find an association between GI symptoms or esophageal motility and *MECP2* mutations may be explained by the limited number of Rett patients and the wide variety of mutation types for the respective proximal and distal *MECP2* mutation groups. It also raises the possibility that alternative genetic mechanisms may be responsible for the clinical GI symptoms seen in Rett syndrome. Future studies with greater numbers of specific mutation types and Rett patients are needed to determine the existence of a genotype-phenotype cor-

relation.

Rett syndrome is an uncommon disorder and our study overall was limited by the small number of patients studied. Our control group (non-GERD, non-dysphagia) consisted of subjects with Rett syndrome and feeding problems. Future studies of patients with feeding disorders without a diagnosis of Rett may allow more specific conclusions about the relationship between autonomic instability and esophageal neuromuscular disorders in this syndrome. In addition, when pH, impedance, or esophageal biopsy results were not available, the diagnosis of GERD was based on symptom history with evidence of improvement during treatment with acid reducing agents. Future studies including pH, impedance, or endoscopic and histologic findings will allow more objective evidence of GERD.

In conclusion, patients with Rett syndrome have a high incidence of GERD and dysphagia, which are associated with abnormal esophageal peristalsis and not hypotensive LES pressures or incomplete relaxation of the LES. Abnormalities in esophageal peristalsis may be secondary to acid and non-acid reflux; however, this study raises the possibility of primary esophageal body dysmotility in some patients with Rett syndrome, which may be attributed to their underlying autonomic nervous system instability. Esophageal motility testing may be considered in children with Rett syndrome and symptoms of GERD or dysphagia and before fundoplication.

ACKNOWLEDGEMENTS

The study is funded by grants from PO1-HD24448, FD-R-002408, MO1RR00052, and the Thomas Wilson Award (AD). The authors would also like to thank Dr. R. Thompson for his statistical analysis.

REFERENCES

1. Kozenietz CA, Skender, ML, MacNaughton

- N, et al: Epidemiology of Rett syndrome: a population-based registry. *Pediatrics* 1993;91:445-450.
2. Amir RE, Van den Veyver IB, Wan M, et al: Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999;23:185-188.
 3. Sirianni N, Naidu S, Pereira J, et al: Rett syndrome: confirmation of X-linked dominant inheritance, and localization of the gene to Xq28. *Am J Hum Genet* 1998;63:1552-1558.
 4. Hoffbuhr K, Devaney JM, LaFleur B, et al: MeCP2 mutations in children with and without the phenotype of Rett syndrome. *Neurology* 2001;56:1486-1495.
 5. Julu POO, Kerr AM, Apartopoulos F, et al: Characterisation of breathing and associated central autonomic dysfunction in the Rett disorder. *Arch Dis Child* 2001;85:29-37.
 6. Motil K, Schultz RJ, Browning K, et al: Oropharyngeal dysfunction and gastroesophageal dysmotility are present in girls and women with Rett syndrome. *J Pediatr Gastroenterol Nutr* 1999;29:31-37.
 7. Naidu S, Bibat G, Kratz L, et al: Clinical variability in Rett syndrome. *J Child Neurol* 2003;18:662-668.
 8. Gold BD: Gastroesophageal reflux disease: could intervention in childhood reduce the risk of later complications. *Am J Med* 2004;117:23S-29S.
 9. Kawahara H, Dent J, Davidson G: Mechanisms responsible for gastroesophageal reflux in children. *Gastroenterology* 1997;113:399-408.
 10. Jadcherla SR: Manometric evaluation of esophageal-protective reflexes in infants and children. *Am J Med* 2003;115:157S-160S.
 11. Massey BT, Simuncak C, Lecapitaine-Dana NJ, et al: Transient lower esophageal sphincter relaxations do not result from passive opening of the cardia by gastric distension. *Gastroenterology* 2006;130:89-95.
 12. Vane DW, Shiffler M, Grosfeld JL, et al: Reduced lower esophageal sphincter (LES) pressure after acute and chronic brain injury. *J Pediatr Surg* 1982;17:960-964.
 13. Tovar JA, Morras I, Arana J, et al: Functional study of gastroesophageal reflux in children with brain disease. *Chir Pediatr* 1986;27:134-137.
 14. Staiano A, Cucchiara S, Del Giudice E, et al: Disorders of oesophageal motility in children with psychomotor retardation and gastroesophageal reflux. *Eur J Pediatr* 1991;150:638-641.
 15. Nissen R: Gastropexy and "fundoplication" in surgical treatment of hiatal hernia. *Am J Dig Dis* 1961;6:954-961.
 16. Filler AM, Randolph JG, Gross RE: Esophageal hiatus hernia in infants and children. *J Thorac Cardiovasc Surg* 1964;47:551-565.
 17. Gibbons TE, Stockwell J, Kreh RP, et al: Population based epidemiological survey of gastroesophageal reflux disease in hospitalized U.S. children, abstracted (#2142). *Gastroenterology* 2001;120:A419.
 18. Gold BD: Outcomes of pediatric gastroesophageal reflux disease: in the first year of life, in childhood, and in adults...oh, and should we really leave *Helicobacter pylori* alone. *J Pediatr Gastroenterol Nutr* 2003;37:S33-S39.
 19. Fonkalsrud EW, Ashcraft KW, Coran AG, et al: Surgical treatment of gastroesophageal reflux in children: a combined hospital study of 7467 patients. *Pediatrics* 1998;101:419-422.
 20. Vecchia LKD, Grosfeld JL, West KW, et al: Reoperation after Nissen fundoplication in children with gastroesophageal reflux: experience with 130 patients. *Ann Surg* 1997;226:315-323.
 21. Subramaniam R, Dickson AP: Long-term outcome of Boix-Ochoa and Nissen fundoplication in normal and neurologically impaired children. *J Pediatr Surg* 2000;35:1214-1216.
 22. Zaninotto G, Molena D, Ancona E: A prospective multicenter study on laparoscopic treatment of gastroesophageal reflux disease in Italy: type of surgery, conversions, complications, and early results. Study group for the laparoscopic treatment of gastroesophageal reflux disease of the Italian society of endoscopic surgery (SICE). *Surg Endosc* 2000;14:282-288.
 23. Leggett PL, Bissell CD, Churchman-Winn R, et al: A comparison of laparoscopic Nissen fundoplication and Rossetti's modification in 239 patients. *Surg Endosc* 2000;14:473-477.
 24. Martinez DA, Ginn-Pease ME, Caniano DA: Sequelae of antireflux surgery in profoundly disabled children. *J Pediatr Surg* 1992;27:267-271.
 25. Diaz DM, Gibbons TE, Heiss K: Antireflux surgery outcomes in pediatric gastroesophageal reflux disease. *Am J Gastro* 2005;100:1844-1852.
 26. Di Lorenzo C, Flores A, Hyman PE: Intestinal motility in symptomatic children with fundoplication. *J Pediatr Gastroenterol Nutr* 1991;12:169-173.
 27. Kawahara H, Nakajima K, Yagi M, et al: Mechanisms responsible for recurrent gastroesophageal reflux in neurologically

- impaired children who underwent laparoscopic Nissen fundoplication. *Surg Endosc* 2002;16:767-771.
28. Water JC: *Classification and Definition of Protein-Energy Malnutrition. Nutrition in Preventive Medicine*. Geneva: WHO; 1976:530-555.
 29. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
 30. Kaler SG, Devaney JM, Pettit EL, et al: Novel method for molecular detection of the two common hereditary hemochromatosis mutations. *Genet Test* 2000;4:125-129.
 31. Erlandson A, Samuelsson L, Hagberg B, et al: Multiplex ligation-dependent probe amplification (MLPA) detects large deletions in the MECP2 gene of Swedish Rett syndrome patients. *Genet Test* 2003;7:329-332.
 32. Sondheimer JM, Morris BA: Gastroesophageal reflux among severely retarded children. *J Pediatr* 1979;94:710-714.
 33. Curci MR, Dibbons AW: Problems associated with a Nissen fundoplication following tracheoesophageal fistula and esophageal atresia repair. *Arch Surg* 1988;123:618-620.
 34. Pearl RH, Robie DK, Ein SH, et al: Complications of gastroesophageal antireflux surgery in neurologically impaired versus neurologically normal children. *J Pediatr Surg* 1990;25:1169-1173.
 35. Richards CA, Milla PJ, Andrews PLR, et al: Retching and vomiting in neurologically impaired children after fundoplication: predictive preoperative factors. *J Pediatr Surg* 2001;36:1401-1404.
 36. Alexander F, Wyllie R, Jirousek K, et al: Delayed gastric emptying affects outcome of Nissen fundoplication in neurologically impaired children. *Surgery* 1997;122:690-698.
 37. Rice H, Seashore JH, Touloukian RJ: Evaluation of Nissen fundoplication in neurologically impaired children. *J Pediatr Surg* 1991;26:697-701.
 38. Esposito C, Van Der Zee DC, Settini A, et al: Risks and benefits of surgical management of gastroesophageal reflux in neurologically impaired children. *Surg Endosc* 2003;17:708-710.
 39. Spitz L, Kirtane J: Results and complications of surgery for gastro-oesophageal reflux. *Arch Dis Child* 1985;60:743-747.
 40. Inge TH, Carmeci C, Ohara LJ, et al: Outcome of Nissen fundoplication using intraoperative manometry in children. *J Pediatr Surg* 1998;33:1614-1617.
 41. Kawahara H, Imura K, Nakajima K, et al: Motor function of the esophagus and the lower esophageal sphincter in children who undergo laparoscopic Nissen fundoplication. *J Pediatr Surg* 2000;35:1666-1671.
 42. Godoy J, Tovar JA, Vicente Y, et al. Esophageal motor dysfunction persists in children after surgical cure of reflux: an ambulatory manometric study. *J Pediatr Surg* 2001;36:1405-1411.
 43. Mattioli G, Gentilino V, Caponcelli E, et al: Effectiveness of esophageal manometry in predicting the outcome of children with primary GER after floppy Nissen-Rossetti wrap. *Surg Endosc* 2004;18:1504-1508.
 44. Ravi N, Al-Sarraf N, Moran T, et al: Acid normalization and improved esophageal motility after Nissen fundoplication: equivalent outcomes in patients with normal and ineffective esophageal motility. *Am J Surg* 2005;190:445-450.
 45. Rydberg L, Ruth M, Lundell L: Does oesophageal motor function improve with time after successful antireflux surgery? Results of a prospective, randomized clinical study. *Gut* 1997;41:82-86.
 46. Munitiz V, Ortiz A, Martinez LF, et al: Ineffective oesophageal motility does not affect the clinical outcome of open Nissen fundoplication. *Br J Surg* 2004;91:1010-1014.
 47. Frantides CT, Carlson MA, Madan AK, et al: Selective use of esophageal manometry and 24-hour pH monitoring before laparoscopic fundoplication. *J Am Coll Surg* 2003;197:358-364.
 48. Velanovich V, Mahatme A: Effects of manometrically discovered nonspecific motility disorders of the esophagus on the outcomes of antireflux surgery. *J Gastrointest Surg* 2004;8:335-341.
 49. Heider TR, Behrns KE, Koruda MJ, et al: Fundoplication improves disordered esophageal motility. *J Gastrointest Surg* 2003;7:159-163.
 50. Miki K, Harada T, Kozaiwa K: Antroduodenal motor function and gastro-oesophageal reflux in neurologically impaired children, adolescents and young adults. *Eur J Pediatr* 1998;157:695-698.
 51. Fonkalsrud EW, Foglia RP, Ament ME, et al: Operative treatment for the gastroesophageal reflux syndrome in children. *J Pediatr Surg* 1989;24:525-529.
 52. Bustorff-Silva J, Fonkalsrud EW, Perez CA, et al: Gastric emptying procedures decrease the risk of postoperative recurrent reflux in children with delayed gastric emptying. *J Pediatr Surg* 1999;34:79-83.