Megaesophagus is a Major Pathological Condition in Rats With a Large Deletion in the Rbm20 Gene

Denise J. Schwahn¹, Jonathan M. Pleitner², and Marion L. Greaser²

Abstract
A spontaneously arising, loss-of-function mutation in the RNA binding motif protein 20 (Rbm20) gene, which encodes a nuclear splicing protein, was previously identified as the underlying reason for expression of an abnormally large TITIN (TTN) protein in a rat model of cardiomyopathy. An outbreak of Pseudomonas aeruginosa led to submission of rats with dyspnea, sneezing, lethargy, nasal discharge, and/or unexpected death for diagnostic evaluation. Necropsy revealed underlying megaesophagus in Rbm20⁻/⁻ rats. Further phenotyping of this rat strain and determination of the size of esophageal TTN was undertaken. The Rbm20-defective rats developed megaesophagus at an early age (26 weeks) with high frequency (13/32, 41%). They also often exhibited secondary rhinitis (9/32, 28%), aspiration pneumonia (8/32, 25%), and otitis media/interna (6/32, 19%). In addition, these rats had a high prevalence of hydronephrosis (13/32, 41%). RBM20 is involved in splicing multiple RNA transcripts, one of which is the muscle-specific protein TTN. Rbm20 mutations are a significant cause of dilated cardiomyopathy in humans. In Rbm20-defective rats, TTN size was significantly increased in the skeletal muscle of the esophagus. Megaesophagus in this rat strain (maintained on a mixed genetic background) is hypothesized to result from altered TTN stretch signaling in esophageal skeletal muscle. This study describes a novel mechanism for the development of megaesophagus, which may be useful for understanding the pathogenesis of megaesophagus in humans and offers insights into potential myogenic causes of this condition. This is the first report of megaesophagus and other noncardiac pathogenic changes associated with mutation of Rbm20 in any species.

Keywords
aspiration, cardiomyopathy, hydronephrosis, megaesophagus, otitis media, rat, rhinitis, striated muscle, titin

Megaesophagus is dilation of the esophagus. It can affect part of or the entire esophagus, may be congenital or acquired, and can reflect peripheral or central nervous system disease (afferent, interneuron, or efferent pathways), neuromuscular junction dysfunction, or muscular disease. In humans, megaesophagus is frequently due to lower esophageal sphincter achalasia (failure to relax), with subsequent retention of ingesta within the distal esophagus, inflammation of the wall (esophagitis), loss of ganglion cells, and dilation. Aspiration pneumonia is an important consequence of megaesophagus in several species and can cause significant mortality. Herein, we describe a hereditary rat model (designated the Rbm20⁻/⁻ rat) that recapitulates many features of megaesophagus, including aspiration pneumonia. Megaesophagus has been described in humans, dogs, cats, horses, cattle, rats, mice, nonhuman primates, ferrets, and camels. Its causes and morphological and functional changes in rats are briefly reviewed here. Idiopathic megaesophagus with mortality has been reported in rats. In the case of esophageal impaction, the rats were of the BHE nonobese diabetic strain, and the caudal half of the esophagus was dilated; there was local myodegeneration attributed to compression atrophy. One strain of Long-Evans rats with a high prevalence of megaesophagus exhibited precordial esophageal dilation, decreased numbers of ganglion cells within the myenteric plexus of both the thoracic and abdominal portions of the esophagus, and decreased muscularis thickness in the thoracic portion. This condition was considered hereditary and neurogenic.

¹Research Animal Resources Center and Muscle Biology Laboratory, University of Wisconsin, Madison, WI, USA.
²Muscle Biology Laboratory, University of Wisconsin, Madison, WI, USA.

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Corresponding Author:
Denise J. Schwahn, Zoetis Research and Development, 333 Portage St, Kalamazoo, MI 49007, USA.
Email: denise.schwahn@gmail.com
Cardiac fibrosis. In addition, RBM20 is a splicing factor for onium chloride, which denervates the esophagus, causing achalasia of a shorter splice isoform (ENH3). Defects in a protein found within striated myofibers, increasing the expression of Enigma homolog 1 (ENH1, PDLIM5, L9, ENH), a scaffolding protein that regulates splicing of multiple cardiac proteins.16 Alternative splicing is an important control mechanism for sarcomeric protein function in the heart.16,48 Mutations in RBM20 are a major cause of dilated cardiomyopathy in humans.16,27,28 Adult Rbm20–/– show altered splicing in this rat strain (herein designated Rbm20–/–). The Rbm20–/– rat was discovered by chance during a developmental study on the giant protein TTN.13 Differently sized TTN isoforms are expressed from a single gene via alternative splicing in an age-dependent manner.13,27 In the developmental study, some rats expressed a much larger TTN than that of age-matched wild-type rats, and all rats with the larger TTN came from the same litter.13 The increased TTN size was due to an autosomal dominant 95-kb deletion in Rbm20, corresponding to the loss of exons 2 to 14 and disruption of its important role in alternative splicing.16 Over 30 different genes show altered splicing in this rat strain (herein designated Rbm20–/–), but the changes in TTN appear to be the most significant.16 The Rbm20–/– rat expresses a markedly enlarged TTN protein in striated myofibers of both cardiac and skeletal muscles. Adult Rbm20–/– rats exhibit left ventricular dilation with increased diastolic parameters but no changes in systolic parameters or contractility.16 Rbm20–/– rats have decreased exercise tolerance, electrocardiographic abnormalities, and a predisposition to arrhythmia and unexpected death, similar to humans with RBM20 mutations.16 Histologically, in Rbm20–/– rats, there is subendocardial fibrosis, and ultrastructurally, there are abnormal myofibril arrangements, Z line streaming, and lipofuscin deposits.15 Elongated and flaccid TTN filaments are thought to lead to reduced myofilament recoil with a compensatory increase in collagen biosynthesis, leading to subendocardial fibrosis.16

The current study was undertaken to more completely characterize the phenotype of a spontaneously arising Rbm20 mutation in rats linked to cardiac disease in humans and assess its suitability as a model of human megaesophagus. We identified a high incidence of megaesophagus with subsequent aspiration pneumonia, rhinitis, and/or otitis media with intraleisional foreign material in Rbm20+/– rats. Megaesophagus is hypothesized to be due to the abnormally large TTN isoform expressed in esophageal skeletal muscle, resulting in elongated sarcomeres, myofibers, and organ dilation. A high incidence of hydronephrosis was also noted.

Materials and Methods

Animals

Rbm20+/– rats were maintained on a mixed genetic background consisting of 50% Brown Norway, 25% Fisher 344, and 25% Sprague-Dawley strains. Rats were cohoused in groups of 2 in open-topped cages in 2 separate conventional facilities with 12/12-hour light/dark cycles and stable temperature (22°C). Teklad standard rodent chow (Harlan, Madison, WI) and water were available ad libitum. Enrichment was provided to dams via a stainless steel loft. Animals housed singly were provided with a PVC tube and Nyla bones. Both colonies were free of rat coronavirus, rat theilovirus, Pseudomocystis carinii, Sendai virus, pneumonia virus of mice, Mycoplasma pulmonis, rat reovirus 3, lymphocytic choriomeningitis virus, cilia-associated respiratory bacillus, Hantaan virus, Clostridium piliforme, mouse adenovirus 1, and mouse adenovirus 2. Positive serologic results were occasionally seen for a generic parvovirus antigen in both colonies. All rats were genotyped by polymerase chain reaction (PCR) as described previously.16 Animal care and use were in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1996), and all experimental procedures were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Case Inclusion Criteria

Thirty-two Rbm20+/– rats of both sexes and ages 6 to 60 weeks, 4 Rbm20+/– rats of both sexes and ages 10 to 47 weeks, and 6 Rbm20+/+ (wild-type) rats of both sexes and ages 6 to 84 weeks were included in this study. Initial cases in all 3 genotypes presented with clinical signs that included dyspnea, sneezing, nasal discharge, head tilt, buphthalmia, lethargy, and unexpected death (Suppl. Table S1). Clinically normal rats of all 3 genotypes were recruited for use as phenotypic comparisons. Rats of any genotype that had been experimentally manipulated in any way were excluded from this study.
 Necropsy, Histology, and Photomicrography

All rats were euthanized via carbon dioxide inhalation. Immediately following sacrifice, rats of all genotypes were examined grossly by a board-certified veterinary pathologist (D.J.S.). Rats with clinical signs were subject to complete necropsy, including microbiological culture of relevant tissues. Rats lacking clinical signs were subject to an abbreviated necropsy procedure, which excluded detailed evaluation of the lower intestinal tract. External esophageal diameters were measured using a ruler on the widest portion of the unopened, flattened esophagus. Megaesophagus was defined as a maximal external esophageal diameter ≥5 mm. Subsequently, the entire esophagus and proximal (squamous) portion of the stomach were opened longitudinally and fixed as a Swiss roll, with the cervical esophagus in the center of the roll. In cases with clinical disease, fresh tissues (including brain, tongue, larynx, trachea, lungs, esophagus and proximal stomach, heart, thymus, entire emptied gastrointestinal tract, kidneys, adrenal glands, reproductive organs, bladder, spleen, preputial/elitoral glands) were placed into 10% neutral-buffered formalin or decalcification solution (head, left pelvic limb; Surgipath Decalcifier I, Leica Biosystems, Buffalo Grove, IL). In cases lacking clinical signs, fresh tissues (lungs, cardiopulmonary hilar lymph nodes, esophagus and cranial stomach, heart, thymus, and any grossly abnormal tissue) were placed into 10% neutral-buffered formalin or decalcification solution (head). After 24 hours of fixation, tissues were trimmed, processed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (HE) or Masson’s trichrome for light microscopic evaluation. A board-certified veterinary pathologist (D.J.S.) examined tissues and graded esophageal thickness and fibrosis from rats of all genotypes. Esophageal wall thickness was measured using a stage micrometer. Fibrosis (blue staining of collagen in all genotypes. Esophageal wall thickness was measured using an Olympus DP26 camera (Olympus, Center Valley, PA) mounted on a Nikon Eclipse 50i microscope (Nikon, Melville, NY).

Agarose Gel Electrophoretic Fractionation of TTN Isoforms

The esophagi of both Rbm20+/+ (n = 4–5) and Rbm20−/− (n = 3) rats were isolated and divided into 3 portions of approximately equal length; the portions were designated the cervical, cranial thoracic, and caudal thoracic segments based on anatomic locations. The external esophageal diameter of all esophagi was <5 mm; megaesophagus was not apparent in any rat. Muscle samples from each segment of each rat were collected and immediately flash-frozen in liquid nitrogen. Tissues were solubilized (1:50 w/v) in a sample buffer consisting of 8 M urea, 2 M thiourea, 3% sodium dodecyl sulfate w/v, 75 mM dithiothreitol, 0.03% bromophenol blue, and 0.05 M Tris-Cl, pH 6.8. Equal quantities were subjected to sodium dodecyl sulfate (SDS) gel electrophoresis in agarose. Sizes were estimated using standards of RBM20+/− cardiac TTN isoform (3.83 MDa) run in its own lane on the same gel and RBM20+/+ adult cardiac TTN N2B isoform (2.97 MDA), which was mixed with the esophageal protein samples. Molecular weights were estimated using the 2 standards and assuming a linear relationship between migration distance and the logarithm of the molecular mass.28,46

Statistical Analysis

Statistical analysis was performed using standard formulas within Microsoft Excel (Microsoft, Redmond, WA). P values were obtained using 2-tailed tests for equal variances.

The histologic slides and additional data analyzed in this study are available by request to the first author.

Results

For this study, 42 rats of all Rbm20 genotypes (32 Rbm20+/+, 4 Rbm20+/−, and 6 wild-type) at 6 to 84 weeks of age were examined grossly and microscopically. Of the 32 Rbm20−/− rats, 8 (25%) had clinical signs, including dyspnea, sneezing, lethargy, nasal discharge, and unexpected death; 24 had no clinical signs. One of the 4 (25%) Rbm20+/− rats presented for sneezing; the others had no clinical signs. Four of the 6 (75%) wild-type rats presented with clinical signs that included sneezing, dyspnea, head tilt, buphthalmia, and unexpected death; the others lacked clinical disease. Megaesophagus was present in 13 of 32 (41%) Rbm20−/− rats and 1 of 4 (25%) Rbm20+/− rats (Fig. 1, Suppl. Table S1). In the 13 Rbm20−/− rats with megaesophagus, the external esophageal diameters ranged from 5 to 17 mm; 8 females and 5 males were affected, and the youngest Rbm20−/− animal with megaesophagus was 26 weeks old.
Wild-type rats and rats without mega-esophagus typically had esophageal diameters of 2 mm (Fig. 1, Suppl. Table S1). Megaesophagus always involved the intrathoracic and intra-abdominal portions of the esophagus and never the cervical portion (Fig. 2). The esophageal diameter in the Rbm20<sup>+/−</sup> rat with megaesophagus was not measured, but dilation was grossly apparent. This rat also presented with sneezing.

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Of the 8 rats (5 Rbm20<sup>−/−</sup>, 1 Rbm20<sup>+/−</sup>, and 2 Rbm20<sup>+/+</sup>) with clinical signs of upper respiratory disease (dyspnea, sneezing, and nasal discharge), all 8 had histologic evidence of aspiration pneumonia, rhinitis, and/or otitis media/interna (Figs. 3–5b, Suppl. Table S1). Three of the 5 rats (60%) that presented for lethargy or unexpected death also had histologic evidence of aspiration pneumonia, rhinitis, and/or otitis media/interna; all were Rbm20<sup>−/−</sup>. Aspiration pneumonia, rhinitis, and/or otitis media/interna were identified histologically in an additional 3 rats (all Rbm20<sup>−/−</sup>) that lacked clinical signs of respiratory disease, lethargy, or unexpected death and were apparently healthy (Suppl. Table S1).

A total of 12 Rbm20<sup>−/−</sup> (11/32; 34%) or Rbm20<sup>+/−</sup> (1/4; 25%) rats had histologic evidence of aspiration pneumonia, rhinitis, and/or otitis media/interna; of these rats, all also had megaesophagus. Megaesophagus was not identified in any Rbm20<sup>+/−</sup> rat. Aspiration pneumonia, rhinitis, and/or otitis media/interna (with intralocular foreign material) are considered secondary to megaesophagus and represent reflux of esophageal contents. In the 14 rats with megaeosophagus, the prevalence of rhinitis was 71%, the prevalence of aspiration pneumonia was 64%, and the prevalence of otitis media/interna was 43%. While gross evidence of megaeosophagus was first seen at 26 weeks of age, the sequelae of aspiration pneumonia, rhinitis, and/or otitis media were not identified microscopically until 36 weeks of age. The most common pathogen isolated from cases of aspiration pneumonia, rhinitis, or otitis media/interna was Pseudomonas aeruginosa.

As megaeosophagus is often associated with esophageal wall thinning, the average thickness of the esophageal wall was measured at 3 different locations (cervical, cranial thoracic, and caudal thoracic) on a Swiss roll section in rats of each genotype (Figs. 6–9). Substantial differences in the thickness of esophageal walls were seen at 26 weeks of age, the sequelae of aspiration pneumonia, rhinitis, and/or otitis media/interna was slightly less collagenous connective tissue staining in Rbm20<sup>−/−</sup> animals with megaeosophagus (Fig. 8b) than in age-, sex-, and genotype-matched controls without megaeosophagus were also examined (Figs. 7, 8). In 3 age- and sex-matched pairs of Rbm20<sup>−/−</sup> rats with and without megaeosophagus, the quantity of blue staining on trichrome-stained Swiss rolls of the esophagus was graded (data not shown). There was slightly less collagenous connective tissue staining in Rbm20<sup>−/−</sup> animals with megaeosophagus (Fig. 8b) than in rats with normal esophagi (data not shown).

Fibrosis may be a component of esophageal wall thinning. To address this possibility, Masson’s trichrome staining with numerical scoring of the amount of collagenous (blue) staining was used. Age-, sex-, and genotype-matched controls without megaeosophagus were also examined (Figs. 7, 8). In 3 age- and sex-matched pairs of Rbm20<sup>−/−</sup> rats with and without megaeosophagus, the quantity of blue staining on trichrome-stained Swiss rolls of the esophagus was graded (data not shown). There was slightly less collagenous connective tissue staining in Rbm20<sup>−/−</sup> animals with megaeosophagus (Fig. 8b) than in age-, sex-, and genotype-matched controls without megaeosophagus.

As megaesophagus is often associated with esophageal wall thinning, the average thickness of the esophageal wall among rats with megaeosophagus was not significantly different between Rbm20<sup>−/−</sup> rats with and without megaesophagus (Fig. 7b), but the difference in fibrous tissue in rats with megaeosophagus may be due to the same quantity of extracellular matrix and collagen spread over a greater area (due to dilation).

Megaesophagus may be due to decreased numbers or function of the submucosal or myenteric plexuses within the esophageal wall. Swiss roll sections of rat esophagi were examined for the presence of both plexuses as well as any histological abnormalities (Figs. 7–9). Submucosal plexuses were difficult to identify with HE or trichrome staining in any of the 47 rats examined, but myenteric plexuses were regularly encountered in rats of all Rbm20 genotypes (Figs. 7a inset, 8a inset, 9 inset, and data not shown). Myenteric plexuses were counted in each animal, and the number of these plexuses did not differ by genotype (data not shown). No histologic abnormalities of the plexuses were noted. There were no differences in the number of ganglion cells within the submucosal and myenteric plexuses in animals with megaeosophagus vs those with normal esophagi (data not shown). Inflammation was not identified in any portion of the esophagus in any rat.

An increased prevalence of hydronephrosis was also noted (Fig. 10, Suppl. Table S1). Either unilateral or bilateral hydronephrosis was identified in 14 rats (33%). Thirteen of these rats were Rbm20<sup>−/−</sup> (7 females, 6 males), and one was Rbm20<sup>+/+</sup> (female). The prevalence of hydronephrosis in Rbm20<sup>−/−</sup> rats was 41% (n = 32), and the prevalence of hydronephrosis in
wild-type rats was 17% ($n = 6$). Hydronephrosis was not seen in $Rbm20^{+/+}$ rats, possibly due to the small sample size ($n = 4$). Interestingly, 6 of the 13 (46%) $Rbm20^{-/-}$ rats with hydronephrosis had concurrent megaesophagus, suggesting the possibility of a functional molecular link between the 2 conditions. Seven of the 14 (50%) rats with hydronephrosis had associated urinary disease, such as urolithiasis (15%) or urinary tract infection (8%). The $Rbm20^{+/+}$ rat with hydronephrosis died of septic pneumonia at 52 weeks of age and did not have evidence of concurrent urinary tract disease.

Figures 7–10. Esophageal Swiss rolls from rats of varying $Rbm20$ genotypes. Figure 7 is from a 52-week-old $Rbm20^{-/-}$ rat without mega-esophagus. Figure 8 is from a 60-week-old $Rbm20^{-/-}$ rat with megaesophagus, and Figure 9 is from a 64-week-old $Rbm20^{+/+}$ rat without megaesophagus. In each case, all layers of the esophagus are present in appropriate ratios (a). Insets show normal ganglion cells (arrow) of the myenteric plexus. Similar amounts of fibrosis (F, blue) are seen in the submucosal regions in both the $Rbm20^{-/-}$ rat lacking megaesophagus (Fig. 7b) and the $Rbm20^{+/+}$ rat with megaesophagus (Fig. 8b); a similar amount of fibrosis was also seen in the $Rbm20^{+/+}$ rat (data not shown). A, Hematoxylin and eosin (HE). B, Masson’s trichrome. E, epithelium; M, muscularis. Figure 10. Hydronephrosis, 52-week-old $Rbm20^{-/-}$ rat with megaesophagus. HE. H, dilated pelvis; M, medulla; P, papilla.
Given the role of RBM20 in splicing Ttn and TTN’s role in determining the length of the sarcomere in striated myofibers, examination of the size of the TTN isoforms in Rbm20+/– rats was undertaken. Large molecule gel electrophoresis of the 3 segments of the esophagi of Rbm20+/– and Rbm20–/– rats was used to determine the size of the TTN isoforms in the cervical, cranial thoracic, and caudal thoracic segments (Figs. 11, 12). The TTN isoform seen in the Rbm20–/– rats was much larger in all segments of the esophagi of Rbm20–/– rats (~3700 kDa) compared with the isoform in Rbm20+/– rats, which further appeared to vary by esophageal segment (~3460 kDa in the cervical portion, ~3490 kDa in the cranial thoracic portion, and ~3590 kDa in the caudal thoracic portion) (Fig. 12).

Discussion

This study was undertaken to further characterize the phenotype of Rbm20–/– rats; a high prevalence of megaesophagus with secondary aspiration pneumonia, rhinitis, and/or otitis media was found. There was a close relationship between the presence of megaesophagus, aspiration pneumonia, rhinitis, and/or otitis media and clinical signs such as dyspnea, sneezing, and nasal discharge. Megaesophagus in Rbm20–/– rats was first identified at 26 weeks of age, while the sequelae of aspiration pneumonia, rhinitis, and/or otitis media were first seen at 36 weeks of age. Megaesophagus in Rbm20–/– and Rbm20+/– rats is likely myogenic, as wall thinning was seen in rats of both genotypes regardless of the presence of megaesophagus. This finding supports the hypothesis that the Rbm20 deletion mutation leads to aberrant Ttn splicing and more flaccid (thinner) myofibers and muscles. There was no evidence of increased esophageal wall fibrosis, histologic abnormalities of the ganglion cells of the submucosal or myenteric plexuses, or inflammation. The primary myogenic mechanism of megaesophagus in Rbm20–/– rats is unique among rats with megaesophagus and also differs from most human cases, which tend to be neurogenic.7

This study also found a similarly high prevalence of both megaesophagus (41%) and hydrencephrosis (41%) in rats with a 95-kb, inactivating, autosomal dominant deletion of the Rbm20 gene. The observed increased prevalence of hydrencephrosis in Rbm20–/– rats may be partly due to their genetic background. Congenital hydrencephrosis is a common strain-related lesion in both Brown Norway and Sprague-Dawley rats, 2 of the 3 strains on which the Rbm20 mutation is maintained, and a small contribution of these genetic backgrounds to the prevalence of hydrencephrosis is expected.38,45 The prevalence of hydrencephrosis was reported to be 67% to 75% in Brown Norway rats43 and 2.0% to 5.1% in Sprague-Dawley rats.5,45 The Rbm20–/– rats are 50% Brown Norway and 25% Sprague-Dawley, so the predicted prevalence of hydrencephrosis in such a cross is approximately 34% to 40%. The observed prevalence of hydrencephrosis in Rbm20+/– rats was 17% (n = 6). The observed prevalence of hydrencephrosis in Rbm20–/– rats was 41%, which is slightly greater than expected, and almost half of Rbm20–/– rats with megaesophagus also had hydrencephrosis. Therefore, a link between loss of Rbm20 function and hydrencephrosis is possible. Interestingly, one of the transcripts spliced by RBM20 is MECP2, a gene whose duplication has been associated with hydrencephrosis in human fetuses.9

RBM20 plays an important role in the development of megaesophagus in these rats, most likely through its action in splicing Ttn. The rat esophageal muscularis is composed entirely of striated myofibers.21,39,36 The TTN isoform seen in the esophagi of Rbm20+/– rats is dramatically larger than the isoform in Rbm20+/– rats; this is similar to what is seen in cardiac muscle and in several skeletal muscles of Rbm20+/– rats.16,27,28,33 Identification of larger TTN isoforms in striated muscles of Rbm20+/– rats strongly suggests that TTN is
improperly spliced in Rbm20<sup>−/−</sup> rats. The larger TTN isoform lengthens individual sarcomeres<sup>14</sup>, which is hypothesized to extend to longer myofilaments and myofibers with less passive tension, allowing the development of megaesophagus with secondary respiratory diseases such as aspiration pneumonia, rhinitis, and otitis media/interna. The failure to observe cervical esophageal dilation is attributed to differences in external esophageal pressures, where the cervical portion has high external pressure, and the thoracic and abdominal portions have low external pressures.

TTN extends the full length of the sarcomere and is physically linked to both the Z and M lines, thereby controlling sarcomere length. Larger TTN isoforms have additional amino acids from the inclusion of alternately spliced exons; the additional amino acids are found in the I band region, giving the myofibers (and muscle) a lower passive tension. TTN has also been suggested to act as a stretch sensor in cardiac and skeletal muscle.<sup>10,12,18,19,24,25,29,30</sup> One signaling pathway involves a complex of TTN, muscle LIM protein (MLP), and telethonin (also called TCP1), through which MLP is translocated to the nucleus in response to stretch.<sup>22,23</sup> Mutations in any of these 3 genes are associated with both dilated and hypertrophic cardiomyopathies in humans.<sup>3</sup> In addition, the larger TTN isoforms expressed in the Rbm20<sup>−/−</sup> rats and in humans lead to dilated cardiomyopathy in both species.<sup>4,16,26</sup> Both decreased passive tension of myofibers containing enlarged TTN isoforms and reduced mechanical signaling by TTN are hypothesized to play roles in the pathogenesis of both cardiac and esophageal dilation in the Rbm20<sup>−/−</sup> rat.<sup>16,26</sup> The findings of this study suggest that expression of a very large TTN isoform (due to a large deletion in the Rbm20 gene leading to failure of proper Ttn splicing) causes megaesophagus, with subsequent aspiration pneumonia, rhinitis, and otitis media/interna in rats. There is primary thinning of the esophageal muscularis. Fibrosis, inflammation, or neurogenic atrophy is not a driver of megaesophagus in Rbm20<sup>−/−</sup> rats. The Rbm20<sup>−/−</sup> rat exhibits a primary myogenic megaesophagus and may be a suitable model for myogenic cases of megaesophagus in humans, although most human cases are neurogenic. This is the first report of noncardiac pathogenic changes associated with an Rbm20 mutation.

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ORCID iD
Denise J. Schwahn https://orcid.org/0000-0001-8665-2402
Jonathan M. Pleitner https://orcid.org/0000-0002-5236-2906

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