Journal of Pediatric Surgery (2013) **48**, 2036–2042

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Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Novel exomphalos genetic mouse model: The importance of accurate phenotypic classification

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Received 17 December 2012; revised 18 April 2013; accepted 21 April 2013

Key words:
Exomphalos;
Gastroschisis;
Abdominal wall defect;
Rodent model;
Scrib;
In amnio micro-MRI;
Interstitial cells of Cajal

Abstract

Background: Rodent models of abdominal wall defects (AWD) may provide insight into the pathophysiology of these conditions including gut dysfunction in gastroschisis, or pulmonary hypoplasia in exomphalos. Previously, a Scribble mutant mouse model (*circletail*) was reported to exhibit gastroschisis. We further characterise this AWD in Scribble knockout mice.

Method: Homozygous *Scrib* knockout mice were obtained from heterozygote matings. Fetuses were collected at E17.5–18.5 with intact amniotic membranes. Three mutants and two control fetuses were imaged by *in amnio* micro-MRI. Remaining fetuses were dissected, photographed and gut length/weight measured. Ileal specimens were stained for interstitial cells of Cajal (ICC), imaged using confocal microscopy and ICC quantified.

Results: 127 fetuses were collected, 15 (12%) exhibited AWD. Microdissection revealed 3 mutants had characteristic exomphalos phenotype with membrane-covered gut/liver herniation into the umbilical cord. A further 12 exhibited extensive AWD, with eviscerated abdominal organs and thin covering membrane (intact or ruptured). Micro-MRI confirmed these phenotypes. Gut was shorter and heavier in AWD group compared to controls but morphology/number of ICC was not different.

Discussion: The Scribble knockout fetus exhibits exomphalos (intact and ruptured), in contrast to the original published phenotype of gastroschisis. Detailed dissection of fetuses is essential ensuring accurate phenotyping and result reporting.

Abdominal wall defects include exomphalos and gastroschisis both of which result in differing pathophysiological consequences such as gut dysfunction in gastroschisis and pulmonary hypoplasia in exomphalos. The cause of these sequelae is not fully understood and of great research

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0022-3468
http://dx.doi.org/10.1016/j.jpedsurg.2013.04.010

interest. Genetic rodent models of abdominal wall defects could provide a useful tool in further understanding these conditions. However, phenotyping can be difficult and requires detailed microdissection to ensure accurate result interpretation and reporting.

1. Background

Ventral abdominal wall defects (AWD) are relatively common and include exomphalos (2.5/10,000 live births) [1] and gastroschisis (4.4/10,000 live births) [2]. Both have a significant clinical impact and distinct phenotypic appearances. Exomphalos is characterised by a ventral wall defect disrupting the umbilical ring resulting in herniation of abdominal viscera including gut and liver into the base of the umbilical cord, contained within a membranous sac. The condition is frequently associated with other abnormalities including pulmonary hypoplasia, structural defects of the heart, diaphragm and limbs, and metabolic and chromosomal disorders. However, gut dysfunction and structural gut defects are rare in exomphalos [3]. On the other hand, gastroschisis is a paraumbilical ventral wall defect, usually (95%) lying to the right of the umbilicus [4], through which bowel and very rarely other organs herniate. Characteristically the peritoneal sac is deficient, placing the bowel in direct contact with the irritant amniotic fluid. Gastroschisis is rarely associated with extra-intestinal abnormalities and the most significant cause of morbidity is that of gut dysfunction, which may require prolonged parenteral feeding and carries the risk of central line infections, sepsis and liver dysfunction [5].

The cause, development and pathophysiology of such conditions still remain controversial and are subject to great research interest. For example, it is not clear why gut dysfunction is found mainly in gastroschisis [6–9] while pulmonary hypoplasia is a characteristic feature of exomphalos [10,11]. Investigating abdominal wall defects (AWD) in robust animal models is essential to further our pathophysiological knowledge and develop improved treatment options. Genetic rodent models are advantageous owing to the short gestational period, similar sequence of abdominal wall closure with resolution of physiological hernia by day 16.5 gestational age [12], large litter sizes and early development of the defect without the need for surgical creation. However, characterization of AWD in rodents is not always accurate and currently there is only one genetic animal model of isolated gastroschisis (mice lacking aortic carboxypeptidase-like protein [ACLP]) reported in the literature [9]. Of note, the ventral wall defect present in the *Alx4*$^{-/-}$ mutant mouse was originally reported in the literature as gastroschisis [13] and then subsequently as exomphalos [14].

Previously, the *circletail* mouse mutant, which carries a single base insertion in the *Scrib* (*scribbled homolog*) gene resulting in a frame shift and premature termination of the Scribble protein, was reported to exhibit gastroschisis in association with craniorachischisis [15]. Subsequently, the Scribble mutant model has been further developed by production of a floxed allele, enabling conditional gene targeting and analysis of *Scrib* gene function in various biological systems [16,17]. Our aim was to fully characterize the AWD that results from Scribble loss of function, using the floxed allele to generate *Scrib* null fetuses.

2. Materials and methods

All experimental protocols were granted Home Office approval under the UK Animals (Scientific Procedures) Act 1986.

2.1. Animals

The floxed *Scrib* (*Scrib*^{fl}) allele was a kind gift from Dr Patrick Humbert. Matings between *Scrib*$^{fl/fl}$ homozygotes and mice expressing the ubiquitously expressed βactin-Cre generated a null allele (*Scrib*$^{-}$). Timed matings between heterozygotes (*Scrib*$^{fl/-}$) generated null fetuses (*Scrib*$^{-/-}$). Pregnant mothers were euthanized by cervical dislocation at two gestational stages: (1) day 17.5 (E17.5) when amniotic fluid volume is greater to aid dissection of the fetus from the amniotic cavity and abdominal wall phenotyping; (2) day 18.5 (E18.5) just before full term for gut length, weight and cellular phenotyping. The fetuses were collected by maternal hysterectomy under a dissecting microscope. The uterine muscle was carefully removed leaving the amniotic membranes intact and the intra-amniotic fetus was photographed under a stereo microscope. The fetuses were then carefully dissected from the amniotic sac, euthanized by cervical dislocation and photographed under a stereo microscope and placed on ice. In randomly selected E18.5 normal and mutant fetuses, the intestine was removed from the gastroesophageal junction to the ileocaecal valve. Gut length (cm) and weight (mg) were measured and weight per unit length was calculated. Data are mean ± standard error of the mean and were compared using t-tests. Randomly selected fetuses were genotyped by polymerase chain reaction (PCR) using DNA from tail biopsies and primers as previously described [17].

2.2. In amnio micro-MRI

Phenotypically normal and mutant fetuses were imaged within intact amniotic membranes. Fetuses were imaged at E17.5 when the physiological hernia has resolved but the amniotic fluid volume is still relatively large to aid visualisation of fine intra-amniotic structures. Intra-amniotic fetuses were fixed in 4% paraformaldehyde (PFA) and then embedded in agarose. T2-weighted, high-resolution (256^3 pixels with a 25.6^3 mm field of view; resolution of 100 μm/pixel) micro-MR images were acquired using a 3D fast spin echo sequence. Images were reconstructed using ImageJ